BOTANICAL RADIATION BREEDING READER

Compiled /// Everest Pipkin /// 04.05.16


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In the 1950's and 60's, after the violence and horror of World War II, nuclear technology was at risk of public denouncement and abandonment. Such sentiments were not ill-founded; radiation is immensely damaging to human bodies and terrestrial ecosystems, and is notoriously difficult to control at large capacities. Such a backlash would mean a dead-end to a considerable amount of energy research, however, and few fields were willing to turn their backs on new information that showed promise across so many disciplines. Instead, there came a concentrated effort to develop methods for using radiation and atomic science for goals other than war; the so-called Atoms for Peace project.

One small, ill-remembered branch of this effort was atomic gardening. The idea was to bombard growing plants with radiation in an attempt to induce mutations, perhaps some of which would be useful or interesting as curiosities for the home gardener.

Radiation gardens were arranged in circular patterns, with wedges of single species extending outwards from a center. In this center was a lead-lined container, in which radioactive materials were housed on rods. These rods could be raised and lowered to expose plants and (hopefully) spare gardeners and researchers the dose of gamma radiation. Plants closest to the radioactive source generally died; the next wedge sported strange tumors and unhealthy growths; those furtherest seemed generally unchanged. There was, however, a small circle of plants that generally exhibited interesting behavior, in the form of odd coloration, differing growth habits, and sometimes extremely useful pest resistance. In short, despite the apparent slap-dash nature of such experiments, it proved fairly successful. This method was rewarded with many viable mutations, several of which are still economically important today- notably, most of the world's peppermint (which developed a fungal resistance), as well as Rio Star grapefruit, known for its red flesh (pre-mutation grapefruits were yellow or white).

In 1956, one could go to garden fair and buy 'atom-blasted' starts, or one could order 'atomically strengthened' seeds from the back of a catalog. The Atomic Gardening Society, started in England by Muriel Howorth, was a community effort at producing new varietals. A retired dentist, C.J. Speas, also produced seeds commercially, in a cinderblock bunker with Cobalt-60. There is limited evidence to suggest that production efforts extended beyond these two commercial capacities, in the form of science fair projects and home-experiments from gardening enthusiasts in many countries. Such varieties (and their off-spring) were poorly documented, and it is not unlikely that there are many plants produced in such capacities still thriving in gardens and the wild; perhaps labeled as an heirloom varietal due to odd growing habits.

Atomic gardening was largely abandoned in the mid 1970s, both due to concerns about exposure and the rise of genetic modification as a commercially viable practice. But there are thousands of known plants produced with this method, and the list grows every year via small-scale experiments by curiosity

breeders who cannot afford to genetically modify plants. Contemporary mutagenesis via chemical exposure or irradiation is also still in practice in areas where GMO foods are rejected by the preferences of a local populace; mutated foods require no additional labeling.

Such historical precedent for new technology is, perhaps, most useful as a lens with which to view our current relationship with biological science. So often we think we hold a scalpel, while in retrospect it is clearly only a butterknife.

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Radiation Induced Mutations for Plant Selection

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The successful use of plant breeding for improving crops requires the existence of genetic variation of useful traits. Unfortunately, the desired variation is often lacking. However, radiation can be used to induce mutations and thereby generate genetic variation from which desired mutants may be selected.

Mutation induction has become a proven way of creating variation within a crop variety. It offers the possibility of inducing desired attributes that either cannot be expressed in nature or have been lost during evolution. More than 1700 mutant cultivars of crop plants with significantly improved attributes such as increased yield, improved quality, disease and stress resistance, have been released worldwide in the last 30 yr. The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has contributed to these achievements through the promotion of research and development in mutation breeding techniques using nuclear and related biotechnological methods. Nuclear technology in plant breeding is then transferred to Member States of the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations through training in mutation breeding methods and the provision of technical advice. Moreover, radiation treatment services are provided to foster applications of nuclear techniques in crop improvement programmes of Member States and more specifically to render direct support to plant breeders by efficient generation of mutations. Plant materials are standardized prior to radiation exposure (usually at $\pm 5\%$ precision in absorbed dose) to warrant reproducibility of the induced effects within practical limits and a radiosensitivity test is implemented to affirm useful doses for applied objectives of a request.

1. Introduction

Genetic diversity among plants in a population is a basic prerequisite for successful plant breeding. Natural genetic variation has been used for a long time as a raw material in plant breeding. Such variation is the product of spontaneous mutation and hybridization, followed by recombination and natural selection. Hybridization has long been used to recombine characters and to provide desired genetic variation for selection. Mutations, however, are only recognized since this century as one of the driving forces of evolution.

Since the discovery of Muller and Stadler that ionizing radiations can induce hereditary alterations and thereby enhance the frequency of mutations many times over the one occurring spontaneously in nature, the breeder is no longer limited to the availability of natural mutations. Initial attempts to induce mutations in plants mostly used x-rays, later more and more γ-rays and also fast and thermal neutrons were used. During this initial phase of mutation induction there was a disappointment about the high detrimental effects of ionizing radiation and the low frequency of valuable mutations; major efforts were devoted to define optimal and reproducible treatment conditions. Research focused on changing "random" mutation induction into directed mutagenesis to obtain more desirable mutations. However, it did not lead to the desired alterations in the mutant spectrum but rather to an improved methodology of mutation induction.

There is no basic qualitative difference between spontaneous and induced mutations. We can induce any mutation that has occurred in nature and may have been lost during evolution. A particular advantage of mutation induction is the possibility of obtaining unselected genetic variation, whereas the available germplasm has already passed effective selection screens by nature or man. Neither natural nor man-made germplasm contain all the possible spontaneous mutations or recombinants. We can then, with appropriate techniques induce and select those mutants suitable for modern agricultural systems rather than being dependent upon those that have survived evolutionary stresses. Mutations can be induced in any gene though at different probabilities. There is sufficient evidence that induced mutations fit Vavilov's law of homologous genetic variation which predicts whether a particular mutation can be expected. Another matter is, of course, whether every

Table 1. Requirements of dosimeter systems for dose assessment in biological objects (e.g. in radiobiology, radiation breeding)

(i) The assessable dose range should be between 10^2 and 10^5 rad or 1–1000 Gy
(ii) The dose measured should be energy independent between 0.3 and 3 MeV
(iii) The precision of measurement should be at least $\pm 5\%$
(iv) The dosimeter system applied should exert a high stability before radiation exposure
(v) After radiation exposure, the measured dose shall maximally change within 24 h by 1%, i.e. the fading should be less than 1%
(vi) Temperature independence should be warranted between 10 and 50°C

A simple and desirable utilization depends on the
(1) Availability of materials and supplies for dosimetry
(2) Availability of functioning measuring devices and/or instruments
(3) Simple production of the dosimeter system of concern
(4) Simple measurement, evaluation and interpretation of data

Useful dosimeter systems can be either:

(1) Liquid systems — inorganic / organic
(2) Solid systems — inorganic (Glass dosimeters) / organic (Polymers)
or (3) Gaseous systems

mutation will express itself phenotypically that it can be selected and eventually used in plant breeding.

2. Efficient Mutation Induction

Appropriate methods for the creation of genetic variation must aim at a high mutagenic efficiency, i.e. a maximum number of desired mutations within a given population size. The mutation effect shall therefore surpass damage effects such as gross physiological aberrations which reduce vitality and may indirectly decrease the number of induced mutations. This has a high impact for mutation breeding and its comparative economic feasibility with other breeding methods (Brunner, 1991).

The dose to be applied for obtaining a high mutagenic efficiency generally depends on the specific properties of the radiation type and the radiation facility characteristics as well as on the biological system to be treated. Hence, dosimetry data have to be established prior to radiation exposure of biological targets to define dose rate and dose distribution and to monitor the exposure time required to obtain an accurate estimate for the dose absorbed in biological materials. Dosimetry for hard x-rays and γ-radiation may be based upon the Fricke dosimeter system (Fricke and Hart, 1966), on ionization chambers and/or on commercially available thermoluminescence detectors (TLD 100 or 700), neutron dosimetry on monitoring systems described in Neutron Irradiation of Seeds (IAEA, 1967, 1968, 1972). General requirements of dosimetry systems for doses applied in radiobiology and mutation breeding are compiled in Table 1 and the sequence of steps required for efficient mutation induction is outlined in Table 2. Among plant objects to be treated are dry, dormant seeds, bulbs, corms, tubers, scions, cuttings, gametophytes and zygotes, pollen grains and tissue or cells in culture ('*in vitro* materials'). Dry, dormant seeds are the most commonly used objects for mutation

Table 2. Steps involved in dose assessment of crop species for mutation breeding

A. Radiation source characteristics	
High or low LET radiation	
Energy distribution	
Degree of contamination with other radiations	
Dose gradients, requirements of dose homogeneity, isodose lines	
Methods to monitor radiation dose and/or dose rate:	
(i) Physical →	Ionization chambers, threshold detectors, etc.
(ii) Chemical →	Determination of ionic (chemical) yield, e.g. Fricke
(iii) Biological →	Determination of an index of primary damage, e.g. seedling height, epicotyl length in comparison with nonirradiated control materials
B. Characteristics of the biological target	
Seeds	Whole plants
Pollen grains	Vegetative organs
Gametophytes and zygotes	Cells and/or tissue in culture ('*in vitro* biotechnology')
Criteria of radiosensitivity	
Biological factors, environmental factors, etc.	
C. Prediction of dose effects	
Early assessable criteria of primary damage in e.g. seedling height of the first leaf, epicotyl length, etc. and their correlation to mutation frequency in M_2, e.g. usually to chlorophyll indicator mutations.	

Table 3. M_1 and M_2 results in *Vicia faba* cv. Wieselburger

Treatment	M_1 results (resp. control = 100)				Frequency of M_2 mutants (‰)					
					Chlorophyll mutants		Morphological mutants field	Morphologic: chlorophyll mutants[2]	Morphologic mutants: non-damage $\times 10^{-2}$	Morphologic mutants × non-damage $\times 10^{-4}$
	Seedling height	Survival	Fertility	Non-damage[1]	Greenhouse	Field				
γ-rays										
0 Gy	100	100	100	100	0.2	0.9	1.8	—	—	—
20	96.5	89.2	92.9	82.9	2.0	5.9	14.0	2.4	1.69	116.1
30	90.3	81.9	74.9	61.3	2.6	7.0	18.0	2.6	2.94	110.3
40	89.9	70.4	62.5	44.0	4.8	8.6	25.6	3.0	5.82	112.6
50	75.2	48.5	59.4	28.8	5.6	13.6	29.3	2.2	10.17	84.4
60	66.8	29.8	50.0	14.9	7.9	13.8	23.8	1.7	15.97	35.5
70	58.6	14.9	38.7	5.8	—	—	—	—	—	—
n_f										
0 Gy	100	100	100	100	0	0.2	2.4	—	—	—
1.0	89.9	87.6	85.1	74.5	4.1	7.5	8.6	1.2	1.15	64.3
1.5	80.2	74.5	76.1	56.7	5.0	9.0	13.5	1.5	2.38	76.5
2.0	72.3	60.4	60.0	36.2	8.2	9.2	14.9	1.6	4.12	53.9
2.5	63.3	48.9	59.3	29.0	8.7	11.3	18.7	1.6	6.45	54.4
3.0	57.4	35.4	52.2	18.5	14.1	14.0	29.0	2.1	15.68	53.6
3.5	47.2	24.7	46.0	11.4	18.0	15.0	32.5	2.2	28.51	37.0
4.0	42.5	15.7	41.3	6.5	16.3	11.7	17.8	1.5	27.38	11.6
4.5	35.2	7.2	34.4	2.5	15.5	11.0	16.5	1.5	66.00	4.1

[1]Survival × fertility × 10^{-2}. [2]Field data.

induction; they contain, in the genetically relevant seed embryos, largely synchronized cell initials in the G_1-phase (the DNA presynthetic gap 1 during interphase). Moreover, a seed moisture equilibration over 60% glycerol to 12–14% minimizes the effects of modifying factors to low LET radiation and warrants reproducibility of parameters of primary damage within practical limits. Since cell initials of metabolically active organs and tissue are commonly asynchronous, the induced radiation effects are frequently not reproducible. Differences in radiosensitivity between and within species can be relatively great though intraspecific or varietal differences are usually smaller than between species (Brunner, 1977). A mutation breeder shall therefore conduct preliminary dose response experiments of a particular cultivar which are to be based on radiosensitivity data valid for the species. Each genotype shall be tested for the optimal treatment within its range of conditions. Dose effects can be predicted by measuring early assessable criteria of primary injury at defined endpoints of initial growth, e.g. relative seedling height of the primary leaf of irradiated seeds compared with nontreated controls in monocots and epicotyl length in dicotyledonous species. These early assessable M_1-criteria of primary damage correlate well with viability, survival and/or sterility. Moreover, the correlation between parameters of M_1-injury with mutation frequency in the M_2-generation, e.g. of chlorophyll indicator mutations, permits an estimate of useful radiation doses for different breeding objectives (M_1 and M_2 refer to the first and second mutation generation, respectively).

Table 3 summarizes M_1 and M_2 data of a mutation breeding experiment with the field bean cultivar Wieselburger. It is clearly shown that M_1 parameters as seedling height, survival and fertility decrease with increasing doses of γ and fast neutron radiation while chlorophyll and morphological mutant frequencies in segregating M_2-populations increase up to a maximum and decrease thereafter due to M_1 injury. Three different indices were established to obtain information on the usefulness of different types and doses of radiations for mutation breeding objectives. The ratio of morphological to chlorophyll mutants reflects Vavilov's law of parallel variability implying that chlorophyll indicator mutations are useful for predicting morphological mutations. The ratio of morphological mutants to nondamage refers to mutagenic effectiveness, the potential of any mutagenic agent to induce mutations irrespective of damage. Since mutation frequency increases linearly at low doses and exponentially at high doses concomitantly with damage, many drastic mutations are detrimental due to the occurrence of multiple mutations and are not useful for plant breeding. The index of morphological mutants times nondamage is an estimate for mutagenic efficiency, the production of desirable changes free from association with nondesirable effects (Konzak *et al.*, 1965). Mutagenic efficiency is usually highest at doses above 30% nondamage. Useful doses for most breeding objectives are estimated by about 25% seedling height reduction at defined endpoints of growth in the greenhouse and 50–60% survival in the field. Mutation breeders should therefore apply doses that generate optimal and not maximal mutation frequencies to achieve a high frequency of useful mutations and minimize the occurrence of drastic and nondesired mutations (Konzak, 1984).

3. Mutation Induction in Vegetatively Propagated Plants (VPP)

Many VPP are perennial plants with complicated physiology (e.g. dormancy, seasonal cycles) and complex genetics (e.g. high degree of heterozygosity, selfincompatible, polyploid, aneuploid, apomictic).

Spontaneous mutations—"sports"—played an essential role in the breeding of new cultivars. Their low-frequency limits effective breeding since the breeding process is accidental and more extensive compared with seed propagated plants (Abbot and Atkin, 1987). Application of radiation enhances drastically the frequency of somatic mutations from which useful traits may be selected. A mutation is a one cell event but multicellular apices generally consist of a number of rather autonomous groups of cell layers such as L_1 (epidermis), L_2 (subepidermis) in the so-called tunica and L_3 in the corpus and have a number of meristematic cells in each layer. Mutagenesis applied to multicellular structures like buds gives rise to mericlinal or sectorial chimeras. However, homohistont shoots can be obtained after several propagation cycles of axillary buds. Irradiation of apical promeristems and high doses increase the probability of occurrence of large mutated sectors and irradiation of adventitious buds that are derived from single, epidermal cells generates homohistont mutants (Broertjes and Van Harten, 1988). Vegetative single cell descending propagules offer a possibility for early screening and fast propagation of mutants for the breeding of commercially improved cultivars.

Difficulties may be due to the isolation of somatic mutations from small and phenotypically not identifiable mericlinal or sectorial chimeras and when the mutated sector comprises few cell layers only. Vast numbers of somatic mutations in layers L_1 and L_3 are lost during crosses since only primordial cells located in the generative L_2 histogen participate in the formation of reproductive organs.

4. *In Vitro* Mutagenesis

Any researcher who wishes to apply *in vitro* mutation breeding has to adapt a general procedure to accommodate particular requirements or characteristics of the plant material and of the treatment methodology towards defined objectives.

Principles of mutation induction *in vivo* apply also to *in vitro*. Cells or tissues can be irradiated either before isolation and explanation or when materials are already in culture. Treatment with high energetic radiations can be done in closed containers, while radiations with low penetration ability must be applied in open vessels and possibly in monolayers to achieve a uniform dose. Irradiation in hormone-free medium followed by a transfer of irradiated *in vitro* material to fresh medium is recommended because of radiation effects on medium components. Other factors such as dose rate, temperature, the stage of cell phase, cell division and development of the genetically relevant cell initials, the influence of modifying factors and the length of recovery period after irradiation must be considered. Generally speaking, irradiation of metabolically active tissue or cells represents a population of asynchronous cells in interphase with different radiosensitivities and reproducibility of the induced effects is usually not warranted. *In vitro* culture in minimal media and/or heat–cold shocks may improve cell synchrony and reproducibility.

Various explants for *in vitro* culture and materials under *in vitro* conditions (e.g. meristems, somatic embryos, calli, cell suspensions, protoplasts) are exposed to different radiation doses of choice and the optimal dose to be applied for a specified objective should be determined after 20 and 40 days *in vitro* culture for assessing fresh or dry matter weight and regeneration ability. As a rule, a useful radiation dose must result in about 30–50% decrease in wet or dry weight compared with non-treated controls though optimal doses depend largely upon breeding objectives.

In case of cell suspension and protoplast culture, where the population density influences the results (e.g. plating efficiency), one must compensate for the lethality induced by the mutagen treatment.

Large populations in *in vitro* materials should be irradiated and a high regeneration ability achieved for the generation of desired genetic variation and selection of a large number of individuals (organs, individuals, cells) under controlled conditions in a small space (Dix, 1990). Mutagenized cell populations must be allowed to undergo a recovery period to "fix" the mutation prior to selection. So far, mutants isolated on a cellular basis from *in vitro* cultured plant material involved mostly biochemical pathways. Their inheritance at the plant level has been cytoplasmic (maternal), dominant, semidominant and recessive (Henke, 1981). Though many advantages could be enumerated of using *in vitro* over *in vivo* techniques, some serious constraints have limited the success for improvement of agronomically important characters. Main reasons are: (i) the technology required to isolate mutants of agricultural importance at the cell level is usually not yet available; (ii) the regeneration potential is frequently rather low; and (iii) mutant traits isolated at the cell level frequently do not express at the plant level (Constantin, 1984). Evidently, knowledge is lacking on gene regulation and gene expression during early stages of ontogenic development to design effective *in vitro* screening procedures for improvement of agronomic traits.

5. Mutation Induction and Selection Methodology

Since the probability of generating desired genetic variation is low, mutation breeding requires induction and screening of large plant populations, which is costly. Research should therefore aim at obtaining many different mutants in a given population size to make mutation breeding more economic. In the 1960s, one thought that this could be achieved by maximal mutation rates. But the disadvantages of

multiple mutations have discouraged the use of high doses since the comparatively small number of beneficial mutations may not be manifested because of a drastic phenotypic effect of a larger number of deleterious mutations (Micke *et al.*, 1990).

Chimera formation is a common phenomenon following irradiation of multicellular cell initials, whether in seeds, buds or tissue culture. Mutation induction in single cells, however, would be ideal to avoid chimeras. If chimerism cannot be avoided, it would be useful to know the chimeric pattern of M_1 plants for a systematic sampling from all mutated sectors of M_1 plants or for a systematic dissolution of chimeric structures in vegetative organs. Selection in seed and vegetatively propagated plant species is mostly based on phenotypic alterations.

Screening under *in vitro* conditions would be advantageous only when the *in vitro* response correlates with the manifestation of the selected characters in the field. Induction methods for *in vitro* generation of enhanced genetic variation with radiation are comparatively simple while *in vitro* selection is still limiting the progress of mutation breeding.

6. Mutagenesis and Biotechnology

In vitro culture provides appreciable advances for plant breeding and specifically for mutation breeding. Micropropagation techniques are already well established and integrated into mutation breeding to accelerate clonal propagation of interesting mutants. Shoot tip culture can additionally provide virus-free propagation. A major problem mostly associated with tissue dedifferentiation during *in vitro* culture is genetic instability often called "somaclonal variation". This genetic variation is disturbing a true to type germplasm preservation and a potential handicap in the use of molecular genetics for plant breeding. Somaclonal variation has gained some importance in practical breeding when used for disease resistance and herbicide tolerance screening (Smith and Chaleff, 1990). Somaclonal changes are unpredictable and uncontrollable and many phenotypic alterations are non-heritable epigenetic changes. Since spectral differences between somaclonal and mutated populations are not high, somaclonal variation will not replace but at best amend the major sources of genetic variation, recombination and induced mutagenesis (Novak *et al.*, 1988). *In vitro* methods yielding large numbers of uniform regenerated plants are to be preferred for mutation breeding programmes. Hence, an unorganized *in vitro* phase (callus, cells and protoplasts) shall be avoided by rapid passage through the meristematic stage (axillary branching and direct formation of somatic embryos or adventitious buds). These stable cultures are not only useful for uniform *in vitro* mutation induction but as well for micropropagation and *in vitro* germplasm preservation; further for mutant screening and genetic confirmation of regenerated plants under field conditions (Novak, 1991).

Haploid techniques may speed up mutation breeding since haploid regenerants derived from anther or microspore cultures of M_1 plants with M_2 gametes might allow a direct selection of mutants without going through the gametophyte phase into the next generation (Szarejko *et al.*, 1991). After chromosome doubling, homozygous mutant lines would increase the efficiency of mutant selection due to a better distinction between mutated and non-mutated M_2 plants.

7. Conclusion

The ultimate aim of mutation induction shall be directed towards procedures yielding the highest possible number of desirable mutations to be used in plant breeding. A concept of optimum mutation frequency should thus replace the concept of maximum mutation rate and a reorientation towards an objective specific dose concept must go along with improved methods of selection. There is no longer a need to prove that radiation can provide useful genetic variation for crop improvement. With the rapid augmentation of technical capabilities in biotechnology and molecular biology, plant breeders will increasingly pay attention to manipulation of individual genes, genome reconstruction and gene mutation in nuclear and extra-nuclear hereditary cell elements. Induced mutations are therefore expected to play in future an even greater role for the success of plant breeding.

References

Abbott A. J. and Atkin R. K. (Eds) (1987) *Improving Vegetatively Propagated Crops*. Academic Press, London.

Brunner H. (1991) Methods of induction of mutations. In *Advances in Plant Breeding* (Mandel A. K., Ganguli P. K. and Banerjee S. P.) (Eds), Vol. 1. CBS, New Delhi, India.

Brunner H. (1977) Radiosensitivity of a number of crop species to gamma and fast neutron radiation. In *Manual on Mutation Breeding*. IAEA, Vienna, TRS 119.

Broertjes C. and Van Harten A. M. (1988) *Applied Mutation Breeding for Vegetatively Propagated Crops*. Elsevier, Amsterdam.

Constantin M. J. (1984) Potential of *in vitro* mutation breeding for the improvement of vegetatively propagated crop plants. IAEA, Vienna, TECDOC-305.

Dix P.J. (Ed.) (1990) *Plant Cell Line Selection*. VCH, Weinheim.

Fricke H. and Hart E. J. (1966) *Chemical Dosimetry, Radiation Dosimetry* (Attix, Roesch, Eds). Academic Press, NY.

FAO/IAEA (1967) *Neutron Irradiation of Seeds I*, TRS 76.

FAO/IAEA (1968) *Neutron Irradiation of Seeds II*, TRS 92.

FAO/IAEA (1972) *Neutron Irradiation of Seeds III*, TRS 141.

Henke R. R. (1981) Selection of biochemical mutants in plant cell cultures: some considerations. *Env. Expl. Bot.* **21**, 347–357.

Konzak C. F., Nilan R. A., Wagner J. and Foster R. J. (1965) Efficient chemical mutagenesis. In *The Use of*

Induced Mutations in Plant Breeding. FAO/IAEA Techn. Meeting Rome, 1964. Pergamon Press, Oxford.

Konzak C. F. (1984) role of induced mutations. In *Crop Breeding, A Contemporary Bases* (Vose P. B. and Blixt S. G., Eds), pp. 216–292. Pergamon Press, Oxford.

Micke A., Donini B. and Maluszynski M. (1990) Induced mutations for crop improvement. *Mutation Breeding Review No. 7.* IAEA, Vienna.

Novak F. J., Daskalov S., Brunner H., Nesticky M., Afza R., Dolezelova M., Lucretti S., Herichova A. and Hermelin T. (1988) Somatic embryogenesis in maize and comparison of genetic variability induced by gamma radiation and tissue culture techniques. *Plant Breeding* **101,** 66–79.

Novak F. J. (1991) Mutation breeding by using tissue culture techniques. In *The 30th Gamma-Field Symp "Biotechnology and Mutation Breeding"*. Inst. of Radiation Breeding, Ibaraki-ken, Japan.

Smith W. and Chaleff R. S. (1990) Herbicide resistance. In *Plant Cell Line Selection* (Dix P.J., Ed.). pp. 151–166. VCH, Weinheim.

Szarejko I., Maluszynski M., Polok K. and Kilian A. (1991) Double haploids in the mutation breeding of selected crops. In *Plant Mutation Breeding for Crop Improvement*, Vol. 2, pp. 355–378. IAEA, Vienna.

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Efects of the treatments with x rays to *Arachis hypogaea* L. in m2 generation in the conditions from Tamburesti Research Station

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Abstract The experience consists in the repeated irradiation treatment of two different groundnut variety seeds with X rays, in doses of 5000-10000R. There were made observations and determinations on various morphological and quantitative characters for M2 generation. From the sow until rising, flowering and maturity the periods were not much affected. For the morphological characters both genotypes showed differential response to the doses of treatment with mutagens.

Significantly reduced height was observed both in irradiated variants and control. Lower doses of treatment with X rays (6000R) proved effective in increasing the number of ramifications to Tamburesti variety. Significant increase in number of ramifications was observed in all variants of treatments to Venus variety. Significant reduction in number of pods/plant was observed in 9000 R dose to Tamburesti variety and 8000R dose to Venus variety. Number of matured pods/plant was found decreased in 5000, 6000 and 9000R dose to Tamburesti variety and 8000-10000R to Venus variety. Seed yield was found decreased in all variants of treatments to both varieties, but one seed mass was found increased in all doses of irradiation.
In the present investigation the treatment with rays in doses of 5000-6000R and even 8000-9000R were found more effective for inducing wide range of mutation in Tamburesti groundnut variety compared to Venus variety.

Key words

X rays treatment, doses of irradiation, groundnut varieties, morphological and quantitative characters, mutation

Mutation induction and some other means of genetic modification, such as genetic transformation, are tools that provide variation in some of the plant characters outlined and hopefully lead to acceleration of domestication. The last 30 years have shown mutations to becoming a useful supplementary tool for the genetic improvement of cultivated plants; the FAO/IAEA Mutant Varieties Database contains over 1737 accessions. The majority of mutant varieties belong to the cereals, although successes have been recorded in the legumes, vegetativelly propagated crops and ornamentals as well (2).

Important methods to artificially induce mutations are the use of chemical and physical agents. Physical mutagens include electromagnetic radiation, such as gamma rays, X rays and UV light and particle radiation such as fast and thermal neutrons, ß and alfa particles.

Mutagenic treatment of seeds is the most convenient because seeds can be treated in large quantities and are easily handled, stored and shipped.

Radiation and chemical mutagenesis were used widely for producing useful mutants with improved characteristics in peanut and many crops (3).

Physical and/or chemical mutagens cause random changes in the nuclear DNA or cytoplasmic organelles, resulting in gene, chromosomal or genomic mutations. Induced mutagenesis is an established method for plant improvement, whereby plant genes are altered by treating seeds or other plant parts with chemical or physical mutagens. Voluminous work has been done worldwide for the improvement of both seed and vegetativelly propagated crops through induced mutation.

These results and those available elsewhere in the literature, clearly show that mutation by using both physical and chemical mutagens has successfully produced quite a large number of new and promising varieties in different seeds and ornamental plants, and is considered to be a most successful tool for breeding ornamental plants (1).

The mutagenic efficiency of physical mutagen depends not only on the properties of the physical agent, but also on the genotype. Published data indicate that different species and even cultivars may respond differently.

Matherial and Method

The experimental material comprises the seed of two different groundnut genotypes obtained in Tamburesti R.S. in different years and different methods. Matured and well dried seeds were used for each dose treatment. The seeds were irradiated with 5000, 6000, 7000, 8000, 9000 and 1000 R dose of X rays at Electroputere Craiova Company.

These iradiated seeds were immediately sown along with one control after treatment on sandy soils from Tamburesti R.S in 2010. From these seeds raised M1 generation. The M1 plants were observed and determined, then harvested, well dried and kept in good conditions. In the spring of 2011 there was made a irradiation with the same doses of the seeds and so raised the M2 generation. Sowing was done in randomized blocks method in four repetitions. The M1 generation was irrigated, not the M2 generation.

The observations and determinations were recorded for many morphological and quantitative characters like days to first flowering, days to maturity, plants height number of branches/plant, number of immature pods/plant, number of mature pods/plant, pod yield, 1000 pods mass (g), 1000 seed mass (g).

Obtained Results

In mutation breeding programme the breeders are more interested in the extent of variability, which is more reflected by the mean between irradiated variants and control.

During vegetation period it were analyzed the variations and other morphological deviations from the normal type. Because the quantity of rainfall was reduced and there was not applied irrigation and the atmospheric temperatures were high from August to harvest in the first days of October, the majority of plants dried and so it was lost the different effect of the mutations spectrum. It could also establish range variability as concern port plants, their height, leaves form in the first part of vegetation. Lethality degree of the plants from the irradiated variants was very low differentiated from control and the frequency of lethal plants was low. Plants fertility was close to all irradiated variants comparative with control.

The length of the vegetation period of the plants from M2 was much influenced by the high temperatures from the August to September. These leaded to the drying of the plants and because of that also the plants height was reduced and even port plants and behavior; it was also influenced the number of ramifications, this being small. To harvest it were analyzed 600 plants from M2 generation, without including the whole material.

The plants situated in two groups as concern the plants height, such as:

- First group included 80-90% plants with small stems, erect behavior and reduced degree of ramification;
- Second group included 24.4% from the total of analyzed plants, plants with middle height, crawling port and higher degree of ramifications.

From the total analyzed plants, 7.2% were forms with total lack of pods (sterile plants), most of them with reduced degree of ramification and low height.

Many researchers consider that the "*mutants*" with lower productivity and vitality are helpful if presents a new character which compensate the decrease of productivity. They refer to some issues which concern the resistance to drought, diseases, shorter period of vegetation.

To the level of the variants irradiated with the different doses of X rays it was establish, that there are earlier variants, with difference of 2-7 days comparative with initially variant, un-irradiated only to Tamburesti variety. To Venus variety the treatment with X rays presented an opposite effect, prolonging the vegetation period with 3-9 days. It did not been observed *mutations* referring to pods color and not even special forms, the only variations representing in the number of seed (1-4). Seed color was a little changed, becoming from the plants from M2 generation brighter, but only in Tamburesti variety.

From the productivity elements point of view, plants from M2 generation presented a higher variability, from plants complete sterile until those with similar productivity as control. If the average number of total pods/plant from irradiated variants was lower than control (7.20 in 9000R dose comparative with 14.20 in control), the weight of one thousand seed mass of the plants from M2 increased comparative with control. So, in control variants, weight of 1000 seed mass varied between 470.3g in 5000R dose and 510.0g in 8000R dose (Ct. = 460.3.) to Tamburesti variety. In irradiated Venus variety variants, total number of pods/plant varied between 8.30 in 8000R dose and 12.70 in 5000R dose (Ct. = 11.10) while the weight of one thousand seed mass varied between 694.8g in 5000R dose and 743.0g in 9000R dose (Ct. = 694.4) (table 1). The changes referring to color, form and seed size was accentuated. Because of that it was easily recognized. Leading from these seeds, in the future we want to analyze the offspring in the next generations.

Chemical composition of the seeds from M2 generation varied in large limits, to both varieties, especially as concern protein content. It is notable the fact that protein percent from the plants from M2 generation increased with 1.4-6.8% and fats content decreased with values until 2.5%.

Choosing the plants and seeds from M2 generation was made with the aim to fallow the offspring with breeding interest.

Table 1

Effect of X rays on groundnut characters in M2 generation of irradiation (2011)

Tamburesti variety							
Character	Ct.	5000R	6000R	7000R	8000R	9000R	10000R
Days to first flowering	40	41	38	38	37	36	40
Days to maturity	147	147	143	145	142	140	150
Plants height (cm)	39.65 32-49	40.10 25.5-48	40.15 26.5-50	33.95 29-38	39.25 27-48	32.60 20-40	33.60 28-52
No. of ramifications	5.60 5-7	5.50 5-8	6.20 4-12	5.70 5-7	5.10 3-6	5.30 3-7	5.20 2-7
No. of mature pods/plant	6.10 4-8	5.30 4-7	5.70 3-12	6.10 4-12	6.20 4-11	4.60 3-4	6.40 4-11
Total no. of pods/plant	14.20 12-18	10.30 5-20	9.40 2-20	9.20 5-15	8.00 6-11	7.20 3-11	8.50 5-19
Yield (Kg/ha)	1937	1575	1425	1300	1100	1072	1287
1000 seed mass (g)	460.3	470.8	475.7	471.0	510.0	498.0	492.0
Venus variety							
Character	Ct.	5000R	6000R	7000R	8000R	9000R	10000R
Days to first flowering	40	41	38	38	37	36	40
Days to maturity	147	147	150	154	154	156	156
Plants height (cm)	26.25 20-30	26.70 20-36	28.75 18-50	27.40 17-39	25.40 20-39	29.20 24-36	26.60 20-37
No. of ramifications	4.90 5-12	9.10 6-13	6.90 5-15	9.10 5-12	9.80 5-14	8.40 5-12	9.20 5-14
No. of mature pods/plant	5.50 3-12	9.10 4-20	10.70 4-20	10.70 3-24	4.20 2-9	7.00 4-18	6.00 1-10
Total no. of pods/plant	11.10 3-18	12.70 7-24	11.00 6-28	16.10 5-28	8.30 5-13	10.80 4-34	10.90 5-16
Yield (Kg/ha)	1837	1950	1800	2125	1400	1625	1687
1000 seed mass (g)	694.4	694.8	717.9	725.0	736.0	743.0	706.2

Fig.1 Plant of Tamburesti variety (first stage of vegetation)

Fig.2 Plant of Venus variety (small stems) (6000R)

Fig.3 Tamburesti plant and pods (6000R)

Fig.4 Dried plant of Venus variety (9000R)

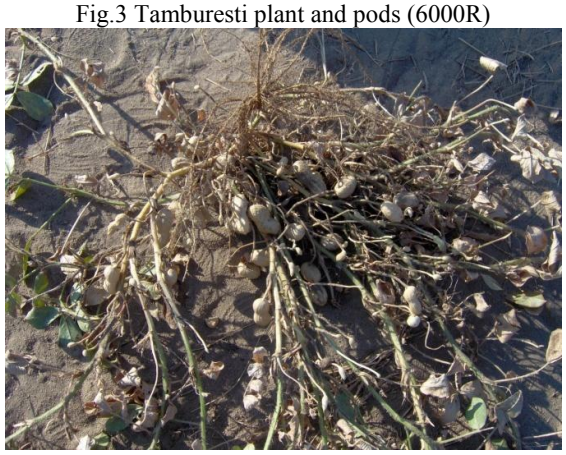

Fig.5 M2 plant with middle height, crawling port and higher degree of ramifications

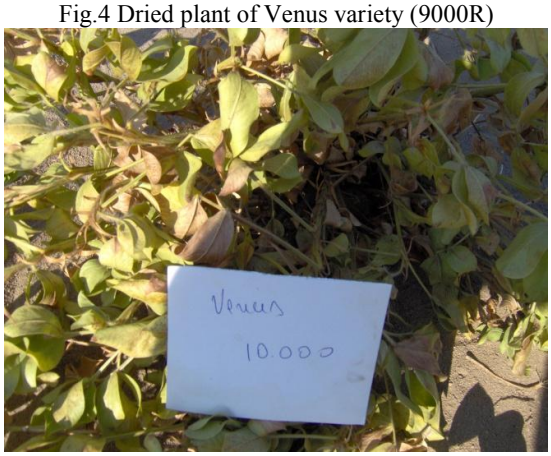

Fig. 6 Plant of Venus variety (longer vegetation period) (10000R)

Conclusions

From the results of the mutagen effect study of X rays upon the groundnut varieties (a small seeded and a large seeded varieties), first it can conclude that the indications from the literature referent to DL 50% for groundnut must be considered as a guide, being strictly dependent of earth-climatic conditions and working variety.

In this experience, even using the dose of 10000R it did not establish DL 50%. The behavior of M2 material was dependent of irradiation dose and research conditions. So, Tamburesti variety proved to be more sensible, comparative with Venus variety, at least in the first period of vegetation.

Risen and number of plants reached to maturity were low inhibited to all doses of irradiation. In M2 generation plants risen was positively stimulated, comparative with M1 generation where was registered a lower percent of risen plants mentioning also that rapport of dependence between the dose of mutagen agent and his effect upon rise as in M1 generation. The problem of mutation supervision, respectively the possibility of establishing some correlations between dose of irradiation and the spectrum of mutations caused are still unsolved.

Acknowledgement

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References

1.Andrea Kodym, 2003 – Physical and chemical mutagenesis. In: Plant Functional Genomics. Methods in Molecular Biology. Vol. 236, III 189-203, DOI:10.1385/1-59259-413-1:189

2.Javed, M.A., Khatri, A., Khan, I.A., Ahmad, M., Siddiqui, M.A., Arain, A.G., 2000 - Utilization of gamma irradiation for the genetics improvement of oriental mustard (Brassica juncea Coss.). Pakstan J. Bot. 32: 77-83.

3.Rehman, A., Das, M.L., Howlidar, M.A.R., Mansur, M.A., 1987 - Promising mutants in Brassica campestris. Mutation Breeding Newsletter. 29: 14-15.

Mutations Caused by X-Rays
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it on with a bit of cotton or soft cloth, and the poison will be oxidized and destroyed. This treatment leaves a brown stain on the skin, which can easily be removed in any one of a number of ways. A one per cent. solution of oxalic acid, Dr. Couch says, is the quickest means. But oxalic acid is a poison, so that if you are afraid of children getting hold of it you may use instead a one per cent. solution of sodium bisulphite, or even just plain soap and water, though the latter is a bit slow in taking off the stain. If the skin has been very much broken by scratching or otherwise and is raw, the oxalic acid will cause a temporary stinging and soap and water is preferable for removing stains from such sensitive surfaces. If the skin is very tender the solution of potassium permanganate may be diluted with water before using.

The permanganate treatment is recommended only as a remedy for poisoning that has already taken place. Persons who know that they are likely to be poisoned may prevent the plant from harming them with a wash devised by Dr. James B. McNair, of the Field Museum, Chicago. This consists of a five per cent. solution of ferric chloride in a fifty-fifty mixture of water and glycerin. This is to be washed on all exposed parts of the skin and allowed to dry there, before going where the dangerous weeds grow. The iron in the chemical combines with the poisonous principle of the ivy and changes it into a harmless, non-poisonous compound.

MUTATIONS CAUSED BY X-RAYS

THE rate at which breeds of animals and plants can be improved will be speeded up over a hundred times if the findings made by Professor H. J. Muller, of the University of Texas, on tiny fruit flies, holds true for other living things. It has been proved in his experiments, carried on at Austin, Texas, during the past nine months, and just reported in *Science*, that in the flies X-rays affect the little particles responsible for heredity in much the same way as a shot-gun fired at a pile of pebbles would affect the pebbles. The hereditary particles become permanently transformed in all sorts of unexpected ways—the changes known as "mutations" are produced in them. Not all of them mutate at once, but here one, there another, in quite a random fashion, and sometimes, too, they are dislodged into new arrangements. Since these hereditary particles, which are known as "genes," are handed down from parent to offspring, and determine the characteristics of the next and later generations, all kinds of new traits are likely to arise among a group of offspring or grand-offspring from parent flies that were treated with X-rays. These new traits are permanent, as they are inherited by succeeding generations.

It has long been known that such "mutations" occasionally happen without X-ray treatment, and so give a chance for the breeder to improve his stock, by breeding from animals that have desirable mutations. In the same way in nature, the "survival of the fittest" mutations is thought to have brought about evolution. But the mutations that happen without X-ray treatment are exceedingly rare and it has never previously been found possible to make them occur oftener. That is why animal and plant improvement has been so slow, and why it has been necessary to raise countless thousands of ordinary individuals for each advantageous mutation that has turned up. Now, if mutations can be produced at will, all this will be changed.

It is true that, even if X-rays can do in cattle and cotton what they do in flies, the kind of mutation that will be produced can not be specified in advance, any more than this could be done in the past. Many different kinds of changes

will be produced, in a hit-or-miss fashion, and the great majority of these will consist of derangements, deformities, and even fatal weaknesses. But this is also true of the mutations that occur naturally. The breeder then has to select from among all these changes the few that happen to be to his advantage, and breed from them only. There is no reason to believe that the number of desirable changes, in comparison with a given number of harmful ones, would be any less after X-rays than naturally. In fact, in the flies, many of the X-ray mutations look just like the natural ones. So the practical effect of the treatment, in producing over a hundred times as many mutations of all kinds in a given number of individuals, would really be to make unnecessary over 99 per cent. of the breeding of ordinary individuals that now has to be carried on before each mutation of a desired type is found.

While it seems a far cry from flies to four-footed beasts, it has always been found in the past that the principles of heredity found in the flies apply also to plants and to higher animals, including man himself. This raises very acutely another question, one that has at times in the past few years been debated among X-ray practitioners—whether, in treating their patients, they are not sometimes producing mutations that may crop up in future generations. If so, some of our medical practices will have to be modified, for there is no doubt that in man, as in flies, most of the mutations produced would be of a detrimental kind.

To scientists, the most interesting aspect of the work will probably be the insight which it may give us into the causes of evolution and into the nature of the little genes themselves. In fact, certain conclusions regarding the structure of genes have been drawn from the work that has already been done. These will be presented by Dr. Muller in a technical paper before the International Genetics Congress, to be held in Berlin this September.

HAFNIUM

THE story of the discovery of helium has been told so often that it is commonplace. The story of hafnium is more recent, and probably less familiar, although not less romantic. It begins with the relation between atomic number and frequency of Röntgen rays, discovered in 1913 by Moseley (England), which definitely fixed the number of possible chemical elements between barium and tantalum as 16 (atomic numbers 57 to 72 inclusive). All but two of these had been identified by the time this law was announced—the unknown elements could only be referred to by their atomic numbers 61 and 72.

In 1878 Marignac (Switzerland) separated from erbium a new earth which he called ytterbium. Auer von Welsbach (Austria) in 1905 found that ytterbium was a mixture of two elements; he proposed the names aldebaranium and cassiopeium. Urbain (France) independently made the same discovery and proposed the names neo-ytterbium and lutecium. Later Röntgen-ray investigations indicated that the atomic numbers of these new elements were 70 and 71. It was then conceivable that the hypothetical element 72 might be present in the final mother liquor from which 70 and 71 were obtained, and both Auer von Welsbach and Urbain looked for it. The former did not find it, but the latter thought he did and announced in 1911 the discovery of a new rare earth which he called celtium.

Eleven years later the quantum theory and explanation of the periodic system of chemical elements was sufficiently developed by Bohr (Denmark) to define very sharply the character of element

two parental types.

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[1] Ingram, V. M., *Biochim. et Biophys. Acta*, **28**, 539 (1958).
[2] Ingram, V. M., *Nature*, **183**, 1795 (1959).
[3] Itano, H. A., and S. J. Singer, these PROCEEDINGS, **44**, 522 (1958).
[4] Hill, R. L., and H. C. Schwartz, *Nature*, **184**, 641 (1959).
[5] Smithies, O., *Biochem. J.*, **61**, 629 (1955).
[6] Markert, C. L., and R. L. Hunter, *J. Histochem. Cytochem.*, **7**, 42 (1959).
[7] Schwartz, D., *Genetics* (in press).
[8] Catcheside, D. G., and A. Overton, *Cold Spring Harbor Symposia Quant. Biol.*, **23**, 137 (1958).
[9] Fincham, J. R. S., *J. Gen. Microbiol.*, **21**, 600 (1959).

*DIVERSE RATIOS OF MUTATIONS TO CHROMOSOME ABERRATIONS IN BARLEY TREATED WITH DIETHYL SULFATE AND GAMMA RAYS**

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The present report demonstrates that a solution prepared by adding diethyl sulfate to water (referred to as diethyl sulfate) induces a high frequency of mutations associated with relatively few chromosomal aberrations. These properties are quite unlike those previously shown for other alkylating agents and radiation.[1]

The mutagenic activity of diethyl sulfate on *Drosophila melanogaster* larvae was reported in 1947 by Rapoport,[2] and was shown recently[3, 4] to produce in barley a slightly higher frequency of mutations than Xrays. By providing different treatment conditions we have observed that the induced mutation frequency for diethyl sulfate may be more than double that previously reported.[3, 4]

Materials and Methods.—Resting barley seeds (*Hordeum vulgare* $2n = 14$, var. Himalaya C.I. 620) selected for uniformity of size and freedom from injury, were stored over a saturated solution of $NH_4Cl + KNO_3$ in a desiccator to stabilize their moisture content. The seeds contained approximately 15 per cent moisture at the time of treatment.

Seeds were treated by immersing them in saturated solutions of diethyl sulfate. The saturated solutions were prepared using 15 ml of diethyl sulfate per liter of oxygen-saturated distilled water at 30°C. The water was saturated with oxygen to assure repeatability of conditions. Recent work, however, has indicated that this factor is not important. After agitating the mixture frequently during a 90-min hydrolysis period, 100 ml aliquots were pipetted into 250-ml Erlenmeyer flasks containing approximately 260 seeds. Six replicates of seeds were immersed for 1-, $1^1/_2$-, and 2-hr treatment periods, then rinsed with distilled water and planted immediately on moist blotting paper. Each replication included: 50 seeds for an analysis of seedling injury; 200 seeds that were germinated 24 hr over moist filter paper, then sown in the field 2-in. apart in 40-ft rows for survival, fertility, and mutation studies; and 10 seeds for cytological examination.

Seedling injury, one criterion of the effect of treatment, was measured in terms of the relative height of treated and control seedlings. Six replicates of 50 seedlings each were grown 7 days on moist filter paper in petri dishes under 400 ft-c of cool white fluorescent light. The temperature during the growth period varied between 20° and 27°C.

In the survival, fertility, and mutation studies, plant survival was determined from the number of plants harvested in proportion to the number of seeds sown for both the chemical and radiation experiments. Fertility was determined from counts of filled and empty florets of spikes from 100 M_1 plants in each of 2 replications of the chemical experiment. In the mutation study, up to 5 of the most mature spikes were harvested from each M_1 plant; thus, presumably all of the spike primordia present at the time of seed treatment were included in the analysis. The spikes from the M_1 plants were laid in steamed-washed sand in a lightly-shaded greenhouse maintained at 18° to 22°C during the early winter of 1959–1960. Each greenhouse bench contained spikes from all treatments of a replication. This design minimized the influence of environment as a variable in the analysis.

The mutation analysis included only chlorophyll-deficient mutations. These mutants were recorded in the seedling stage according to the system of Stadler[5] and Gustafsson,[6] and matched with actual key-type samples. Mutant and non-mutant seedlings were recorded for each plant and each spike, thereby providing mutation data on the plant, spike, and seedling basis. Since precise methods of mutation analysis are still under development, several methods were compared in the present study. Inherent in each of the methods is a certain bias to the estimate of the induced mutation frequency.

The mutations per plant method of analysis tends to underestimate the true value because multiple events of the same mutation type are not recognized. The spike method of analysis may underestimate or overestimate the mutation frequency depending on the recognition given to different spikes carrying the same mutation type. According to Gaul,[7] the mutant seedling method, also used here, seems to be less affected by the above factors but does not appear to show the relative number of mutational events induced. An attempt was made to correct the bias in the spike method of analysis by the following means: two spikes of a plant that carried the same type of mutation were recorded as one mutation on the plant basis; however, for the spike, only one mutation was recognized if either of the two spikes showed a segregating ratio of 3 to 1. In this case, it was assumed that both spikes contained mutations which were the result of the same mutational event. A 3 to 1 ratio would be expected if a tiller originated from a mutated sector. On the other hand, when neither spike showed a segregating ratio approximating 3 to 1, their mutations were assumed to be independent and were recognized as two mutations.

To compare mutation spectra, the chlorophyll mutations for the chemical and radiation treatments were grouped in the following phenotypic categories: (1) albina, near absence of yellow and green pigments; (2) viridis, green-yellow pigments distributed uniformly or in a gradient; (3) xantha, yellow pigment distributed uniformly; (4) tigrina, transverse destruction of pigments in yellow or green leaf; and (5) striata, longitudinal stripes of yellow or white. The very small number of other types was ignored for this comparison. The mutation spectrum for each

agent included the percentage of spikes segregating in each of the phenotypic classes. Confidence limits for the comparison of mutation spectra were set according to tables presented in Steel and Torrie.[8]

Chromosome aberrations were analyzed by the aceto-orcein smear technique in the diethyl sulfate experiment, and by the aceto-carmine smear technique in the radiation experiment. Previous results have shown these techniques to be comparable. The aberrations were scored at anaphase in the somatic cells of the shoots from germinating seeds and were recorded as dicentric bridges and acentric rod, isodiametric, and dot fragments. Mitotic shoot tip analyses were conducted on seeds from the field experiment. The studies were expanded later with additional and more severe diethyl sulfate treatments for cytological observation of cells in the first, second, and third mitotic division cycles in seeds fixed during periods from 24 to 72 hr germination at 25°C. Induced chromosome translocations were scored in pollen mother cells from plants of this separate experiment using the acetocarmine smear technique.

The field planting of the diethyl sulfate study was completed May 29, 1959, somewhat late for a normal barley season, but the growth and general vigor of the crop was satisfactory. The gamma radiation experiment was completed the previous season. The radiation treatments used for comparison were those regarded as the most effective developed in our research program. In this study, the seeds were frozen in dry ice (−78°C) prior to and dur-

TABLE 1

SURVIVAL, FERTILITY, AND MUTATION RESPONSE OF BARLEY TO DIETHYL SULFATE AND RADIATION TREATMENTS

Treatment	Number examined: Plants M_1	Number examined: Spikes M_1	Number examined: Seedlings M_2	Number mutated Plants	Number mutated Spikes	Number of M_2 seedling mutants	Per cent mutated Plants	Per cent mutated Spikes	Per cent M_2 mutant seedlings	Seedling response ht. per cent of control	Per cent plant survival	Per cent spike fertility
						Diethyl Sulfate						
1 hr*	646	1,969	56,192	214	220	463	33.1	11.2	0.82	84.1	78.7	85.0
1½ hr	604	1,700	40,460	327	338	652	54.1	19.9	1.6	71.5	75.1	74.5
2 hr	437	1,045	17,744	290	298	603	66.4	28.5	3.4	31.8	54.6	55.4
						Water Control						
1 hr	750	2,433	87,588	4	4	4	0.53	0.16	0.004	..	89.2	87.0
1½ hr	706	2,373	80,682	4	4	4	0.57	0.17	0.008	..	86.3	86.2
2 hr	731	2,376	80,784	8	8	12	1.1	0.33	0.015	..	89.3	85.6
						Gamma Radiation						
0 Kr	922	2,278	65,465	7	7	23	0.76	0.31	0.035	..	80.2	..
40 Kr	612	1,674	32,644	134	140	430	21.9	8.4	1.32	94.8	81.6	..
60 Kr	720	1,941	25,518	234	236	592	32.5	12.6	2.32	76.8	75.8	..
80 Kr	605	1,294	14,206	161	163	440	26.6	12.6	3.10	63.3	52.6	..
100 Kr	141	206	1,673	21	21	63	14.9	10.2	3.76	45.4	12.3	..

* It should be noted that steps taken recently to purify the commercial diethyl sulfate have indicated that the chemical used for this study contained decomposition products. Recent experiments using purified samples of diethyl sulfate revealed that shorter seed treatment times were necessary to produce the same seedling growth responses reported here.

ing irradiation, then hydrated in distilled water at 32°C for 2 hr before sowing. Other conditions were reasonably comparable to those of the diethyl sulfate study.

Results.—The M_1 seedling injury response to diethyl sulfate treatment was non-linear in contrast with the linear response for similar material exposed to gamma radiation (Table 1). The leaves of seedlings treated with diethyl sulfate were shortened but did not show the flecking reaction typical for irradiated material.

Plant survival was reduced both by the diethyl sulfate treatments and the irradiation. Differences in the survival for the two agents were not especially notable, except in relation to the comparable seedling injury data. Here, plant survival from chemically treated seeds was higher at a given seedling injury value than was observed with radiation treatments.

The mutation data showed that the frequency of mutations induced by the diethyl sulfate solution increases with time of treatment. A similar response was shown for the mutation frequency based on mutations per plant, mutations per spike, and per cent mutant seedlings in M_2. Deviation from linearity was noted in the mutation frequency-curves for diethyl sulfate treatment after plotting the data for mutations per plant progeny and per cent mutant seedlings in M_2. However, the deviations were in opposite directions for these two measurements.

The same methods of analysis applied to the data obtained from the gamma radiation experiment showed that a near-linear relationship was obtained for the increase of mutation frequency with dose only for the percentage of mutant seedlings in M_2. Both mutations per spike and per plant showed non-linearity with doses above 60 Kr. Greatest differences between the mutation rates determined by the different methods were at the highest radiation dose. Here, sterility due to induced translocations was greater, and the plant survival was lower than for other doses. The proportion of mutant seedlings was noticeably higher among the 80 and 100 Kr treatments, and reflected in the values obtained for the per cent of mutant seedlings.

Diethyl sulfate treatments appeared to induce a different mutation spectrum than gamma radiation (Table 2). At the 99 per cent confidence interval, differences were shown for the albina, xantha and striata categories, while similarities were observed for the viridis and tigrina classes.

TABLE 2

COMPARISON OF MUTATION SPECTRA INDUCED BY DIETHYL SULFATE AND GAMMA RADIATION

Phenotypic categories	Number of mutations: Gamma radiation	Number of mutations: Diethyl sulfate	Per cent of total mutations: Gamma radiation	Per cent of total mutations: Diethyl sulfate	99% confidence interval: Gamma radiation	99% confidence interval: Diethyl sulfate
Albina	272	262	48.6	30.3	43.2–54.8	24.8–35.5
Viridis	211	387	37.7	44.8	31.5–42.8	39.2–50.9
Xantha	25	88	4.5	10.2	2.2– 6.7	6.8–14.0
Tigrina	33	52	5.9	6.0	3.4– 9.0	3.6– 9.2
Striata	19	75	3.4	8.7	1.8– 6.1	6.1–12.8
Total	560	864				

Notable also was the fact that the survival value for the 80 Kr radiation treatment was about the same as for the 2-hr diethyl sulfate treatment, and the mutation frequency, measured as per cent mutant seedlings in M_2, was similar. Compared on the basis of mutations per plant and per spike progeny, the mutagenicity of the

two treatments appeared to be very different. Sixty-six per cent of the diethyl sulfate treated plants showed mutations, compared with only 26.6 per cent of those exposed to 80 Kr of gamma rays.

Cytological analyses of over 300 first mitotic anaphase cells from shoot tips of seeds treated with diethyl sulfate for the field experiment revealed no visible chromosome structural changes. However, the mitotic analysis of 591 anaphase cells from seeds given a more severe chemical treatment than seeds in the field experiment showed a frequency of 0.11 chromosome fragments and 0.02 bridges per cell.

Similar results have been obtained also in studies on *Crepis capillaris*, which has only 3 pairs of large, distinct chromosomes. Among 275 root-tip metaphase cells from seeds treated with a saturated solution of diethyl sulfate, only 12 chromosome fragments were observed. Three fragments were observed in a similar number of cells from nontreated seeds. In contrast, the cytological analysis of shoot tips from barley seeds exposed at 60 Kr gamma radiation showed 3.4 rod and dot fragments, and 0.52 bridge per cell from 300 anaphase cells.

The meiotic analysis of M_1 plants from a severe chemical treatment of barley showed 4 spikes with chromosome interchanges among 175 examined. Similar studies on spikes from the 60 Kr gamma radiation treatment showed 46 interchanges among 175 spikes.

Discussion.—Differences in the biological effects of diethyl sulfate and gamma radiation were observed in the following: (1) frequency and spectrum of mutations, (2) chromsome aberrations, (3) leaf-flecking reaction, (4) survival in relation to the seedling injury test, and (5) causes of semi-sterility.

According to Heslot and Ferrary[3] and Ehrenberg,[4] diethyl sulfate was more effective than radiation for producing mutations in barley. However, evidence presented here showed that diethyl sulfate was at least twice as effective as these workers reported. This difference in magnitude in the activity of diethyl sulfate might be due to experimental conditions.

In this study, the treatment conditions using diethyl sulfate differed in two respects from that previously reported. Firstly, the temperature used was 30°C as compared to 3° and 24°C; and secondly, saturated solutions were used instead of 0.1 and 0.2 per cent solutions.

The difference in temperature was probably not the most important factor influencing the mutation rate since Heslot and Ferrary found no appreciable difference when 3° and 24°C temperatures were used. The possibility exists that high temperatures increase metabolism and, in combination with an active mutagen, produce higher mutation rates.

The second difference concerns the concentration of the active mutagen in the treatment solution. It appears reasonable to suppose that frequency of mutation would be increased through use of higher concentrations of mutagen. Diethyl sulfate is rapidly hydrolyzed to ethyl-sulfuric acid and alcohol followed by much slower hydrolysis to sulfuric acid and alcohol. Thus, the larger amount of diethyl sulfate and the shorter exposure times used in our experiments should have insured a greater concentration of active mutagen available to the barley seeds.

Although the differential action of mutations to chromosome aberrations has been reported for many mutagenic agents,[9] it has been shown for the first time that diethyl sulfate induces high rates of mutations accompanied by few chromosome

structural aberrations. Thus, the ratio of mutations to chromosome aberrations for diethyl sulfate is extraordinarily high. This demonstrates that diethyl sulfate possesses properties quite unlike radiation and most other alkylating agents. With other agents this ratio may vary from practically zero for 8-ethoxycaffein to infinity for nebularine, with most alkylating agents and ionizing radiation taking an intermediate position.[4]

Information obtained from the cytological analysis indicates that the partial sterilities induced by diethyl sulfate treatment and by radiation exposure are different. The high frequency of chromosome translocations induced by radiation would help to account for the semi-sterility. On the other hand, the few chromosome translocations and little other structural damage induced by diethyl sulfate solutions indicates that semi-sterility in this case may not be due to the same cause. It is possible that some of the sterility induced by the diethyl sulfate treatments is due to induced gene mutations.

The frequencies of induced chromosome damage may be correlated with the leaf flecking which appear as patches of injured or necrotic cells. This is supported by the fact that severely injured seedlings from seeds immersed in a saturated diethyl sulfate solution showed no leaf flecking, a reaction typical for moderate to severe radiation treatments. Hence, this evidence seems to substantiate data obtained from the cytological analysis.

Another difference is that the survival of seeds severely injured by diethyl sulfate was greater than for radiation. In this respect, it is noteworthy that other workers[10,11] have shown recently that diethyl sulfate treatments produce high mutation rates with high survival in bacteria.

Moreover, the spectrum of mutation types induced by diethyl sulfate and gamma rays also may be different. The chemical treatments appeared to induce fewer albinas, and more xanthas and striatas than gamma radiation. The proportion of viridis and tigrina types was similar for the two agents. Heslot and Ferrary[3] had reported earlier that the spectra of mutation types was similar for the two agents, but the larger population of mutations grouped into a broader number of categories used in the present study may have revealed differences.

Preliminary studies indicate that dimethyl-sulfate and ethyl-methane-sulfonate also induce few chromosome aberrations in barley.

These results have far-reaching fundamental and practical significance. They demonstrate for a highly effective mutagen that the mechanisms responsible for induced mutations are distinct from those responsible for chromosome structural changes. Breeders can produce, at little cost, large numbers of induced mutations, with minimum disruption of chromosome complements. This should both simplify and increase the efficiency of mutation plant breeding.

Summary.—Treatment with a diethyl sulfate solution caused injury to barley seeds, but the injury differed from that caused by radiation in that the characteristic leaf flecking of M_1 seedlings was not observed. The relative absence of leaf flecking appeared to be correlated with the observed low frequency of chromosome structural changes at the first mitosis in treated seeds and microsporocytes of M_1 plants. In contrast, radiation treatments produced abundant chromosome structural damage which could be measured at both stages of plant growth. Moreover, the semi-sterility of M_1 spikes of diethyl-sulfate-treated barley could not be accounted

for on the basis of induced chromosome interchanges. In addition, it was found that diethyl sulfate treatments induced a high frequency of mutations, and further investigation revealed that the spectrum of mutation types appeared to be different for the two mutagenic agents being compared. The distinct lack of association of chromosome structural aberrations with mutations for a highly effective mutagenic agent has broad fundamental as well as practical implications.

The writers wish to acknowledge that the selection of diethyl sulfate as a mutagen for study was made as a result of a suggestion from Dr. Mogens Westergaard, Copenhagen, Denmark. Correspondence with Dr. James MacKey was helpful in developing experimental techniques. Discussions with Drs. R. F. Foster and Kermit Groves contributed much to the design of the experiments. We are indebted also to Miss Edith Froese-Gertzen, Mrs. Sally Wilbur, and Mrs. Diann Robbers who assisted in preparation of certain treatments or in cytological analyses. The helpful suggestions of many others at W. S. U. are also gratefully acknowledged.

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[1] von Wettstein, D., *Arbeitsgemeinschaft für Forschung des Landes Nordrhein-Westfalen* (Köln und Opladen: Westdeutscher Verlag) Heft **73**, 7–60.

[2] Rapoport, A., *Dok. Vsesoyuz. Akad. Sel'sko-Khoz Nauk V. I. Lenina*, **12**, 12 (1947).

[3] Heslot, H., and R. Ferrary, *Ann. Inst. Nat. Agron.*, **44**, 3 (1958).

[4] Ehrenberg, L., in *Proceedings of the Symposium on Chemical Mutagens* (held at Gatersleben, Germany, August 1959, in press).

[5] Stadler, L. J., *Missouri Agr. Expt. Station, Res. Bull.* **204**, 29 (1933).

[6] Gustafsson, A., *Lunds Univ. Arsskr. N. F. Avd.* 2, Bd. **36**, Nr. 11, 1–40 (1940).

[7] Gaul, H., in *Proceedings of the Sixth Congress of the Societa Italiana di Genetica Agraria* (held at Forli, Italy, October 4–6, 1959, in press).

[8] Steel, R. G. D., and J. H. Torrie, *Principles and Procedures of Statistics* (New York: McGraw-Hill, 1960), p. 456–7.

[9] Ehrenberg, L., *et al.*, in *Conference on Chromosomes* (Zwolle, the Netherlands: W. E. J. Tjeenk Willink, 1956), pp. 131–159.

[10] Westergaard, M., *Experientia*, **13**, 224–234 (1956).

[11] Loveless, A., and S. Howarth, *Nature*, **184**, 1780–1782 (1959).

THE THERMODYNAMICS OF SEA WATER

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In a recent volume dealing with the physics and chemistry of sea water,[1] Fofonoff[2] presents a systematic application of the equations of thermodynamics to an ocean water system. Few subjects are more fundamental to the study of the sea and more neglected in application, reflecting the inherent difficulties in treating a

Attempts were also made to confer active protection upon mice with doses of oxidized cotton ranging from 0.005 mg. down to 0.00001 mg.,[11] but the material failed to protect against 10 to 100 minimal lethal doses of an extremely virulent strain of Type VIII pneumococcus. However, the same amounts of the Type III and Type VIII specific polysaccharides also failed to give protection against this strain.

The precipitation data with the oxidized cotton again emphasize the strict correlation between chemical constitution and immunological specificity and show that predictions as to reactivity may be made when the constitution of the repeating unit responsible for that reactivity is known.

[1] Heidelberger, M., and Avery, O. T., *Jour. Exp. Med.*, **38**, 73 (1923); **40**, 301 (1924). Avery, O. T., and Heidelberger, M., *Ibid.*, **42**, 367 (1925).

[2] Heidelberger, M., and Goebel, W. F., *Jour. Biol. Chem.*, **70**, 613 (1926); **74**, 613 (1927).

[3] Hotchkiss, R. D., and Goebel, W. F., *Ibid.*, **121**, 195 (1937). Reeves, R. E., and Goebel, W. F., *Ibid.*, **139**, 511 (1941). Adams, M. H., Reeves, R. E., and Goebel, W. F., *Ibid.*, **140**, 181 (1941).

[4] Goebel, W. F., *Ibid.*, **110**, 391 (1935).

[5] Heidelberger, M., Kabat, E. A., and Shrivastava, D. L., *Jour. Exp. Med.*, **65**, 487 (1937).

[6] Sugg, J. Y., Gaspari, E. L., Fleming, W. L., and Neill, J. M., *Ibid.*, **47**, 917 (1928). Cooper, G., Edwards, M., and Rosenstein, C., *Ibid.*, **49**, 461 (1929). Also Ref. 5. Anti-pneumococcus Types III and VIII horse sera show this cross-precipitation more regularly than do the corresponding rabbit sera.

[7] Yackel, E. C., and Kenyon, W. O., *Jour. Am. Chem. Soc.*, **64**, 121 (1942). Unruh, C. C., and Kenyon, W. O., *Ibid.*, **64**, 127 (1942).

[8] Heidelberger, M., Kendall, F. E., and Soo Hoo, C. M., *Jour. Exp. Med.*, **58**, 137 (1933). Heidelberger, M., and Kendall, F. E., *Ibid.*, **61**, 559; **62**, 697 (1935).

[9] Heidelberger, M., Kabat, E. A., and Mayer, M., *Ibid.*, **75**, 35 (1942).

[10] After removal of antibodies precipitated by pneumococcus C-substance.

[11] Schiemann, O., and Casper, W., *Zeitschr. f. Hyg.*, **108**, 220 (1927). Felton, L. D., *Jour. Infect. Dis.*, **56**, 101 (1935).

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*THE GENETIC NATURE OF X-RAY INDUCED CHANGES IN POLLEN**

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Ample evidence exists in genetic literature to suggest that many more pollen characters are genetically self-determined in the pollen than have already been reported. Some of these cases—for example, the genes determining small pollen in *Zea*—were discovered by virtue of the fact that

small pollen is less viable and hence causes distorted ratios of more conspicuous characters to which the genes for small pollen size are linked.[1] Some other respects in which genetic autonomy occurs in pollen include: chemical composition,[2] viability in the many instances of semi-sterility and behavior as in the many examples of oppositional self-sterility allelomorphs.

With the known mutation inducing agents available, it should be possible to demonstrate whether or not certain characters are determined in this manner. Since size is usually governed by a large number of genetic factors, each of relatively small effect and each integrated with others in its action, mutation in a single gene of this type in pollen would be very difficult to detect. Mutation of a large number, however, in pollen where the haploid condition would permit expression of recessive as well as dominant changes, should be readily detectable as an increase in variation of size. Accordingly, experiments were performed to detect whether or not x-rays would induce any genetic response in size of pollen. Lethal effects, i.e., changes resulting in abortion of pollen, were also studied.

Genetically self-determined pollen abortion, whether conditioned by chromosomal deficiencies and duplications or by gene mutation, has been observed frequently. There are also reports in the literature of a similar control of microspore and pollen size.[3] Nevertheless, pollen size may also be determined by the sporophyte producing the pollen instead of by the gametophyte itself.[4]

Typical of pollen measurements in general, the intra-treatment variability of sample values was much greater than would be expected on the basis of pure chance variation. In order to cope with this natural fluctuation in comparing different treatments statistically, many samples of small size were taken and n was taken as the number of samples, instead of the total number of grains observed, as the basis for comparisons between treatments. Pollen abortion, mean length of grain and variance of those lengths were calculated for each sample. Means and standard errors were then calculated for the distribution of these sample values. In the following report a random sample of 25 was adopted for measurement of lengths, and 100 for frequency of pollen abortion.

The pollen collections were examined in aceto-carmine in order to estimate pollen abortion in the same collection used for measurement of lengths. Examination was made immediately after mounting. A pollen grain was considered aborted if it was devoid of cytoplasmic contents as indicated by the aceto-carmine stain. Length of grain is the most satisfactory dimension for measurement of the elongate grains of *Tradescantia* and *Pisum* lines used here. Lengths were measured by means of an ocular micrometer.

Diploid-Tetraploid Comparisons.—A comparison between diploid and closely related autotetraploid lines should offer a test of the hypothesis of

x-ray induced mutations in pollen grains. Stadler[5] has demonstrated conclusively that in the cereals the rate of x-ray induced mutation drops rapidly with increasing degree of polyploidy. The pollen produced by autotetraploids is diploid, consisting of two identical sets of chromosomes. Since every gene exists in duplicate, a mutation from the dominant to recessive condition of any gene will gain no phenotypic expression because of the presence of the dominant allele except in the very rare event that the same recessive mutation is induced in both alleles. On the other hand, the haploid pollen produced by diploids should show phenotypic expression of recessive mutations. Since the great majority of x-ray induced mutations are recessive, this comparison should be illuminating.

In the x-ray treatments clones of *Tradescantia* species were used because of the data available on the timing of the cycle of development of the male gametophyte.[6] Collections were made from the eight to eleventh day after the material had been irradiated. In terms of the developmental cycle these collections were taken from buds in which the pollen mother cells had just completed meiosis and the microspores were experiencing the first half of the post-meiotic resting stage at the time of treatment. The conditions of radiation are given in table 1.

TABLE 1

COMPARISON OF X-RAY EFFECTS ON ABORTION OF POLLEN, MEAN AND VARIANCE OF LENGTH OF GRAIN IN DIPLOID AND AUTOTETRAPLOID *Tradescantia* SPECIES

200 kv., 15 ma., 81 cm., 1/2 mm. copper

SPECIES	TREATMENT	NUMBER OF SAMPLES	POLLEN ABORTION, %	LENGTH OF POLLEN GRAIN MEAN, μ	LENGTH OF POLLEN GRAIN VARIANCE, μ^2
T. paludosa					
Diploid	Control	16	4.99 ± 0.49	49.11 ± 0.19	0.742 ± 0.042
	400 r	18	10.98 ± 0.83	47.89 ± 0.24	2.163 ± 0.158
T. canaliculata Autotetraploid	Control	20	13.10 ± 0.90	57.24 ± 0.27	1.322 ± 0.062
	400 r	18	13.20 ± 0.79	56.34 ± 0.45	1.308 ± 0.138
Clone No. 374*					
Diploid	Control	10	22.2 ± 1.2	49.38 ± 0.26	2.549 ± 0.037
	200 r	10	28.87 ± 1.43	47.99 ± 0.14	3.043 ± 0.149
T. virginiana Autotetraploid	Control	19	26.03 ± 0.92	56.38 ± 0.33	1.879 ± 0.115
	400 r	19	26.84 ± 1.25	59.13 ± 0.20	1.940 ± 0.088

* A segregate from the cross, *T. canaliculata* x *T. humilis*.

Two clones of diploid and two of tetraploid *Tradescantia* species were used in this study. Evidence of the autotetraploid nature of the tetraploid species, *T. virginiana* L. and *T. canaliculata* Raf., has been presented by

Anderson and Sax.[7] Of the diploid clones used, one was identified as *T. paludosa* Anderson and Woodson and the other was a segregate from the cross, *T. canaliculata* Raf. x *T. humilis* Rose. The diploid *T. paludosa* and the tetraploid *T. canaliculata* were irradiated simultaneously and grown under the same conditions. The other clones were treated at different times.

The results are summarized in table 1. In the diploid clones the radiation induced a very significant increase in pollen abortion and in variability of length and a decrease in mean length. These changes showed a slight gradual increase during the four-day period of collections, but in comparison with measurements of collections on preceding days, these values are at a new high level and for purposes of comparison can be safely considered as a unit. This pattern of x-ray effect is typical of many that have been investigated here in diploid *Tradescantia*.

This response to x-ray treatment of the haploid microspores would have occurred if the radiation induced mutations (chromosomal aberrations as well as gene mutations in the strict sense) which were immediately expressed in the size and viability of the microspores. The great preponderance of x-ray induced mutations reported in the literature has been of the negative type; therefore, it is no surprise that the changes encountered here have been mostly, if not entirely, in the direction of smaller grains, and that the percentage of aborted grains showed a significant increase. The changes involved will be described in more detail in a later publication.

The effect on the pollen of autotetraploid clones is strikingly different. The rate of pollen abortion and the variance of pollen grain length remain very little affected by radiation which caused quite significant changes in the diploid, thus bearing out the genetic interpretation. Mean length of grain seemed to be affected, in one instance to a nearly significantly lower level and in the other to a very significantly higher level. No explanation would seem to account for these peculiar trends. Of the three values, mean length in general shows the greatest fluctuations and lengths from the same plant may be subject to substantial increases and decreases over a period of time. Since the changes here are both positive and negative, it is doubtful whether they are related to the x-ray treatment.

In a comparison of the x-ray sensitivity of microspore chromosomes, Sax and Swanson[8] found the rate of aberration in chromosomes of diploid *Tradescantia* microspores to be only half that of the haploid. These results were expressed on a chromosome basis; if expressed as frequencies per cell, the sensitivity of haploid and diploid would be about equal. Yet, even if this might indicate a gene sensitivity reduced to one-half in the diploid microspore, the differences in table 1 would remain relatively unaffected. For instance, the negative changes in the autotetraploid clones

must be increased by factors of 3 to 25 before the differences reach the five per cent level of significance.

TABLE 2

COMPARATIVE EFFECTS OF X-RAYS APPLIED AT 3°C. AND 33°C. ON MEASUREMENTS OF POLLEN GRAINS

134 kv., 10 ma., 50 cm., no filters

Ten samples per treatment

TREATMENT NUMBER	RADIATION	TEMPERATURE, °C.	POLLEN ABORTION, %	LENGTH OF POLLEN	
				MEAN, μ	STANDARD DEVIATION, μ
20*	150 r	3	11.5 ± 0.9	47.11 ± 0.63	2.758 ± 0.096
21*	150 r	33	10.2 ± 0.9	47.67 ± 0.54	2.492 ± 0.082
24	None	3	9.5 ± 0.6	46.57 ± 0.59	1.836 ± 0.099
19	None	33	.....	46.75 ± 0.48	2.027 ± 0.082

* Lots 20 and 21 were irradiated simultaneously.

Effect of Temperature on X-ray Induced Changes.—Only one trial has been made of the effect of temperature. The pollen test of mutation rate offers advantages of rapidity and ease of manipulation for tests of this sort and might prove useful in future experiments where the contributory effects of dosage, intensity, temperature and other factors are studied. Inflorescences of a diploid clone of *Tradescantia paludosa* were irradiated simultaneously in cardboard containers of warm and cold water. The temperature differences were maintained during the period of treatment and for several hours afterward. The results and other conditions of the experiment are given in table 2. Temperature influenced the values significantly only in the case of variation of length and here the P value of the difference is 0.036, but the effect of radiation at low temperature is consistently more intense in each of the three measures. From the standpoint of direction and consistency alone these results are well within the realm of chance occurrences and larger samples will be needed from material treated with heavier doses before it can be said with certainty that low temperature enhances the x-ray effect in all respects.

The increased variability induced at 3° over 33°C. would be rather difficult to account for in terms of a physiological response other than a genetic one. The genetic interpretation is admittedly far from satisfactory, yet the point of interest here is that this response in variability of pollen size agrees with the response found in most other experiments, the genetic nature of which is undisputed. For instance, the frequency of x-ray induced chromosomal aberrations in *Tradescantia*[9] and in *Drosophila*[10] is greater when treatments were applied at lower temperatures. A similar response has been found in lethal mutations in *Drosophila*[11] and in chlorophyll deficient mutants in *Hordeum*[12] but these reports are contradicted

by the finding of no temperature effect by others.[13] The picture conveyed by the literature then is confused but there is agreement to the effect that there is no positive temperature coefficient of x-ray induced mutation and in this respect the response in pollen resembles mutation.

Inheritance of Pollen Size in Pisum sativum L.—As mentioned above there is ample evidence in the literature of genes governing size, viability and physiological activity of pollen, which segregate at meiosis and gain expression immediately in the grains to which they are contributed. In these cases the segregating types have been sharply distinguished and only one or a few gene pairs have been concerned in their determination. Although it did not seem unreasonable to suppose that pollen size could be regulated by multiple factors, it seemed highly desirable to have some such example in untreated material to compare with the x-ray treatments.

The naturally self-pollinated legumes offer a source of lines which are homozygous yet do not suffer the usual effects of inbreeding. In the garden pea, *Pisum sativum* L., varieties were found which differed in length of pollen grain. Crosses were made between large and small types and the F_1 hybrids were grown simultaneously with selfed progeny from the plants used as parents. For comparative data, samples were taken from the selfed progenies of the parents rather than from the parent plants themselves in order that the parent lines be grown under the same conditions as the hybrids. The homozygous condition of the parent plants attested by the uniformity of their progeny justified this measure.

TABLE 3

MEASUREMENTS OF POLLEN IN F_1 AND PARENTAL LINES OF GARDEN PEA

NUMBER OF LINE	DESCRIPTION	NUMBER OF SAMPLES	POLLEN ABORTION, %	LENGTH OF POLLEN GRAIN MEAN, μ	LENGTH OF POLLEN GRAIN VARIANCE, μ^2
42 *Ps* 8	F_1, 3 × 13	18	2.68 ± 0.55	50.68 ± 0.32	1.267 ± 0.105
42 *Ps* 18	F_1, 25 × 26	9	2.49 ± 0.72	52.13 ± 0.58	1.860 ± 0.271
42 *Ps* 22	F_1, 11 × 25	19	3.71 ± 0.44	51.25 ± 0.46	1.511 ± 0.075
42 *Ps* 28	F_1, 31 × 25	11	1.94 ± 0.46	51.05 ± 0.34	1.270 ± 0.070
42 *Ps* 3	P_1	19	2.42 ± 0.35	55.58 ± 0.21	0.602 ± 0.038
42 *Ps* 11	P_1	18	2.89 ± 0.30	54.39 ± 0.29	0.590 ± 0.040
42 *Ps* 13	P_1	18	5.91 ± 0.72	49.67 ± 0.37	0.545 ± 0.043
42 *Ps* 25	P_1	25	5.27 ± 0.44	49.57 ± 0.32	0.685 ± 0.045
42 *Ps* 26	P_1	20	3.17 ± 0.39	54.01 ± 0.14	0.535 ± 0.023
42 *Ps* 31	P_1	3	10 ±	53.76 ± 0.42	0.552 ± 0.050

Ample data for statistical comparisons are available from four different F_1's and their parents. The observations are presented in table 3 and figure 1. In every case pollen of the hybrid was intermediate in size between its two parents; furthermore, the variation of the hybrid pollen grain length significantly exceeded that of each parent in every case. In the case of

least difference, i.e., the F_1, 42 *Ps* 18 and its parent, 42 *Ps* 25, the t value of the difference is 2.63 with a corresponding P value of less than 0.01. This is precisely the effect expected if size of pollen were to some extent governed by the genotype of the pollen itself and if a large number of genes, each having a small effect, were involved. A possible source of internal instability might be hybridity for chromosome rearrangement, say inversions or translocations. Sufficient buds of the hybrids were not available to permit a cytological study to detect such hybridity but the four pea hybrids show no increased percentage of pollen abortion which is characteristic of inversion and translocation heterozygotes.

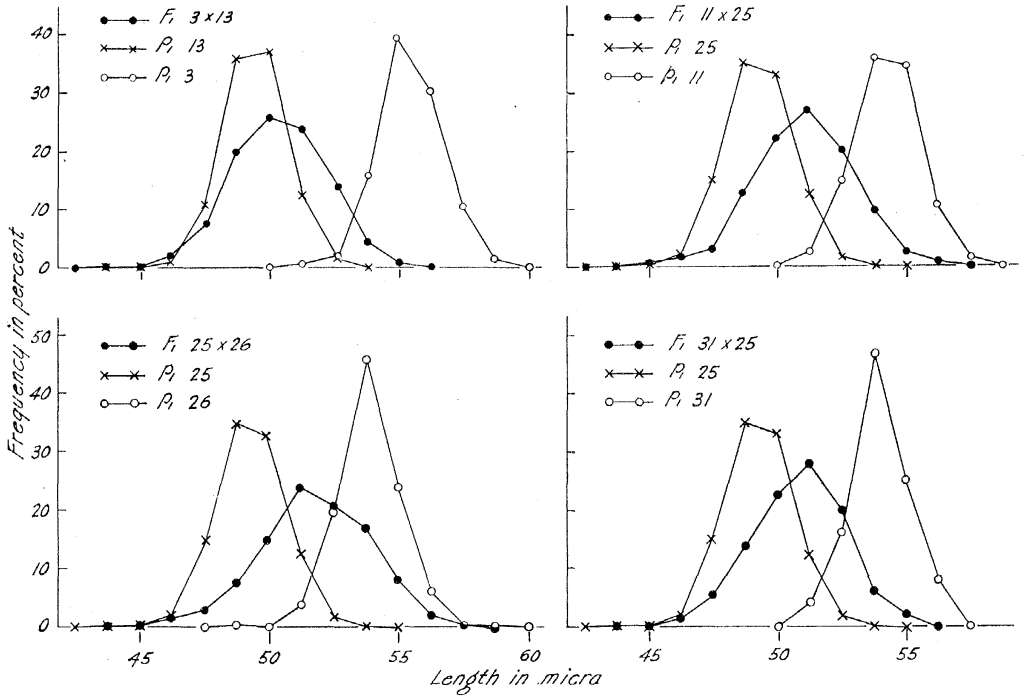

FIGURE 1
Frequency distributions of length of pollen in F_1 and parental lines of garden pea.

Unless a very great number of factors interact to determine size differences in these hybrids, the distribution of lengths in the hybrids might be expected to completely cover the range of either parent. Also, the presence of only a few determiners of small size in the larger parent or vice versa might lead to considerable transgressive variation. Since these F_1 distributions are all contained within the range of either parent and one in particular, 42 *Ps* 8, fails to cover the entire range of its parents, it is possible that even in these hybrids size is determined maternally to a limited extent. Nevertheless, the consistent tendency of all hybrids to be more variable than either parent certainly points to a multifactorial genetic determination of size within each grain.

Summary.—X-rays applied to microspores of diploid species of *Tradescantia* shortly after meiosis cause a significant increase in variability of length of the subsequently developed pollen grains and in the percentage of aborted pollen. There is also a significant decrease in the mean lengths of grains. Similarly treated autotetraploid species of *Tradescantia* show no significant changes. This difference is interpreted to indicate that in the diploid pollen of autotetraploids any recessive mutation is masked by its dominant allele.

X-rays induced significantly greater variability of pollen length when applied to diploid *Tradescantia* at 3° than when applied at 33°C.

In consideration of these results it is concluded that size and viability of pollen are, at least in part, genetically self-determined and that the changes observed are the consequence of mutations induced by the x-ray treatment.

These conclusions are supported by an analysis of genetic variation in pollen length in *Pisum sativum*.

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[1] Mangelsdorf, P. C., *Jour. Hered.*, **23**, 289–295 (1932); Rhoades, M. M., and Rhoades, Virginia H., *Genetics*, **24**, 302–314 (1939).

[2] Brink, R. A., and MacGillivray, J. H., *Amer. Jour. Bot.*, **11**, 465–469 (1924); Demerec, M., *Ibid.*, **11**, 461–464 (1924); Parnell, F. R., *Jour. Genet.*, **11**, 209–212 (1921).

[3] Mangelsdorf, P. C., loc. cit.; Renner, O., *Zeit. Bot.*, **11**, 305–380 (1919); Satina, Sophia, and Blakeslee, A. F., *Amer. Jour. Bot.*, **24**, 518–527 (1937); Sax, K., *Genetics*, **22**, 523–533 (1937); Sinotô, Y., *Cytologia*, **1**, 109–191 (1929).

[4] Bateson, W., Saunders, E. R., Punnett, R. C., and Hurst, C. C., *Rept. Evol. Com. Roy. Soc. Rept.*, **2**, 154 pp. (1905). Another example of sporophytic control is the difference in size and color of pollen maintained in anthers of different levels in the flower of *Lythrum Salicaria* L.

[5] Stadler, L. J., these PROCEEDINGS, **15**, 876–881 (1929).

[6] Sax, K., *Genetics*, **23**, 494–516 (1938).

[7] Anderson, E., and Sax, K., *Bot. Gaz.*, **97**, 433–476 (1936).

[8] Sax, K., and Swanson, C. P., *Amer. Jour. Bot.*, **28**, 52–59 (1941).

[9] Fabergé, A. C., *Jour. Genet.*, **39**, 229–248 (1940); Rick, C. M., *Genetics*, **25**, 466–482 (1940); Sax, K., and Enzmann, E. V., these PROCEEDINGS, **25**, 397–405 (1939).

[10] Mickey, G. H., *Genetica*, **21**, 386–407 (1939).

[11] Medvedev, N. N., *Compt. Rend. Acad. Sci. U.R.S.S.*, **4**, 283–285 (1935); Medvedev, N. N., *Ibid.*, **19**, 301–302 (1938); Timoféeff-Ressovsky, N. W., *Mutationsforschung in der Vererbungslehre*, 184 pp., Theodor Steinkopf, Dresden (1937).

[12] Rick, C. M. (unpublished).

[13] Stadler, L. J., *Jour. Hered.*, **21**, 3–19 (1930); Stadler, L. J., *Sci. Agr.*, **11**, 645–661 (1931); Timoféeff-Ressovsky, N. W., und Zimmer, K. G., *Biol. Zbl.*, **59**, 358–362 (1939).

SOMATIC MUTATIONS IN THE CARNATION, DIANTHUS CARYOPHYLLUS L.

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Introduction.—So-called "bud sports" have long been known in horticulture. In fact, in plants where vegetative reproduction is readily practiced, such sports have been widely utilized to extend the range of variation, especially with respect to fruit, flower, and foliage colors.[1] It is generally held in horticulture that such sports are not transmitted through seeds.[2] The results obtained by Clausen and Goodspeed[3] in their thorough study of two bud sports in *Nicotiana* hybrids support this view. However, considering the frequency of bud sports in horticultural plants, too little experimental work has been done to verify this point, probably because most sports have been observed in woody plants which generally require a rather long time from seed to maturity or in plants which from their origin might be expected to be highly heterozygous and consequently might be expected to give complex segregations.

PLATE I

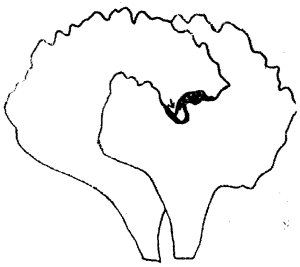

Fig. 1*a*.

Fig. 1*b*.

Fig. 2.

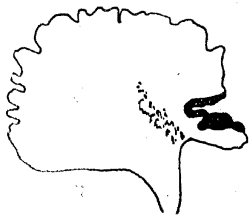

Fig. 3.

Petal diagrams showing types of streaking and blotching on white and flesh-pink flowers. Black areas represent red color.

Fig. 1*a*.—Two overlapping petals fused at point indicated by arrow. $\times 5/3$.

Fig. 1*b*.—Separated petals from Fig. 1*a*; point of former attachment indicated by arrows. $\times 5/3$.

Fig. 2.—Petal with large and small streaks and irregular marginal marking. $\times 5/3$.

Fig. 3.—Petal with "dot series" and marginal blotch. $\times 2$.

In commercial carnation culture, bud sports occur rather frequently, and many have been widely propagated. Most of these have been sports differing from the parent-plant in color only, but others have been an improvement in shape or degree of doubleness. Occasionally, sports have been found differing from the parent in more than one characteristic.

In 1946 a red-flowered carnation variety, William Sim, was introduced. It soon became the leading red-flowered variety, and when both white and flesh-colored mutants appeared in 1949 and 1950, respectively, the Sim varieties soon became the leading ones, not only in this country but in some European ones as well. During the last three or four years, at least a dozen additional color sports have been registered with the American Carnation Society. The most unusual of these is of a clear buff-orange color, rarely seen in carnations.

Methods and Material.—Because most carnations are diploid ($2n = 30$)[4] and earlier experiments had established the main genes concerned with flower color[5, 6] it was decided to ascertain whether or not these mutations are inherited in sexual reproduction. For this purpose, cuttings were obtained of the red-flowered parent-form and four of its mutants, all of which are described in Table 1. Plate I shows the streaks and blotches which are characteristic of the white and pink forms. The buff-colored form was not available at the time these experiments were begun but has now been added.

TABLE 1

CLONE	POSTULATED GENETIC COMPOSITION	COLOR
William Sim	*A I Y S r m*	Bright red, with occasional white streaks
Pink Sim	*A I Y s r m*	Light salmon (flesh pink), with occasional red streaks and blotches
White Sim	*a I Y S r m* or *A I y S r m*	White, with occasional red streaks and blotches (see Pl. I)
Peppermint Sim	a^v *I Y S r m*	Red stripes on white ground
Skyline Frosted Sim	?	Red stripes on white or lightly flushed ground

The postulated genetic composition (Table 1) is based on previous data[5, 6] which might be summarized as follows:

A is the basic gene for anthocyanin; *a*-plants develop no anthocyanin pigment in any part. One or more intermediate alleles a^v produce pencil striping on white ground.

I determines the color of the anthoxanthin; I = white, i = yellow. One or more intermediate alleles, i^v, produce broad indefinite stripes of anthoxanthin and anthocyanin.

Y determines the extent of coloration; Y = full color, y = very little color, mainly in anthers and style tips, and, at times, a light flush in petals. A series of intermediate alleles, y^n, produce intermediate patterns.

S controls concentration of anthocyanin; S = deep colors, such as red, deep pink, crimson, and magenta; s = dilute colors such as salmon, light pink, and lavender.

R determines the kind of anthocyanin; R = cyanin, r = pelargonin.

M determines the number of sugar molecules attached to the anthocyanin. M = diglycoside: srM = light pink, SrM = deep pink, sRM = lavender, SRM = magenta; m = monoglycoside: srm = salmon, Srm = red, sRm = lavender, SRm = crimson.

TABLE 2

PEDIGREE	FLOWER COLOR	GENOTYPE
51169-1, -7, and -12	Salmon	*AA II YY ss rr mm*
51622-3 and -7	*a*-White	*aa II YY SS rr mm*
51632-3	*y*-White	*AA* Ii^v *yy SS rr MM*
51640-5	*y-a*-White	*aa* Ii^v *yy SS rr MM*
51640-13	Pale yellow	*aa* i^vi^v *yy SS rr MM*

William Sim and its four mutants were crossed to test lines of known genotypes, described in Table 2. The results of these crosses are summarized in Tables 3–7. It would have been desirable to self-pollinate all the Sim forms, but this could not be done readily, since all were male sterile, producing anthers but very rarely. From such occasional anthers one self-population of twenty plants was obtained from Pink Sim.

TABLE 3

PARENTAGE		RESULTS
William (Red) Sim	× Salmon *AA II YY ss rr mm*	74 Red
Pink Sim	× Salmon *AA II YY ss rr mm*	80 Red
White Sim	× Salmon *AA II YY ss rr mm*	96 Red
Peppermint Sim	× Salmon *AA II YY ss rr mm*	79 Red
Skyline Frosted Sim	× Salmon *AA II YY ss rr mm*	86 Red
Total		415 Red

TABLE 4

PARENTAGE		RESULTS
Pink Sim	× *a*-White *aa II YY SS rr mm*	45 Red
White Sim	× *a*-White *aa II YY SS rr mm*	45 Red
Peppermint Sim	× *a*-White *aa II YY SS rr mm*	25 Red
Skyline Frosted Sim	× *a*-White *aa II YY SS rr mm*	5 Red
Total		120 Red

TABLE 5

PARENTAGE		RESULTS: Deep Pink *SrM*	White *yy*	Orange Var. *ii^v*	Pale Yellow *yyii*	*Total*
William (Red) Sim	× *y*-White *AA Ii^v yy SS rr MM*	13	15	5	4	37
Pink Sim	× *y*-White *AA Ii^v yy SS rr MM*	11	12	2	3	28
White Sim	× *y*-White *AA Ii^v yy SS rr MM*	17	11	7	3	38
Peppermint Sim	× *y*-White *AA Ii^v yy SS rr MM*	15	11	5	1	32
Skyline Frosted Sim	× *y*-White *AA Ii^v yy SS rr MM*	14	12	3	0	29
Observed totals		70	61	22	11	164
Theoretical ratio		3	3	1	1	8
Calculated totals		61.5	61.5	20.5	20.5	164

$\chi^2 = 5.68$; $P = 0.13$

TABLE 6

PARENTAGE		RESULTS: Deep Pink *SrM*	White *yy*	Orange Var. *ii^v*	Pale Yellow *iiyy*	Total
White Sim	× 51640-5 *aa Ii^v yy SS rr MM*	4	2	0	1	7
Peppermint Sim	× 51640-5 *aa Ii^v yy SS rr MM*	11	11	3	4	29
Skyline Frosted Sim	× 51640-5 *aa Ii^v yy SS rr MM*	11	13	6	6	36
Observed totals		26	26	9	11	72
Theoretical ratio		3	3	1	1	8
Calculated totals		27	27	9	9	72

$\chi^2 = 0.52$; $P = +0.8$

TABLE 7

PARENTAGE		RESULTS: Deep Pink *SrM*	White *yy*	Orange Var. *ii^v*	Pale Yellow *iiyy*	Total
William (Red) Sim	× 51640-13 *aa i^{v}i^v yy SS rr MM*	1	3	4	5	13
Pink Sim	× 51640-13 *aa i^{v}i^v yy SS rr MM*	8	10	7	8	33
White Sim	× 51640-13 *aa i^{v}i^v yy SS rr MM*	9	9	4	7	29
Skyline Frosted Sim	× 51640-13 *aa i^{v}i^v yy SS rr MM*	10	11	9	9	39
Observed totals		28	33	24	29	114
Theoretical ratio		1	1	1	1	4
Calculated totals		28.5	28 5	28.5	28.5	114

$\chi^2 = 1.44$; $P = 0.69$

Results and Discussion.—In explanation of Tables 5–7, it should be stated that all the whites were pure white, except for an occasional colored streak and the small amount of pigment sometimes seen in *y*-plants, such as slightly colored anthers, style tips, and petals. There were no sinus blotches or large streaks such as those shown in Plate I. White streaks in the colored segregates were small and usually limited to one to five per flower, while in William Sim the number at times was as high as ten or more per flower. The variegation in the orange groups was such as might be expected from the gene *i'* introduced through the test plants.

The results show two interesting things. First, they are the same regardless of which form of the Sim carnation was used. Second, there was no segregation for Salmon (Table 3). Within the limits of random segregation, all five forms bred as if the genotype was *AA Ii Yy SS rr mm*. The only exception is Skyline Frosted in Table 5, but, when these results are added to those from the same form in Table 6, the observed ratio of 25:25:9:6 does not differ significantly from the expected 3:3:1:1. In other words, the four mutant forms apparently represent somatic changes only, not involving any germinal tissue.

Were it possible to propagate carnations from root cuttings, it could probably be shown that the four mutant forms used in this study are periclinal chimaeras in which the mutant condition does not extend deeply enough to affect the formation of gametes, as Clausen and Goodspeed found in *Nicotiana*.[3]

This conclusion raises the question as to how the "Pink Sim" originated from a plant not heterozygous for *S*. The simplest explanation would be to assume that the light salmon or flesh-pink color in Pink Sim is due to a gene different from that represented by the test plants 51169-1, -7 and -12. However, evidence from two sources indicates that this is not so. First, in the large numbers of progenies grown earlier in order to determine the genetics of flower color in carnations, not more than one main gene concerned with the production of salmon or flesh color from red was ever identified.[6] Second, the segregation in the self-population of 20 plants from Pink Sim was 13 red, 5 white, 1 orange, and 1 pale yellow, but no flesh-colored.

Unless F_2 data, when it becomes available at the end of this summer, indicates that the pink flower color in Pink Sim is determined by a gene distinct from *s*, we must assume either that Pink Sim arose from Red Sim (William Sim) in somatic tissues by a series of two successive mutations involving the *S* locus in both homologous chromosomes or that, following one mutation *S* to *s* in one chromosome, somatic segregation took place, producing tissues which are *SS* and *ss*, respectively.

It is generally agreed in the trade that Pink Sim differs from the red and white forms in that it has a greater tendency to split its calyx. Since splitting of the calyx in many varieties is due to abnormally high petal numbers, a comparison of Pink Sim with the other forms is now being made. Segregation of single to semidouble in the crosses between the salmon-colored single-flowered plants and Pink Sim (Table 3) disclosed no significant discrepancy in this respect, as the ratio was 200 single to 215 semidouble.

Variations in chromosome numbers have been claimed by Dowrick[7] to be the reason for, or associated with, color mutations in *Chrysanthemum*. Chromosome determinations made on the five forms involved in this experiment failed to disclose any variation from the normal diploid number of $2n = 30$. However, *Dianthus*

chromosomes are rather small, so it is quite likely that minor chromosome deficiencies or deletions would not be detected.

Recently, we have obtained from commercial sources two spontaneous chromosome aberrants, one of which appears to be wholly tetraploid, while the other is chimaeric, containing diploid as well as tetraploid tissues. The first named is known to be, and the other probably is, a bud sport from William Sim. Both are red-flowered. Both seem to be identical in appearance with colchicine-induced tetraploids produced by Stewart[8] and kindly shared with us. These aberrants will be subjected to genetic analysis as soon as suitable tetraploid test plants are available. Diploid test plants cannot be used so readily for this purpose because of very low seed production in crosses between tetraploids and diploids.

Summary.—These results indicate that the carnation William Sim and four mutant clones, Pink Sim, White Sim, Peppermint Sim, and Skyline Frosted Sim, all have the same genotype: *AA Ii Yy SS rr mm.* The mutant clones thus are somatic variants, probably in the nature of periclinal chimaeras, with the mutant condition on the outside, but not deep enough to affect the formation of gametes.

[1] M. B. Crane and W. J. C. Lawrence, *The Genetics of Garden Plants* (New York: Macmillan Co., 1947); D. F. Jones, *Bot. Rev.*, **7** (No. 6), 291 (1941); A. D. Shamel and C. S. Pomeroy, *J. Heredity*, **27**, 487 (1936).

[2] M. G. Kains and L. M. McQuesten, *Propagation of Plants* (New York: Orange Judd Co., 1949), p. 138.

[3] R. E. Clausen and T. H. Goodspeed, *Genetics*, **8**, 97–105 (1923).

[4] G. A. L. Mehlquist, *Proc. Amer. Soc. Hort. Sci.*, **46**, 398 (1945).

[5] T. A. Geissman and G. A. L. Mehlquist, *Genetics*, **32**, 410 (1947).

[6] G. A. L. Mehlquist and T. A. Geissman, *Ann. Missouri Bot. Gard.*, **34**, 39 (1947).

[7] G. J. Dowrick, *Gard. Ill.*, **69** (No. 12), 308 (1952).

[8] R. N. Stewart, *Proc. Amer. Soc. Hort. Sci.*, **57**, 408 (1951).

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*A PARTIAL MAP OF LINKAGE GROUP D IN NEUROSPORA CRASSA**

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In connection with investigations of the genetic behavior of pyrimidine mutants in linkage group D (group IV of Barratt and Garnjobst[1]), data were obtained on linkage of other mutants in this group. This paper reports these data, together with results from additional crosses. Although these results are not entirely unambiguous, they appear to indicate the order of eleven genes located on one arm of the chromosome.

Description of Mutants.—The biochemical mutants used are, as follows: *arg* (33442), arginine;[2] *pdx* (37803), pyridoxine, not pH-sensitive;[3] *pyr 1* (263) and *pyr 2* (38502), pyrimidine, and *pyr 3* (37815), pyrimidine, temperature-sensitive;[3] *ad* (28610), adenine; *hist* (C141), histidine;[4] *pan* (34556), pantothenic acid.[3]

Of the three visible mutants, one, *co* (70007), "colonial," has been described.[2, 5] A second, *cot* (C102), "colonial," temperature-sensitive, has been described only

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Full Length Research Paper

Effects of electron beam radiation on trait mutation in azuki bean (*Vigna angularis*i)

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Dry seeds of azuki bean (*Vigna angularis*i), Jingnong 6 and Hebei 801 varieties were irradiated by electron beam of 100, 300, 600, 700 and 900 Gy, respectively. Mutations of leaf shape and color, seed size and shape, trailing, more branching, dwarfing, early or late flowering time and high yield were created in M_2 and M_3 generations. There were richest variations in Jinnong 6 treated with 600 Gy. Heibei 801 was more sensitive to electron beam radiation than Jingnong 6; more mutation types were produced at 100, 300 and 600 Gy. The pod number per plant, seed number and yield per plant of Jinnong 6 displayed a strikingly negative correlation to radiation dose, while the pod length, pod width, and 100-seeds weight of progenies from Hebei 801 had a significantly negative correlation with the radiation dose, but pod number per plants showed significantly positive correlation. Few of the normal phenotype plant in M_2 generation derived mutants of new leaf yellowing, narrow leaf, clustering flower and leaf, kidney or sword leaf in M_3 generation. Mutants of kidney and sword leaf, early flowering time from M_2 generation could be stably inherited in M_3 generation.

Key words: Azuki bean (*Vigna angularis*i), electron beam radiation, trait mutation.

INTRODUCTION

Azuki bean (*Vigna vulgaris* Ohwi and Ohashi) originated in China (Vavilov, 1935). It had been cultivated more than two thousand years. Azuki bean is one of the important food legumes in China. Its area is 2.5 to 3.0 million hm^2 and total yield is 3 to 4 million ton per year, ranking first in the world. Radiation breeding induce plant mutation; by X-ray, γ-ray, ion beam, laser beam, neutron and electron beam, which result in gene mutation and chromosome aberration, and then gain new variety (Chen, 2002). Calaldecatt (1955) firstly treated barleys using 2 MeV electrons beam and showed that electron radiation induced high mutation rate and wide mutation spectrum. Most of research reports of electron beam radiation breeding were published in China; the earliest is in the 1980s. Some researches revealed that electron beam radiation holds small physiological damage in M_1 generation and wide mutation frequency in M_2 generation.

5 MeV electrons and ^{60}Co-gamma-radiation were used to irradiate dry seeds of rice. The results showed that electron beam possess lower damage, higher mutagen frequency, and wider mutagen spectrum than ^{60}Co-gamma-radiation (Guo et al., 1982). The optimum doses for germinating seeds and dry seeds of rice were 50 and 150 Gy, respectively. Indica was more sensitive to electron beam than *Japonica* (Shu et al., 1996). In barley, the half lethal dose (LD_{50}) of electron beam radiation was 2.5×10^4 to 3.5×10^4 rad, lethal dose (LD_{100}) of 6.3×10^4 rad, and 1.0×10^4 to 2.5×10^4 rad was the appropriate inducing dose (Xu et al., 1983).

The dry seeds of 4 barley cultivars were irradiated by electron beam with doses from 100 to 300 Gy. The variation lines of high yield, dwarf and large 1000-grain weight were gained (Rui et al., 1995). The mutants of chlorophyll and growth period were created in M_2 generation of soybean treated by electron beam, and the appropriate radiation dose was 2.7×10^4 to 4.4×10^4 rad.

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(Li et al., 1988). LD_{50} was 40 Gy in M_1 generation of sorghum radiated by electron beam, different color sorghum seeds had different sensitivity on electron beam radiation, and the appropriate dose of white seed was from 30 to 50 Gy, while it was from 100 to150 Gy in red seed (Lu et al., 1995). 22 seedless mutants were selected from electron beam-induced orange bud. The suitable dose of treating sweet orange bud was around 5.0×10^3 rad and mandarin oranges was about 3.0×10^3 rad (Zhou et al., 1995). Electron beam radiation was also used in ornamental plants breeding. The mutants of flower color, flower petal, and flowering time were produced in tissue culture seedlings of chrysanthemum treated with 30 to 50 Gy electron beam (Lin et al., 2000). The percentages of bud formation of 100 Gy electron beam-irradiating Mauve and Indikon lines were 3.7 and 11.3%, respectively in African violet (*Saintpaulia ionahta*), and doses from 40 to 60 Gy were suitable for leaf tissue (Zhou et al., 2006). Electron beam inhibited growth and development of plants and resulted in flower mutation in cockscomb (*Celosia cristata* L). The mutation rate was between 0.5 to 2%. 150 Gy was appropriate dose of treating dry seed of cockscomb (Wang et al., 2006). LD_{50} and LD_{100} of electron beam-radiating scarlet sage were 55 and 85 Gy, respectively (Huang et al., 2007).

Electron beam radiation could significantly inhibit the growth and development of M_1 generation plants of Gladiolus in the seedling and initially flowering period; LD_{50} of treating corm of Super rose cultivar was 240 Gy, but LD_{50} of Beauty queen was greater than 240 Gy (Zhang and Wang 2008). Until now, the report of electron beam mutagenesis in azuki bean is still not published. In this research, azuki bean varieties Jinnong 6 and Hebei 801 were treated with different dose electron beam. The effects of mutation were analyzed for exploring an optimum inducing dose in azuki bean and creating more mutants. It is very useful in acquiring mutants for gene mapping, cloning and breeding of azuki bean.

MATERIALS AND METHODS

Azuki bean Jinnong 6 and Hebei 801 varieties were supplied by College of Plant Science and Technology in Beijing University of Agriculture. Jinnong 6 was bred by Beijing University of Agriculture. Whole growth period of Jingnong 6 was from 90 to 95 days. Its average plant height is 50 cm (Jin et al., 2000). Hebei 801 with big seed was bred by Hebei Province, and its average 100-seed weight is more than 20 g.

Electron beam radiation treatment

In 2007, dry seeds of Jingnong 6 and Hebei 801 were irradiated with electron beam of 100, 300, 600, 700 and 900 Gy dose, respectively (5 MeV, BF-5 electron linear acceleratorelectric current intensity 0.2 mA, 4 Gy/min) in Institute of Low-Energy Nuclear Physics of Beijing Normal University. 1800 seeds of Jinnong 6 were treated with 600 Gy dosage, and the rest doses treated 220 dry seeds of Jinnong 6, respectively. Each of HB801 220 dry seeds was radiated by 100, 300, 600, 700 and 900 Gy, respectively. The controls were non treated Jinnong 6 or HB801 dry seeds.

Planting

The electron beam-treated Jingnong 6 and Hebei 801 seeds were planted in the experimental field of Beijing University of Agriculture on June 13th 2007. Germinating rate of M_1 generation was investigated and calculated. Every plant was separately harvested in the autumn of 2007. On June 16th 2008, all seeds from M_1 generation were planted according to the individual plant. The row length was 3 m, and 35 seeds were sown in each row, and a row of control was planted per 10 rows. During the whole growing stage, the traits of plant architecture, leaf shape, leaf color, flowering time, pod color resistant and susceptible disease, and growth period in M_2 generation were investigated. Every single plant of trait mutation was recorded and tagged, respectively. All tagged morphological mutation plants were harvested individually in mature period, and then their branch number on main stem, plant height, pod length and width, seed color and shape, 100-seeds weight, seed number and yield per plant were tested. The data was analyzed statistically. On June 13, 2009, the seeds of tagged each mutant and a part of seeds from the non variational trait plants in M_2 generation were planted. One row contrast was grown at every 20 rows. The phenotype traits and growth period were extensively surveyed and tracked during the whole growing period in M_3 generation. Mutants were further identified. The data was statistically analyzed.

Statistical analysis

Average is $x^{\wedge} = \sum x / N$, in which, $x^{\wedge}$ delegates mean value, x the observed value, and N is the number of observed value. Coefficient variation $(CV) = \sigma / x^{\wedge}$, in which σ stands for standard difference, and CV is the statistics for elevating variation degree of all observed values. Correlation analysis is conducted using DPS analysis soft.

RESULTS

Impacts of radiation doses on germination rate of M_1 generation

Germination rate of Jingnong 6 and Hebei 801 radiated by electron beam decreased with the increase of radiation dosage (Table 1). No one seed of Jingnong 6 germinated at doses of 700 and 900 Gy. Hebei 801 had relatively higher germination rate than Jingnong 6, indicating that Hebei801 is more tolerant to electron beam radiation. It is evident that sensitivity of different azuki bean variety is different under the electron beam radiation. LD_{50} of electron beam radiating azuki bean is approximately 132 Gy.

Mutation types and frequency of M_2 generation

Jingnong 6 has phenotype of ovate leaf of deep green color and determined growth. The mutants of kidney leaf, sword leaf, lanceolate leaf, small heart-shaped leaf, light

Table 1. Germination rate of M_1 generation induced by electron beam in azuki bean.

Treatment	Dosage (Gy)	Number of seed	Number of seedlings (%)	Germination rate (%)	Relative germination rate[a] (%)
Jingnong 6 control	0	360	242	67.20	100.00
	100	220	43	19.55	29.09
	300	220	19	8.63	12.84
Jingnong 6	600	1800	81	4.50	6.70
	700	220	0	0.00	0.00
	900	220	0	0.00	0.00
Hebei 801 control	0	70	48	68.60	100.00
	100	220	108	49.09	71.56
	300	220	66	30.00	43.73
Hebei 801	600	220	11	5.00	7.29
	700	220	7	3.18	4.64
	900	220	8	3.64	5.31

Relative seedling rate = seedling rate of induced plants/ seedling rate of control plants × 100%.

Table 2. Mutation frequency of M_2 generation induced by electron beam in azuki bean.

Mutant trait	Jingnong 6 (%)			Hebei 801 (%)				
	100 Gy	300 Gy	600 Gy	100 Gy	300 Gy	600 Gy	700 Gy	900 Gy
Dwarf	-	3.23	0.58	0.27	0.75	1.67	-	-
Kidney leaf	-	-	0.58	1.64	3.01	1.67	-	-
Sword leaf	-	-	0.49	-	-	-	-	-
Small leaf	2.94	-	7.51	-	1.5	1.67	6.25	4.17
Small heart-shaped leaf	-	-	0.58	-	-	-	-	-
Early flowering	-	-	-	0.27	0.38	-	-	-
Late flowering	-	-	-	1.09	0.38	1.67	-	-
Light green leaf	-	-	9.83	2.73	3.38	6.67	-	-
Dark green leaf	-	-	-	-	-	-	6.25	-
Yellowing leaf	-	-	0.58	0.27	0.75	-	-	-
More branches	-	-	1.16	0.82	1.13	6.67	-	-
Trailing	-	-	-	0.82	1.13	6.67	-	-
susceptible mosaic virus	5.88	-	-	0.82	0.75	-	-	-
High yield	-	-	1.73	-	-	-	-	-

green and yellowing leaf, trailing, multi-branch, susceptible mosaic virus, dwarf and high yield were produced in M_2 generation (Table 2, Figures 1 and 2). Hebei 801 showed the phenotype of heart-shaped leaf and determined growth. Variations of dwarf, kidney leaf, small leaf, early or late maturing, light and dark green leaf and trailing in M_2 generation were created (Table 2, Figures 1 and 3). Electron beam radiation had better efficiency to Hebei 801 than to Jingnong 6. The most mutation types of Jingnong 6 were obtained at 600 Gy doses, while more variation types of HB801 were gained at 100, 300 and 600 Gy.

Impacts of electron beam radiation on agronomic traits of M_2 generation

Plant height, 100-seed weight and average node number of main stem increased in M_2 generation compared to Jingnong 6 control. Pod number per plant, seed number per plant and yield of single plant at low radiation dose increased and decreased at high radiation dose; both the

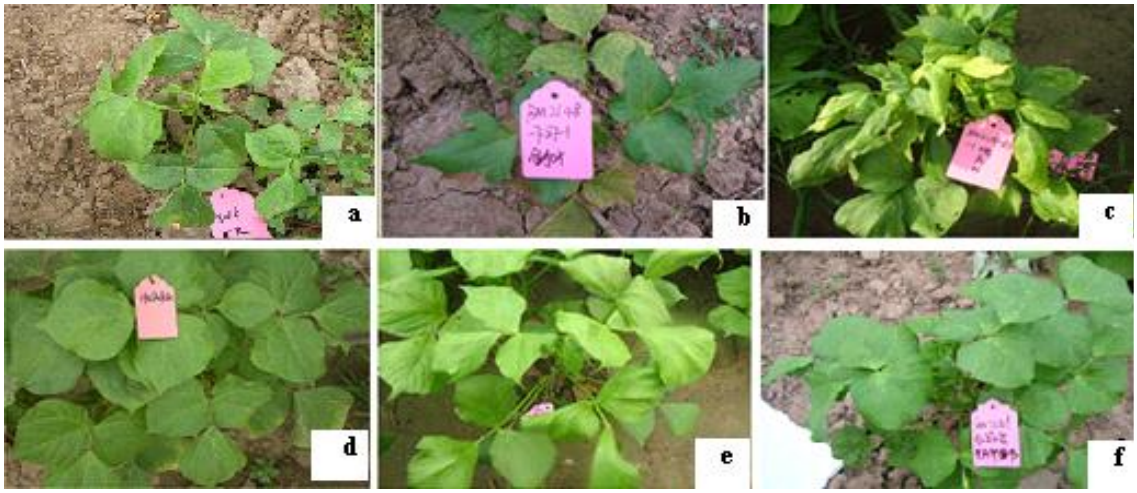

Figure 1. Mutants of leaf shape induced by electron beam. (a) Jingnong 6 control. (b) Sword leaf (600 Gy from Jingnong 6). (c) Lanceolate leaf (600 Gy from Jingnong 6). (d) Hebei 801 control. (e) Kidney leaf (100 Gy from Hebei 801). (f) Oval leaf (600 Gy from Hebei 801).

Figure 2. Mutant of yellowing leaf in Jingnong 6 treated by electron beam. (a) Jingnong 6 control. (b) Mutant of yellowing leaf (600 Gy).

Figure 3. Mutant of plant shape in Hebei 801 treated by electron beam. (a) Hebei 801 control. (b) Mutant of plant architecture and compound leaf (100Gy).

Table 3. The difference comparison of main agronomic traits in M_2 generation treated by electron beam with controls.

Trait	Jingnong 6			Hebei 801				
	100 Gy	300 Gy	600 Gy	100 Gy	300 Gy	600 Gy	700 Gy	900 Gy
	M-CK	M-CK	M-CK	M-CK	M-CK	M-CK	M-CK	M-CK
Plant height (cm)	6.14	1	1.6	2.31	-0.86	21.54	-3.54	-7.28
Length of pod (cm)	-0.23	-0.27	-0.11	0.16	-0.43	-3.07	-1.19	0.44
Width of pod (cm)	0.05	0.05	0.03	-0.04	-0.06	-0.16	-0.09	-0.05
Pod number per plant	4.46	0.75	-5.91	2.88	1.79	6.24	5.55	7.02
Seed number per plant	12.8	-9.6	-37.1	16.07	8.27	2.23	3.88	30.92
Yield per plant (g)	4.39	0.82	-4.95	0.77	-2.18	-5.72	-3.64	3.89
100 seed weight	2.35	2.85	3.59	-2.57	-4.21	-8.33	-6.16	-2.58
Mode number of main stem	2.01	2.36	0.76	1.28	0.45	2.78	0.22	0.82

M-CK, the average of mutants subtract the average of control.

Table 4. Coefficient of variation of main agronomic traits in M_2 generation induced by electron beam.

Trait	Jingnong 6				Hebei 801					
	Control	100 Gy	300 Gy	600 Gy	Control	100 Gy	300 Gy	600 Gy	700 Gy	900 Gy
Plant height (cm)	27.51	20.07	23.18	22.61	23.58	28.25	36.06	22.71	37.16	15
Node number of main stem	22.91	16.07	16.75	21.21	13.52	47.72	17.75	18.07	19.4	12.63
Pod length (cm)	10.87	23.13	15.68	18.01	12.35	58.36	17.13	28.69	28.53	12.28
Pod width (cm)	7.69	9.43	7.514	9.87	8.033	10.95	11.78	9.8	11.06	10.35
Pod number per plant	54.37	71.57	58.08	85.24	49.24	75.8	82.02	91.51	96.9	52.51
Seed number per plant	49.76	80.83	57.66	87.26	54.11	79.53	86.94	98.1	89.09	48.29
Yield per plant (g)	55.15	81.99	60.44	87.21	54.88	76.21	79.44	94.92	81.84	51.68
100-seed weight (g)	8.29	14.41	15.37	94.11	4.65	31.12	29.4	21.96	27.84	25.51

pod length and pod width were proximate to the contrast (Table 3). Plant height, 100-seed weight, node number of main stem, pod width were close to the contrast Hebei 801's in M_2 generation induced with different dose electron beam, while pod length and yield per plant increased. It is clear that same character of different azuki bean variety had differential sensitivity at same radiation dose.

Analysis on the coefficient of variation of agronomic characters in M_2 generation

On the whole, Jingnong 6 treated with 600 Gy dose had the max coefficient of variation (CV) in, pod number per plant, seed number per plant, yield per plant and 100-seed weight. Hebei 801 treated with 700 Gy recorded the max CV in plant height and pod number per plant, the most CV of node number of main stem, pod length and 100-seed weight at 100 Gy, as well as the most CV of seed number per plant and yield per plant at 600 Gy (Tables 4 and 5). The correlation between main agronomic characters of Jingnong 6, Hebei 801 and electron beam radiation dose was analyzed (Table 6). Pod number per plant, seed number per plant and 100-seed weight of Jingnong 6 had significantly negative correlation to the radiation dose; the higher the dose was, the higher the negative impact on these characters was. The pod length, pod width and yield per plant of Hebei 801 showed significantly negative correlation to radiation dose, indicating that pod length, pod width and per 100-seed weight decreased significantly under high radiation dose, while the pod number per plant increased obviously. It is evident that same character of different cultivars had different correlation to the radiation dosage, while the different character of same variety had different correlation to the radiation dosage.

Mutation and heredity in M_3 generation mutagenized by electron beam

The phenotypes of kidney leaf, sword leaf and early or late flowering mutants from M_2 generation can be stably inherited in M3 generation (Figures 4 and 5). However, some variation traits of the leaf color and susceptible mosaic virus could not stably be inherited or segregated in M_3 generation, presumably these characters are

Table 5. The difference of variation coefficient of the main agronomic traits between progenies of M_2 and controls.

Trait	Jingnong 6			Hebei 801				
	100 Gy	300 Gy	600 Gy	100 Gy	300 Gy	600 Gy	700 Gy	900 Gy
Plant height (cm)	-7.44	-4.33	-4.9	4.67	12.48	-0.87	13.58	-8.58
Node number of main stem	-6.84	-6.16	-1.7	34.2	4.23	4.55	5.88	-0.89
Pod length (cm)	12.26	4.81	7.14	46.01	4.78	16.34	16.18	-0.07
Pod width (cm)	1.74	-0.18	2.18	2.92	3.75	1.77	3.03	2.32
Pod number per plant	17.2	3.71	30.87	26.56	32.78	42.27	47.66	3.27
Seed number per plant	31.07	7.9	37.5	25.42	32.83	43.99	34.98	-5.82
Yield per plant (g)	26.84	5.29	32.06	21.33	24.56	40.04	26.96	-3.2
100-seed weight (g)	6.12	7.08	85.82	26.47	24.75	17.31	23.19	20.86

Table 6. Correlation analysis between main agronomic traits of electron beam radiating M_2 generation and radiation doses.

Agronomic trait	Rediation dosage (Jingnong 6)	Rediation dosage (Hebei 801)
Plant height (cm)	-0.06	0.07
Node number of main stem	-0.09	0.01
Pod length (cm)	0.03	-0.15**
Pod width (cm)	-0.01	-0.27**
Pod number per plant	-0.23**	0.08*
Seed number per plant	-0.28**	0.01
Yield per plant (g)	0.04	-0.21**
100-seed weight (g)	-0.25**	-0.06

*p < 0.05, **p < 0.01.

Figure 4. The heredity of kidney leaf mutant. (a) Phenotyepe of kidney leaf mutant BM2015 in M_2. (b) Phenotype of kidney leaf mutant BM2015 in M_3.

sensitive to environmental effects. Several mutants of kidney leaf, sword leaf, new leaf yellowing, plant yellowing in M_3 generation were separated from normal morphologic plants of M_2 generation (Figures 6, 7 and 8). Variational types of crimping leaf, clustering leaf or flower, poor fertility and less pod number from normal plant of M_2 generation were derived in M_3 generation (Figure 9). More mutants were segregated from normal

Figure 5. The heredity of sword leaf mutant. (a) Phenotyepe of sword leaf mutant BM2148 in M_2. (b) Phenotype of sword leaf mutant BM2148. in M_3.

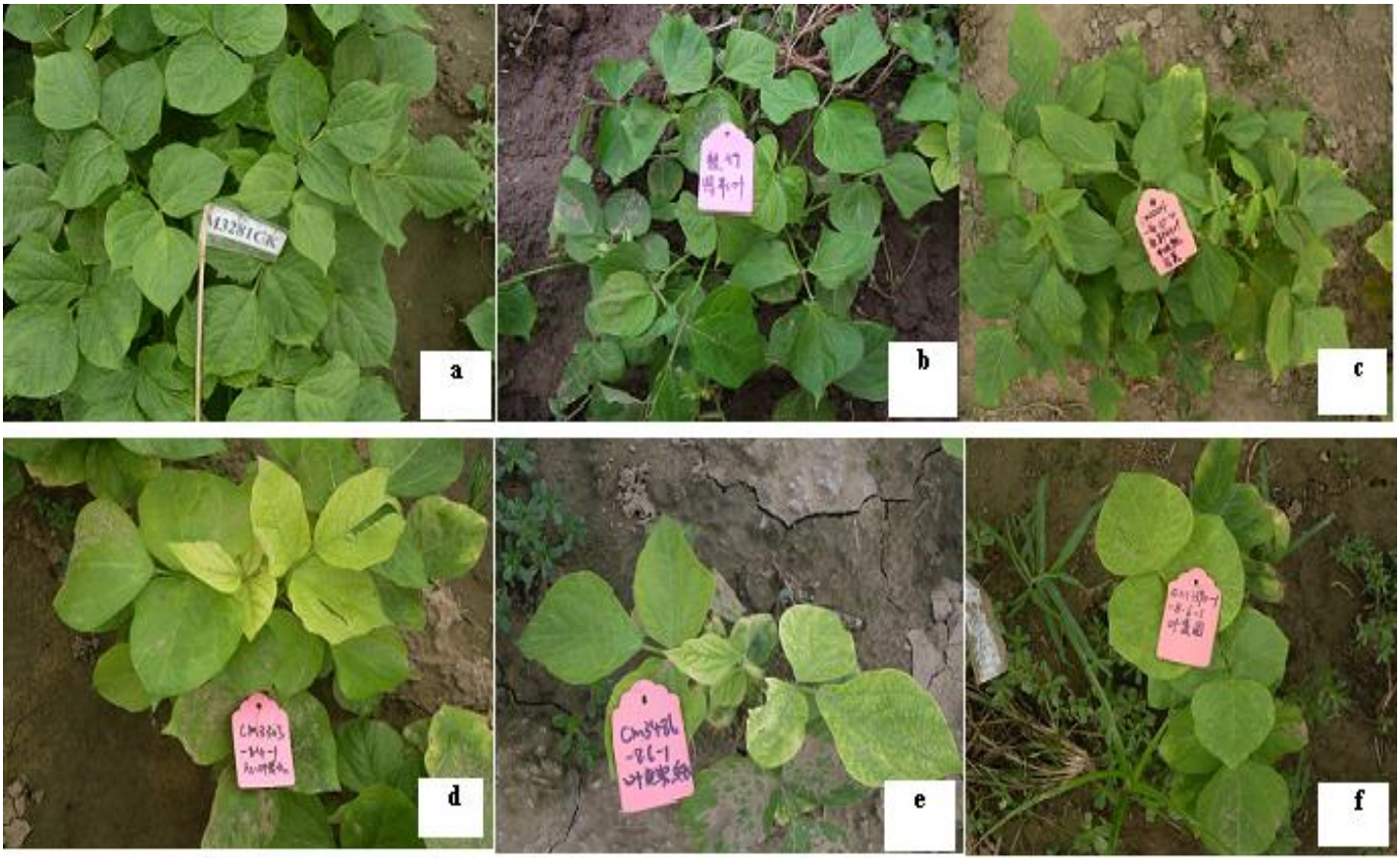

Figure 6. Segregative mutants of M_3 generation from M_2 wild phenotyepe plant of Hebei 801 induced by electron beam. (a) Control Hebei 801. (b) Kidney leaf (100 Gy), (c) Sword leaf (100 Gy); (d) New leaf yellowing (100 Gy), (e) Narrow leaf (100 Gy). (f) Heart-shape leaf (600 Gy).

Figure 7. Segregative mutants of M_3 generation from M_2 wild phenotyepe plant of Jingnong 6 induced by electron beam. (a) Control Jingnong 6. (b) Sword leaf mutant (600 Gy). (c) New leaf yellowing mutant (100 Gy).

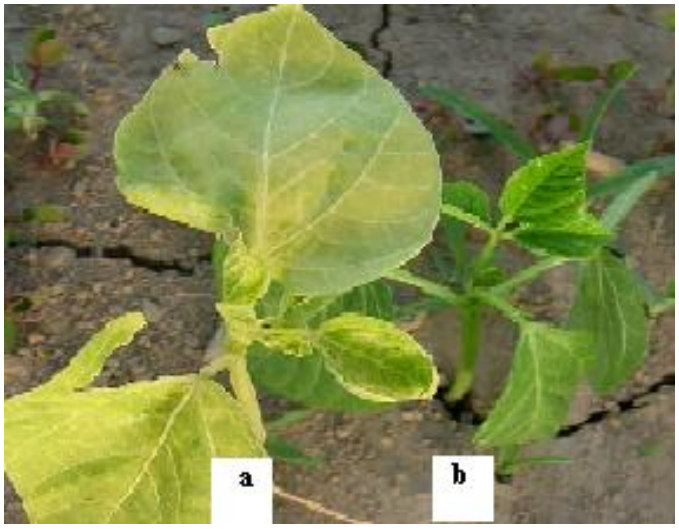

Figure 8. Segregated yellowing and leaf mutant of M_3 from M_2 wild phenotyepe progeny of HB801 induced by electron beam. (a) Yellowing and compound leaf-free mutant (300 Gy). (b) Normal phenotype plant.

phenotype plants of Hebei 801 than Jingnong 6. Continuous investigation will be done whether this mutant phenotype could stably be inherited.

Seed size and shape mutants in M_3 generation

Seed size and shape mutants were gained in M_3 generation. The average 100-seed weight of Jingnong was around 16 g; big and small seeds with average 100-seed weight of 24.0, 15.0, 9.2 and 5.6 g, respectively in M_3 generation (Figure 10). Jingnong 6 seed is big and elliptical; the round and short cylinder seeds were obtained in M_3 generation (Figures 11 and 12). Hebei 801's 100-seed weight was above 20 g. The mutants of medium and small seed size were got in M_3 generation. 100-seed weight of medium or small seed mutants was 15.0 and 9.5 g, respectively (Figure 13). Hebei 801 seed was long and cylindrical, and round and short-cylinder seeds were produced in M_3 generation (Figures 14 and 15).

DISCUSSION

Creating variation is the prerequisite of breeding new cultivars, mapping gene, and map-based cloning. Azuki bean is a cleistogamous plant with extremely low crossing and variation rate in natural environment. Electron beam radiation has little influence on the function of plasma membrane and protein, while it results in gene mutation through inducing much DNA damage of single strand breaks (SSB) and double strand breaks (DSB). The *G*-value for DSB formation of electron beam

Figure 9. New mutant from M_3 progeney of Hebei 801. (a) Clustering plant mutant (300 Gy); (b) Clustering flower mutant (300 Gy); (c) Control Hebei 801; (d) Flower of control Hebei 801.

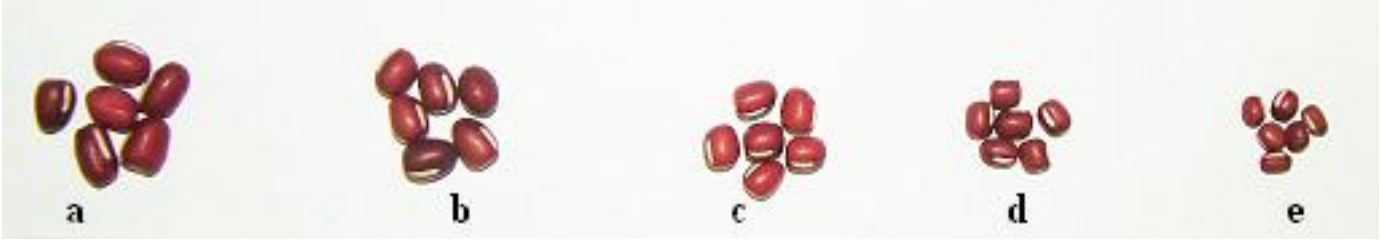

Figure 10. Mutants of seed size from Jingnong 6; (a) big seed (300 Gy); (b) control Jingnong 6; (c) middle seed (100 Gy); (d) small and short cylinder seed (100 Gy); (e) smallest seed (600 Gy).

radiation in aqueous solution was 5.7 times higher than that caused by 60 Co-gamma rays (Zhu et al., 2008). Electron beam radiation has higher efficiency variation, low cost, safety and smaller radiation damage. Weng et al. (1974) thought that more mutants were segregated in electron beam-irradiated M_2 generation of soybean. This research indicates that electron beam irradiation result in many types of mutations in M_2 and M_3 generations of

Figure 11. Round seed mutant of Jingnong 6. (a) Control Jingnong 6. (b) Round seed mutant (600 Gy).

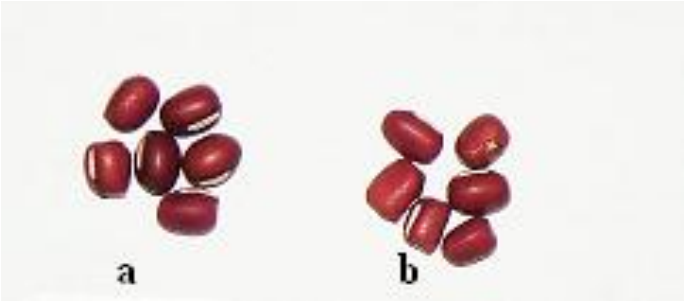

Figure 12. Cylinder seed mutant from Jingnong 6. (a) Control Jingnong 6. (b) Columnar seed mutant (100 Gy).

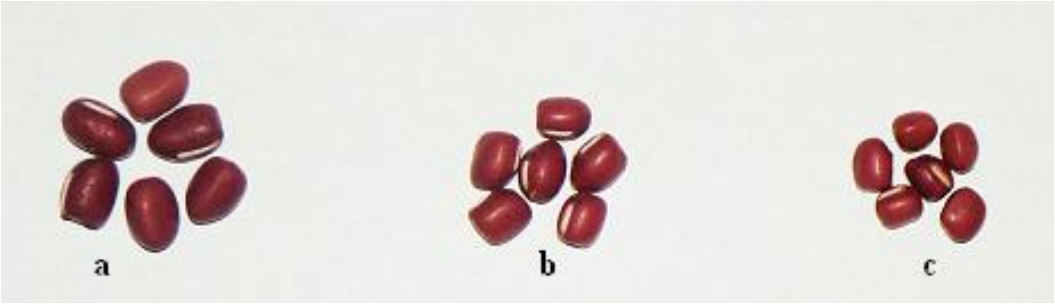

Figure 13. Mutant of seed size from Hebei 801. (a) Control Hebei 801; (b) Middle seed mutant (100 Gy); (c) Small seed mutant (100 Gy).

Figure 14. Round seed mutant from Hebei801. (a) Control Hebei 801; (b) Round seed mutant (300 Gy).

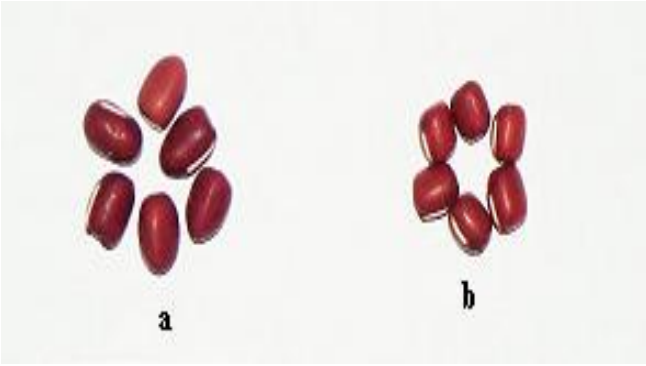

Figure 15. Short cylinder seed mutant of Hebei 801. (a) Control Hebei801; (b) Short cylinder seed mutant (100 Gy).

azuki bean.

Variation in M_2 generation originates from radiation and environmental effect. The CV of contrast is the reaction of environmental effect, while the difference between CV of M_2 generation and contrast reflects to radiation effect (Jin et al., 2000); because different varieties has significant difference in electron beam irradiation, therefore different azuki bean variety should be treated with its appropriate radiation dose for gaining the best mutation. Phenotype of sword leaf, kidney leaf, early and late flowering mutants can be stably inherited. Some variational characters of leaf color, susceptible mosaic virus in M_2 generation segregate failed to be inherited in M_3 generation; maybe these characters are controlled by recessive genes or are susceptible to environment. A few of normal phenotype plants in M_2 generation segregate out variations of narrow leaf, new leaf yellowing, clustering flower and leaf, kidney leaf, sword leaf in M_3 generation. These segregated mutants will be further identified in later generations. Mutation frequency and variational types induced by electron beam are overall lower than ethyl methane sulfonate (EMS) mutagenesis in azuki bean (Tong et al., 2010), but more mutants of seed size and shape are obtained. Electron beam mutagenesis is very useful for breeding, gene mapping, gene cloning and functional analysis in azuki bean.

Conclusion

Electron beam mutagenesis are effective in azuki bean and can create mutations of leaf shape and color, seed size and shape, plant architecture, plant height, early and late flowering time, trailing and high yield etc., especially to induce more mutants of seed size and shape. LD_{50} is about 132 Gy in azuki bean. Different azuki bean variety has different sensibility to electron beam radiation. There are most variation types in 600 Gy irradiating Jingnong 6, and 300 Gy treating Hebei 801. The mutants of kidney leaf and sword leaf, early or late flowering time from M_2 generation, can be stably inherited in M_3 generation.

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REFERENCES

Calaldecatt RS (1955). The effects of X-rays, 2-mev electrons, thermal neutrons, and fast neutrons on dormant seeds of barley. New York Acad. Sci. 59:514-535.

Chen ZY (2002). Prospect of plant breeding by spaceflight from the views on development of irradiation breeding in China. Acta Agriculturae Nucleatae Sinica 16(5):261-263.

Guo BJ, Wu YY, Ruan JH (1982). Studies on the mutagenic effect of 5MeV electron irradiation on rice. Acta Genetica Sinica 9(6):461-467.

Huang HT, Wang D, Zhou LJ, Li WF, Song H (2007). Effect of combined treatments of electron beam radiation and GA on growth and developmemt of M_1 of Scarlet sage dry seeds. Acta. Agriculturae Boreali-occidentalis Sinica 16(4):253-256.

Jin WL, Chen XZ, Yu SF (2000). A study on the radiative mutation on the seeds of Adzuki bean by ^{60}Co γ ray, Acta Agriculturae Nucleatae Sinica 14(3):134-140.

Li GQ, Yu SH, Li M (1988). Study of the effect of electron beam on Soybean radiation mutation. Acta Agriculturae Universitatis Jilinensis 10(3):57-62.

Lin ZJ, Sun JX, Cui GQ, Liu XB, Xin GS, Wang JL (2000). Study on technique of mutation breeding by electron beam in chrysanthemum. Shandong Agric. Sci. 5:10-11.

Lu W, Su YM, Song GY (1995). Comparison of electron beam and γ ray irradiated seeds of Sorghum. J. Nucl. Agric. Sci. 16(4):160-163.

Rui JY, Jin JL, Ma XS, Huang PZ, Ma JH (1995). Studies on the mutagenic effect of the electron beam in Barley(*Hordeum Vulgarel.*)-the expression of mutagenic mutants in M_3 and M_4 generations. J. Radiat. Res. Radiat. Proc. 13(3):170-172

Shu QX, Li WH, Xia YW, Wu DX, Rui JY (1996). Study on the M_1 generation of the biological effects of electron beam implanted seeds of rice. J. Nucl. Agric. Sci. 17(4):162-164.

Tong X, Zhao B, Jin WL, Zeng CW, Liu HX, Wu BM, Pu SJ, Chen XZ, Pan JB, Wan P (2010). Identification of mutants from Azuki bean (*Vigna angularisi*) Jingnong6 seed induced by physical and chemical agents. Acta Agronomic Sinica 36(4):565-573.

Vavilov NI (1935). Theoretical basis of plant breeding. VIR, St. Petersburg, USSR.

Wang D, Ren SX, Li WF, Su Q, SU J (2006). Plant growth of E-beam irradiated or ion implanted seeds of ornamental Celosia. J. Radiat. Res. Radiat. Proc. 24(3):188-192.

Weng XY, Wang BR, WU HL, Wang LZ, Chen Y, Xu XC (1974). Radiation breeding of Soybean. Acta Genetica Sinica 1(2):157-169.

Xu XC, Zhou XH, Li GZ, Dai GQ, Wang PD, Wang BL (1983). Inducing mutation by electron beam in Barley. Appl. Atomic Energy Agric. 4:44-50.

Zhang ZW, Wang D (2008). Effect of electron beam irradiation on growth and development of induced Gladiolus. J. Northeast Forestry Univ. 36(1):26-27.

Zhou LB, Li WJ, Ma S, Dong XC, Li Q, Gao QX (2006). Effect of X-ray and electron beam irradiation on adventitious bud regeneration

from *in vitro* leaf explants of *Saintpaulia ionahta*. Radiat. Res. Radiat. Proc. 24(1):53-58.
Zhou YB, Peng DX, Zhou ZX, Zhang JY, Dai ZG (1995). Studies on the mutagenic effect of electron beam on Citrus budwood. China Citrus 24(1):7-10.
Zhu H, Xu JZ, Li SQ, Sun XY, Yao Side, Wang SL (2008). Effects of High-energy pulse electron beam radiation on Biomacromolecules. Science in China Series B: Chem. 51(1):86-91.

SPECIAL ARTICLES

MUTATIONS IN BARLEY INDUCED BY X-RAYS AND RADIUM

AT the Nashville meeting of the American Association last December I reported the occurrence of mutations in barley following X-ray treatment.[1] The experiments, which were independent of and coincident with those of Muller,[2] though by no means so comprehensive and thorough, confirm Muller's discovery of the power of X-rays to induce mutation and show its application to plants. They show also that mutations may be induced similarly by radium treatment.

The treatments were applied to germinating seeds, and the induced mutations recorded were in all cases somatic mutations affecting the progeny of only part of the plant. The experiments were set up in this way in order to exclude the possibility that new characters appearing after treatment might be ascribable to some irregular segregation from hybrid ancestry. The barley plant produces several tillers from axillary buds, each tiller terminating in an inflorescence of about thirty self-fertilizing flowers. In the dormant embryo the first three or four leaves are already differentiated, and the cells from which the tillers will be developed are separated. A mutation occurring in one of these cells, therefore, will affect only one tiller, and, whether dominant or recessive, will segregate in the progeny of only one head.[3] Its absence in the progeny of other heads of the same plant shows that the genetic change occurred during the development of the plant treated, for any ancestral character, however inherited, would affect all head progenies of the individual similarly.

The X-ray treatments were applied intermittently in twelve equal exposures at one-hour intervals, while the seeds were germinating under optimum conditions. A 30 m.a. "radiator-type" self-rectifying Coolidge tube was used, operated at a tube current of 5 m. a. Treatments were made at seventy-eight kilovolts (peak), two and three quarter minutes per exposure, and at fifty-four kilovolts, five and one half minutes per exposure, in an attempt to apply approximately equal quantities of radiation differing somewhat in quality. At each voltage a "heavy" and a "light" treatment were applied simultaneously, at target distances of 22.7 and 45.4 cm, respectively. The radiation passed through two samples of seed at shorter distances, and the filtering effect of the wet blotters and seeds must be considered in computing dosage. Ionization measurements made later showed that this reduced the intensity of the radiation at the higher voltage by about 52 per cent., and of that at the lower voltage by about 65 per cent. The relative ionizing intensity of the heavy and light treatments at the higher voltage and the heavy and light treatments at the lower voltage was in the ratio 100: 21: 50: 9. The so-called heavy doses were not heavy enough to reduce viability appreciably, but a dose of approximately three times this intensity, with the higher voltage, was found to be partially lethal. I am indebted to the department of physics of this university for the use of the X-ray equipment, and particularly to Mr. R. T. Dufford, of that department, for much advice and assistance and for the construction of an ionization chamber of the Duane type,[4] with which the dosage measurements above were made.

The radium treatments were applied under similar conditions, using as a source 50 mg of radium in the form of radium sulfate, sealed in a thin glass tube within a tube of silver 1 mm thick. Dr. Dudley A. Robnett, of Columbia University, generously lent his personal supply of radium for the treatments. The seeds, germinating in stacked watch glasses, were exposed continuously for twelve or twenty-four hours at distances ranging from one and a half to eleven cm. The maximum dose (applied to seeds in the dishes immediately above and below that containing the radium tube) was well below the limit of tolerance.

The mutations previously reported were three seedling chlorophyll defects, "white," "virescent," and "yellowing." White seedlings are colorless from emergence, and die in two to three weeks. Virescent seedlings are colorless at emergence, but gradually develop a pale green color. With care they may be kept alive for a long period, but they grow very slowly. Both white and virescent seedling types have previously been reported as Mendelian characters in barley. Yellowing seedlings are green and apparently normal at emergence, but about a week later they pass through intermediate shades to full yellow and die soon after.

These three mutations were found among seventy-seven head progenies, representing twenty-six X-rayed plants. Each mutant type made up one fourth to one eighth of a single head progeny from a plant of which other head progenies were entirely normal. If the segregation is due to a mutation affecting one head

[1] Experiments on the effects of X-rays on crossing over and chromosome distribution, reported in the same communication, have been published separately. (*Proc.* Nat. Acad. Sci., 14: 69–75, 1928.)

[2] SCIENCE, 66: 84–87, 1927.

[3] Under favorable conditions tillers are sometimes developed from the axillary buds of earlier tillers. In such cases more than one head may be affected by the same mutation.

[4] *Am. Jour. Roent. and Rad. Therapy,* 10: 935–943, 1923.

or part of a head, some of the normal plants from this head should segregate the same mutant character in the following generation, while none of the plants from the unaffected heads should do so. This expectation was realized in the next generation, in which eight of the sixteen normal plants tested from the head which segregated white seedlings were found to be heterozygous for white, seven of the twelve plants tested from the head which segregated virescent were heterozygous for virescent, and three of the seven plants tested from the head which segregated yellowing were heterozygous for yellowing. In each case about fifteen plants from unaffected heads of the mutating plant were tested, and all gave entirely normal progeny.

The remaining progenies have since been planted and seedling segregations noted. The total frequency of mutations resulting in definite and conspicuous seedling characters is shown below:

	Total number of head progenies examined	Number segregating mutant seedling characters
X-ray treated:		
Higher voltage		
Heavy dose	210	6
Light dose	259	1
Lower voltage:		
Heavy dose	494	6
Light dose	280	1
Total X-rayed	1,243	14
Radium treated:		
Total for all doses	1,039	3
Untreated	1,341	0

The majority of the mutations listed were white seedlings. Tests of genetic identity have not yet been made, but it is probable that many of these represent mutations at different loci. At least three genetically distinct white seedlings have previously been reported in barley, and in maize, in which genetic analysis has been more intensive, dozens of genes for white seedling are known.

The mutations following radium treatment included two whites and one virescent. Since the radiation passed through 1 mm of silver and at least 5 mm of glass before reaching the seeds, it consisted largely of gamma rays. The fact that mutation frequency was lower in the radium series than in the X-ray series does not imply that radium is less effective than X-rays in inducing mutation, since the intensity of the radium treatment was probably much lower. All three mutations occurred in the plants receiving the heavier doses.

A preliminary trial was made also of the possibility of increasing the effectiveness of X-ray treatment by impregnating the seeds with salts of heavy elements. Since in general X-ray absorption increases approximately in proportion to the fourth power of the atomic number of the absorbing element, it may be increased materially by the presence of even a small amount of some heavy element within the cell. The seeds were soaked for seven hours in M/5 solutions of the salts listed below. The X-ray treatment, which began fifteen hours later, was similar to that described above as heavy dosage at the higher voltage, but the intensity was about 40 per cent. higher. The frequency of mutation for distinct seedling characters is shown below:

Treatment		Number of head progenies	Number of mutations
Radiation	Chemical		
X-rayed	$Ba(NO_3)_2$	136	9
X-rayed	$Pb(NO_3)_2$	133	9
X-rayed	$UO_2(NO_3)_2$	194	11
X-rayed	none	72	2
Not X-rayed	$UO_2(NO_3)_2$	53	0
Not X-rayed	none	76	0

Although the numbers are too small to justify a final conclusion, the results suggest that the chemical treatments increased the effectiveness of irradiation. The mutations included, in addition to types already noted, the following seedling characters: "yellow," "pale yellow," "yellow-green," "banded" (transverse white bands), "striped" (two distinct patterns) and "tapering" (a morphological peculiarity). Several possible mutations for less conspicuous seedling characters are omitted from the summary pending further investigation.

In all, forty-eight mutations causing distinct seedling characters have been found following irradiation. These include almost all the seedling characters of barley previously reported and several not previously described. No mutations have yet been found in untreated plants, of which about fifteen hundred head progenies have been examined.

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THE NUCLEAR CONDITIONS IN THE SPERMATOCYTES OF DROSOPHILA MELANOGASTER

IT is a surprising fact that very little has been known until recently concerning the nuclear condi-

Each year as funds permitted the division has undertaken conferences for the initiation and coordination of chemical research. Such conferences have been held on the subjects of permanence of printed records, on the coordination of chemical literature, on biological nomenclature, and on farm waste and chemistry of soils. The results of these meetings have been intangible in part, but the mere bringing together of eminent men interested in a certain phase of chemistry is important.

The division has from time to time collected pertinent data relating to chemistry. Since 1922, the division has each year made a census of graduate students in chemistry throughout the country, and this census has been published in the journals. The division also conducted a study of conditions of chemical research in the Southern States. The report of this study was published by the Chemical Foundation. Other activities include cooperation between academic and industrial research, and a list of research problems in various fields of chemistry.

One of the important activities of the division has been the administration of the grants in aid of research. During the past four years 42 grants have been made to 37 individuals. Many papers have been published as a result of these grants and many capable research workers have been encouraged in their work. Particularly during the last few years, the grants-in-aid have proved invaluable to research workers who have found themselves handicapped through lack of funds.

Another important activity of the Research Council and of the Division has been in connection with the National Research Fellowships. Since their foundation, 257 fellowships in chemistry have been awarded to 150 individuals. While it is true that some will profit more than others from the opportunities afforded by these research fellowships, there can be no question that the National Research Fellowships have produced tangible results.

Through meetings of the division and of committees, and through other contacts afforded by the division, those interested in various fields of chemistry are brought together and contacts are established. It is from these contacts that we may expect some of the major benefits in the course of time.

SCIENTIFIC EVENTS

CHICAGO PROGRAM ON RADIATION AND PLANT LIFE

THE American Society of Plant Physiologists has joined with Sections G and O and their affiliated organizations in the programs of June 20 and 21. On the morning of June 22, however, a special symposium has been arranged for plant physiologists who are attending the meetings. The meeting will be held in the Civic Opera Building, Chicago, in the rooms of the Lighting Institute at 10:00 A. M. The meeting will be open to all visiting botanists and to professional growers of plants. The titles of the papers are as follows:

"Influence of Radiation on CO_2 Absorption by Plants": W. H. Hoover, Smithsonian Institution.

"The Interrelated Effects of Light and Temperature on Plant Growth": A. D. Davis, University of California.

"Growth as a Criterion for Physiologic Response to Radiations": E. S. Reynolds, Missouri Botanic Garden.

"Photoperiodism and its Practical Application to Greenhouse Crops": Alex Laurie, Ohio State University.

"Responses of Certain Plants to Artificial Radiation Factors Applied as Supplements to Daylight": R. B. Withrow, Purdue University.

"The Response of Greenhouse Plants to Electric Light Supplementing Daylight": Laurenz Greene, Purdue University.

"Experimental Work at Pennsylvania State College on Radiation as Applied to Plants": H. W. Popp, Pennsylvania State College.

"Chemical Responses of Certain Plants to Solar Ultra-violet Radiation": W. E. Tottingham, University of Wisconsin.

"Some Growth Responses of Plants to X-ray Treatments": C. A. Shull, University of Chicago.

The program will be continued in the afternoon, if necessary, followed by a round table discussion.

CHARLES A. SHULL

MATHEMATICS AT THE CHICAGO MEETING

THE preliminary program of the Chicago meeting of the American Association for the Advancement of Science and Associated Societies, printed in the issue of SCIENCE for May 19, contains information concerning the programs of the different sections, but it may be well to repeat here a summary of the plans of the mathematicians given in the announcement of the American Mathematical Association.

It reports that the American Association and associated societies will present unusually attractive programs in connection with the Century of Progress, the week of June 19 being devoted chiefly to pure science and the next week to applied science. There will be numerous addresses by prominent foreign scientific men who have been specially invited for

The Cytological Effects of Low-Intensity Radiation[1]

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The effects of low intensities of ionizing radiation are of interest in relation to the incidence and nature of induced mutations. The effects of long-continued radiation at low intensities are also of interest from the standpoint of atomic energy programs in times of peace or war. Little is known about the cumulative effects of exposure over long periods of time.

The early work by Muller and by Timofeeff-Ressovsky showed a linear relationship between x-ray dosage and mutation frequency in *Drosophila*. It was also found that the induced mutation rate was independent of radiation intensity. From these observations it was concluded that the x-ray-induced mutations are produced by single "hits," and that there is no threshold effect. Spencer and Stern (*2*) found no increase over the spontaneous mutation rate by irradiating *Drosophila* for 21 days at 2.5 r/day, but later experiments by Uphoff and Stern (*3*) indicated that low intensities are effective.

Further studies on x-ray-induced mutations by Stadler showed that such "mutations" are usually, if not always, caused by aberrations of the chromosomes. The aberrations usually involve deficiencies, but inversions and translocations may also produce "mutations." The frequency of simple deletions is directly related to x-ray dosage, but the aberrations involving two breaks—rings, dicentrics, translocations, and presumably inversions—increase in frequency in proportion to the square of the dosage when the time of exposure is constant.

At very low intensities of irradiation, the simple deletions constitute the great majority of all chromosome aberrations in *Tradescantia*. A total dose of 150 r of γ rays at an intensity of 0.05 r/min produced 5% of deletions, but only 0.6% of translocations and dicentrics in Tradescantia chromosomes. The same dose of x-rays at an intensity of 40 r/min produced essentially the same percentage of simple deletions, but 10% of rings and dicentrics.

Although low intensities of ionizing radiation are less effective, the accumulation of aberrations and lethal "point mutations" over a long period of time could be just as deleterious as smaller doses given at high intensities. The effects of long-continued exposure of low-intensity radiation have been studied by exposing potted plants of *Tradescantia paludosa* (Clone 3) to low intensities of γ radiation for several months. The results are shown in Table 1.

The control plants show considerable variability in spontaneous chromosome aberrations. The average percentage of chromosome breaks for the total of all controls was .08%. If, however, plants removed from the radium beam for 4 weeks or longer are included among the controls, the spontaneous aberration frequency is reduced to .06% of breaks.

TABLE 1

CHROMOSOME ABERRATIONS AND POLLEN STERILITY INDUCED BY 1.7 R/DAY OF γ RADIATION

Weeks of exposure	Controls		Irradiated	
	No. chromosomes	% breaks	No. chromosomes	% breaks
1	2,052	.10	7,380	.06
2	1,480	.07	5,010	.18
3	2,190	.14	5,460	.57
4	2,280	.09	5,820	.53
6	2,166	.09	4,830	.33
11	4,350	.07	2,322	.52
22	6,180	.05	3,300	.52
Total	20,698	.08 (av.)	21,732*	.49 (av.)

* Total for 3–22 weeks.

Continued exposure to 1.7 r of γ rays/day increased the aberration frequency at the end of the second week, and a continued increase at the end of 3 weeks' exposure. There was, however, no further increase in aberration frequency following continued exposure. At the end of 22 weeks the plants had received 262 r of γ radiation, but showed only 0.5% of breaks in the microspore chromosomes. This dose of x-rays, given in a few minutes at the prophase stage, would have produced more than 30% of breaks.

The increase in aberration frequency during the first few weeks of exposure is due to the accumulation of aberrations produced during microspore development. During the fall and winter months, when this study was made, the duration of the microspore cycle from meiosis to the division of the microspore nucleus is about 12 days. Aberrations produced at late meiosis may be passed on to a viable microspore, but most detectable aberrations produced at the first meiotic division or earlier are not recovered at the microspore nucleus division, because of lethal deficiencies that prevent microspore development.

The failure of a cumulative effect of the γ radiation could be attributed to the screening-out of chromosome aberrations at meiosis and/or to differential development of normal and aberrant cells in premeiotic development. These alternatives were tested by a study of pollen sterility and by a chromosome analysis of plants removed from the field of radiation. Plants that had received 1.7 r/day for 2 months were removed from the beam, and microspore chromosomes were examined during subsequent weeks. The data are shown in Table 2. There was some decrease in chromosome aberrations after a week, and after the third week the chromosome aberration frequency was reduced to the spontaneous level. If the lack of this cumulative effect is due only to the screening of chromosome aberrations at meiosis, the pollen sterility should increase with continued exposure, and eventually the plants should be completely sterile. Pollen sterility counts were made at weekly intervals from plants exposed to 1.7 and 8.0 r/day for 12 weeks.

[1] This work was supported by the Office of Naval Research, Contract number N.R. 164,823. Henry Luippold, technician.

TABLE 2

FREQUENCY OF CHROMOSOMAL ABERRATIONS AFTER REMOVING PLANTS FROM 2 MONTHS' EXPOSURE TO 1.7 r OF γ RADIATION. RECOVERY PERIODS, 1 WEEK TO 4 MONTHS

Recovery time	No. chromosomes	Chromatid breaks	Chromosome breaks	Total % breaks
1 week	3,150	7	3	.32
2 weeks	4,320	4	0	.09
3 "	5,760	4	4	.14
4 "	2,910	1	0	.03
6 "	1,800	0	0	.00
4 months	6,180	3	0	.05

Counts from control plants were made at the same time. The normal sterility varies considerably, presumably in response to environmental conditions of temperature and light, and ranged from 5 to 14%. The percentage of sterility in the controls was deducted from the sterility of the exposed plants to give the net sterility due to radiation effects. The data are shown in Table 3.

TABLE 3

POLLEN STERILITY INDUCED BY CONTINUOUS RADIATION AND THE RECOVERY OF POLLEN FERTILITY SUBSEQUENT TO 5 WEEKS OF EXPOSURE

No. weeks exposure	Net pollen sterility			
	During exposure		After exposure	
	1.7 r/day	8 r/day	1.7 r/day	8 r/day
1	6	−1	28	42
2	11	4	28	42
3	29	3	18	48
4	37	14	18	56
5	24	35	4	37
6	35	37	3	33
7	31	50		50
8	42	53		53
9	25	48		18
10	27	43		9
11	21	55		9
12				2
13				0

At an intensity of 1.7 r/day the pollen sterility increased during the first 3 weeks and then leveled off at about 30%, although there was considerable variability from week to week. At 8 r/day the maximum sterility was not reached until about the sixth or seventh week, presumably in consequence of the retarding effect of the greater amount of radiation; but after 6 weeks' exposure, the pollen sterility remained at about 50% during the subsequent weeks. At this intensity there was considerable inhibition of floral development, and very few flowers were produced after 2 months of exposure.

After 5 weeks of exposure some of the plants were removed from the field of radiation in order to see how long the pollen sterility would continue. At 1.7 r/day the pollen fertility at 5 weeks after exposure was practically normal. At 8 r/day the plants did not recover normal fertility until about 12 weeks after removing them from the beam of γ rays. This greater delay in recovery is attributed to the greater retardation of growth of the plants at the higher intensity.

The lack of a cumulative effect in the production of microspore chromosome aberrations and pollen fertility after several weeks' exposure to low intensities of γ rays, and the recovery of normal microspore chromosomes and pollen fertlity after the plants are removed from the radiation field, indicate that cells containing chromosome aberrations do not continue to divide or are outgrown by the normal cells. Earlier work (*1*) has shown that, if sufficient radiation is given to produce chromosomal aberrations in nearly all cells, the plant dies. At low intensities of radiation many cells are not permanently affected, and presumably these cells are the ones that produce the normal microspore chromosome complements and the fertile pollen grains. Continuous exposure to several roentgens per day does not seriously reduce pollen fertility or seed set, although it is possible that some deleterious mutations may appear in later generations. The fact that both chromosome aberrations and pollen sterility level off after a few weeks of exposure indicates that the plants can survive and reproduce after months, or perhaps even years, of exposure.

These results indicate that Tradescantia plants, and probably most plants, can survive continuous radiation at the rate of several roentgens per day. Unfortunately, they cannot be expected to apply to the higher animals, including man. The factors of determinate growth, high sensitivity of critical tissues, the absence of haploid mitosis in gametophytic development, and the lack of rapid somatic divisions preceding egg formation, should render animals much more vulnerable to low intensities of ionizing radiation.

References

1. SAX, K. *Genetics,* 1941, **26,** 418.
2. SPENCER, W. P., and STERN, C. *Genetics,* 1948, **33,** 43.
3. UPHOFF, D. E., and STERN, C. *Science,* 1949, **109,** 609.

A Comparison of the Response of Normal and Hypothyroid Mice to Acute Whole Body Roentgen Radiation[1]

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In 1949 Blount and Smith (*1*) showed that premedication with thiouracil slightly decreased the mortality of mice subjected to acute whole body roentgen ray irradiation. This would indicate that the hypothyroid state was conducive to survival after roentgen ray irradiation. Shortly thereafter Patt *et al.* (*6*) reported significant decreases in radiation mortality in animals premedicated with cysteine. It was postulated that the beneficial effect

[1] This paper is based on work performed under Contract AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

Effects of Ionizing Radiation on Terrestrial Ecosystems

Experiments show how ionizing radiation may alter normally stable patterns of ecosystem behavior.

George M. Woodwell

During the past two decades man has had the capacity to increase levels of ionizing radiation in the environment by almost any magnitude and on a global scale. No other environmental factor is yet subject to such manipulation, and no other factor appears to have quite the same potential for producing both genetic and somatic effects in living systems. Preoccupation with the potential effects on man has led to concentration of research in environmental biology on the possibility of contamination of man's food chain with radioactive isotopes and to neglect of the potential effects of radioactivity on ecological systems. The recent discovery that certain plants are damaged by total exposures in the same range as those which cause damage in mammals emphasizes the possibility that substantially higher levels of ionizing radiation in the environment would be not only a direct hazard to man but also would cause changes in the ecological systems of which man is but a part. The nature of the potential changes in terrestrial ecosystems and the exposure levels at which they occur is a topic of vital current interest, bearing not only on the possible aftermath of war but also on the feasibility of large-scale peaceful use of ionizing radiation.

Sensitivity of Primary Producers

Plants, the primary producers of all ecosystems, are subject to damage from ionizing radiation at lower levels than was previously thought to be the case (*1*). The gymnosperms include some of the most sensitive of plants; the algae and bacteria, some of the most resistant. Sensitivities within this range vary by a factor of the order of several thousand (*2*). For example, exposure of pitch pine (*Pinus rigida* Mill.) to average levels of less than 5 roentgens per day for several years has killed more than 90 percent of these trees, while exposures in the range of 1 to 3 roentgens per day inhibit growth in diameter (*3*) and needle growth (*4*). Recently Miksche *et al.* (*5*) demonstrated that a total exposure of 82.5 roentgens at a rate of 3.75 roentgens per day damages *Taxus* buds. Near the other extreme of sensitivity among the higher plants, *Arabidopsis* survives long-term exposures of several thousand roentgens per day. Bacteria, algae, and fungi are in many instances still more resistant. In general, the trend of research on both the somatic and the genetic effects in higher plants is toward recognition of effects at lower and lower exposures.

Differences in sensitivity are not restricted to differences between species; sensitivity varies during the life cycle of an organism. Sparrow and I have suggested (*6*) that reproductive stages in plants are generally more sensitive than vegetative stages and that lethal effects occur during flowering and seed set at approximately one-fourth the exposure necessary to cause 100-percent mortality in mature plants. In animals, especially in insects, variations in sensitivity at different stages have been recognized for many years (*7*).

The mechanisms which appear to account for the effects of ionizing radiation on the growth of plants, as well as the effects themselves, have been reviewed recently by Read (*8*), by Sparrow and Evans (*2, 9*), by Gunckel and Sparrow (*10*), and by Sparrow and me (*6*). The primary site of damage appears to be the chromosome, and the great differences in sensitivity among organisms are attributable to differences in chromosome number and size. Organisms with few, large chromosomes may lose a significant portion of their genome from one chromosome break, while organisms with many, small chromosomes may suffer only minor genetic damage from a single break. Sparrow and Miksche (*11*) have shown that this relationship between sensitivity and chromosome size and number holds for several plant species.

Effects on Organisms and Ecosystems

The effects of exposure of plants to ionizing radiation range from death, through varying degrees of growth inhibition, to effects on reproductive capacity and to even more subtle genetic effects recognizable only in subsequent generations. Numerous instances of stimulation of growth have been reported, especially in the Russian literature (*12*). Additional effects are recognizable in animals, including shortening of the life span (*13*).

In general, the research which has elaborated these effects in plants and which has yielded estimates of sensitivities has been carried out on small populations under conditions of cultivation in greenhouses or gamma-radiation fields—under conditions specifically designed to reduce the variability attributable to environmental stress. Introduction of the various forms of environmental stress characteristic of natural ecological systems can be expected to intensify the damage from exposure to ionizing radiation and to produce measurable effects at lower exposure levels (*6, 14*), possibly to produce additional effects not recognized previously.

Virtually all of the effects recognized at the organismal and cellular levels have implications at the population and ecosystem levels; combined, they present a bewildering array of possibilities at these higher levels. For simplicity I divide possible effects into short-term and long-term effects, assuming *short-term* to mean less than 2 years. In most terrestrial ecosystems the short-term effects are dominated by the consequences of differential sensitivities; the long-term effects, by these consequences plus effects on re-

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productive capacity and genetic effects. I dwell here principally on the short-term effects.

Two types of short-term effects would be expected from long-term irradiation of an ecosystem: (i) selective mortality of sensitive species, due to direct and immediate effects of exposure, and (ii) shifts in the relative importance of species populations through alteration of the biological interactions which normally contribute to a stable pattern of ecosystem behavior. These interactions include not only the many vaguely defined inter-organism relationships commonly lumped as "competition" but also parasite-host and predator-prey relationships. There are numerous models suggesting the potential consequences of such shifts in biological interactions. Some of these have been summarized by Elton (*15*) and Andrewartha (*16*).

Exposures Necessary To Produce Effects on Ecosystems

Research on the effects of ionizing radiation on organisms living in natural arrays is complicated by the variability of these arrays and the necessity for recognizing slight effects caused by exposure to the low-level radiation present. In addition, the effects of exposure are usually confounded with the factor of location, making clear separation of radiation effects from other environmental influences difficult. The lowest levels of long-term ionizing radiation at which nongenetic effects on higher plants had been observed, approximately 2 roentgens per day, were estimated by Sparrow and me (*6*) to be 8000 times greater than the highest exposure levels from fallout in New York City in 1958 (*17*). It is probable that effects on stem diameter and needle growth in pine could be observed at levels perhaps half those used in our calculations, and it is true that in some areas levels of fallout radioactivity are higher than they are in New York City; nevertheless, a large gap exists between present general radiation levels and the lowest level necessary to produce a measurable effect in a sensitive plant. There is, therefore, little reason to believe that radiation effects can be seen now in natural ecosystems other than ecosystems exposed to local fallout from experimental bomb bursts, as suggested by reports such as those of Fosberg (*18*), Palumbo (*19*), and Shields and Wells (*20*) and ecosystems such as that adjacent to the Lockheed reactor in Georgia (*21*). To produce observable effects even in ecosystems containing pines, which are among the most sensitive plants known, long-term exposures in the range of 1 to 5 roentgens per day would be necessary, while to produce parallel effects in oak, minimum exposures of 10 roentgens per day would be required. Much higher levels would be necessary to kill these plants within a short period and to produce presently recognizable morphological effects in other, more resistant species. Miller and I (*3*) and McCormick and Platt (*14*) have presented data indicating that environmental stress increases the damage in plants caused by exposure to ionizing radiation at any level, and Sparrow and I (*6*) have suggested one mechanism in explanation of this effect. We suggest that damage on a unit-cell basis is the principal factor governing response, and that any increase in the exposure of a cell prior to division increases damage. Cells which divide slowly are exposed to more radiation prior to division, and sustain greater damage, than those which divide rapidly. Any environmental factor which reduces the rate of cell division increases the exposure on a unit-cell basis and thereby increases the effects. In any case it seems possible that exposure to ionizing radiation reduces tolerance to environmental stress, and that ionizing radiation kills or damages plants at lower levels in irradiated ecosystems than under conditions of cultivation. We would, therefore, expect to find nongenetic effects in the most sensitive plants in natural arrays at long-term exposure rates of the order of 1 roentgen per day.

Table 1. Vegetation zones around the gamma-radiation source and the approximate exposures each zone received during the first 6 months of the experiment. The zones remained stable in general throughout the summer, but they are expected to recede during the second year of the experiment.

Vegetation zone	Daily exposure rates (r)	Approximate total accumulated exposure (r)
Oak-pine forest	Background*	
Oak forest	20– 60	3,600–11,000
Gaylussacia-Vaccinium heath	60–150	11,000–27,000
Carex zone	150–350	27,000–63,000
Zone in which all higher plants died	>350	>63,000

* Tree growth was inhibited in this zone at exposures as low as 2 r/day (Fig. 3).

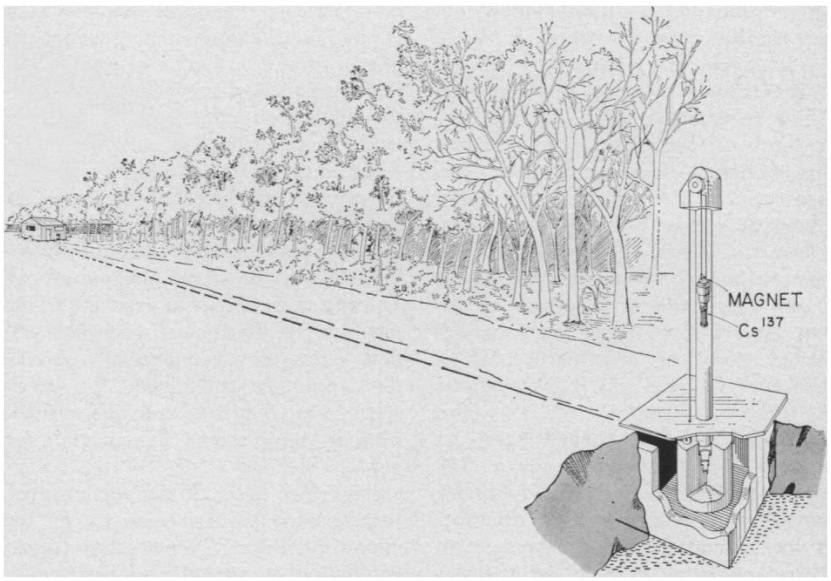


Fig 1. Mechanism for controlling the gamma-radiation source used in irradiating a forest ecosystem at Brookhaven National Laboratory. The source can be raised or lowered into a lead-shielded container through operation of a winch in the building a safe distance away.

Experimental Approach

These difficulties dictate an experimental approach to the quantitative study of effects at community and ecosystem levels. For such an experiment the radiation levels used must vary from levels lethal to most organisms through low levels approximating background. Gamma radiation from a central point seems most appropriate for experimental purposes because, with a relatively small quantity of radioactive material, an intense radiation source can be conveniently provided. In addition, there is no activation problem as there is with neutrons.

Such a radiation facility has been established at Brookhaven National Laboratory, specifically to provide opportunity for systematic study of the effects of ionizing radiation on a terrestrial ecosystem and its components. The ecosystem chosen for this experiment supports a stand of the Long Island oak-pine forest, with *Quercus alba, Q. coccinea*, and *Pinus rigida* the principal tree species.

The source of radiation is cesium-137 (9500 curies), a gamma emitter, centrally located; it can be shielded, when shielding is desired, through operation of a winch (Fig. 1). Rates of exposure around this source vary from several thousand roentgens per day within a few meters to about 2 roentgens per day at 130 meters. The source is exposed 20 hours per day, and has been exposed on this schedule since 22 November 1961.

Two broad research programs designed to elucidate effects at the ecosystem level are being carried out with this radiation facility. One involves measurement of changes in the populations of species which form the ecosystem; the other, measurement of the rates of energy fixation and the paths of energy movement through the system. The first of these programs includes study of short-term changes induced by direct and indirect effects on present populations and long-term effects of genetic changes and of changes in reproductive capacity. The second program is designed to provide a more nearly precise measure of effects on the system through measurement of the energy-fixing capacity of the system and of its components. Although use of this facility is by no means restricted to studies involved in these two programs, the programs form the core around which research on the behavior of the overall system is organized.

Prior to installation of the source, detailed information on the species composition of the vegetation and on the size and vigor of individual plants within the vegetation were obtained through the technique of Woodwell and Hammond (*22*). Less detailed data on insect, bird, and mammal populations were also obtained. These, plus data from other, similar stands remote from the source, are the control data for the experiment.

The required size of the gamma source was estimated from the correlation between radiosensitivity and chromosome number and size shown by Sparrow and his associates (*1, 6, 9*). A source size was selected which was estimated to be large enough to produce effects in the first year, through an area of approximately ½ hectare, ranging from mortality through inhibition of growth of most species in the vegetation.

Early Effects of Exposure

At the time of budbreak in the spring, approximately 6 months after irradiation was started, effects were obvious as far as 40 meters from the source (Fig. 2), where exposure rates were approximately 40 roentgens per day. Differences in sensitivity among plant species produced a zonation of vegetation, five zones being clearly defined (Table 1): a zone of total kill of all higher plants; a sedge zone; a heath-shrub zone; an oak zone; and, at lower levels of radiation, the oak-pine forest.

The striking differences in sensitivity of primary producers is indicated by the growth curves of Fig. 3 for white oak and pitch pine, which show severe inhibition of shoot elongation in oak at exposures above 35 roentgens per day and in pine at 15 roentgens per day. These curves approximate closely the responses predicted for these species by Sparrow and me on the basis of chromosome size and number (*6*).

A further effect of differential sensitivity among species is shown by the curves of Fig. 4, which show insect defoliation, expressed as a percentage of the leaves present on white oak, plotted against exposure rate. Defoliation by insects was approximately 10 times more severe on trees damaged by radiation than in the nonirradiated forest. This increase in damage was probably due not to an increase in the abundance of insects but, rather, to a decrease in the number of leaves available to the endemic populations. The populations of leaftiers (*Psilocorsis* spp.) leaf rollers (primarily *Argyrotoxa semipurpurana*), leaf beetles (Chrysomelidae), and loopers (Geometridae) which caused most of the damage shown (Fig. 5) were aparently more resistant to damage than their host trees.

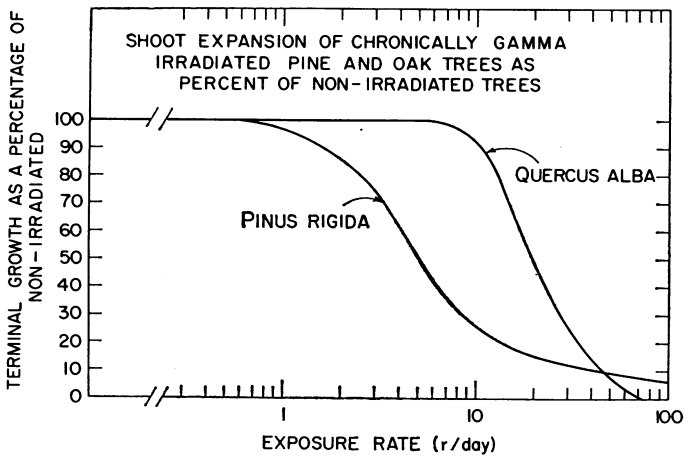
These early observations illustrate the types of short-term changes which exposure to high levels of radiation in the general environment can be expected to produce in a forest ecosystem. While such high levels are above present levels of radiation from world-wide contaminants by many orders of magnitude, they are well within the range of exposures associated with local fallout from bombs (*23*). From Table 1 and Fig. 3 it is clear that contamination-producing exposures in excess of 1000 roentgens delivered over any period of less than 6 months would cause severe damage to pitch-pine forests and probably to other gymnosperm forests as well, while parallel damage would occur in oak forests at exposures in excess of 10,000 roentgens.

Furthermore, it is clear that ionizing radiation may alter such host-parasite relationships as those existing between a plant and its insect defoliators, and that radiation-damaged plants will suffer greater insect damage than plants not damaged by radiation. While the assumption that all host-parasite relationships will be affected in this direction is not justified, the hypothesis seems tenable that small organisms with wide ecological amplitudes and high rates of reproduction—in short, weeds and other organisms frequently considered pestiferous because of their persistence under persecution—have survival advantage under conditions of long-term exposure to ionizing radiation over large organisms with longer life cycles.

Discussion

If we consider from a very fundamental and practical standpoint the general problem of contamination of the environment with radioactive debris, it is clear that two types of con-

Fig. 2. The forest within 40 meters of the source after 6 months' exposure to ionizing radiation. The source is cesium-137 (9500 curies), suspended in the tower at right. The numbers indicate approximate daily exposure in roentgens, at the point indicated.



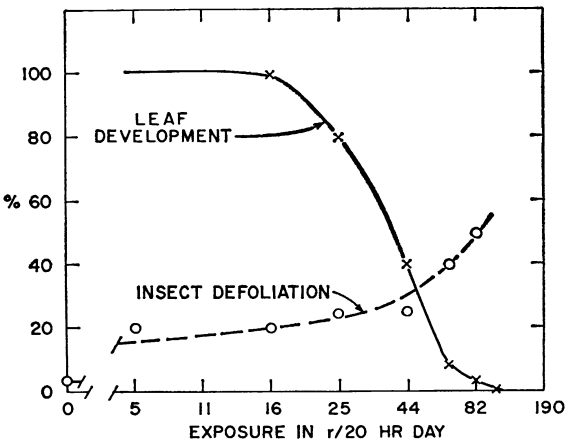


Fig. 3 (left). Growth of white oak and pitch pine in an irradiated forest, at various rates of exposure. Fig. 4 (right). Variation in the intensity of insect defoliation among white oaks damaged by ionizing radiation.

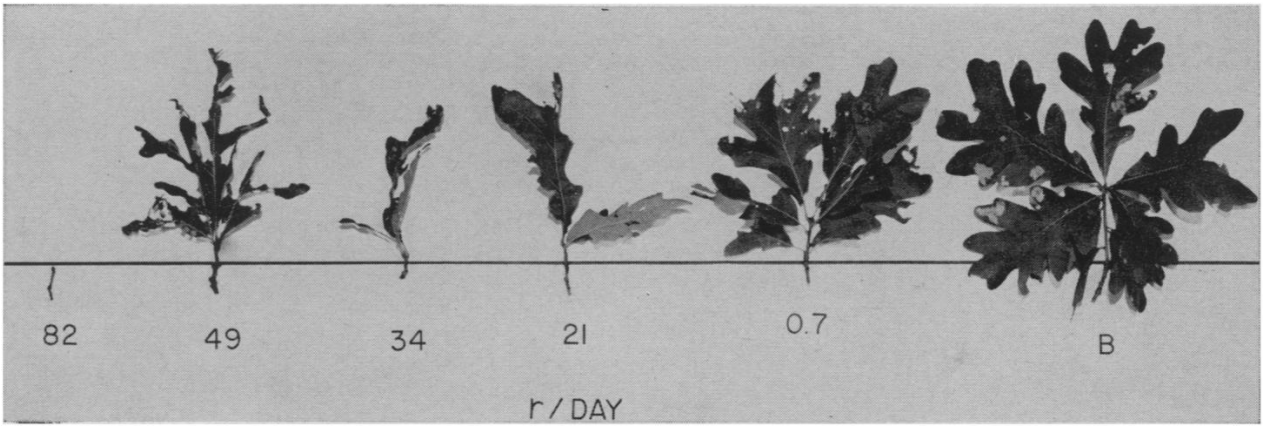


Fig. 5. Insect defoliation and damage from ionizing radiation in white oak. 30 July 1962.

tamination are possible; first, the severe contamination from heavy local fallout associated with bomb bursts; second, the much less intense long-term and world-wide contamination from sporadic bomb testing, from accidents, and from wastes originating from peaceful uses of atomic energy. These two situations are fundamentally different, the one involving large, short-term effects principally from external emitters, the other, long-term effects from both internal and external emitters. Both situations present problems which are difficult, and many of their finer points may be susceptible of only limited, empirical solution. Nonetheless, certain principles seem to bear on the general problem, and certain questions seem answerable within broad limits.

Numerous radiobiological studies emphasize that the principal damage incurred by an organism exposed to ionizing radiation occurs in the nucleus (*8*), and more recent work shows that the sensitivity of an organism is related to the size and number of chromosomes present (*2*). This relationship is now well enough established to be used as a basis for predicting the sensitivity of organisms to radiation of any level (*6, 24*). Although the technique lacks precision, it is useful; for instance, through this technique accuracy in predicting the range of sensitivity for a higher plant is increased to a point where predicted values deviate from experimental values by a factor of 4 or less instead of a factor of about 500. Further refinement of this technique should increase its precision greatly. At present it is obviously useful in predicting not only effects on individual plants but also the gross, short-term effects of heavy fallout on the plants of any ecosystem.

Long term effects of chronic exposures on organisms living in natural arrays are dependent to a higher degree on the nature of the contamination and on an additional set of biological factors. Such long-term effects are necessarily the result of exposure from both internal and external emitters, and it is clear that to predict effects of exposure for any type of intensity of contamination, the mineral cycles and periods of residence of isotopes in various organisms must be known. Great progress is being made in defining these cycles and their biological implications (*25*).

Less progress has been made in defining the biological considerations which are important in determining potential long-term effects. These considerations seem to be three.

1) Ionizing radiation is generally deleterious to living systems, and exposure can be expected to reduce physiological tolerances to environmental stress. Although there are notable exceptions to this generality (*12*), especially as a result of clever genetic manipulations by man (*26*), evidence from animals (*27*) and an increasing body of evidence from plants indicate strong interactions between stress and radiation exposure (*3, 14*). Sparrow and I have suggested (*6*) that relative sensitivity among species to this type of radiation damage probably parallels radiosensitivity shown by morphological characteristics. The extent to which this is true remains to be seen.

2) Variation in sensitivity to damage during the life cycle of an organism may be extreme, the population as a whole thus being much more sensitive than the mature stages of single organisms. In general, reproductive processes are most sensitive to damage, vegetative or mature stages least sensitive. On the other hand, there is no threshold exposure for the production of mutations.

3) Selective removal or differential inhibition of species will alter biological interactions, potentially upsetting the usual patterns of species abundance and ecosystem stability. This type of distrubance can have several forms including alteration of intra- and interspecific interactions among plants, shifts in the host-parasite balance, and shifts in predator-prey relationships. There are abundant models for disturbances of these types, ranging from the removal of chestnut from the extensive oak-chestnut forests of eastern North America by the fungus *Endothia parasitica* (*28*) to disturbances shown in numerous animal-population studies (*16*).

All of these changes produce potential instabilities in ecosystems, ranging from the initiation of a new successional sequence only slightly different from the old one to violent oscillations in population density which can result in extinction or in population explosions.

The research needed for elaboration of these large and complex problems is itself large and complex, involving the delineation of model systems and the analyses of these systems from numerous standpoints. Perhaps the most successful ecological study of this type is the series of studies of the spruce budworm in eastern Canada, carried out over more than two decades and involving many scientists (*29*). Although ionizing radiation presents a set of problems different from those

posed by the budworm, the work in Canada emphasizes the need for long-term, integrated approaches to such large-scale and fundamental biological problems. One technique for analyzing certain aspects of the potential effects of ionizing radiation is outlined here. Installations such as that at Brookhaven, established within major vegetation types, with their control ecosystems, provide one type of model. A second type of model has been provided by chance at Rongelap Atoll and on neighboring atolls in the Pacific, and at the White Oak Lake Bed at Oak Ridge, Tennessee. Similar models must now exist in the Russian Arctic. The partially shielded Lockheed reactor in Georgia has provided a most useful model of an irradiated ecosystem. Use of these models as they become available, in conjunction with experiments involving mineral cycling and the effects of internal emitters not only on organisms but on populations and ecological systems as well, will provide at least an understanding of what is happening to the environment, if not the wisdom to control it (*30*).

References and Notes

1. A. H. Sparrow, R. L. Cuany, J. P. Miksche, L. A. Schairer, *Radiation Botany* **1**, 10 (1961).
2. A. H. Sparrow and H. J. Evans, *Brookhaven Symp. Biol.* **14**, 76 (1961).
3. G. M. Woodwell and L. N. Miller, *Abstr. Bull. Ecol. Soc. Am.* **43**, 81 (1962).
4. A. H. Sparrow, personal communication.
5. J. P. Miksche, A. H. Sparrow, A. Rogers, *Radiation Botany*, in press.
6. A. H. Sparrow and G. M. Woodwell, *ibid.* **2**, 9 (1962).
7. C. Packard, *Radiology* **45**, 522 (1945).
8. J. Read, *Radiation Biology of* Vicia faba *in Relation to the General Problem* (Blackwell, Oxford, England, 1939).
9. H. J. Evans and A. H. Sparrow, *Brookhaven Symp. Biol.* **14**, 101 (1961).
10. J. E. Gunckel and A. H. Sparrow, in *Encyclopedia of Plant Physiology*, W. Ruhland, Ed. (Springer, Berlin, 1961), vol. 16.
11. A. H. Sparrow and J. P. Miksche, *Science* **134**, 282 (1961).
12. L. P. Breslavets, in *Plants and X-rays*, A. H. Sparrow, Ed. (American Institute of Biological Sciences, Washington, D.C., 1960).
13. Z. M. Bacq and P. Alexander, *Fundamentals of Radiobiology* (Academic Press, New York, ed. 2, 1961).
14. F. J. McCormick and R. B. Platt, *Radiation Botany*, in press.
15. C. Elton, *The Ecology of Invasions by Animals and Plants* (Methuen, London, 1958).
16. H. G. Andrewartha, *Introduction to the Study of Animal Populations* (Methuen, London, 1961).
17. W. R. Collins, Jr., G. A. Welford, R. S. Morse, *Science* **134**, 980 (1961).
18. F. R. Fosberg, *Atoll Res. Bull.* **61**, 1 (1959); *Nature* **183**, 1448 (1959).
19. R. F. Palumbo, *Radiation Botany* **1**, 182 (1961).
20. L. M. Shields and P. V. Wells, in *Radioecology*, V. Schultz and A. Klement, Jr., Eds. (Reinhold, New York, 1962).
21. R. B. Platt, *ibid.*; R. A. Pedigo, *ibid.*; C. P. Daniel, *ibid.*
22. G. M. Woodwell and E. C. Hammond, "A Descriptive Technique for Study of the Effects of Chronic Ionizing Radiation on a Forest Ecological System," *Brookhaven National Laboratory Publ. No. BNL 715 (T-251)* (1962).
23. S. Glasstone, Ed., *The Effects of Nuclear Weapons* (U.S. Atomic Energy Commission, Washington, D.C., 1962).
24. A. H. Sparrow, *Abstracts of Papers, 2nd International Congress of Radiation Research, Harrowgate* (1962), p. 178.
25. V. Schultz and A. W. Klement, Jr., Eds., *Radioecology* (Reinhold, New York, in press); ———, proceedings of the 1st National Symposium on Radioecology, Fort Collins, Colo. (1961); A. W. Klement, Jr., "Radioactive Fallout from Nuclear Weapons Tests," *U.S. Atomic Energy Commission Publ. No. TID-7632* (1962) (proceedings of a conference held November 1961 in Germantown, Md.).
26. S. Shapiro, "The role of radiation in the production of new plant varieties," testimony presented before the Subcommittee on Research and Development of the Joint Committee on Atomic Energy, 28 March 1961.
27. S. E. Bernstein, *Science* **137**, 428 (1962).
28. C. Keever, *Ecology* **34**, 44 (1953).
29. R. F. Morris, *Can. Entomologist Suppl.*, in press.

30. The research described is being carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission. Many of my associates have contributed to this work in various ways. I particularly want to thank Dr. A. H. Sparrow, whose continued and vigorous interest made the project possible.

News and Comment

Administration Sees No Ground for Jubilation as Missile Episode Is Brought to a Calm Conclusion

The administration is not encouraging any cheering over its success in thwarting the Soviet missile gambit in Cuba.

For one thing, the strong medicine that the United States employed in Cuba could have distant and unforeseen side effects, and jubilation is therefore considered to be premature. No matter how Khrushchev may euphemize the incredible events of the past two weeks, he, in effect, dismantled some of his own political and military prestige when he agreed to dismantle his Cuban missile launchers; it is not unreasonable to assume that he is looking to recoup his losses, and the administration is eager to refrain from any words that may irritate him toward accomplishing that quest.

Furthermore, the administration desires to make it clear, especially to American audiences, that it successfully responded to the Soviet threat, not with a bludgeon, but with carefully measured words and a minimum application of force. Thus it was no accident that the Navy employed binoculars, rather than a boarding party, to inspect the first Soviet-owned vessel—a tanker—that crossed the quarantine line. A Defense Department spokesman explained that an external examination had satisfied the Navy that the vessel was not carrying prohibited material. It would seem that this was more of an educated guess than a substantiated conclusion, but it had the merit of keeping armed American naval personnel from forcing their way onto to what is legally the equivalent of Soviet soil. When an actual boarding did take place, it was on a Lebanese vessel under charter to the Soviets. In this fashion, the highly provocative fact of the quarantine was tempered through judicious execution, and the Soviets cooperated by reversing the course of those vessels whose cargoes fell under the ban.

Although "hard-liners" are now praising the administration for taking the advice they were giving all along, the response employed in Cuba was quite different from what the jingoists were recommending. From the onset of the crisis, the administration set a course aimed at *convincing* the Soviets that the U.S. would use force to achieve the removal of the missile launchers if the Soviets did not remove them first. To get this idea across, it had to come perilously close to employing

22. L. G. Sillén, "Hydrolytic aqueous species," lecture presented at Robert A. Welch Foundation Conferences on Chemical Research, Houston, Texas, 26–28 November 1962.
23. ———, *Acta Chem. Scand.* **8**, 318 (1954).
24. I thank Prof. L. G. Sillén and Dr. G. Lundgren for stimulating discussions of uranyl-ion chemistry, and for informing me of their unpublished findings. I thank Prof. Sillén, the John Simon Guggenheim Foundation, and the U.S. Geological Survey for making possible a year in residence at the Royal Institute of Technology in Stockholm. Publication of this report has been authorized by the director, U.S. Geological Survey.
25. W. H. Zachariasen, *Acta Cryst.* **7**, 788 (1954).
26. S. Samson and L. G. Sillén, *Ark. Kemi, Mineral. Geol.* **25**, No. 21 (1947).
27. D. E. Appleman, *Geol. Soc. Am. Bull.* **68**, 1696 (1957).
28. M. Ross and H. T. Evans, Jr., *J. Inorg. Nucl. Chem.* **15**, 336 (1960).
29. D. E. Appleman and H. T. Evans, Jr., *Acta Cryst.* **10**, 765 (1957).
30. C. L. Christ, J. R. Clark, H. T. Evans, Jr., *Science* **121**, 3144 (1955).
31. G. M. Dieke and A. B. F. Duncan, *Spectroscopic Properties of Uranium Compounds* (National Nuclear Energy Series III–2, McGraw-Hill, New York, 1949), chap. 2.
32. J. E. Fleming and H. Lynton, *Chem. Ind. (London)* **1960**, 1416 (1960).
33. ———, *ibid.* **1959**, 1409 (1959).
34. D. E. Appleman, *Geol. Soc. Am. Bull.* **67**, 1666 (1956).
35. F. Mazzi and F. Rinaldi, *Acta Cryst.* **13**, 1139 (1960).
36. W. H. Zachariasen, *Acta Cryst.* **1**, 265 (1948).
37. ———, *ibid.* **1**, 281 (1948).

16 May 1963

Corn Seeds Affected by Heavy Cosmic Ray Particles

Abstract. *Corn seeds of a special genetic stock were recovered from two satellite flights and the plants grown from them were examined for abnormalities. Some evidence for a slight increase in chromosomal deletions was observed, which was predicted from the flux of heavy cosmic ray primary particles. Nothing unexpected was observed.*

The ionizing radiations which exist at altitudes greater than 120 km, where the earth's atmosphere is very sparse or nonexistent, have been extensively studied in recent years with the aid of balloons and satellites. The biological effect of these radiations has been predicted from the physical measurements alone, but it seems desirable to test these predictions with appropriate live material.

The present experiments employed corn seeds as the test material. The seeds were flown in satellites and returned to the laboratory, where they were germinated and grown. Certain of the early leaves were examined for abnormalities which would indicate radiation damage to the embryo. If radiation causes genetic damage in one or more individual cells of a leaf primordium of the seed embryo, then for each mutated cell a change can be observed at a corresponding point in the leaf of the plant.

The radiations can be divided into three categories, as follows. (i) Electromagnetic radiations such as x- or gamma rays which would be expected to have a negligible effect on the seeds at the radiation levels encountered. (ii) Electron and proton radiations which comprise by far the most abundant type of radiation and would be expected to cause genetic damage in individual scattered cells of the seed embryos. Readings from ionization chambers in the satellites, together with experience gained from previous exposures to known radiation sources, permit an accurate estimate to be made of the frequency of leaf sectors to be expected from this source. (iii) Heavy cosmic ray particles, consisting of stripped atomic nuclei having masses as high as iron and traveling at very high speeds, may produce a very broad path of dense ionization as they enter matter. As the particle slows down the track increases in ionization density, and just before its end it becomes very broad and dense. This section, known as a thindown, may be as much as 25 μ in diameter in tissue and several millimeters long. Since the particles are traveling very fast in outer space, almost none of them would be expected to be slowed down in a small object, like a package of seeds, sufficiently to form a thindown. However, behind rather thick shielding or when the particles have penetrated the atmosphere some distance, thindowns should be encountered rather frequently. Schaefer (*1*) has shown the maximum number to occur at an altitude of about 40 km and to decrease sharply at higher altitudes, reaching zero in an unshielded situation in outer space beyond about 80 km.

These high-energy heavy particles cannot be produced in the laboratory, so there has been no direct biological experience with them. Thus the chief interest in this experiment was with this type of radiation, and corn seeds were chosen as the test object because they would be expected to respond to it in an observable way.

In general, the biological damage produced is proportional to the ionization produced, and for the heavy particles this ionization is concentrated in very small volumes, except for the thindown portion of the track. The diameter of this part of the track may be wide enough to hit several cells of the corn embryo, and within its core the ionization would be very dense. If such a track went through an embryo, one would expect damage to the primordia of all leaves through which it passed. The plant grown from such a seed might show damaged areas in several leaves, and from the positions of these one should be able to estimate the course and extent of original damage to the embryo as it was traversed by the particle. The very-high-speed heavy particles encountered would be expected to cause occasional damaged cells in the seeds which would show as mutant streaks in mature leaves. It was this speculation that the experiment was designed to verify.

The corn seeds used in these experiments have embryos in which six leaves or leaf primordia are present in various stages of development. Observations on leaves 3 and 4 were used in these experiments to obtain quantitative data on genetic damage. These leaves are most easily scored because of the size and frequency of mutant sectors produced, which, in turn, are due to the particular combination of numbers of target cells in the embryonic initials and the amount of cell division and expansion that occurs in subsequent growth. The seeds employed were of a genotype that is heterozygous for alleles controlling green (Yg_2-dominant) versus yellow-green (yg_2-recessive) color of the leaf. The larger the dose of radiation delivered to one of the cells of an embryonic leaf, the greater is the probability that the cell will undergo chromosome breakage, and the higher is the frequency of loss of the allele (Yg_2) responsible for green color. As a consequence of such a loss, this altered cell and all its progeny will fail to form the fully green chlorophyll of normal leaf cells. Thus, a single "mutation" in an embryonic leaf cell in this stock will show up in the growing plant as a yellow-green streak or sector in the mature leaf (*2*). A microbeam of deuterons from the Brookhaven cyclotron has been developed as a tool for simulating the biological effects of the thindown particles (*3*) and the effects of these beams on this genetic stock of corn have been described (*4*) and these results have been used to predict the appearance of a thindown hit in this material.

Seeds were flown and successfully retrieved from two satellites: Discoverer 32 launched on 13 September 1961, and a satellite launched in midsummer 1962 (*5*). These satellites were in polar

orbit with an average altitude of about 280 km for 27.3 and 49.6 hours, respectively. In addition to the seeds, the second flight had some nuclear track plates packed in the corn.

After recovery, the corn was grown in a controlled environment room, along with control samples which had been sent to Vandenberg Air Force Base with the flight samples but not flown. The results from coded scoring of the frequency of yg_2 streaks on leaves 3 and 4 are presented in Table 1. An analysis of variance of the data from the first flight, utilizing the approximate proportionality of the number of leaves scored, gave no evidence for a significant increase in the frequency of streaks in the corn recovered from the flight as compared to the controls. An analysis of variance of the data from the second flight, based on the method of weighted squares of means, revealed a significant increase in sector frequency for flight seed scored for leaf 4 but not for leaf 3. On the other hand, it can be seen from the data that, in the ten comparisons made (five seed lots, two leaves each) for the two flights combined, in eight of these the sector frequency for the flight seed was greater than for the controls, and in one it was the same. Friedman's (*6*) rank sum test applied to the pooled data gives evidence that there is significantly more sectoring, but only at the 5-percent level ($\chi^2_1 = 4.9$), in plants grown from the flight seed. The conclusion reached from this analysis is that there was little, if any, increase in sector frequency due to the flights.

An exceptional yg_2 sector frequency observed in one plant may have been due to a thindown particle hit on Discoverer 32. In one-half of leaf 3 there were six separate yg_2 streaks, in leaf 4 there were two such streaks, and one also appeared in leaf 5. These leaves overlap in the embryo so that this pattern of hits may indicate a single major thindown traversal. With this possible exception there was no conclusive evidence of more radiation damage in the flight samples than in the controls.

In addition to the data taken on the frequency of yg_2 streaks, observations were also made on the occurrence of cut or notched leaves, aborted shoot apexes, and files of dead leaf cells in both the genetic stock and a commercial hybrid corn that was used as packing in the first flight. These abnormalities were found to be no more abundant in the flight samples than in the controls.

The numbers of yg_2 sectors to be expected were estimated in several ways. The U.S. Air Force included ionization chambers on these two flights, and they recorded about 15 mrad and 3 rad, respectively. This ionization would be due almost entirely to electrons, protons, and x-rays. This amount of radiation would cause a negligible amount of radiation damage in these seeds. Thus any damage observed must have been due to heavier particles.

Table 1. Frequency of yg_2 sectors.

Seed lot	Leaf 3				Leaf 4			
	Control		Flight		Control		Flight	
	No. of leaves	yg_2 per leaf	No. of leaves	yg_2 per leaf	No. of leaves	yg_2 per leaf	No. of leaves	yg_2 per leaf
				First flight				
A	89	0.034	90	0.056	89	0.022	91	0.022
B	94	.085	92	.120	94	.043	90	.067
$\bar{m}$		.060		.088		.033		.044
				Second flight				
A	93	.086	206	.116	93	.032	206	.048
B	86	.081	187	.070	86	.035	187	.054
C	121	.050	162	.056	121	.016	162	.037
$\bar{m}$		.070		.083		.027		.047

The flight film and control plates, were developed along with an identical plate which had been exposed to 44-mev alpha particles as a reference for relatively heavy particle tracks. The plates were carefully scanned for heavy particle tracks, and any tracks heavier than the alpha tracks were scored. In all, 64 cm^2 of emulsion were scanned on both flight and control plates and the flight plates showed a track frequency of two tracks per square centimeter for the 49.6-hour flight. None was found on the controls. This agrees well with results obtained by Hewitt and Campbell (*7*). However, none of these tracks was more than about twice the ionization density of an alpha track, so that all were from particles traveling at such velocities that they did not produce tracks heavy enough to be classed as thindowns. The one apparent thindown hit observed in one seed from the first flight was probably a real effect, since there was certainly a finite probability of receiving such a hit.

These emulsions gave information only on the very heavy ionization tracks, that is, those above alpha track ion densities. It is possible to compute from the work of Schaefer (*1*) the total number of heavy particles passing through the samples; this amounts to about 380 and 670 per square centimeter, respectively for the two flights. There would also be some slow protons which would cause dense ionization tracks, but it is very difficult to estimate their numbers. Calibration measurements have indicated that a low-energy proton flux having about the same ionization density as the very energetic heavy particles will produce about one streak on leaf 3 per seed for a flux of 10^6 protons per square centimeter. For the first flight, on this basis, one might expect for the 182 seeds no significant increase in mutant streaks, but for the second flight, with 555 seeds, there might be an increase between 0.001 and 0.01 streak per leaf. This is at best a rough approximation, but is accurate enough to explain the apparent slight increase in streaks found in this experiment. It clearly indicates that if the very heavy particles are traveling at very high velocities, they can pass through living cells and produce little damage. This is in accord with radiobiological expectations.

Particles producing ionization tracks such as these are known to be very effective relative to sparsely ionizing tracks in producing chromosome breaks in corn seeds, and this is the reason one would expect a slight increase in streaking even though the ionization chamber readings were very low.

The real purpose of this experiment was to verify the predicted radiobiological effect of the heavy cosmic ray primary particles, and to test whether any unexpected biological phenomena existed in a satellite environment. Within the limits of this biological system, nothing unexpected was found (*8*).

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References and Notes

1. H. J. Schaefer, *U.S. Navy School Aviation Med., Res. Rept. NM001-059.13.04* (1952).
2. O. L. Stein and D. Steffensen, *Am. J. Botany* **46**, 485 (1959).
3. C. P. Baker, H. J. Curtis, W. Zeman, R. G. Woodley, *Radiation Res.* **15**, 489 (1961).

4. H. H. Smith, H. J. Curtis, R. G. Woodley, O. L. Stein, *Radiation Botany* **1**, 255 (1962).
5. These flights were made available by the Space Systems Division of the Air Force Systems Command.
6. M. Friedman, *J. Am. Stat. Assoc.* **32**, 675 (1937).
7. J. E. Hewitt and M. M. Campbell, *Air Force Aerospace Med., Report 62-62* (1962).
8. Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

31 May 1963

Radioprotection by Pressor Amidines

Abstract. *In the mouse, radioprotection is not always associated with the effect of hypertensive amidines and related amines. The protection resulting from this group of agents follows the pharmacological reduction of intercellular oxygen tension.*

After the observation that simple *S*-alkyl isothiuronium salts decrease radiosensitivity, Ashwood-Smith (*1*) tested some of its homologs in an attempt to relate structure to radioprotective action and to discover more promising agents. He found that activity diminishes rapidly as the *S*-alkyl substituent is lengthened beyond three carbon atoms. It is interesting that Fastier (*2*), in his excellent review of the structure-activity relationships of amidines, describes a loss of pressor action for *S*-alkyl isothiuroniums with alkyl substituent longer than three carbon atoms. The possible correlation of chemical structure, pressor activity, and radioprotection by these amidine derivatives led to a study of the effects of pressor amidines and pharmacologically related amines on the radiosensitivity of mice.

Table 1. Thirty-day survival data of mice receiving single doses of related pressor amines and amidines before irradiation to lethal doses of Co^{60} (1000 r).

Intraperitoneal administration		Animals (No.)	Survival (%)
Dose (mg/kg)	Time (min)		
	Controls		
		290	0
	2-Methylpseudo urea		
500	15	20	50
	Methyl guanidine		
150	5	20	5
	2-Amino pyridine		
25	15	30	0
	4-Amino pyridine		
3	15	15	0
	n-*Pentylamine*		
50	15	10	0
	n-*Hexylamine*		
40	15	10	0
	S-*ethyl isothiuronium*		
150	30	40	98
150	15	80	90
75	15	30	90
20	15	20	60
	*Papaverine · HCl**		
325	30	10	10
*Papaverine · HCl,** *plus* S-*ethyl isothiuronium*			
325	30		
150	15	26	40
	Hypoxia†		
		42	5
Hypoxia, plus S-*ethyl isothiuronium*†			
150	15	20	5

* Subcutaneous administration. † Irradiated with 2200 r Co^{60}.

Young female mice (Bagg Swiss), weighing 20 to 25 g, were used. Ten control mice were irradiated simultaneously with each treated group and thereafter both groups were housed jointly. The radiation was done in a specially designed cobalt-60 irradiator which contained about 1200 curies of cobalt-60, half above and half below the radiation chamber. The mice were exposed in a plexiglass box which rotated through a flat radiation field of about 100 r/min. In the experiment with hypoxia, two treated and two control mice were irradiated simultaneously in a cobalt-60 Gammacell-220 (*3*) at about 1800 r/min. The irradiation chamber was gassed before and during exposure with a mixture of 5 percent oxygen and 95 percent nitrogen.

Each of the chemicals tested is known to increase blood pressure (*2*), but only two of these offered significant protection against lethal radiation. The survival data in Table 1 indicate that radioprotection by amidines is not directly associated with their pressor activity. In an attempt to explain this disparity, additional investigations were conducted with *S*-ethyl isothiuronium as a test compound.

The results in Table 1 show that *S*-ethyl isothiuronium is radioprotective when used over a wide dose range and for a considerable period of time. Also, papaverine, a known pharmacological antagonist (*2*) significantly reduced the protective effect of a massive dose of *S*-ethyl isothiuronium. Other agents—reserpine, atropine, phenergan, and dibenzyline—had no influence on *S*-ethyl isothiuronium action. The favorable therapeutic ratio and the response to a specific antagonist are parallel to actions established for serotonin (*4*), which is thought to decrease radiosensitivity through oxygen-dependent pathways. A similar mechanism may explain the action of *S*-ethyl isothiuronium since our data show that it fails to increase the radioprotection afforded mice by the optimal reduction of intercellular oxygen.

The experimental results suggest that pressor amidines offer radioprotective activity through a pharmacological mechanism which leads to a lowered oxygen tension of radiosensitive tissues.

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References

1. M. Ashwood-Smith, *Intern. J. Radiation Biol.* **2**, 233 (1960).
2. F. Fastier, *Pharmacol. Rev.* **14**, 37 (1962).
3. F. Rice, *Ind. Eng. Chem.* **52**, No. 5, 47A (1960).
4. H. van den Brenk, *Intern. J. Radiation Biol.* **3**, 73 (1961).

15 April 1963

Glycogen Deposition in the Liver Induced by Cortisone: Dependence on Enzyme Synthesis

Abstract. *The deposition of liver glycogen in starved rats given a single dose of cortisone is inhibited by puromycin and actinomycin. The former agent interferes with induced enzyme formation in general, and the latter with the cortisone-induced rise in liver enzyme levels. The results suggest that the regulatory effect of cortisone on carbohydrate metabolism may be brought about by its action on the cellular concentration of certain enzyme proteins.*

Adrenocortical hormones, which influence the rate of certain metabolic processes in vivo, do not appear to act as simple inhibitors or activators of enzymic reactions in vitro. Therefore, Knox, Auerbach, and Lin (*1*) suggested that hormone action may be brought about by changes in the actual concentration of the protein moiety of specific enzyme systems. The dependence on enzyme synthesis of the acute stimulation of glycogen deposition by cortisone in the liver of starved rats has now been tested.

Recent data suggest that the rise of enzyme activity induced by cortisone reflects an increase in the rate of *de novo* enzyme synthesis. The accumulation of liver tyrosine transaminase (*2*), glutamic-alanine transaminase (*3*), and tryptophan pyrrolase (*4*) has been measured immunochemically. Correspondingly, the administration of an inhibitor

5. S. Kit, D. R. Dubbs, L. J. Piekarski, T. C. Hsu, *Exptl. Cell Res.* **31**, 297 (1963).
6. J. W. Littlefield and P. F. Sarkar, *Federation Proc.* **23**, 169 (1964).
7. J. W. Littlefield, unpublished material
8. W. Szybalski, E. H. Szybalska, G. Ragni, *Natl. Cancer Inst. Monograph* **7**, 75 (1962).
9. G. Pontecorvo and E. Käfer, *Advan. Genet.* **9**, 71 (1958).
10. The frequency of 10^{-10} was estimated from previous work (*4*) and unpublished studies.
11. Supported by USPHS grant CA-04670-05. This is publication No. 1173 of the Cancer Commission of Harvard University. We acknowledge the assistance of Susan J. Whitmore.

4 May 1964

Seed Radiosensitivity: A New Constant?

Abstract. *Dormant seeds of different species tolerate varying amounts of ionizing radiation, species having smaller nuclei in the apical meristem generally withstanding greater exposure. Nuclear volume (in μ^3) multiplied by radiation exposure (in roentgens) equals a constant, estimated from 12 species to be $(10.14 \pm 1.17) \times 10^6$. From nuclear volumes alone, predictions of radiation response for two unknown species were made; experimental values in both cases fell below the 95 percent but within the 99 percent confidence intervals of the predictions.*

Sparrow *et al.* (*1*) observed that sensitivities of 16 actively growing plant species to acute x- or γ-irradiation may vary up to 125-fold as measured by total exposure, but only 4-fold when the criterion is energy absorbed per chromosome at the lethal exposure. The implication is that a similar quantity of energy is absorbed for a similar amount of nuclear damage regardless of total radiation exposure. This striking concept was hinted at in earlier papers (*2*) describing high positive correlations of nuclear volume (or DNA content) with sensitivity of growing plants to chronic irradiation and with frequencies of somatic mutation and chromosome aberration. Similar correlations exist for the sensitivity of dormant (seed) embryos to acute irradiation (*3*). We here derive a constant with which seed radiosensitivity can be predicted from nuclear volume of certain embryonic cells.

Controlling important modifiers such as seed moisture (*4*), we have found that most interspecies differences in radiosensitivity are attributable to nuclear volumes in the apical initial cells of shoot meristems (*5*), although eight additional measurements are required to account for all genetic variability (*6*). For comparisons between species, we calculated the maximum radiation exposure tolerated by seeds before the dry weight of seedlings grown in controlled-environment rooms was reduced by 50 percent ("50 percent exposure"). When growth values were transformed to probits, an almost linear response was obtained with the logarithm of the radiation exposure. Nuclear volumes were determined for dormant seeds stored at 35 to 60 percent relative humidity; volume has been found to remain unchanged within this range (*4*). Embryos were excised from dormant seeds, fixed in chrom-acetic-formalin, infiltrated with tertiary butyl alcohol, and embedded in paraffin. They were then sectioned at 10 μ, stained with warm safranin, and counterstained with fast green in clove oil. Cells in the apical meristem region were examined at $\times$ 930 with an ocular micrometer, and two measurements at right angles were made for each nucleus: the longer axis was designated "*a*" and the other "*b*." After all nuclei of the meristems of several embryos were measured, "*a*" and "*b*" values were averaged and average nuclear volume, *V*, was computed from the ellipsoid formula:

$$V = \frac{4}{3}\pi\left(\frac{a}{2}\right)\left(\frac{b}{2}\right)\left(\frac{a+b}{4}\right)$$

Chromosome numbers were obtained from Darlington and Wylie (*7*) except for *Festuca elatior*, which was determined in our laboratory from root tips.

The nuclear measurements, 50 percent exposures, and calculated values for energy absorption for 12 species from 10 botanical families are listed in Table 1. The second column from the right is comparable to the pertinent numbers of Sparrow *et al.* (*1*), except for our using the 50 percent rather than the lethal exposure. In our computations, however, a spread of more than 11-fold was obtained despite a range of only 10-fold in tolerance as measured in radiation units (kr, third column from the right). When comparisons were made on a per-nucleus rather than a per-chromosome basis (right-hand column), a spread of about 3.3-fold was found.

The data from reference (*1*) were used to determine energy absorbed per nucleus at the lethal exposure, and a 28-fold range was found; however, the range of the nine polyploid species was only 8-fold and that of the seven diploid species was less than 5-fold. The apparent lack of concordance between the two series of experiments may be ascribed to the facts that, in the experiments of Sparrow *et al.*, actively growing meristems were irradiated to the lethal point and most species—9 out of 16—were polyploid while, in our experiments, dormant meristems were irradi-

Table 1. Test of the hypothesis that in dormant seeds of quite different sensitivity, as measured by total radiation exposure, similar or identical sensitivity exists as measured by energy absorbed per chromosome or per nucleus. (SE, standard error.)

Plant group and chromosome number	Average nuclear volume ($\mu^3 \pm$ SE)	Energy per chromosome per roentgen (ev)*	Energy per nucleus per roentgen (ev)	50% exposure (kr $\pm$ SE)†	Energy per chromosome at 50% exposure (Mev)	Energy per nucleus at 50% exposure (Mev)
1. *Cucumis sativus* (14)	117 $\pm$ 2.2	502.9	7,041	46.3 $\pm$ 0.21	23.28	326.0
2. *Trifolium incarnatum* (14)	126 $\pm$ 1.4	541.6	7,583	135.0 $\pm$ 3.74	73.12	1023.7
3. *Brassica napus* (38)‡	125 $\pm$ 0.2	198.0	7,522	142.2 $\pm$ 8.11	28.16	1069.6
4. *Linum usitatissimum* (30)	164 $\pm$ 3.8	329.2	9,870	71.3 $\pm$ 5.91	23.47	703.7
5. *Lycopersicon esculentum* (24)	193 $\pm$ 5.5	483.8	11,615	47.5 $\pm$ 2.01	22.98	551.7
6. *Lactuca sativa* (18)	193 $\pm$ 0.3	645.1	11,615	47.3 $\pm$ 3.66	30.51	549.4
7. *Arachis hypogaea* (40)‡	249 $\pm$ 2.9	224.5	14,985	29.3 $\pm$ 0.82	6.58	439.1
8. *Festuca elatior* (42)‡	435 $\pm$ 7.1	623.5	26,178	14.0 $\pm$ 0.69	8.73	366.5
9. *Hordeum vulgare* (14)	467 $\pm$ 2.1	2,007.6	28,104	25.9 $\pm$ 1.99	52.00	727.9
10. *Allium cepa* (16)	901 $\pm$ 21.0	3,388.7	54,222	13.0 $\pm$ 0.38	44.05	704.9
11. *Gossypium arboreum* (26)	435 $\pm$ 3.6	1,006.8	26,178	16.8 $\pm$ 0.27	16.91	439.8
12. *Daucus carota* (18)	114 $\pm$ 1.8	379.7	6,830	61.8 $\pm$ 2.32	23.47	422.1
				(Averages)	(29.44)	(610.4)

* Based on 1.77 ionizations per cubic micron of tissue per roentgen and 34 ev per ion pair; for the computations it is assumed that nuclei are composed entirely of chromosomes. † Maximum exposure to seeds causing 50 percent reduction in seedling dry weight. ‡ Polyploids.

ated to a sublethal endpoint and most species—9 out of 12—were diploid.

The search for a unifying concept of seed radiosensitivity can be carried one step further. The values in the last column of Table 1 may be estimates of a single number, representing the maximum energy (in Mev) which can be absorbed by a dormant nucleus in the apical meristem before growth of the ensuing seedling will be reduced by 50 percent; the average value is 610.4 ± 70.4 Mev. The only variables making up this "constant" value (k) are the nuclear volume and the 50 percent exposure; thus either could be used to estimate the other. Since the experimenter is usually interested in predicting radiation tolerance, it would be relatively simple to section a few embryos and measure apical nuclei. It follows that

$$\frac{k}{\text{nuclear volume}} = \frac{}{\text{the 50 percent exposure in roentgens}} \quad (1)$$

for dormant embryos in their most resistant state, and

$$k = \frac{610.4 \pm 70.4 \text{ Mev/nucleus}}{(1.77)\ (34)\ \text{ev}/\mu^3/\text{roentgen}} = \frac{}{(10.14 \pm 1.17) \times 10^6} \quad (2)$$

therefore

$$\frac{(10.14 \pm 1.17) \times 10^6}{\text{average nuclear volume } (\mu^3)} = \frac{}{\text{the 50 percent exposure in roentgens}} \quad (3)$$

for dormant embryos in their most resistant state.

This method was tested on the last two species of Table 1 prior to the performing of dose-response experiments. For the first 10 species, average energy per nucleus at the 50 percent exposure was 646.3 ± 80.1 Mev, hence k was calculated to be $(10.74 \pm 1.33) \times 10^6$. The 50 percent exposure for *Gossypium arboreum*, with an average nuclear volume of 435 μ^3, was thus predicted to be 24.7 kr with a 95 percent confidence interval of 17.5 to 32.1 kr and a 99 percent confidence interval of 14.8 to 34.6 kr. The experimental value (Table 1) was 16.8 ± 0.27 kr.

The data from *G. arboreum* were then added to the preceding 10 species and the average energy per nucleus at the 50 percent exposure became 627.5 ± 74.8 Mev, and k was thus $(10.43 \pm 1.24) \times 10^6$. *Daucus carota* (average nuclear volume 114 μ^3) was predicted to have a 50 percent exposure of 94.2 kr, with a 95 percent confidence interval of 65.2 to 119.4 kr and a 99 percent confidence interval of 57.0 to 125.9 kr. The experimental value (Table 1) was 61.8 ± 2.32 kr. Thus in both tests the observed values fell below the 95 percent but within the 99 percent confidence interval (*8*).

Since this report was first submitted, we have been permitted access to relevant unpublished data from two Spanish authors (*9*). Their study provides nuclear volume and LD_{50} (lethal dose to 50 percent of the population) values for 20 species. Pertinent technical features are: all species were from the family *Cruciferae* and 16 of the 20 species were diploid; dormant seeds were equilibrated at 70 percent relative humidity then x-irradiated at 1200 r/min; exposures reducing survival by 50 percent were determined after 2 months of growth; and nuclear volumes of apical meristems were measured in sprouted seedlings. Average nuclear volumes ranged from 25 to 270 μ^3; chromosome numbers, from 10 to 64; and LD_{50}'s, from 15 to 240 kr. Ranges of energy per chromosome and per nucleus at the LD_{50} were inconclusive, being 6.6-fold in the former case and 5.4-fold in the latter. Means and standard errors were $(24.97 \pm 2.99) \times 10^6$ ev per chromosome and $(553.4 \pm 65.5) \times 10^6$ ev per nucleus, values quite in agreement with ours of Table 1. From their data one obtains a k value of $(9.20 \pm 1.09) \times 10^6$, which compares favorably with our value of $(10.14 \pm 1.17) \times 10^6$.

The theoretical significance of such a constant is obscure, but the practical importance is clear. Heretofore a person embarking on a radiation study with dormant seeds of an untested species could not predict whether his material would be devastated by 1 kr or be unaffected by 100 kr. Now a few microscopic measurements and some easy arithmetic will reveal the approximate amount of radiation he can expect the seeds to tolerate before a significant reduction in growth will occur.

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References and Notes

1. A. H. Sparrow, L. A. Schairer, R. C. Sparrow, *Science* **141**, 163 (1963).
2. A. H. Sparrow and J. P. Miksche, *ibid.* **134**, 282 (1961); A. H. Sparrow, R. S. Cuany, J. P. Miksche, L. A. Schairer, *Radiation Botany* **1**, 10 (1961).
3. H. J. M. Bowen, *Radiation Botany* **1**, 223 (1962).
4. T. S. Osborne, A. O. Lunden, M. J. Constantin, *ibid.* **3**, 19 (1963).
5. *Univ. of Tennessee Agricultural Research Laboratory Semi-Annual Progress Report Jan. 1–June 30, 1963, ORO-614* (1964).
6. A. O. Lunden, *Radiation Botany*, in press.
7. C. D. Darlington and A. P. Wylie, *Chromosome Atlas of Flowering Plants* (Allen, London, 1955).
8. It is possible that the numbers in the last column of Table 1 are estimates, not of a single value, but of a distribution having a rather small variance. It may be that further work will reveal different k values for different classes of seeds—for example, different ploidy levels.
9. C. Gómez-Campo and L. Delgado, *Radiation Botany*, in press
10. We thank C. Gómez-Campo and L. Delgado for permission to use unpublished data, D. A. Gardiner and R. J. Monroe for statistical advice, and A. H. Sparrow for helpful discussions.

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Protein Synthesis During Development: Control through Messenger RNA

Abstract. *Utilization of long-lived messenger RNA appears to be the exception rather than the rule in cells which are differentiating and synthesizing large amounts of specialized product at the same time. The fact that polyribosomes synthesize protein after RNA synthesis is turned off by actinomycin D is used to demonstrate messenger RNA of long half-life. The data suggest that most tissues examined have short-lived messenger RNA's, but the ocular lens can synthesize protein after an incubation of 24 hours in 40 μg of actinomycin D per milliliter. A common basis for the presence of long-lived messenger RNA in the cells of the lens, the feather, and in reticulocytes is discussed.*

A mark of the differentiated cell is its capacity to synthesize structural or enzymatic cell specific proteins. Some cells, such as skin, liver, muscle, connective tissue, reticulocyte, pancreas, and thyroid, produce large amounts of one or a few kinds of protein. We have asked whether all or only some differentiating cells synthesize their specialized product on messenger RNA which has a long half-life. It has already been shown that hemoglobin (*1*) and feather

close to the range of values for natural radiation background. Visible changes in the skeleton have been reported only after hundreds of rep were accumulated and tumors only after 1500 or more [were accumulated].

In relation to world-wide contamination, food chains are important. Fallout contaminates plants through ground and leaf deposition; animals eat these plants. Therefore, milk and cheese are human sources of radiostrontium, being high in calcium. Throughout this chain, strontium is discriminated against relative to calcium, which reduces the hazard somewhat. It must be remembered that in regions where soil and water are low in calcium, calcium and strontium will be more readily taken up.

Therapy of radiation injury: while treatment is difficult, some success has been achieved with antibiotics and properly timed blood transfusions. Shielding of a portion of the body appears to give a degree of protection disproportionately large for the mass shielded. Experiments set up to explain this fact may help in developing a rational treatment. Also, various forms of treatment given immediately before radiation have been devised, but do not appear in any sense practical. Studies of this sort may, however, provide a basis for future discoveries. . . .

Agriculture, Food Supplies, and Atomic Radiation

The committee interpreted its task as requiring its members to survey the scientific aspects of that great sequence of events which precedes the delivery of food items to the ultimate consumer, and to do so from two separate viewpoints. These were (i) the beneficial effects that may result from the deliberate involvement of radiation of any sort with constructive intention, or what has been spoken of so frequently as the "peaceful uses of atomic energy," and (ii) the harmful or disadvantageous effects of radiation of any sort due to nuclear warfare, to accidents involving atomic power plants, or even to a slowly rising background of radiation that conceivably may follow as a result of atomic technological developments in industry.

Public and private funds are currently being expended in the United States for research in agriculture and food processing at a rate in the vicinity of $300 million annually. An undeterminable but not insignificant fraction of this considerable body of research involves radiation or radioistotopes. Members of the committee did not believe it to be incumbent upon them to defend or justify, to criticize, or to challenge applications of atomic radiation to agriculture that have been developed or are under discussion. They did not wish to evaluate the programs of particular agencies or groups, but instead with judicial mind to examine the accomplishments and the potentialities, the implications and the limitations of radiation as related to the production and processing of agricultural products.

One broad conclusion is that there is not imminent any drastic change in *agricultural production* as a result of the application of radiation. However, radiation techniques provide new tools for research and may aid agricultural production by improving and enhancing the efficiency of production methods.

The committee is strongly of the view that the applications of radiation will be of far greater immediate consequence to agricultural research than directly to agriculture, and that most of the benefits that may arise to agriculture, as manifest in the availability of an adequate and varied supply of wholesome food for man, wherever he may be, will come as a summation of many improvements, small and large, in materials, in plants and animals, and in the technology of husbandry and processing developed through programs in agriculture and food processing research.

Changes therefore may be expected to come in a series of little steps, none of which in themselves may be of great impact, but which, through the years, are likely to be impressive in their total.

Another broad conclusion is that the slowly rising background of radiation caused by weapons testing in peacetime at the present rate is not likely to impair or interfere with food production. Levels of radiation considered tolerable by man are below those believed to have effects in plants or animals that would place food production in jeopardy. However, the high levels of radiation which might develop in small or large areas as a result of [the use of] atomic or thermonuclear weapons in wartime, or from mishaps with nuclear power plants in peacetime, could have catastrophic effects on agricultural production that might be of long duration, because of injury to personnel and animals, disruption of services, and contamination of soil, vegetation, and water supplies.

Tracer Studies in Agriculture

In the consideration of the beneficial effects of radiation, the committee endeavored, not wholly successfully, to separate in its thinking those benefits that may arise from additions to the pool of basic knowledge about plants and animals and their welfare from those more direct effects that may specifically result from the exposure of plants, animals, or agricultural products to radiation. Tracer studies in the biological sciences have already been enormously fruitful in aiding the elucidation of essential metabolic processes in plants and animals and may be expected to be increasingly so as the number and diversity of such experiments increases. When there is knowledge and understanding of a process, then comes the opportunity to control it for a desired end; in this way the art of agriculture is transformed to the science of agriculture.

The committee endeavored to make the separation mentioned above because

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This article is, with some shortening of the subheads, the text of the summary report of the Committee on the Effects of Atomic Radiation on Agriculture and Food Supplies. The report is one part of a study of the Biological Effects of Atomic Radiations conducted by the National Academy of Sciences with the support of the Rockefeller Foundation. The full report will be published in monograph form by the NAS. The committee members are A. G. Norman, University of Michigan, *chairman*; C. L. Comar, Oak Ridge National Laboratory; George W. Irving, Jr., U.S. Department of Agriculture; James H. Jensen, Iowa State College; J. K. Loosli, Cornell University; Roy L. Lovvorn, North Carolina State College; Ralph B. March, University of California, Riverside; George L. McNew, Boyce Thompson Institute for Plant Research; Roy Overstreet, University of California, Berkeley; Kenneth B. Raper, University of Wisconsin; H. A. Rodenhiser, U.S. Department of Agriculture; W. Ralph Singleton, University of Virginia; Ralph G. H. Siu, Office of the Quartermaster General; G. Fred Somers, University of Delaware; and George F. Stewart, University of California, Davis.

of the conviction that there is nothing unique about radioistotopic studies as applied to agricultural research. Tracer techniques, however, frequently permit answers to be obtained to questions which seemed previously unanswerable by conventional experimentation. The involvement of isotopes puts a new dimension into metabolic studies, and areas, formerly dark, may now stand out in relief.

It is worthy of comment that many of the applied problems involved in the arts or technology of agriculture are as susceptible to study by procedures involving radioisotopes as are those more basic questions of plant and animal physiology or nutrition. Excellent examples of this type of employment of isotopes are to be found in work on the placement and recovery of phosphorus fertilizers in soils, the efficiency of various methods of application of insecticides, fungicides, and herbicides, the determination of post-harvest residues of such chemicals, the extent of utilization of feed components by animals, and so forth. It is to be anticipated that there will be greatly increased use of tracer radioisotopes in the solution of such applied problems and that the immediate dividends from such research may be considerable. Further, it is likely that new methods of employing isotopes advantageously will be developed; the ingenuity of investigators in this field should not be underestimated.

Because of the unanimity of their views as to the enormous potentialities of isotope tracers as a research tool in agricultural science and biology generally, the committee gave some consideration to whether there are limitations in facilities for training or funds for specialized equipment for such studies. The consensus seemed to be that motivation for the use of such techniques must come from individual investigators themselves, that the necessary know-how is to be found in almost all research institutions, and that progress in agricultural research is not at the moment limited by inadequacies in dissemination of knowledge and techniques. There was, however, a feeling that much of the graduate training in this field is rather informal, that more universities might consider establishing courses in which the methodology, techniques, and principles of this new and powerful science are expounded, and that there is an additional need for an advanced training program for specialists in radiochemistry and radiobiology who may be developers of new techniques or interpreters of new applications of potential value in agricultural research.

Crop Production

It is abundantly established that mutations can be induced in many plant species by exposure to x-radiation, gamma radiation, and other forms of radiation. The changes which result are possibly due to chromosome deletions or aberrations. There is some difference of opinion whether radiation-induced mutants intentionally obtained are qualitatively identical with those which occur spontaneously from naturally occurring mutagenic agents, but there is no doubt that their frequency is increased. Even so, the mutation rate in most species is still very small, and furthermore most mutations are disadvantageous. The investigator seeking to exploit this phenomenon must expect to have to handle very large populations, and so far he has been able to look only for desirable changes that are reflected in morphology or appearance and therefore can readily be seen, or for changes which can be recognized by some blanket method such as inoculating all irradiated plants with disease organisms in the hope of finding one or more exhibiting resistance to infection.

It is likely that characters at present unrecognized also undergo change and that there are unexplored potentialities for effecting improvement in quality that may alter the demand for the plant, or in physiological properties that may alter the relationships of the plant with its environment.

It would be a mistake to imply that this new development has greatly simplified the tasks of those involved in crop improvement. On the contrary, it has made them more complex, but, by extending the boundaries, offers many new possibilities. It is not to be expected that acceptable new agronomic varieties can be obtained by simple irradiation of present varieties, though this is possible if large enough populations are examined. In general, however, back-crossing and recombination are needed to add the new characteristic to a crop plant acceptable in other repects.

As yet relatively few new varieties of economic plants, developed from radiation-induced mutants, have actually been introduced and widely planted. These, however, do attest to the potentialities of this procedure. Much of the research effort in this field has properly been devoted to the investigation of techniques, to such vital questions as the determination of the particular stage of development at which radiation exposure may be most effective, and to the comparative mutability of crop species. It appears that different species cannot be expected to respond in an identical manner. More perhaps is known about this aspect of corn genetics than of any other major crop plant.

Mutations in microorganisms may similarly be induced by exposure to various types of radiation, though at considerably higher radiation levels than with crop plants. The changes induced have been shown to include the degree of virulence and host range of certain pathogenic fungi. The suggestion has repeatedly been made that the plant pathologist should examine this phenomenon so as to anticipate disease-resistance requirements in a breeding program. As yet, however, there have been no significant results along these lines. Considerable success has been achieved in the development of greatly enhanced antibiotic production by some molds through radiation-induced mutation and selection. Similar genetic changes in the case of other microorganisms have produced information about the likelihood of genetic control of metabolic processes.

There is considerable evidence that bud mutations or somatic mutations can be induced by radiation and that this phenomenon can be exploited in the development of new strains of crop plants that are normally propagated by cuttings and grafting. This may be of special value in the improvement of some such crops, but as yet there have been no striking accomplishments in this direction. Progress in such studies is, however, inevitably slow because of the nature of the materials and the length of time necessary to recognize a desirable change and to produce the stocks necessary for field evaluation.

Since the mutation rate of plants may be enhanced by radiation, presumably there is some possibility of the appearance of undesirable mutants in areas where the background radiation becomes higher than normal for any reason. This may be of some significance in connection with waste-disposal practices or atomic accidents. There is, however, no evidence of such changes in areas containing radioactive springs or ores. This may be due to lack of intensive examination of the vegetation of such areas, and such surveys are to be encouraged. However, the likelihood of appearance of undesirable lines under radiation levels that would be tolerated on other grounds seems small.

There is no evidence that plant growth is stimulated or crop yields increased by exposure to low levels of radiation, despite earlier well-publicized claims to this effect. Radioactive fertilizers, used in a conventional manner, produce yield increments no greater than expected from ordinary fertilizers.

Plants accumulate nutrient elements present in the root zone in solution or absorbed onto soil colloids, but nonnutrient elements are not excluded and may similarly be taken up. The availability of radioisotopes has greatly improved the understanding of plant nutrition and soil-plant relationships and may be expected to aid substantially in the improvement of cultural practices, as indicated earlier.

Through the use of isotopes it has been demonstrated unequivocally that certain elements can enter the plant through the leaves. This is of some consequence in relation to fallout. Radioisotopes of long life or high activity if deposited in fallout from an atomic or thermonuclear incident are likely to be accumulated in crop plants by root uptake from the soil and entry through the foliage. Some of the products deposited may be initially quite insoluble, but may become soluble through weathering. Others, initially soluble, may be irreversibly fixed by many soils in a form not readily available to crops. It appears at present that strontium-90 and iodine-131 are the chief radioactive elements which are of concern in such circumstances. The subsequent use of such crops presents a great diversity of problems depending on the level of radioactivity, its nature, and the specific use of the crop. The committee was interested to learn that the Department of Agriculture is preparing for farmers some informational material relating to these problems.

The committee desires to examine further the available information on the interactions of fallout components with soil, their entry and accumulation in crop plants in order to determine whether there is available the necessary basic information from which appropriate agronomic recommendations could be formulated for agricultural operations in areas that may have undergone any likely level of contamination.

Animal Production

Whereas it appears that crop improvement programs may be considerably aided by the availability of radiation-induced mutants that may have certain desirable characteristics capable of incorporation into an agronomically acceptable variety, currently available evidence does not suggest that a similar approach with animals would be so rewarding. This statement is made not from a belief that farm animals are inherently less responsive to radiation than plants, but because physical differences of size, cost, generation time, and so forth militate against extensive studies with animals and act as obstacles that cannot readily be overcome. Probably only with poultry and to a lesser degree with swine would it be possible to handle large enough populations, and even here, if one extrapolates from the smaller laboratory animals, the chances of improvement seem slim. At present, one such study, with chickens, is known to be underway.

Limited whole-body exposure studies with farm animals have primarily been carried out to investigate physiological and pathological changes, often with the intention of transferring the information by analogy to problems of responses in man. The sequence of changes induced in most farm animals by heavy radiation exposures has been well defined. There are one or two examples, however, of the use of radiation exposure as a research tool for inhibiting certain functions in animals. For example, various functions in the oviduct of poultry can be blocked by proper radiation techniques, thereby permitting a study of the contribution made by the parts of this organ.

Much of the work with radioisotopes in the animal field centers around problems of animal nutrition and metabolism, and substantial progress has been made both in the elucidation of fundamental problems of animal physiology as well as in those of a more applied character, such as the utilization of feed constituents and the incorporation in animal tissues of inorganic constituents of forages. The experimenters in this field at present encounter one serious difficulty, which in the case of the larger farm animals greatly limits the scale of activity. This is the problem of the salvage or disposal of animals after use in experiments involving radioisotopes or radiation exposure. Even in the case of short half-life isotopes and at tracer levels only, the animals cannot be marketed through the usual outlets. This problem is of course much more serious with dairy or beef cattle than with hogs or poultry because the cost to the program is so much greater. Moreover, this limitation tends to restrict undesirably the scale and scope of such experiments, with the result that the conclusions may be less surely established than if the numbers of animals used were larger.

It appeared to the committee, therefore, that essential research on farm animals using radioisotopes or radiation is being discouraged by the high costs involved because animals must be destroyed at the termination of experiments. It recommends that a special committee be appointed to study this problem and to develop procedures and standards that, if followed and enforced, would adequately protect the consumer but permit the marketing of animals that in experimentation have been brought into contact with radioactive substances or exposed to radiation.

The welfare of the livestock population is enhanced if troublesome insect pests can be controlled or eradicated. As mentioned earlier, insecticide studies have been greatly aided by the availability of radioisotopes as tracers, but in addition there may be certain opportunities for control of insect pests by taking advantage of radiation-vulnerable stages in their life cycles. Eradication of the screw-worm fly from the southeastern United States is to be attempted, based on the virtual elimination of this fly from the island of Curaçao by the release of males rendered sterile by radiation exposure. This technique may not be generally applicable to all insect pests.

Radioisotopes in Agricultural Products and Foods

The committee discussed in detail some of the difficult problems that may arise because of the presence of a radioisotope burden in agricultural products and foods higher than that "naturally occurring." The applicable legislation in this area is clouded with uncertainties because the very possibility was not envisaged by those who enacted the laws and defined the responsibilities of the agencies that protect the public food supply. There are no permissible limits for radioisotopes in foods; any burden above the "natural" is regarded as undesirable. The current interpretation of the law places isotopes in the same category as poisonous additives. It is difficult, however, to be wholly consistent in this, inasmuch as the normal radioisotope burden varies considerably in different agricultural products, and in the same product from different locations. Moreover, the testing of atomic and nuclear weapons is placing in soil, water, and air, the world over, radioisotopes not formerly present, though at extremely low levels. The "natural content" of foods now consumed by animals and man is not the same as in the preatomic age. Though extremely small, the increment is measurable and inescapable.

It is to be anticipated that there will be in the years ahead a slowly rising background of radiation manifest in agricultural and food products by the presence of the isotopes of elements not previously found therein or of "unnatural" levels of radioactivity. Atomic warfare might greatly increase the rate of this development. As pointed out earlier in this report, radiostrontium is particularly the element which would cause concern in the latter event. Forage directly contaminated with fallout, if consumed by farm animals soon after deposition, might cause radiation injury from the presence of insoluble radioactive products. Strontium is metabolically similar to calcium and moves into bone and other calcium-accumulating tissues or fluids. Much is known of the relative behaviors of calcium and strontium, but there appears to be no way of wholly preventing strontium retention. There is some evidence that poultry may "decontaminate" or "detoxify" themselves by reason of a continued dilution through transfer to eggshell. In meat animals, certain tissues might be consumable if boned out, but

such an expedient would be beyond the ordinary scope of meat inspection. Dairy products would contain radiostrontium for some considerable time after cows had ingested strontium-containing forage. Moreover, all available feeds, in heavily contaminated areas, might contain significant levels of radiostrontium, perhaps for years.

At present it is not possible to say at what level a food, otherwise wholesome, becomes unwholesome or deleterious by reason of the presence of an unnatural burden of radioactivity. There is a great deficiency of requisite data on the long-term biological effects that may follow the ingestion of such foods by animals and man. Situations in which such information might be of great public importance are not inconceivable and possibly inevitable.

The committee therefore urgently recommends that appropriate experimentation be immediately activated to provide specific information about possible total or cumulative biological effects that might follow the ingestion of such foods. It further urges that the planning of such experiments be broadly based and that the development of the experimental designs and details of their subsequent execution be most carefully considered in order that the emerging data will be acceptable as a basis for the crucial decisions that ultimately will have to be taken, and directly of value to the regulatory agencies charged with the protection of the public interest.

Environmental Changes and Ecological Studies

In the decades ahead there is a strong possibility that the general background of radioactivity in agricultural areas will rise. Contributing to this would be fallout, if weapons testing continues, and wastes from nuclear power plants or isotope processing plants. As indicated in the report of another committee, every effort will have to be made to contain radioactive wastes. Atomic warfare or accidents involving nuclear power sources could of course greatly augment the background and pose difficult problems of land use for agricultural purposes. Limited ecological studies are in progress in the vicinity of certain AEC installations, but it may be wise to consider this general problem somewhat more widely and to attempt to establish, through careful sampling, the present background in representative agricultural areas and in their chief crop and livestock products.

Research activities might appropriately be carried out on areas near weapons test sites where substantially greater changes in background would be anticipated. The distribution in the environment, in the soil at various depths, in the vegetation, in the wildlife, in the streams, and so forth would all be pertinent. The rate of accumulation in soil as affected by land use ought to be studied. Forested land, range land, rotation grassland, and plowland, irrigated and nonirrigated, may each present a different situation. It is possible that certain of the state agricultural experiment stations might be in a position to undertake limited surveys of this type on areas likely to be under their control for some considerable time in the future.

The committee recognized clearly that sustained monitoring and ecological research activities of this type are expensive and are not apt to be professionally rewarding to the individuals participating therein because trends and conclusions would emerge only slowly. However, to be able to recognize changes in the levels of radioactivity in the environment and in products removed therefrom, and to follow movements in the system, may well be in the public interest from a long-range viewpoint.

Food Processing

A recent development in food technology, potentially of considerable and possibly of dramatic significance, is the recognition of the fact that radiation can be used as a means of preserving certain foodstuffs or of lengthening shelf life, either unrefrigerated or refrigerated. The radiation source may be gamma rays or high-energy electron beams. No radioactivity is induced in the irradiated material. Feeding experiments to date indicate that foods so irradiated will prove to be suitable and safe for consumption by man. Parasites in meat and meat products can be killed by exposure to penetrating radiation, and undesirable postharvest changes in plant products, such as the sprouting of potatoes, can be delayed.

The prime objective in radiation processing is to destroy microorganisms, or so greatly to reduce the microbial population (radiation pasteurization) that spoilage is long delayed. To accomplish this, very heavy radiation exposures are necessary because microorganisms are much less sensitive to radiation than are animals and higher plants. The food processor is particularly attracted by the fact that the radiation exposure can and should be carried out after packaging.

The acceptability of some radiation-sterilized foods is open to doubt because of the development of off-flavors and changes in odor or in the texture of the tissues. Much of the developmental work in this field, however, has been of a rather empirical nature, and it is possible that through research means may be found to repress some of these undesirable changes.

Although the feasibility of radiation sterilization has been amply demonstrated, the economics of the various processes have not yet been established. This development has largely been financed by the military with the Army Quartermaster Corps as the primary agency involved, but there has been a broad basis of cooperation in industry and elsewhere, with some technical guidance and evaluation by advisory committees of the National Academy of Sciences. Having in mind the magnitude and coherence of the current broad programs in this area, the committee was of the opinion that the potentialities of this use of radiation are being thoroughly explored and that the interests of the food consumer will be adequately protected. At a later date, the committee expects to review particularly the evidence of wholesomeness and acceptability of irradiated foods.

It is commonly said that P. G. Tait laid down the length of a drive on mathematical principles which could not be exceeded, and that his son drove the ball farther. But at that time Tait had not realized the full effect of spin on the ball.—OLIVER LODGE, *in Past Years, an Autobiography. (Young Tait was a golf champion.)*

Banff National Parks, Alberta. Natl. Mus. Canada, 38 p.

Retzer, J. L. 1956. Alpine soils of the Rocky Mountains. J. Soil Sci. 7:22-32.

———. 1962. Soil survey Fraser alpine area Colorado. U.S. Dept. Agr. For. Serv. and Soil Conserv. Service Series 1956, No. 20, 47 p.

Richards, L. A. 1949. Methods of measuring soil moisture tension. Soil Sci. 68:95-112.

Tedrow, J. C. T., and **D. E. Hill.** 1955. Arctic brown soil. Soil Sci. 80:265-275.

Thompson, W. F. 1960. The shape of New England mountains. Part I. Appalachia 33:145-159.

———. 1961a. The shape of New England mountains. Part II. Appalachia 33:316-335.

———. 1961b. The shape of New England mountains. Part III. Appalachia 33:458-478.

Torrey, R. H. 1927. Contrasts between alpine floras of the eastern and western U.S. as observed by an amateur botanist. J. New York Botan. Gard. 23:242-246.

U.S. Weather Bureau. 1962. Local climatological data with comparative data 1961. Mount Washington, New Hampshire. U.S. Dept. Comm., Weather Bureau. Asheville.

VEGETATIONAL RECOVERY ON ATOMIC TARGET AREAS IN NEVADA[1]

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INTRODUCTION

Shields and Wells (1962) appraised the effects of fission-type nuclear detonations on perennial plant cover at the Nevada Test Site in the northern Mohave Desert. The present paper resumes the account of denuding and recolonization, by annual species in particular, in the vicinity of seven ground zeros. Observations dated from Operation Plumbbob (1957), the last full-scale surface nuclear test series in Nevada, through the 1961 growing season.

The area under consideration is Yucca Flat, an arid internal drainage basin extending approximately 20 miles in a north-south direction and varying from 16 to 18 miles latitudinally. A playa marks the lowest section, altitude 3,915 feet, somewhat off-center toward the southeast foothill zone. The interrupted surrounding mountains reach a height of 7,300 feet to the north. The Sonoran and transitional vegetation types in Yucca Flat grow at moderate daytime and low night temperatures during the winter and spring. Summer noonday temperatures regularly reach 110-115°F. Sporadic precipitation amounts to 4 to 5 in. annually at the lower elevations, approximately one-third falling as snow.

METHODS

The area of complete initial denuding was established at five tower-shot sites and two balloon ground zeros (Fig. 1). In late April or early May, 1958-61, during the height of flowering in annuals, plant cover was determined on tower

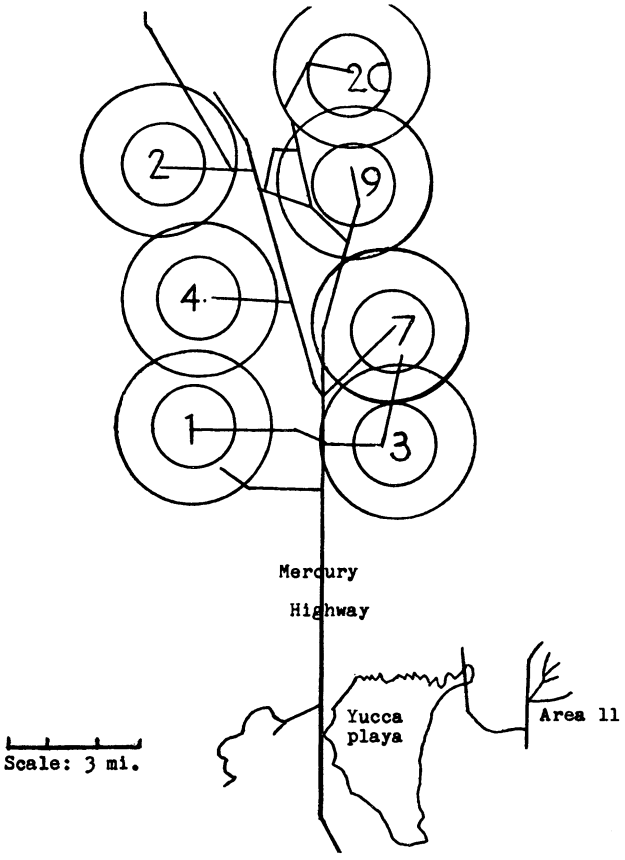


FIG. 1. Approximate locations of ground zero sites, Yucca Flat, Operation Plumbbob, 1957: tower target areas 1, 4, 2, 2c, and 3; balloon sites 7 and 9.

[1] This investigation was supported by contract AT(29-2)517 between the U.S. Atomic Energy Commission and New Mexico Highlands University. Logistic support in the field was provided by the U.S. Atomic Energy Commission's Civil Effects Test Operations.

target areas by a modification of the Braun-Blanquet cover-class method developed by Daubenmire (1959). Permanent 100-ft lines were spaced at 0.1-mile intervals to a distance of 1 mile in one direction from each of five ground zeros and at 0.3, 0.6, and 1 mile in a second direction from two. Cover by species was estimated within each of fifty 2- by 5-dm frames placed at 2-ft intervals along the 100-ft tape. For a particular species the cover-class estimate within each frame was converted to its mean percentage value (1 = 2.5%, 2 = 15%, 3 = 37.5%, 4 = 62.5%, and 5 = 87.5%). Data for 50 frames were totaled and averaged to obtain cover percentages by that species. The sum of these species averages constituted the cover percentage along the 100-ft line. Since this cover-class method of expressing plant cover applies to the canopy of each species, overlapping of canopies may allow total cover to exceed 100%. During the same period total vegetative cover was measured annually on control plots as well as on areas denuded by blading to depths of 3-12 in. in March 1958. In July of 1959 and 1960, percentage cover by *Salsola kali* (Russian thistle) was determined within 0.3 to 0.5 mile of each of five tower sites.

Vegetational Character and Test History of Target Sites

Target areas 1 and 4 were located in a predominantly *Grayia spinosa-Lycium andersonii* shrub type. A *Larrea divaricata* remnant fringed area 1 to the south and east. Tower ground-zeros 2 and 2c, as well as balloon sites 7 and 9, were in the *Coleogyne ramosissima* zone typical of the basin margins and foothills. An *Atriplex confertifolia-Kochia americana* stand north of the playa surrounded ground-zero 3.

Certain target areas had served as shot sites in successive test seasons ordinarily scheduled in alternate years. Of the five tower ground zeros studied in Yucca Flat, 1, 2, and 4 each had been the site of four detonations at altitudes ranging from 300 to 500 ft. Balloon shots, frequently of a higher energy yield, usually were fired from a height of 1,500 ft. The two balloon ground zeros in Yucca Flat, 7 and 9, served repeatedly as detonation sites during each test season.

From the time of the first shot at each target area until the suspension of testing, vegetational recovery had not been allowed to proceed beyond the second year. Previous to the 1957 test series, the second-year vegetation within 0.6 mile of ground-zeros 1 and 4 had consisted in August of a 35-50% cover of *Salsola kali* (Fig. 2). At ground-zero 2, in the northwest quadrant of Yucca Flat, the surrounding area was barren except for sparse small plants of *Oryzopsis hymenoides, Salsola kali,* and *Eriogonum nidularium.* This ground zero was at the periphery of overlapping zones denuded earlier in 1957 by two detonations (0.5 mile northeast and 0.5 mile southeast). Two remaining tower target areas, 2c and 3, were initial shot sites.

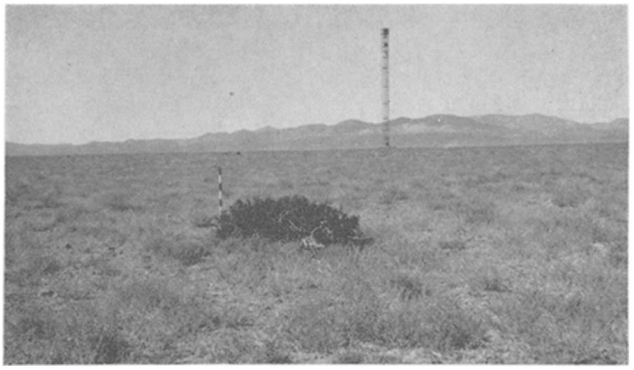


FIG. 2. *Larrea divaricata* surrounded by second-year stand of *Salsola kali* 0.5 mile from ground-zero 1. Photograph taken in August 1957, preceding September detonation.

The following list includes the plant species of the Nevada Test Site which are referred to in this report. Nomenclature follows Munz and Keck (1959).

Shrub species
- *Atriplex canescens* (Pursh) Nutt. (Four-wing saltbush)
- *Atriplex confertifolia* (Torr. and Frem.) S. Wats. (Shadscale)
- *Coleogyne ramosissima* Torr. (Black brush)
- *Grayia spinosa* (Hook.) Moq. (Hop-sage)
- *Hymenoclea salsola* T. and G. (Burrobrush)
- *Kochia americana* Wats. (Gray molly)
- *Larrea divaricata* Cav. (Creosote bush)
- *Lycium andersonii* Gray (Desert thorn)
- *Menodora spinescens* Gray (Ground thorn)

Tree species
- *Yucca brevifolia* Engelm. (Joshua tree)

Perennial grasses
- *Oryzopsis hymenoides* (Roem. and Schult.) Ricker (Indian rice grass)
- *Stipa speciosa* Trin. and Rupr. (Needlegrass)

Other non-shrubby perennials
- *Sphaeralcea ambigua* Gray (Globe mallow)
- *Stanleya pinnata* (Pursh) Brit. (Desert plume)

Annual species
- *Amsinckia tessellata* Gray (Fiddleneck)
- *Bromus rubens* L. (Brome grass)
- *Chaenactis* spp.
- *Chaenactis stevioides* Hook. and Arn. (Esteve pincushion)
- *Cryptantha circumscissa* (H. and A.) Johnston
- *Cryptantha nevadensis* Nels. and Kenn.
- *Cryptantha* spp. (Forget-me-not)
- *Eriogonum nidularium* Cov. (Wild buckwheat)
- *Erodium cicutarium* (L.) L'Her. (Storksbill, desert filaree)
- *Gilia latiflora* A. Gray (Gilia)
- *Gilia* spp.
- *Malacothrix glabrata* (Gray) Gray

Mentzelia albicaulis Dougl. (Stickleaf)
Phacelia fremontii Torr.
Salsola kali L. (Russian thistle)

Results

Specific patterns of injury during the 1957 test season

Blast and shock effects.—A typical tower shot of approximately a 20-kiloton yield (1957) eliminated all desert shrub vegetation within 0.5 mile (Fig. 3). The seed-containing layer of topsoil

Fig. 3. Same area as shown in Fig. 2, seen from 0.4 mile north of ground-zero 1 in December 1957, 3 months following nuclear detonation.

was removed within the 0.1- to 0.3-mile radius. Somewhat nearer to ground zero the remaining substrate was considerably loosened by soil displacement. Stem damage from blast and root injury from the shock wave traveling through the ground possibly account for most of the gross effects on vegetation beyond the perimeter of complete denudation (Shields and Wells 1962). Mechanical damage to perennials was selective, tending to vary with stem rigidity and to extend asymmetrically to a greater distance on less consolidated substrata. At approximately 0.6 mile, the burned crowns of bunchgrasses, *Oryzopsis hymenoides* and *Stipa speciosa,* remained alive (Fig. 4). Flexible-stemmed or low shrubs, as *Larrea divaricata* and *Menodora spinescens,* persisted at around 0.7 mile, sometimes forming a distinct shrub line. Beyond 0.8 to 1.0 mile most perennial species remaining after the 1957 test series showed no gross damage. In places at a distance of 1.2 miles, blast had snapped the mature *Yucca brevifolia.* The brittle wood of *Grayia spinosa* was also especially susceptible to mechanical injury.

Thermal effects.—Thermal damage from tower detonations in the 1957 test series, ranging from approximately 11- to 43-kiloton yields, typically extended out somewhat beyond 0.6 mile as evidenced by unilateral searing of surviving bunchgrasses. Normally a thermal energy of 5-7 cal/cm^2 will ignite dry grass, and 10-15 cal/cm^2 will char vegetation (Glasstone 1962). At ground-

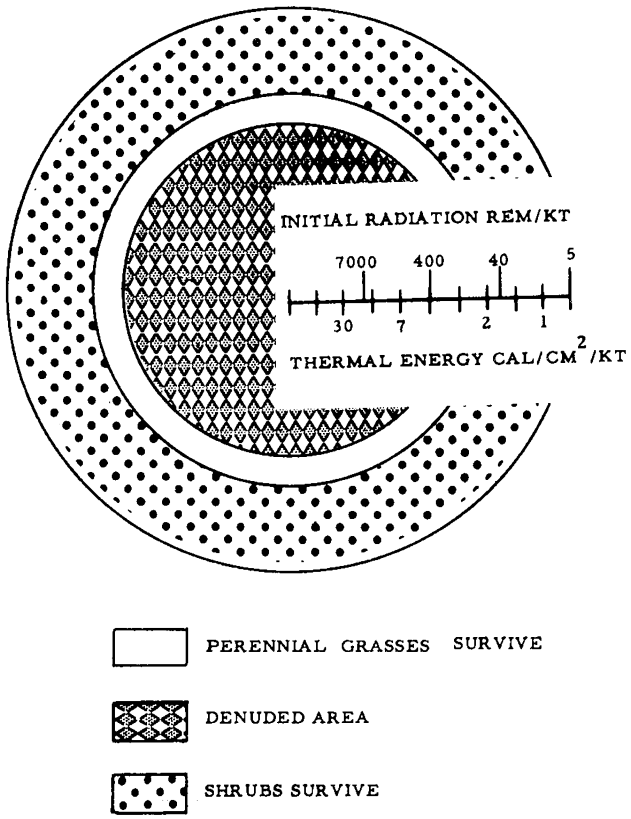


Fig. 4. Generalized diagram showing nuclear effects on vegetation and approximate thermal and ionizing radiation yields per kiloton 1 minute after burst within 1.0 mile of tower ground zeros at the Nevada Test Site. Ionizing radiation levels appear in roentgen equivalents for mammals per kiloton yield, and thermal energy is given as cal/cm^2. For the typical tower detonation (1957), each of the above figures would be multiplied by 20. A fission-type nuclear device has been calculated to release 50% of its energy as shock, 35% as heat, and 15% as other forms of radiation (Glasstone 1962).

zero 1, for four shots totaling 93 kilotons (11- to 43-kiloton individual yields), the integrated thermal levels averaged 2.8 cal/cm^2 per kiloton at 0.6 mile, ranging at this distance for the four tests from 31 cal/cm^2 (11-kiloton) to 120 cal/cm^2 (43-kiloton).

Ionizing radiation effects.—Except for the destruction of plant cover by thermal and mechanical damage within the 0.5-mile radius, gross radiation effects would doubtless be in evidence at all ground zeros. At ground-zero 1 the estimated acute gamma exposure at 0.15 mile averaged around 30,000 r per kiloton yield.[2] At 0.6 mile, where the estimated integrated gamma dosage was on the order of 14,000 r from the total 93-kiloton yield of the four shots, averaging 155 r per kiloton, the acute gamma exposure would have ranged for individual shots from 1,705 r (11-kiloton) to

[2] Private communication, calculations by Jerry G. Lackey, Santa Barbara Laboratory of Edgerton, Germeshausen, and Grier, Inc.

6,665 r (43-kiloton). The calculated neutron dosages (inrads) amounted to approximately 43% of the gamma levels. While the kiloton yield of a detonation cannot be directly extrapolated to the energy delivered to a particular area, the plant survivors nearest to ground zeros were subjected to intense radiation exposures. In July, when this study was initiated in 1957, most shrubs were in a state of summer dormancy. The reproductive cycle of spring annuals had been completed. Consequently, the full potential impact of ionizing radiation on vegetation may not have been observed. More subtle radiation effects may have existed undetected or have been lost. A degree of recovery by vegetative growth in shrub survivors might be expected during the 2-year interval between test series. Sustained residual radiation from fallout, as much as 1 r per hour 5 days after detonation, might have caused asymmetrical damage to vegetation at certain ground-zero areas. A number of shrub species recovering from mechanical damage at distances of 0.7 to 0.8 mile from ground-zero 1 now produce an abundance of viable seeds. Ionizing or thermal radiation or both may have exercised a selective action on seeds in the soil within 0.6 mile of all target areas.

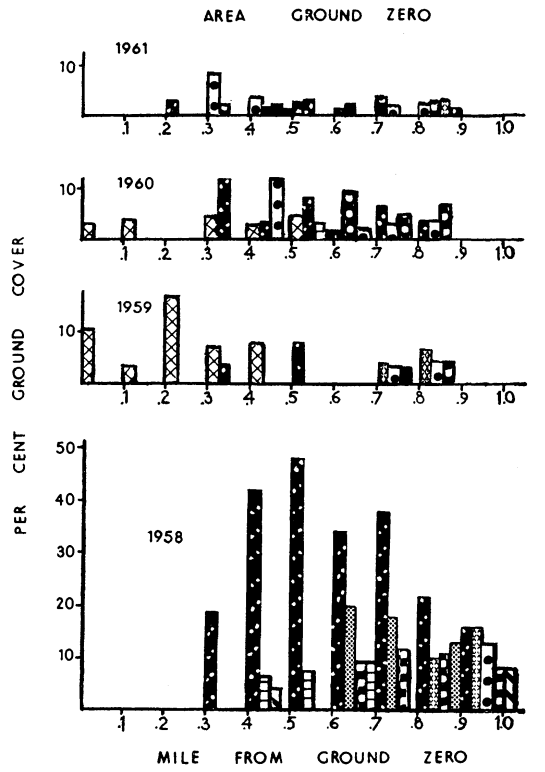
Recovery by annual species at ground zeros

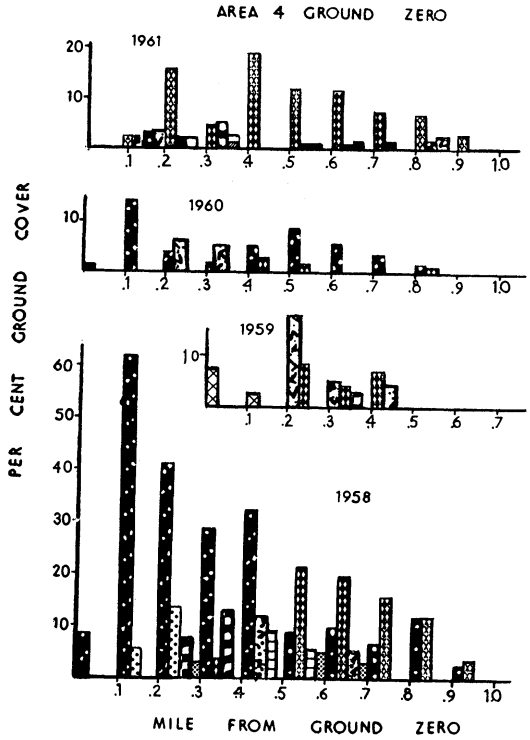
First-year recovery patterns.—The pattern of initial vegetational recovery was similar on all areas bared by tower shots (Figs. 5, 6, 7, 1958 values). The zone within 0.1 to 0.3 mile from different ground zeros remained essentially barren during the spring months of the year following detonation. A sparse representation of spring annuals developed in this area from seeds introduced naturally. Between the outer perimeter of pronounced soil removal, 0.1 to 0.3 mile, and 0.6 mile, two annual species contributed the bulk of the total plant cover, *Mentzelia albicaulis* alone at sites 1, 3, and 4 and associated with *Erodium cicutarium* at 2 and 2c. At all five tower shot sites, coverage by *Mentzelia* within 1.0 mile (peak 35 to 62% at four sites) was greater than for any other species. *Mentzelia* attained maximum coverage at distances varying from 0.2 (area 4) to 0.6 mile (areas 1 and 3) from different ground zeros. Beyond 0.4 to 0.6 mile, cover by certain other annuals increased, in particular, *Gilia latiflora, Chaenactis stevioides, Amsinckia tessellata, Malacothrix glabrata, Cryptantha* spp., and *Bromus rubens.* With the exception of target area 3, the number of annual species varied from 12 to 19 at 0.6 mile and from 17 to 25 at 1.0 mile. In May 1958 coverage by spring annuals between 0.4 and 0.8 mile from target areas exceeded total cover in the surrounding undamaged vegetation, reaching a peak of from 70 to 117% between 0.4 and 0.8 mile at four target areas, excluding ground-zero 3 with a peak of 12%. The compact soil at ground-zero 3 largely accounts for this low coverage by annual species. In contrast, total coverage by annuals for 16 control plots in Yucca Flat ranged from 12 to 47%. In vegetation not subject to nuclear disturbances, *Chaenactis stevioides* was the dominant annual species, followed by *Mentzelia* with a 3-11% cover. The spring annuals in the vicinity of ground zeros were all native or naturalized components of the indigenous vegetation patterns. Except at ground-zero 1, the total vegetative cover for all target sites decreased toward the 1.0-mile perimeter. At this distance the species composition approached that of areas entirely removed from atomic testing.

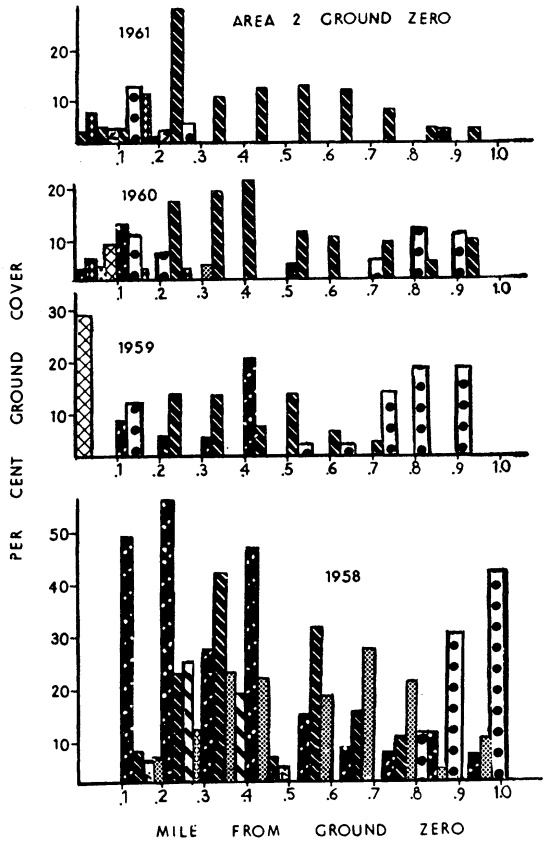
As spring annuals at target sites completed their life cycles and died in late May and early June, a summer-maturing species, *Salsola kali,* was developing into a widely-spaced stand in the area within 0.1 to 0.3 mile of four ground zeros. By September these plants had matured to a size of 1-2 m in diameter. The widely-spaced stand and its appearance within the radius of greatest soil removal indicate that *Salsola* is an invader. In the balloon test areas, subjected to repeated disturbance during each test season, recovery vegetation in the first year following detonations and in subsequent years (1958-61) was limited to the development of *Salsola* stands during the summer months.

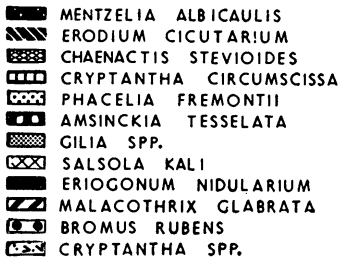
Second-, third- and fourth-year recovery patterns.—The high coverage by spring annuals in 1958 was associated with a favorable growing season. A decrease in the second-, third- and fourth-year cover at target areas (Figs. 5, 6, 7, 1959-1961 values) must be attributed largely to climatic factors. In 1959 dimensions of all annual species were greatly reduced. At four tower ground zeros the total spring annual cover amounted to one-seventh to one-third that of 1958, and the number of species encountered decreased to one-third to one-half that of the previous year. Though the 1960 and 1961 growing seasons were also dry, the percentage ground cover was higher (Figs. 5, 6, 7); but, as in the control vegetation, it more nearly approached the 1959 than the 1958 values. During the second year of recovery (1959), the *Mentzelia* stand narrowed centripetally by approximately half, and *Salsola* within 0.5 mile of ground zeros formed a dense cover of smaller, sparsely-branched plants which did not achieve the tumbling habit.

In 1959 *Mentzelia* tended to be replaced by *Erodium* at one target area and by *Chaenactis* spp elsewhere. *Mentzelia* in 1960 was again the









FIGS 5-7. Percentage cover by more abundant species at approximately 0.1-mile intervals in one direction from three tower ground zeros. Data obtained in late April or early May of 1958 (first year of recovery) and in three subsequent less favorable growing seasons (1959 through 1961). Fig. 5, upper left, west of ground-zero 1; Fig. 6, upper right, east of ground-zero 4; Fig. 7, lower left, west of ground-zero 2; Key to figures 5, 6, and 7, lower right.

dominant annual between 0.3 and 0.6 mile from ground zeros. In 1961, however, cover by *Mentzelia* was less than that by *Chaenactis* or *Bromus*. *Salsola* was represented by only scattering individual plants on all target areas in 1961. During the 4-year period since the last nuclear test, *Chaenactis* and *Bromus* have invaded progressively in the direction of ground zeros (Figs. 5, 6, 7). In May 1961, stands of *Bromus* in low places in area 4 resembled the grassland scars of natural burns in the *Coleogyne-Grayia* vegetation type.

Patterns of invasion by three annual species.— Of the many diverse patterns of invasion evidenced by different species at ground-zero areas, a few may be chosen to illustrate certain of the trends recorded. Fig. 8 plots percentage cover by *Amsinckia tessellata* at 0.1-mile intervals from ground-zero 4 during 1958-61. This species occurred in greatest numbers in a restricted portion of the transect (viz. 0.4 mile from ground zero) in the first growing season (1958) after the last detonation at this site. During subsequent drier years this pattern remained constant, with a general decrease in density of cover. In contrast, *Chaenactis stevioides* (Fig. 9) showed a 1958 peak at 0.6 mile from the same ground zero followed by a centripetal invasion with a major peak as near as 0.3 mile in 1961. The pronounced trough at 0.4 mile, which marks the peak occurrence for *Amsinckia,* may have some significance. Fig. 10 shows the distinctive invasion pattern for the exotic *Bromus rubens* at ground-zero 2, where it is abundant. In 1958 *Bromus* was present in small numbers in the central portion, but at a distance of 1.0 mile from ground zero this species constituted the bulk of the cover. During subsequent years, it abruptly invaded the heavily disturbed central area, with a peak at 0.2 mile, while progressively declining in numbers at the 0.8-1.0 mile distance. The well-marked trough at 0.4-0.7 mile is a byproduct of this invasion pattern.

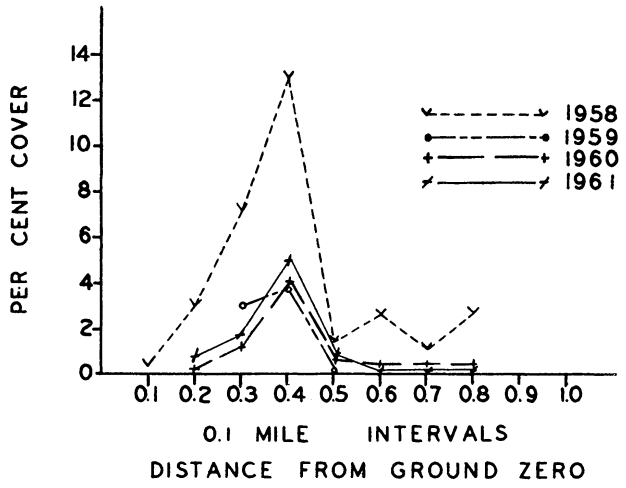


FIG. 8. Coverage by *Amsinckia tessellata* at ground-zero 4, 1958-1961.

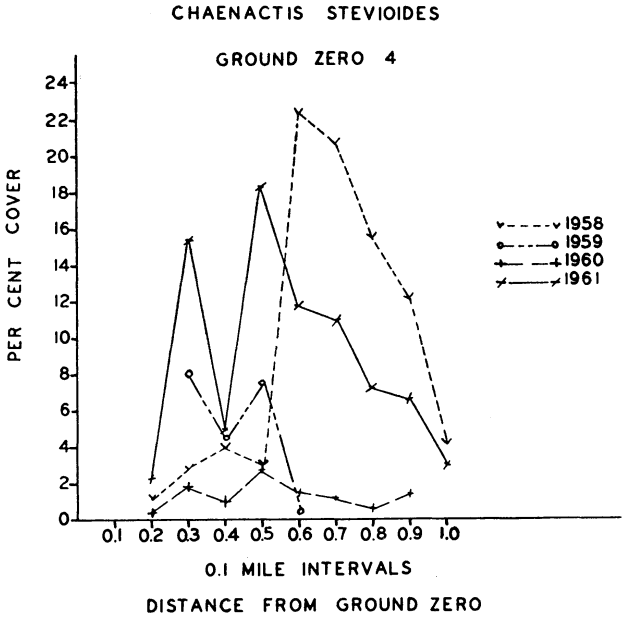


FIG. 9. Coverage by *Chaenactis steviodes* at ground-zero 4, 1958-61.

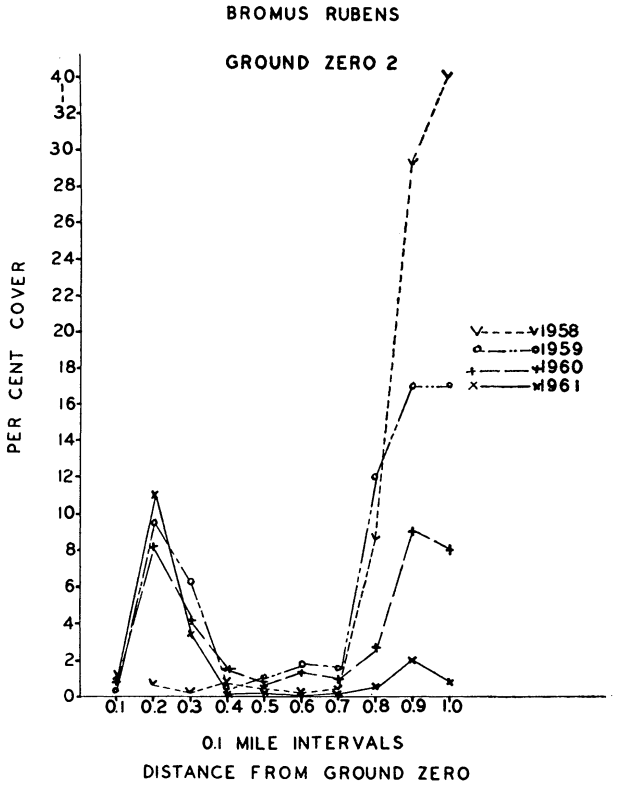


FIG. 10. Coverage by *Bromus rubens* at ground-zero 2, 1958-1961.

Recovery by annual and perennial species on plots denuded by blading

In general, similar annual and perennial species constituted the initial vegetation on comparable habitat types bared mechanically by blading and by nuclear detonations. *Mentzelia* and *Salsola* were prominent on bladed plots while *Chaenactis* was more prevalent along lines measured on the adjacent unbladed area. As at tower ground zeros, coverage values as well as the number of species encountered were higher on bared areas than in the adjoining shrub cover.

Recolonization, as expressed by percentage of annual cover, proceeded much more slowly on bladed areas because of the compact nature of the substrate and removal of the seed-containing layer throughout. Recovery of perennials, on the other hand, was more rapid than at ground zeros because underground parts of a number of shrubs and grasses survived the blading and sprouted the following spring. As at ground zeros (Shields and Wells 1962), recovery on bladed plots after 4 years is evident in the crown sprouting of several shrub species (*Larrea divaricata, Menodora spinescens*), in the appearance of weedy perennials (*Hymenoclea salsola, Sphaeralcea ambigua*), and in the prominence of bunchgrasses (*Stipa speciosa, Oryzopsis hymenoides*).

Discussion

Palumbo (1962) observed that, following the 1954 Nectar detonation, fallout at the Eniwetok atoll produced local effects in vegetation on Belle Island. Essentially all of the damage to land plants, however, could be attributed to heat and blast rather than to ionizing radiation. The initial gamma dose of 30 r (400 r total accumulated dose 200 days post detonation) delivered at Belle Island was only a fraction of that received by perennials at the 0.6-mile perimeter from tower shot sites at the Nevada Proving Ground.

In experimental acute exposures from a mobile Co-60 unit at the Nevada Test Site, a total dosage of 10,000 r at the source produced no visible gross effects on vegetative or reproductive structures of a *Larrea divaricata* shrub or on *Stanleya pinnata* plants (Brandenburg et al. 1963). Following exposure the *Larrea* shrub produced an abundance of viable seeds, as did shrubs recovering from integrated gamma dosages of about 14,000 r at 0.6 mile from ground-zero 1. An acute Co-60 exposure of a young mature pine, *Pinus monophylla,* however, killed actively growing regions receiving 500 to 600 r. Lower dosages inhibited growth, and approximately one-fourth of the needles formed on apices exposed to 50-100 r showed a reduction of vascular tissue or developed a double vascular strand (Brandenburg et al. 1963). Dose rates far below those which cause severe growth inhibition or lethal effects will visibly reduce seed production and viability (Sparrow and Woodwell 1963). Radiosensitivity varies among species of higher plants at least 500-fold (Sparrow and Miksche 1961, Sparrow and Evans 1961), many having an LD_{50} of less than 600 r when exposed to x-rays or gamma rays for a period not exceeding a few days (Sparrow and Schairer 1963). A highly sensitive plant, *Pinus strobus,* and a highly resistant plant, *Gladiolus,* show severe growth inhibition at about 10 and 5000 r per day, respectively (Sparrow and Woodwell 1963). Plants severely damaged by radiation, however, can apparently undergo complete recovery with respect to general appearance and vigor.

The composition of the recovery vegetation on target sites is determined primarily by the survival of nuclear effects by seeds and underground propagative structures and by the reproductive capabilities of surviving plants in the vicinity. At four of five tower ground zeros, situated in areas dissimilar in vegetation type, edaphic conditions, and topography, the numerically significant common denominator among spring-maturing annuals in the first year of recovery was the *Mentzelia* stand extending from the 0.1- to 0.3-mile perimeter of extensive soil disturbance out to 0.6 to 0.9 mile. Early invasion of bladed plots by *Mentzelia* establishes a weedy character for this species compared to *Chaenactis* spp. The prevalence of *Mentzelia,* however, within 0.6 mile of target areas in the initial recovery patterns, its greatly decreased coverage in the three subsequent dry growing seasons, and the extension of this species farther inward in places less subject to soil removal suggest that *Mentzelia* seeds may have survived in the soil surrounding the 1957 tower ground zeros. Ionizing or thermal radiation may have exercised a selective effect on relatively unshielded seeds which at 0.3 mile were subjected to estimated 50,000 r (11-kiloton) to 200,000 r (43-kiloton) levels of acute gamma radiation during different individual shots and to 131 cal/cm^2 (11-kiloton) to 512 cal/cm^2 (43-kiloton) thermal exposures. Radiation may exert a stimulatory as well as an inhibitory effect upon seeds (Platt 1963).

In the desert, where small differences in the amount of moisture are of great significance, the numerical relations among species are not constant from year to year or necessarily correlated with the seed supply (Tevis 1958b). Of four Death Valley soils tested, all contained more seeds than were observed to germinate under any combination of moisture and temperature levels. If the

ratios among different kinds of seedlings are determined in part by fluctuation in temperature, a species which germinates abundantly in 1 year may be relatively scarce in another when winter rain is followed by warmer or colder weather (Tevis 1958a).

The cycle in the development and decline of *Salsola* stands on the innermost portion of ground zeros over a 4-year period coincides with that observed on abandoned fields (Piemeisel 1938). Seed dissemination by the tumbling habit, known to advance *Salsola* 5 to 10 miles in a single season (U.S. Dept. of Agriculture 1893), gives this species an initial advantage in seeding the denuded target areas, averaging 1.0 mile in diameter. *Salsola* became best established in the central zone of greatest soil disturbance where the widely-spaced plants in the first-year stand attained a large size.

The encroachment of *Chaenactis* spp. and *Bromus rubens* toward ground zero, apparent in 1959, was marked by 1961 (Figs. 6, 7). The increasing cover by *Bromus* in the innermost zone of complete denudation, the recovery of crown-sprouting shrubs about the perimeter, and the encroachment of bunchgrasses and weedy perennials provide the basis for a short-term prediction as to the future composition of the perennial vegetation at target areas within the zone now dominated by annual species. *Atriplex canescens* and *Hymenoclea salsola* probably will be important in the shrub stratum. Stands of *Oryzopsis* and *Stipa* bunchgrasses will assume prominence, especially in the near future. Ultimately, a gradual invasion of the dominant shrub species of the surrounding terminal vegetation should follow (*Grayia spinosa-Lycium andersonii, Coleogyne ramosissima* or *Larrea divaricata*). In how long a time can the original climax vegetation be expected? From evidence provided by older disturbances in this vicinity, including the streets of a ghost town 33 years old (Wells 1961), invasion by most woody species is slow and is preceded by bunchgrasses.

Summary

The typical (1957) nuclear detonation at the Nevada Test Site, an airburst of a 20-kiloton yield, denuded a concentric zone of desert shrub vegetation ca. 0.5 mile in radius. Selective shock and blast damage to perennials extended asymmetrically to beyond 1 mile in certain cases. During the first recovery year the area within 0.1 to 0.3 mile of ground zeros remained essentially barren until *Salsola kali* formed a widely spaced summer stand. Cover by spring annuals between 0.4 and 0.8 mile from different tower detonation points exceeded total cover in the control vegetation. One species, *Mentzelia albicaulis*, contributed the greater part of the cover within 1.0 mile of five tower sites. In the surrounding vegetation *Chaenactis stevioides* was the predominant annual. A marked decrease in the second-, third- and fourth-year cover at target areas was associated with a less favorable climatic regime. *Chaenactis* spp. and *Bromus rubens,* however, have invaded progressively in the direction of ground zeros. Marginal to the denuded areas, certain perennials are recovering by crown sprouting, and weedy shrubs are appearing. More subtle radiation effects may possibly have existed undetected or have been lost. Gross damage to vegetation beyond the perimeter of complete denudation, however, appears to be attributable to mechanical and thermal injury.

Literature Cited

Brandenburg, M. K., H. L. Mills, W. H. Rickard, and **L. M. Shields.** 1963. Effects of gamma radiation on growth and morphology in *Pinus monophylla* Torr. and Frem. (pinyon pine). Radiation Botany **2**:251-263.

Daubenmire, R. 1959. A canopy-coverage method of vegetation analyses. Northwest Science **33**:43-64.

Glasstone, S. (Ed.). 1962. The effects of nuclear weapons. U. S. Govt. Printing Office, Washington. 661 p.

Munz, Philip A., and **D. D. Keck.** 1959. A California flora. Univ. of California Press, Berkeley and Los Angeles. 1550 p.

Palumbo, R. F. 1962. Recovery of the land plants at Eniwetok Atoll following a nuclear detonation. Radiation Botany **1**:182-189.

Piemeisel, R. L. 1938. Changes in weedy plant cover on cleared sagebrush land and their probable causes. U.S. Dept. Agr. Tech. Bull. **654**, 44 p.

Platt, Robert B. 1962. Radiation and plant life. Discovery, November.

Shields, L. M., and **P. V. Wells.** 1962. Effects of nuclear testing on desert vegetation. Science **135**: 38-40.

Sparrow, A. H., and H. J. Evans. 1961. Nuclear factors affecting radiosensitivity. I. The influence of nuclear size and structure, chromosome complement and DNA content. Brookhaven Symposia in Biology **14**:76-100.

Sparrow, A. H., and **J. P. Miksche.** 1961. Correlation of nuclear volume and DNA content with the tolerance of plants to chronic radiation exposures. Science **134**:282.

Sparrow, A. H., and **L. A. Schairer.** 1963. The tolerance of 200 species of higher plants to chronic exposure of gamma radiation from Co^{60}. Radiation Botany (in press).

Sparrow, A. H., and **G. M. Woodwell.** 1963. Prediction of the sensitivity of plants to chronic gamma irradiation. p. 257-270. *In Radioecology.* Vincent Schultz and Alfred W. Klement, Jr., Editors. Reinhold, New York.

Tevis, L., Jr. 1958a. Germination and growth of ephemerals induced by sprinkling a sandy desert. Ecology **39**:681-688.

———. 1958b. A population of desert ephemerals germinated by less than one inch of rain. Ecology **39**: 688-695.

U.S. Dept. of Agriculture. 1893. The Russian thistle and other troublesome weeds in the wheat region of Minnesota and North and South Dakota. Farmer's Bull. **10**, 16 p.

Wells, P. V. 1961. Succession in desert vegetation on streets of a Nevada ghost town. Science **134**:670.

A BACTERIOLOGICAL STUDY OF AN ARCTIC COASTAL LAKE[1]

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INTRODUCTION

Many lakes of Northern Alaska have been found to support the growth of various forms of life. The limnological work has been largely restricted to the Point Barrow area with the Arctic Research Laboratory as the base of operations. Lake Imikpuk, which is adjacent to the Laboratory, has been shown to contain a population of copepods (Comita 1956), species of *Daphnia* (Edmondson 1955), and several species of algae (Prescott 1953). A lake a few miles to the south (Ikroavik) at one time supported a population of sticklebacks (Wohlschlag 1953), and other lakes west of Point Barrow have been shown to contain black fish (Ostdiek and Nardone 1959). The presence of plants and higher animals in lakes of this arctic region is indirect evidence for including bacteria in the aquatic microflora which is important in the metabolic cycling of different compounds and in the maintenance of the various natural food cycles.

The saprophytic bacteria of lakes in the North American Arctic have not been studied extensively. McBee and McBee (1956) reported on the incidence of coliform and thermophilic bacteria in a number of different materials including water from some of the lakes of the Point Barrow area. Most of the work on the aquatic bacterial population has been concerned primarily with demonstrating the presence of certain pathogenic groups, especially the gram-negative enteric pathogens responsible for typhoid fever, paratyphoid fever, and bacillary dysentery, which are endemic to this area. Pauls (1953) reviewed the incidents of enteric diseases in Alaska and reported that epidemics of bacillary dysentery at Barrow Village in 1948 and at Anaktuvik Pass in 1949 were the results of contaminated water supplies.

[1] These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the Arctic Institute of North America. Reproduction in whole or in part is permitted for any purpose of the United States Government.

This 20-month study gives the seasonal changes in the number of bacteria in Imikpuk Lake and a partial analysis of the species of this lake and other lakes along the Arctic Coast. Distribution of bacteria in soil and animal and bird feces was also investigated. Data on seasonal changes in the chemical composition of this lake have been published previously (Boyd 1959).

METHODS

Water samples were collected at a depth of 8 feet near the center of the lake or at the runoff stations shown in Fig. 1, employing the techniques described by Boyd (1959). The presence of coliform bacteria was determined on five 10-ml por-

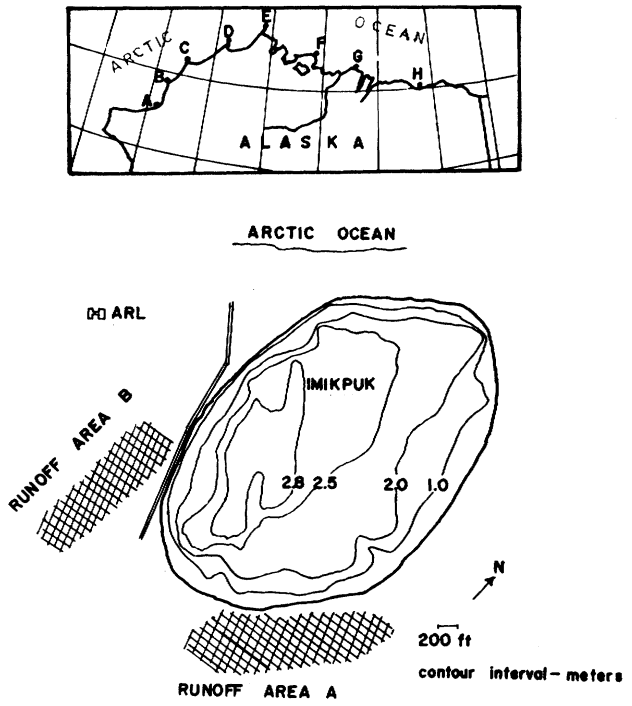

FIG. 1. Sketch map of Imikpuk Lake including the major runoff areas (after Comita 1953). Inset, map of the Alaskan Arctic Coastal Plain. A. Cape Beaufort; B. Point Lay; C. Wainwright; D. Point Franklin; E. Point Barrow; F. Cape Simpson; G. Oliktok Point; H. Barter Island.

suggest that the Cuterebrid botflies and small mammals have established a fairly stable relationship and the parasites do not greatly reduce their host populations.

Acknowledgments

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Literature Cited

Buckner, C. H. 1958. Cuterebrids (*Cuterebra grisea* Coq.) attacking small mammals. Bi-monthly Progress Report. Forest Biology Div. Can. Dept. Agr. **14**: 2-3.

Clough, G. C. 1965. Viability of wild meadow voles. Ecology **46**: 119-134.

Haas, G. E. and R. J. Dicke. 1958. On *Cuterebra horripilum* Clark (Diptera: Cuterebridae) parasitizing cottontail rabbits in Wisconsin. J. Parasitol. **44**: 527-540.

Sealander, J. A. 1961. Hematological values in deer mice in relation to botfly infection. J. Mammal. **42**: 57-60.

Test, F. H. and A. R. Test. 1943. Incidence of dipteran parasitosis in populations of small mammals. J. Mammal. **24**: 506-508.

Wecker, S. C. 1962. The effects of bot fly parasitism on a local population of the white-footed mouse. Ecology **43**: 561-565.

GROWTH RESPONSE OF TWO SEDGES INHABITING A RADIOACTIVE WASTE DISPOSAL AREA[1]

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Abstract. An observed increase in the size of the inflorescences of two sedges inhabiting a radioactive waste disposal area is correlated with the complex interaction between various edaphic factors that contribute to plant growth and an "effect" of ionizing radiation. The perennial sedges, *Carex frankii* and *Carex vulpinoidea,* tolerate a continuous dose rate generally in excess of 2 R/day at ground level. Although radiation has been associated with excessive plant growth, it is not entirely independent of other factors, such as the combined contribution of potassium and organic matter, soil pH, calcium, as well as other unmeasured edaphic conditions.

Introduction

Ionizing radiation in some of our environments is increasing both from weapons' fallout and from waste disposal. However, radiation-induced changes in natural populations living in contaminated environments have been reported only infrequently, although in designed experiments the effects of radiation have been demonstrated using various types of radiation, species of organisms, and environmental conditions. On White Oak Lake bed, an area contaminated with radioactive wastes, sedges (*Carex frankii* Kunth and *C. vulpinoidea* Michx.) were found to have longer inflorescences than sedges growing on nearby uncontaminated sites (Plummer 1960). Radiation dose rates approached 40 milliroentgen/hour, which is well below dose rates usually employed in experimental work. Since there were no control areas comparable in site quality to White Oak Lake bed, further sampling was conducted in which various soil factors were measured as well as sedge inflorescences. Analyses and interpretation of the results are presented in this paper.

White Oak Lake bed is a unique ecological system of about 48 acres, which once served as a final holding basin in the Oak Ridge National Laboratory's low-level radioactive waste disposal system. In 1955 the lake was drained, and during the spring season that followed plants and animals invaded the radioactive sediments. Soils contained Sr^{90}, Cs^{137}, Co^{60}, Ru^{106}, and other radioisotopes. History and characteristics of developing soils have been published elsewhere (Shanks and DeSelm 1963).

Materials and Methods

Mature culms of both species of *Carex* were collected from three areas on White Oak Lake bed and from four noncontaminated areas in the Oak Ridge vicinity during mid-June 1960. In each locality plants were collected from both "wet" and "dry" habitats, a wet habitat being defined as the one with the highest water table in each study area. The wettest places occurred at the lower end of the lake bed where the soil pH ranged from 8.0 to 8.4; the driest site was on the upper lake bed with a pH of 7.0 to 7.4. The other sites were on the Oak Ridge Atomic Energy Commission Reservation but were not radioactive; the soils generally were acid (pH 5.0 to 6.5) but the plant communities appeared to be similar to those on White Oak Lake bed. The lengths of each of 350 flowering heads were measured as an index of plant response to the environmental factors. Soil samples taken at each site were analyzed for salt pH and conductivity, calcium, phosphorus, potassium, and total organic matter (Graham 1959).

Results

Analyses of the inflorescence lengths confirmed the results of a previous study (Plummer 1960) that flowering heads were longer on sedges growing on White Oak

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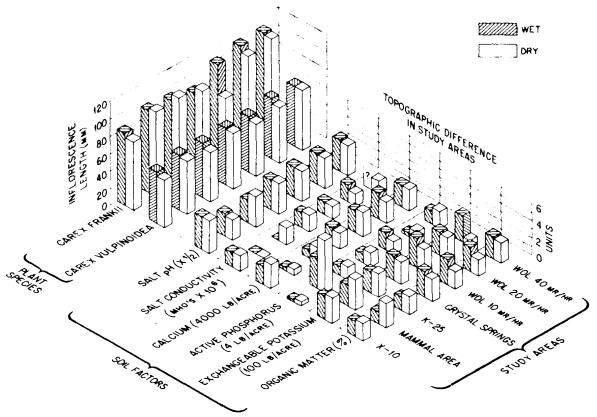

FIG. 1. Inflorescence lengths and soil factors in *Carex* communities. WOL 40, 20, and 10 mR/hr are study areas on White Oak Lake bed; Crystal Springs, K-25, mammal area, and X-10 are control study areas.

Lake bed than on those from noncontaminated sites. Fig. 1 shows the site-to-site variation in inflorescence lengths for both sedge species on wet and dry sites, as well as variation in soil factors. The longer inflorescences from sedges growing on White Oak Lake bed were significantly correlated ($P = 0.05$) with the air dose rates (10, 20, and 40 mR/hr) of radiation. The locations of the three lake bed sites, and the appearances of the inflorescences for the two sedge species, are shown in Fig. 2.

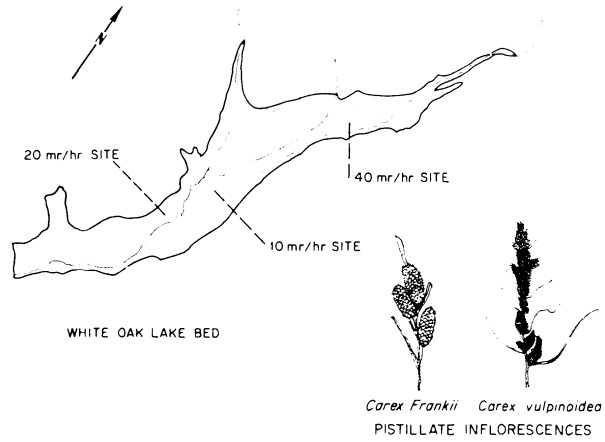

FIG. 2. Locations of *Carex* communities on White Oak Lake bed, and sketches of inflorescences.

The contribution of the soil factors to inflorescence length and their relation to radiation dose rate could not be evaluated with the usual regression analyses and tests of significance. The data consisted of multiple observations on seven distinct points (for the seven sites) in the six-dimensional factor space of pH, calcium, active phosphorus, exchangeable potassium, organic matter, and radiation dose rate. Our purpose in examining these soil factors was not to discover whether they were significantly correlated with inflorescence lengths or with each other, but to examine the possibility that the soil factors, singly or together, could account for the observed longer inflorescences on the lake bed sedges. As a first approximation to a function relating these factors to inflorescence length we considered the linear function

$$Y = \alpha + \sum_{i=1}^{k} \beta_i X_i$$

in which Y represents inflorescence length, α is a constant, β_i is a regression coefficient for the ith factor X_i is an amount of the ith factor, and $k = 0, 1, 2, 3, 4, 5,$ *or* 6. There are 2^6, or 64, possible models of this type but in one of them ($k = 6$) the model would pass through the seven averages and leave no means for assessing the validity of the model. We examined the remaining 63 models with a computer program, which selected the best model with one variable only, the best with two variables, and so forth. Measurements from "wet" habitats for both *Carex frankii* and *C. vulpinoidea* were used.

Although this may not be the place to discuss the advantages and disadvantages of an alternative technique known as stepwise regression, a few remarks about stepwise regression may be in order. It is not a very well known fact that stepwise regression will not necessarily choose a group of k variables which give a smaller residual sum of squares than any other group of variables. Hamaker (1962), however, demonstrates this quite clearly. That is, if three factors, A, B, and C, are candidates for a two-term regression model, it is possible that stepwise regression would choose the A-B pair, even though the A-C pair or the B-C pair would do a better job. The only sure way to choose the best k factors is to examine all possible combinations of k factors as we have done.

Tables I and II summarize the results of one- to five-term models derived for inflorescence length in *Carex frankii* and *C. vulpinoidea*, respectively. In both cases radiation dose rate gave the best single-term model. For *C. vulpinoidea* it appears that the best general model is

$$Y = 63.55 + 0.2091\,R$$

TABLE I. Summary of models relating soil factors and radiation dose rate to inflorescence length in *Carex frankii* (R = radiation dose rate, OM = organic matter)

	Number of terms in model				
	1	2	3	4	5
Variables	R*	R*	R*	R*	R*
	—	K	pH*	pH*	pH*
	—	—	Ca*	Ca*	Ca*
	—	—	—	K	K*
	—	—	—	—	OM
Error mean square	833.63	833.63	833.63	833.63	833.63
Lack of fit mean square	2,059.6	2,085.7	1,260.1	928.4	489.1
Lack of fit significant	Yes	No	No	No	No

*Significant coefficient.

TABLE II. Summary of models relating soil factors and radiation dose rate in inflorescence length in *Carex vulpinoidea* (R = radiation dose rate, OM = organic matter)

	Number of terms in model				
	1	2	3	4	5
Variables	R*	R*	R*	R*	R*
	—	OM	OM	OM*	OM*
	—	—	K*	K*	K*
	—	—	—	pH	pH
	—	—	—	—	Ca
Error mean square	135.62	135.62	135.62	135.62	135.62
Lack of fit mean square	212.60	196.98	111.14	4.57	0.00
Lack of fit significant	No	No	No	Yes**	Yes**

*Significant coefficient.
**Significantly *less* than expectation.

where Y = inflorescence length (mm) and R = radiation dose rate (mR/hr). The standard error for the coefficient of R is 0.04414 (343 degrees of freedom).

For *C. frankii* the data suggest that the best general model would be the three-term model:

$$Y = 157.83 + 0.5827\,R - 14.87\,\text{pH} + 0.4362\,\text{Ca}$$

where pH represents hydrogen-ion concentration in soil and Ca represents exchangeable calcium (lb/acre). The standard errors for the coefficients carry 343 degrees of freedom and are 0.1344, 5.36, and 0.1607 for the coefficients of R, pH, and Ca, respectively. There was an indication of a potassium contribution in the two- and the four-term models, but the contribution did not become significant until potassium and organic matter were considered jointly. No such conflicting effects arose with *Carex vulpinoidea*.

Discussion

The air dose rate of gamma radiation measured at 1 m above ground surface provided the best correlation with inflorescence length for the two sedge species. Although soil factors considered here were found to affect inflorescence length, those influences were far from adequate to explain the increase in length in the radioactive areas. The variable R in our models doubtless includes many more factors than radiation, however, and it still remains likely that other soil characteristics—nitrates and sulfates in particular—associated with inorganic constitutents of industrial wastes, influence the lengths of inflorescences.

The absorbed radiation dose (in rads) delivered to the plants is doubtless greater than the air dose measurements (in mR) would estimate. Beta radiation dose to underground parts and from internally deposited radioisotopes would have to be considered. Kaye (1965) estimated that the exposure dose rate 3 cm below soil surface is approximately five times the exposure dose rate above the soil surface, so that the underground parts would have received a greater dose than did the aerial parts.

Even at minimal estimates, the dose rates on White Oak Lake bed are sufficient to induce both genetic and physiological disturbances in plants. Chromosome aberrations have been noted in significant numbers at dose rates below 1R/day although few plants show morphological changes at less than 14 R/day (Sparrow and Singleton 1953), one exception being pine trees which may be affected by 2 R/day delivered during the growing season (Woodwell 1962). Stimulation of plant growth with ionizing radiation has been demonstrated experimentally for both chronic and acute exposures (McCormick and Platt 1962, Sax 1963).

Considerable attention has been given recently to relative radiosensitivity among plants as related to chromosome numbers and nuclear volumes, and to the prediction of plant radiosensitivity (Sparrow, Schairer, and Sparrow 1963). For the most part, radiosensitivity is expressed in terms of lethal exposure doses, so that at any other exposure dose it is difficult to know what to expect from *Carex* spp. in the way of morphological variation. No morphological distortions were seen in the plants on White Oak Lake bed; except perhaps for being larger and greener they seemed identical with controls. Gunckel and Sparrow (1961) list no less than 75 known chemical and physical factors affecting the tolerance or radiosensitivity of plants.

An experimental approach (in both laboratory and field) would seem to be needed to resolve whether the increase in lengths of inflorescences for the *Carex* spp. is a direct response to irradiation, an indirect response, or is associated with some purely chemical aspect of industrial waste disposal. In any case a radiation response in natural environments is not likely to be entirely independent of other factors.

Literature Cited

Graham, E. R. 1959. Soil testing. Univ. Missouri Agri. Exp. Sta. Bull. 734, 20 p.

Gunckel, J. E., and A. H. Sparrow. 1961. Ionizing radiations: biochemical, physiological and morphological aspects of their effects on plants. Encyclopedia of Plant Physiology, **16**: 555-611.

Kaye, S. V. 1965. Use of miniature glass rod dosimeters in radiation ecology. Ecology **46**: 201-205.

Hamaker, H. C. 1962. On multiple regression analysis. Statistica Neerlandica **16**: 31-56.

McCormick, J. F., and R. B. Platt. 1962. Effects of ionizing radiation on a natural plant community. Radiation Botany 2(3/4): 161-188.

Plummer, G. L. 1960. Biometric analysis of a growth response of two plant species in a radioactive waste disposal area. U. S. Atomic Energy Comm. Rept. ORNL-2903 (Oak Ridge National Laboratory). Office of Tech. Serv., Washington, D. C.

Sax, Karl. 1963. The stimulation of plant growth by ionizing radiation. Radiation Botany 3(3): 179-188.

Shanks, R. E., and H. R. DeSelm. 1963. Factors related to concentration of radiocesium in plants growing on a radioactive waste disposal area, p. 97-101. *In* V. Schultz and A. W. Klement, Jr., [ed.] Radioecology, Reinhold Publ. Co., New York, and AIBS, Washington.

Sparrow, A. H., and W. R. Singleton. 1953. The use of radiocobalt as a source of gamma rays and some effects of chronic irradiation on growing plants. Amer. Naturalist **87**: 29-48.

Sparrow, A. H., L. A. Schairer, and R. C. Sparrow. 1963. Relationship between nuclear volumes, chromosome numbers and relative radiosensitivities. Science **141**: 163-166.

Woodwell, G. M. 1962. Effects of ionizing radiation on terrestrial ecosystems. Science 138: 572-577.

Induced Gene and Chromosome Mutants
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Induced gene and chromosome mutants

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Plant scientists, including breeders, can use an arsenal of physical and chemical mutagens and appropriate selection techniques to 'manufacture' in their experimental plots gene and chromosome mutants to compensate for the erosion of natural sources of genetic variability. They also have the capability of generating in this type of genetic manipulation the entire array of genetic variation inherent in all loci controlling each plant trait, and thus in a relatively short time producing most, if not all, of the genetic variants that have ever occurred in the evolution of a given agricultural plant.

This capability is required not only for the breeder concerned with developing new cultivars to meet the numerous and varied demands of the modern farmer, processer and consumer, but also for the geneticist, physiologist, anatomist and biochemist concerned with unravelling important plant processes and their genetic control. In short, these scientists need inexhaustible supplies of genetic variability, often never before selected in Nature or by earlier plant breeders.

Numerous experiments demonstrate that induced mutants have considerably extended the genetic variability of a phenotype. An outstanding example is *eceriferum* ('waxless' plant surfaces) in barley. Spontaneous mutations produced several well known variants controlled by about six loci. Genetic analyses of over 1300 induced and the few spontaneous mutants have determined that this trait is controlled by at least 77 loci (Lundqvist 1976, and personal communication). There are numerous alleles at some of these loci. Other examples are described in this paper.

The quantity and quality of artificially induced genetic variability in plants is in no small part due to the contributions of improved mutagens, mutagen treatments and selection techniques. A new potent and unique mutagen, sodium azide, is particularly successful in inducing putative point mutations. Recent experiments with barley and *Salmonella* have revealed that it is not azide *per se* but an activated metabolite that is the mutagenic agent. The metabolite has been isolated and crystallized and can now be synthesized *in vitro*. These findings usher in a new category of mutagens and suggest new avenues for understanding the interaction of mutagens with chromosomes and genes and for greater control of the induction of genetic variability in plants.

The considerable success of varietal development through induced mutants is well documented: 465 culvitars of sexually and vegetatively reproducing crops have been released that owe some of their production advantage to an induced gene or chromosome mutant. These cultivars have led to considerable economic impact in a number of countries.

In breeding research, induced mutants are indispensable for probing and elucidating the pathway and genetic control of important plant processes such as wax synthesis and deposition (von Wettstein-Knowles 1979), nitrogen assimilation (Kleinhofs *et al.* 1980), photorespiration and different facets of photosynthesis (Somerville & Ogren 1980; Miles *et al.* 1979; Simpson & von Wettstein 1980).

In the manipulation of plant genes (genetic engineering) in breeding research, it becomes increasingly necessary to pinpoint these genes on chromosomes. For this endeavour, an abundant array of induced chromosome mutants such as trisomics, telotrisomics, acrocentrics, inversions, translocations and deletions is required. This important activity can now be complemented by ever-improving chromosome banding techniques.

Introduction

Genetic variability, as derived to a greater extent from mutations comprising extragenic and possibly intragenic events (Nilan & Vig 1976; Brock 1980), and to a lesser extent from chromosome mutations (changes in chromosome number and structure), is the raw material of plant improvement. Until recently, genetic variability was secured by the plant breeder entirely from 'Nature's improvement programme' (rarely arising spontaneous mutations, subsequent recombination and natural selection) through collections of crop germ plasm, and wild species and even genera. In Nature, the genetic variants are end-products of thousands of years of evolution and were selected primarily for survival and reproductive capability.

Presumably during evolution, myriads of variants resulted from the variability potential of every locus and every chromosome break and many of the variants now needed by the plant breeder probably occurred. However, most were discarded since they were of little value for the plant's survival and fecundity. Evolutionary processes were not concerned with preserving the numerous traits that are now required from plants by the modern farmer for higher yield and adaptation to advanced farming practices and to new environmental niches; by the modern processor of food and fibre; and by the animal and human consumers who require an increased variety of foods with improved nutrition, palatability and attractiveness. These requirements are increasing while at the same time natural genetic variability in some crops is eroding.

The plant breeder needs now, and will do more so in the future, a broad array of genetic variation, possibly for every locus, whether it be a structural or a regulatory gene, for every plant process and phenotype. This is required most assuredly for the relatively few plants upon which we now base our production of food and fibre and for those plants that have the potential of broadening the food and fibre base and for fulfilling special needs. This genetic variation, plus all the conceivable changes in plant karyotypes that can be achieved through chromosome mutants, will eventually be required to reconstruct, so to speak, plants for man's needs. Obviously, the breeder cannot wait for new and usable spontaneous mutations.

Fortunately, plant geneticists and breeders, using an arsenal of physical and chemical mutagens and appropriate selection techniques, can now 'manufacture' gene and chromosome mutants to compensate somewhat for the depletion of natural sources of genetic variability. They also have the capability in this type of genetic manipulation of generating experimentally the entire array of genetic variation inherent in all loci controlling each plant trait, and thus in a relatively short time producing most, if not all, of the genetic variants that have ever occurred or may yet occur in the evolution of agricultural plants. The bases for the above concepts have been previously recounted (Brock 1980; Nilan *et al.* 1977).

This paper considers the current and future impact of induced gene and chromosome mutants on plant improvement. It also examines the impact of induced mutants on breeding research, especially towards providing new knowledge about the genetics, physiology, anatomy and biochemistry of cellular processes that produce all the traits so necessary for successful cultivars now and in the future.

Induced and spontaneous mutations

A breeder contemplating induced mutations might first ask, 'Do induced and spontaneous mutations and mutants differ?' and 'What is the value of induced mutants when they are so "raw" and have not been moulded by evolution and the various recombination and selection

processes that have developed spontaneous mutants into useful adapted complexes?' It has been amply demonstrated in a wide variety of organisms, including plants, that there are actually no major detectable differences between induced and spontaneous gene and chromosome mutations (Brock 1980; Nilan *et al.* 1977; Nilan & Vig 1976; Konzak *et al.* 1977). Of interest to the breeder is that the older spontaneous mutants have been moulded by recombination and natural selective forces into useful co-adaptive complexes. Newly arisen spontaneous mutants have not had time to be moulded into such complexes. However, the artificial acceleration of recombination plus refined and discrete selection techniques in the hands of the plant breeder can soon lead to useful trait complexes whether the origin of the trait is by induced or spontaneous mutation. Indeed, Brock (1980), on several pieces of evidence, questions the value of co-adapted complexes in plant improvement.

Induced 'new' variability

Another question often asked by plant scientists contemplating the use of induced mutations is, 'Can induced mutations produce "new" forms of traits that have not been observed among the spontaneous genetic variability?' The answer is that they can. Artificial mutagens can produce mutants that have not arisen in recent evolutionary history and thus have never been encountered by the breeder. However, they are probably not 'new' to a given plant because such variants may have occurred during its evolution.

In plants such as barley and peas, and to a lesser extent maize and wheat, that have been used extensively in basic and applied mutagenesis research, the extension of genetic variability by induced mutations and mutants is well documented. In these examples, induced mutants have uncovered hitherto unknown or 'new' loci controlling a phenotype and have revealed much about the potential variability (alleles) of many loci.

One of the most striking examples of the induction of 'new' variability and loci for a phenotype is represented by variants for the *eceriferum* ('wax-less' plant surface) phenotype of barley. Natural variability for this trait was confined to a few spontaneous mutants at six controlling loci. By using a wide variety of mutagens, 1302 mutants for this trait have been induced (Lundqvist 1976; Lundqvist *et al.* 1968; Lundqvist, personal communication). These numbers of independent gene changes differentially affect the wax composition of the leaf blade and sheath, spike and stem. They also lead to remarkable and distinct differences in fine structure and chemical composition of the surface wax molecules (von Wettstein-Knowles 1976, 1979). Appropriate genetic tests of the induced and spontaneous mutants have revealed at least 77 loci mapped to each arm of the seven barley chromosomes and numerous alleles, over 100, occurring at each of several loci. Similarly, in barley, induced chlorophyll-deficient mutants have revealed 600–700 loci controlling chlorophyll development (von Wettstein *et al.* 1974; Nilan & Velemínský 1981; Simpson & von Wettstein 1980) and 26 loci, some with numerous alleles, for the *erectoides* trait (Persson & Hagberg 1969). Moreover, genetic variability has been greatly broadened through induced mutation techniques for such phenotypes in barley as anthocyanin development (Jende-Strid 1978), nitrate reductase (Kleinhofs *et al.* 1980), mildew resistance (Jørgensen 1976), spike development (Gustafsson & Lundqvist 1980), and for lysine content (Doll *et al.* 1974). That the examples above are in barley testifies to the fact that among all of the crop plants, and indeed higher plants, there has been no other plant that has received so much investigation in the area of mutagenesis. In short, it has been the plant model of choice for experimental mutagenesis and mutation breeding. Examples of how induced

mutants have revealed 'new' loci in other plants have been given previously (Nilan *et al.* 1977; Konzak *et al.* 1977; Brock 1980).

Improved techniques

The increasing success of induced gene and chromosome mutants in breeding and breeding research can be attributed to improvements in mutation induction and selection techniques. These, along with relevant literature and descriptions of the most useful physical and chemical mutagens, recipes for their use on appropriate plant parts, e.g. seeds, buds, pollen, tissue and cells, and techniques for inducing and selecting mutants in sexually and vegetatively reproducing crops, are presented in the International Atomic Energy Agency's *Manual on mutation breeding* (1977).

There are numerous data and well documented technology that can lead to greater mutagen effectiveness (frequencies of mutations per dose of mutagen), efficiency (frequencies of desired events such as gene mutations in relation to such undesirable or unwanted events as sterility and, in some cases, chromosome aberrations), and specificity (group mutability (spectrum alteration), interlocus and non-random chromosome breakage) (Konzak *et al.* 1965; Nilan 1972; Brock 1980). With judicial selection of mutagens and manipulation of mutagen treatments, the breeder can influence the kind of genetic events that he may wish to induce as sources of genetic variability for his improvement programme. For instance, all of the physical mutagens such as X-rays, γ-rays and neutrons, as well as certain chemicals such as myleran, can induce high ratios of chromosome aberrations to mutations. There are other mutagens, e.g. ethyleneimine, that can induce about equal frequencies or proportions of both. Finally, there are mutagens such as ethyl methanesulphonate, diethylsulphate, sodium azide and certain base substitution and nitroso compounds that appear to induce higher proportions of mutations to chromosome aberrations (Konzak *et al.* 1977). As more knowledge is obtained about the mechanism, action and specificity of mutagens, and the nature of the mutations that they induce, the breeder will acquire even more precision for advantageously inducing and manipulating mutants in plant improvement.

At Pullman, 10 years of extensive basic research has developed a relatively new mutagen, sodium azide (Sideris *et al.* 1973; Nilan *et al.* 1973), which is one of the most potent available for higher plants. The research with this mutagen has been recently summarized by Kleinhofs *et al.* (1978*a*).

Azide is unique in that it induces in plants very high frequencies of gene mutations but is ineffective in producing major chromosomal changes. Experiments with bacteria indicate that azide is a base substitution mutagen, and in eukaryotes it appears to induce changes on the order of point mutations. Whether these mutations are small deletions or true base changes has not yet been resolved. We are attempting to answer this question by mapping numerous alleles induced by azide at the waxy pollen locus of barley (Rosichan *et al.* 1981; Nilan *et al.* 1981). Here the nature of the mutant alleles can be genetically resolved to near the base-pair level, since rare interallelic recombination events can be detected on a per million pollen basis. Preliminary results suggest that distances between alleles of about 50 base pairs can be detected, indicating that at least some mutational events do not involve large DNA deletions.

Recently, we have determined that it is not the inorganic azide *per se* but an organic metabolite synthesized in azide-treated barley and bacteria cells that is the mutagenic agent (Owais *et al.* 1978, 1979). This metabolite has been isolated, purified, crystallized and partly

characterized chemically (Owais *et al.* 1981 *b*) and its *in vitro* synthesis from cell-free extracts of *Salmonella typhimurium* has been accomplished (Owais *et al.* 1981 *a*). Furthermore, the pathway by which this metabolite is synthesized is being revealed (Owais *et al.* 1981 *c*; Cieśla *et al.* 1980).

Although activation of numerous chemicals to mutagenic metabolites is well known in mammalian mutagenesis, very little research in this area has been conducted in plants. Indeed, the azide metabolite is one of only three (atrazine (Plewa & Gentile 1976) and 1,2-dibromoethane (Scott *et al.* 1978) being the others) that have been detected, and the only one in plants that has been isolated and purified to crystal form and about which knowledge of its synthesis is becoming available. It is now suspected that additional chemicals may act the same way in plants.

This type of research is providing a greater insight about the interactions of mutagens with genes and chromosomes and the nature of induced genetic change. It is also providing the breeder with new knowledge and technology with which he can 'manufacture', with considerable deliberation, greater genetic variability.

Induced mutants for cultivar improvement

There is now overwhelming evidence that induced mutants have contributed most significantly to breeding new cultivars of crops (Sigurbjörnsson 1976; Gustafsson 1975; Sigurbjörnsson & Micke 1974; Broertjes & Van Harten 1978; A. Micke, personal communication). By September 1980, at least 224 cultivars of self and cross-pollinating crop species had been released for commercial production around the world (table 1). These cultivars, possessing at

Table 1. Released induced mutant cultivars
(September 1980.)

	number	
type of crop	direct	cross
cereals	74 (total)	57 (total)
bread wheat	12	5
durum wheat	5	7
rice	28	9
barley	25	33
oats	4	3
legumes	18	10
fruit trees	8	1
other crops	46	10
total crops	**146**	**78**
ornamentals	237	4
total	**383**	**82**

After Sigurbjörnsson & Micke (1974), Sigurbjörnsson (1976), and A. Micke (personal communication).

least one improved trait due to an induced mutation, include 131 cereals, 28 legumes, and 9 fruit trees. In addition, 241 new strains of vegetatively reproducing species, mostly ornamental have been released. Cultivars that owe their advantage to induced mutants have been developed in 37 countries and grown successfully on millions of hectares, and thus have had considerable economic impact in numerous countries. In some countries induced mutant cultivars have enjoyed most of the acreage devoted to a given crop species.

The techniques for utilizing induced mutant genes for both qualitatively and quantitatively inherited traits and chromosome mutants in breeding have been adequately described in numerous reviews (for instance Brock 1980; Gaul 1964; Nilan *et al.* 1965) and publications from the International Atomic Energy Agency, especially the *Manual on mutation breeding* (1977).

In sexually propagating species, induced mutants can be used in two principal ways: directly, or in crosses or hybridization. In the former, a mutant that exhibits at least one improved trait with no new undesirable traits as a result of the induced genetic changes is multiplied directly. Once the mutant has been sufficiently tested with positive results, then it can be released to growers as a new cultivar. Among the 224 crop cultivars developed through induced mutants, 78 have been developed by direct multiplication of the mutant line (table 1). One advantage of the method is the short time required for developing a new cultivar. An example is the breeding of the six-row winter barley 'Luther' at Washington State University. Only 6 years elapsed from the time of mutagen treatment of seeds of the parent cultivar 'Alpine' to release of the mutant cultivar to growers in the Pacific Northwest of the U.S.A.

The plant breeder also can effectively use induced mutants in crosses – a necessity when the desired improved trait is closely linked or associated with undesirable spontaneous or induced traits. Furthermore, even directly useful mutant cultivars have proved to be outstanding parents for cross-breeding. The latter is well illustrated by barley. Of the 58 barley cultivars released with an induced mutant in their backgrounds, 33 have resulted from crossing mutants with other varieties or lines (table 1). Six successful cultivars were developed from the Swedish mutant cultivar Mari (Gustafsson 1975). In our barley breeding programme, the mutant cultivar Luther has been the parent of one released cultivar in Washington and of several advanced selections pending release in the states of Oregon, Washington, and Idaho.

In vegetatively propagated species (ornamentals, including cut flowers, bulbs, trees and shrubs; fruits; potato; sweet potato; sugar cane; cassava), much plant improvement has been based on the selection of 'sports' or spontaneous mutants. Thus, induced mutants are an obvious supplementary source of genetic variability. According to Broertjes & Van Harten (1978), the main advantage of mutation induction in vegetatively propagated plants is the ability to change one or a few characteristics of an outstanding genotype or cultivar without altering the remaining phenotype. In such plants, selection and propagation of useful mutants is relatively easy and development of mutant cultivars quite rapid.

The decision to use induced mutants in breeding will depend on the available supply of natural variability (and for some crops this is rapidly being depleted), the potential for success, and the effort and cost, especially where the utility of induced mutants are compared with securing the needed variants from related species or genera. These aspects of mutation breeding have been thoroughly analysed and discussed by Brock (1971, 1980).

Induced mutants for breeding research

The molecular basis of the genetic, biochemical, physiological and anatomical processes leading to those traits that comprise a successful crop cultivar are little understood. Yet the plant breeder today, and especially in the future, must learn to control and manipulate these processes if new cultivars to meet the requirements of the farmer, processer and consumer are to be met. Some of the progress and problems in understanding these processes, which are in the realm of plant molecular biology, have been recently reviewed (Walbot 1980) and are described elsewhere in this symposium.

In microorganisms, and even certain well studied animal species, one of the requirements for advancing knowledge about the molecular bases of cell processes is an array of mutant lines, often induced, that modify or block steps in the process under study. Until recently, this approach has been neglected in plants and probably accounts for the lack of knowledge and slow development of technology in plant molecular biology.

Examples of the role of induced mutants in probing developmental and cellular processes and their genetic control use a broad spectrum of induced mutants with specific defects. Some facets of this approach have been reviewed by Rice & Carlson (1975) and Scholz & Böhme (1980). The former also present some valuable ideas about the use of induced mutants in analysing seed development and relevant biochemical and physiological processes.

The use of numerous *eceriferum* ('waxless' plant surface) mutants (von Wettstein-Knowles 1979) in barley is elucidating the pathway of wax synthesis and deposition; mutants lacking in serine–glyoxylate aminotransferase activity in *Arabidopsis* are permitting an understanding of photorespiration and its genetic control and regulation (Somerville & Ogren 1980); detailed genetic, biochemical and ultrastructural analyses of innumerable chlorophyll-deficient mutants in barley (von Wettstein *et al.* 1974; Simpson & von Wettstein 1980), and of high chlorophyll fluorescence mutants in maize (Miles *et al.* 1979), are providing an understanding of the regulation, genetic control and metabolic pathways involved in various facets of photosynthesis; ten induced nitrate-reductase deficient mutants in barley are permitting biochemical, genetic and physiological investigations toward an understanding and control of the nitrate assimilation pathway (Kleinhofs *et al.* 1978*b*; Warner & Kleinhofs 1974; Kleinhofs *et al.* 1980); waxy pollen mutants in barley are being used to probe with classical and molecular genetic techniques the nature of induced mutations, the composition of a eukaryotic locus and the synthesis and deposition of starch, and to develop a mutagen monitoring system (Rosichan *et al.* 1981; Nilan *et al.* 1981; Hodgdon *et al.* 1981); and numerous induced anthocyanin-free mutants in barley are helping us to understand the pathway and genetic control of anthocyanin synthesis and to develop strains free of proanthocyanidins, which are responsible for permanent chill haze and instability in beer (von Wettstein *et al.* 1980; von Wettstein 1979; Jende-Strid 1978; R. A. Nilan & A. L. Hodgdon, unpublished).

Success in using induced mutants in cell cultures, protoplasts and pollen for analysing basic processes has been very limited. Recent developments, and the problems inherent in mutant induction and selection, and especially plant regeneration, have been reviewed (Rice & Carlson 1975; Brock 1980) and are described in more detail by Davies (this symposium).

Another important area of breeding research involves chromosome mutants and the location of genes on chromosomes. The efficient assembly of necessary genotypes for analyses of biochemical and physiological processes and progress in manipulating genes in breeding and breeding research (genetic engineering) requires that each gene or set of genes contributing to a trait be pinpointed on the chromosome. Success in this endeavour will require a vast array of induced chromosome mutants and improved chromosome banding techniques. In short, this area of cytogenetics should become as important for plant improvement as it has been for advancing knowledge and technology in human genetics and medicine.

Locating genes on a specific chromosome is facilitated by trisomics (Khush 1973; Lewis *et al.* 1980; Tsuchiya 1969), monosomics (Kimber & Sears 1980; Law *et al.*, this symposium) and translocation break points. The latter may be recognized and used in mapping through partial sterility (Ramage *et al.* 1961) or cytologically (Tuleen 1971). In barley, over 300 translocations,

mostly induced, are available for cytologically locating genes (Nilan 1974). Induced translocation break-points can often provide cytological markers in chromosome regions lacking suitable genes.

To locate genes on a specific arm, telocentrics (Sears & Sears 1978; Kimber & Sears 1980; Singh & Tsuchiya 1977) and translocation break points are indispensable. In maize, numerous induced B–A translocations have been useful (Beckett 1978). To pinpoint genes cytologically within chromosome arms, translocation break-points as well as deletions, as so elegantly demonstrated in tomato by Khush & Rick (1968), and acrocentrics (Tsuchiya & Hang 1980), are necessary. Chromosome banding, now being used for locating genes (Kimber & Sears 1980; Linde-Laursen 1979) will be a powerful complementary tool in this endeavour.

Conclusion

Improved mutagen treatments, along with increased precision in selection of resulting mutants, are rapidly increasing the use and success of induced mutations in plant improvement. Such mutations are substituting for and even extending the variability obtained from natural germ plasm sources. As natural variability becomes further depleted and a much greater supply of variants is needed to create new cultivars of the future, artificially induced variability will assume greater importance. Indeed, the relative ease of producing and the suitability of induced variability for some crops may reduce or even negate the need for collection and preservation of natural germ plasm.

Induced gene and chromosome mutants are already proving indispensable for elucidating new basic knowledge about physiological, biochemical and genetic processes composing phenotypes and their control and for pinpointing genes on chromosomes.

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References (Nilan)

Beckett, J. B. 1978 B–A translocations in maize. I. Use in locating genes by chromosome arms. *J. Hered.* **69**, 27–36.

Brock, R. D. 1971 The role of induced mutations in plant improvement. *Radiat. Bot.* **11**, 181–196.

Brock, R. D. 1980 Mutagenesis and crop improvement. In *The biology of crop productivity* (ed. P. S. Carlson), pp. 383–409. New York: Academic Press.

Broertjes, C. & Van Harten, A. M. 1978 *Application of mutation breeding methods in the improvement of vegetatively propagated crops.* Amsterdam: Elsevier Scientific Publishing Company.

Cieśla, Z., Filutowicz, M. & Klopotowski, T. 1980 Involvement of the L-cysteine biosynthetic pathway in azide-induced mutagenesis in *Salmonella typhimurium. Mutat. Res.* **70**, 261–268.

Doll, H., Køie, B. & Eggum, B. O. 1974 Induced high lysine mutants in barley. *Radiat. Bot.* **14**, 73–80.

Gaul, H. 1964 Mutations in plant breeding. *Radiat. Bot.* **4**, 155–232.

Gustafsson, Å. 1975 Mutations in plant breeding – a glance back and a look forward. In *Radiation research (Proc. Fifth Int. Cong. Radiat. Res.)* (ed. O. F. Nygaard, H. I. Adler & W. K. Sinclair), pp. 81–95. New York: Academic Press.

Gustafsson, Å. & Lundqvist, U. 1980 Hexastichon and intermedium mutants in barley. *Hereditas* **92**, 229–236.

Hodgdon, A. L., Marcus, A. H., Arenaz, P., Rosichan, J. L., Bogyo, T. P. & Nilan, R. A. 1981 The ontogeny of the barley plant as related to mutation expression and detection of pollen mutations. *Environ. Hlth Perspect.* **37**. (In the press.)

Jende-Strid, B. 1978 Mutations affecting flavonoid synthesis in barley. *Carlsberg Res. Commun.* **43**, 265–273.
Jørgensen, J. H. 1976 Identification of powdery mildew resistant barley mutants and allelic relationship. In *Barley genetics III* (ed. H. Gaul), pp. 446–455. Munich: Karl Thiemig.
Khush, G. S. 1973 *Cytogenetics of aneuploids.* New York: Academic Press.
Khush, G. S. & Rick, C. M. 1968 Cytogenetic analysis of the tomato genome by means of induced deficiencies. *Chromosoma* **23**, 452–484.
Kimber, G. & Sears, E. R. 1980 Uses of wheat aneuploids. In *Polyploidy: biological relevance* (ed. W. H. Lewis), pp. 427–443. New York: Plenum Publ. Corp.
Kleinhofs, A., Kuo, T. & Warner, R. L. 1980 Characterization of nitrate reductase-deficient barley mutants. *Molec. gen. Genet.* **177**, 421–425.
Kleinhofs, A., Owais, W. M. & Nilan, R. A. 1978*a* Azide. *Mutat. Res.* **55**, 165–195.
Kleinhofs, A., Warner, R. L., Muehlbauer, F. J. & Nilan, R. A. 1978*b* Induction and selection of specific gene mutations in *Hordeum* and *Pisum*. *Mutat. Res.* **51**, 29–35.
Konzak, C. F., Nilan, R. A. & Kleinhofs, A. 1977 Artificial mutagenesis as an aid in overcoming genetic vulnerability of crop plants. In *Genetic diversity in plants* (ed. A. Muhammed, R. Aksel & R. C. von Borstel), pp. 163–177. New York: Plenum Publ. Corp.
Konzak, C. F., Nilan, R. A., Wagner, J. & Foster, R. J. 1965 Efficient chemical mutagenesis. In *The use of induced mutations in plant breeding* (*Radiat. Bot., Suppl.* **5**), pp. 49–70. Oxford: Pergamon.
Lewis, E. J., Humphreys, M. W. & Caton, M. P. 1980 Chromosome location of two isozyme loci in *Lolium perenne* using primary trisomics. *Theor. appl. Genet.* **57**, 237–239.
Linde-Laursen, I. 1979 Giemsa C-banding of barley chromosomes. III. Segregation and linkage of C-bands on chromosomes 3, 6 and 7. *Hereditas* **91**, 73–77.
Lundqvist, U. 1976 Locus distribution of induced *eceriferum* mutants in barley. In *Barley genetics III* (ed. H. Gaul), pp. 162–163. Munich: Karl Thiemig.
Lundqvist, U., Wettstein-Knowles, P. von & Wettstein, D. von 1968 Induction of *eceriferum* mutants in barley by ionizing radiations and chemical mutagens. II. *Hereditas* **59**, 473–504.
Manual on mutation breeding 1977 Tech. Reps Ser. no. 119, 2nd edn. Vienna: I.A.E.A.
Miles, C. D., Markwell, J. P. & Thornber, J. P. 1979 Effect of nuclear mutation in maize on photosynthetic activity and content of chlorophyll-protein complexes. *Pl. Physiol.* **64**, 690–694.
Nilan, R. A. 1972 Mutagenic specificity in flowering plants: Facts and prospects. In *Induced mutations and plant improvement*, pp. 141–151. Vienna: I.A.E.A.
Nilan, R. A. 1974 Barley (*Hordeum vulgare*). In *Handbook of genetics* (ed. R. C. King), vol. 2, pp. 93–100. New York: Plenum Press.
Nilan, R. A., Kleinhofs, A. & Konzak, C. F. 1977 The role of induced mutation in supplementing natural genetic variability. *Ann. N.Y. Acad. Sci.* **287**, 367–384.
Nilan, R. A., Konzak, C. F., Wagner, J. & Legault, R. R. 1965 Effectiveness and efficiency of radiations for inducing genetic and cytogenetic changes. In *The use of induced mutations in plant breeding* (*Radiat. Bot., Suppl.* **5**), pp. 71–89. Oxford: Pergamon.
Nilan, R. A., Rosichan, J. L., Arenaz, P., Hodgdon, A. L. & Kleinhofs, A. 1981 Pollen genetic markers for detection of mutagens in the environment. *Environ. Hlth Perspect.* **37**. (In the press.)
Nilan, R. A., Sideris, E. G., Kleinhofs, A., Sander, C. & Konzak, C. F. 1973 Azide – a potent mutagen. *Mutat. Res.* **17**, 142–144.
Nilan, R. A. & Velemínský, J. 1981 Mutagenicity of selected chemicals in barley test systems. In *Proc. Comparative Chemical Mutagenesis Workshop* (ed. F. J. de Serres), ch. 11. (In the press.)
Nilan, R. A. & Vig, B. K. 1976 Plant test systems for detection of chemical mutagens. In *Chemical mutagens. Principles and methods for their detection* (ed. A. Hollaender), vol. 4, pp. 143–170. New York: Plenum Press.
Owais, W. M., Kleinhofs, A. & Nilan, R. A. 1979 In vivo conversion of sodium azide to a stable mutagenic metabolite in *Salmonella typhimurium*. *Mutat. Res.* **68**, 15–22.
Owais, W. M., Kleinhofs, A. & Nilan, R. A. 1981*a* Effects of L-cysteine and *O*-acetyl-L-serine in the synthesis and mutagenicity of azide metabolite. *Mutat. Res.* **80**, 99–104.
Owais, W. M., Kleinhofs, A. & Nilan, R. A. 1981*b* In vitro synthesis of the mutagenic metabolite by cell-free bacterial extracts. (Submitted.)
Owais, W. M., Kleinhofs, A., Ronald, R. C. & Nilan, R. A. 1981*c* Isolation of an azide mutagenic metabolite in *Salmonella typhimurium*. *Mutat. Res.* (In the press.)
Owais, W. M., Zarowitz, M. A., Gunovich, R. A., Hodgdon, A. L., Kleinhofs, A. & Nilan, R. A. 1978 A mutagenic in vivo metabolite of sodium azide. *Mutat. Res.* **50**, 67–75.
Persson, G. & Hagberg, A. 1969 Induced variation in a quantitative character in barley. Morphology and cytogenetics of *erectoides* mutants. *Hereditas* **61**, 115–178.
Plewa, M. J. & Gentile, J. M. 1976 Mutagenicity of atrazine: a maize–microbe bioassay. *Mutat. Res.* **38**, 287–292.
Ramage, R. T., Burnham, C. R. & Hagberg, A. 1961 A summary of translocation studies in barley. *Crop Sci.* **1**, 277–279.
Rice, T. B. & Carlson, P. S. 1975 Genetic analysis and plant improvement. *A. Rev. Pl. Physiol.* **26**, 279–308.
Rosichan, J., Arenaz, P., Blake, N., Hodgdon, A., Kleinhofs, A. & Nilan, R. A. 1981 An improved method for the detection of mutants at the waxy locus in Hordeum vulgare. *Environ. Mutagenesis.* **3**. (In the press.)

Scholz, G. & Böhme, H. 1980 Biochemical mutants in higher plants as tools for chemical and physiological investigations – a survey. *Kulturpflanze* **28**, 11–32.

Scott, B. R., Sparrow, A. H., Schwemmer, S. S. & Schairer, L. A. 1978 Plant metabolic activation of 1,2-dibromoethane (EDB) to a mutagen of greater potency. *Mutat. Res.* **49**, 203–212.

Sears, E. R. & Sears, L. M. S. 1978 The telocentric chromosomes of common wheat. In *Proc. 5th Int. Wheat Genet. Symp.*, New Delhi, pp. 389–407.

Sideris, E. G., Nilan, R. A. & Bogyo, T. P. 1973 Differential effect of sodium azide on the frequency of radiation-induced chromosome aberrations vs. the frequency of radiation-induced chlorophyll mutations in *Hordeum vulgare*. *Radiat. Bot.* **13**, 315–322.

Sigurbjörnsson, B. 1976 The improvement of barley through induced mutation. In *Barley genetics III* (ed. H. Gaul), pp. 84–95. Munich: Karl Thiemig.

Sigurbjörnsson, B. & Micke, A. 1974 Philosophy and accomplishments of mutation breeding. In *Polyploidy and induced mutations in plant breeding*, pp. 303–343. Vienna: I.A.E.A.

Simpson, D. J. & von Wettstein, D. 1980 Macromolecular physiology of plastids. XIV. *Viridis* mutants in barley: genetic fluoroscopic and ultrastructural characterisation. *Carlsberg Res. Commun.* **45**, 283–314.

Singh, R. J. & Tsuchiya, T. 1977 Morphology, fertility, and transmission in seven monotelotrisomics of barley. *Z. PflZücht.* **78**, 327–340.

Somerville, C. R. & Ogren, W. L. 1980 Photorespiration mutants of *Arabidopsis thaliana* deficient in serine-glyoxylate aminotransferase activity. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2684–2687.

Tsuchiya, T. 1969 Status of studies of primary trisomics and other aneuploids in barley. *Genetica* **40**, 216–232.

Tsuchiya, T. & Hang, A. 1980 Acrocentric chromosome $4S^{4L}$ in barley. *Barley Genet. Newsl.* **10**, 72–73.

Tuleen, N. A. 1971 Translocation-gene linkages from F_2 seedlings in barley. In *Barley genetics II* (ed. R. A. Nilan), pp. 208–212. Pullman: Washington State University Press.

Walbot, V. 1980 Molecular biology of higher plants. In *The biology of crop productivity* (ed. P. S. Carlson), pp. 343–409. New York: Academic Press.

Warner, R. L. & Kleinhofs, A. 1974 Relationships between nitrate reductase, nitrite reductase, and ribulose diphosphate carboxylase activities in chlorophyll-deficient mutants of barley. *Crop Sci.* **14**, 654–658.

Wettstein, D. von 1979 Biochemical and molecular genetics in the improvement of malting barley and brewers yeast. In *Proc. 17th Congr. European Brewery Convention*, West Berlin, pp. 587–629.

Wettstein, D. von, Jende-Strid, B., Ahrenst-Larsen, B. & Erdal, K. 1980 Proanthocyanidin-free barley prevents the formation of beer haze. *MBAA tech. Q.* **17**, 16–23.

Wettstein, D. von, Kahn, A., Nielsen, O. F. & Gough, S. 1974 Genetic regulation of chlorophyll synthesis analyzed with mutants in barley. *Science, N.Y.* **184**, 800–802.

Wettstein-Knowles, P. von 1976 Tracking down β-diketone synthesis with the aid of the *eceriferum* mutants. In *Barley genetics III* (ed. H. Gaul), pp. 20–22. Munich: Karl Thiemig.

Wettstein-Knowles, P. von 1979 Genetics and biosynthesis of plant epicuticular waxes. In *Advances in the biochemistry and physiology of plant lipids* (ed. L.-Å. Appelqvist & C. Liljenberg), pp. 1–26. Elsevier/North-Holland Biomedical Press.

Cell and Tissue Culture: Potentials for Plant Breeding
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Cell and tissue culture: potentials for plant breeding

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For the plant breeder, one of the objectives of cell culture systems should be their exploitation for the induction and isolation of mutant cells, which can then be regenerated as mutant plants. While a number of mutations have been recognized in plant cells *in vitro*, few have had any significance for plant breeding. There are currently a number of constraints to the exploitation of this technology, some of which are related to methodological limitations; these are likely to be overcome, but others, which relate to the nature of the attributes that the plant breeder seeks to modify, are much more intractable. There is scope for exploiting cell cutures as genetic tools, as has already been done with animal cell cultures. In contrast, the culture of organized tissues in the form of meristems or small shoots has begun to be useful a technique for plant breeders, and examples of diverse applications will be discussed. Most exploit the rapid rates of multiplication, and the assured health status of the propagules, that can be attained in culture; there is also the possibility of manipulating the genotype of these tissues. Finally, organ culture, and it is the culture of embryos that is of most interest to the plant breeder in this context, is considered; the value of embryo cultu:e as a means of producing novel interspecific and intergeneric hybrids is well recognized. In addition, cultured embryos can be used as experimental systems for studying the biochemistry and molecular biology of storage product synthesis and accumulation.

The totipotency of plant cells and the relative ease with which they can be cultured *in vitro* have engendered a degree of optimism that cell and tissue culture can provide a useful new technology for plant breeders, but in only a few instances and only for particular kinds of application has this optimism been justified. I shall discuss some of the achievements as well as the limitations of cell tissue culture, excluding from consideration pollen and protoplast cultures, as these topics are discussed elsewhere in this symposium (by Hermsen & Ramanna and by Cocking), and deal first with single-cell culture, then culture of cell groups, of organized tissues and finally of plant organs.

Cell culture

One of the objectives of cell culture systems in a plant breeding context is the induction and isolation of mutant forms and the regeneration of plants from such mutants. Single-cell culture has been successful in few instances; the production of embryoids from single carrot cells was noted by Steward *et al.* (1958), of plantlets from single cells of *Macleaya cordata* by Kohlenbach (1965), and a few other examples are known (see Narayanaswamy 1977). From an analytical point there would be advantages in being able to generate embryoids or colonies from single cells, preferably plated at low densities; there also could be other benefits in avoiding the mixture of cells of differing genotype that can occur within a group, since in these circumstances a mutant can be swamped by faster-growing wild-type cells surrounding it, or be killed by the lytic products of dying cells around it.

The culture of groups of cells growing as callus masses in liquid or on solid media has formed

the basis of most of the experimental work that has been undertaken, and the cells of a very large number of plant species can now be cultured in this manner. This system suffers from the cells' need to be grown at high densities, their tendency to genetic instability and the difficulty of regenerating plants at high frequencies. The two last problems may well be a function of the cell type that tends to occur in many callus cultures; this often consists of large highly vacuolated cells, whereas it is a general experience that small highly meristematic cells are less prone to these problems. There is an important area of research in the role of cell geometry, of the cell wall and of cell–cell relations in genetic stability and differentiation in plant cells. However, the technical problems of genetical stability and ease of differentiation will be overcome as more appropriate media and conditions are defined. It is a moot point whether another technical limitation, the inability to replica-plate plant cells, will be overcome.

Another kind of limitation has been noted in some experiments: the differential expression of characters in cells grown *in vitro* and those *in vivo*. For example, Widholm (1980) recently quoted four examples in which aspects of amino acid biosynthesis were different in cultured cells from those in the plants from which they were derived, or the plants to which they gave rise. One example was that of 5-methyltryptophan-resistant tobacco cells that had an altered anthranilate synthetase; plants regenerated from resistant lines selected *in vitro* did not show the altered enzyme, although cultures derived yet again from these plants once more had the modified enzyme. This limitation is certainly not ubiquitous, and examples of consistency of expression in cells *in vitro* and *in vivo* are known (see Maliga (1978) for references). Examples are nicotine content (Kinnersley & Dougall 1980), resistance to valine (Bourgin 1978), to picloram (Chaleff & Parsons 1978), to 5-bromodeoxyuridine (Márton & Maliga 1975) and to the fungal pathogen *Phytophthora parasitica* in tobacco (Helgeson *et al.* 1976) and *P. infestans* in potato (Behnke 1980). Two well documented examples of the exploitation of cell culture of significance to plant breeding are those in which *Pseudomonas tabaci* resistant tobacco strains (Carlson 1973) and *Helminthosporium maydis* resistant forms of *Zea mays* (Gengenbach *et al.* 1977) were produced. Attempts are also being made to select in culture for altered levels of specific constituents of crop plants, those of nicotine in tobacco (Collins & Legg 1979) and of urease in soybean (Polacco 1976) being good examples.

Although there are a few examples of potentially useful classes of mutants being selected, there are severe limitations on the kinds of selective techniques that can be exploited and thus of mutant forms that can be recognized *in vitro*. The complexity of many of the attributes that we seek to improve in our crops, and our ignorance of that which underlies them at a cellular and biochemical level, are severe constraints in this context. For example, while we can isolate disease-resistant cell lines when this is based on resistance to a pathogen-produced toxin (see Earle 1978), in the vast majority of instances we have insufficient understanding of that which underlies resistance to allow us to derive a selection régime. Such limitations to our understanding of the molecular biology of the components of plant productivity are likely to be greater barriers to progress in the near future than the technical problems of cell culture; the same is true of the techniques of genetic engineering in plants.

For those attributes that are only expressed in particular differentiated organized tissues, and they currently constitute a substantial proportion of those in which the plant breeder is interested, cell culture can at present offer us little. It has been considered by some that one exception to this might be storage proteins; it is assumed that they are only synthesized in seed. In these proteins, particular amino acids may be deficient, e.g. lysine in barley and rice, and methionine in legumes.

It has been suggested that if mutant cells could be isolated in culture that overproduce the required amino acid, it is possible that the seed proteins might also contain more of that particular amino acid. This is a tenuous argument, and Boulter & Crocomo (1979) have stated that in legumes there is as yet no evidence that protein quality is dependent on the supply of particular amino acids. Chaleff & Carlson (1975) isolated mutant cultures of rice, on the basis of their resistance to the lysine analogue *S*-β-aminoethylcysteine, that overproduced lysine, but as no plants could be differentiated from the cultures the consequence of these mutational changes on the seed protein was not tested. More recently, Hibberd *et al.* (1980) have generated *Zea mays* lines able to grow on normally inhibitory levels of lysine plus threonine and found that the selected cultures had increased concentrations of particular amino acids; for example, in one line the lysine was twice, and in another the methionine concentration 3.8 times, as high as in the control. Only free aspartate-derived amino acids were increased. Plants were regenerated from these cultures, but no further generations could be derived and so once again the consequences in terms of seed protein could not be evaluated.

One of the problems of callus cultures, their proneness to genetic instability, has been turned to advantage in sugar cane, where variants have proved useful (Nickell 1977). Forms showing improved resistance to a virus and to fungal pathogens have been obtained; it has to be noted, however, that sugar cane is a vegetatively propagated species, with very high chromosome numbers and tolerant of chromosomal changes.

It is difficult to summarize a topic that has generated on average one symposium per year in the last few years, but while a great deal of attention has been drawn to the potential role of this technology in plant breeding, the achievements are minimal as yet. The extent to which this will alter will depend in part on our ability to improve the technology of cell culture and attain a greater expression of characters *in vitro* than is now possible. Cell cultures have not yet been exploited as genetic tools in plants, for mapping, and for complementation and linkage studies, as they have been in human cell systems, for example (McKusick & Ruddle 1977). Neither have we examined whether it is feasible to induce, and if so what might be the consequences of duplicating, certain chromosomal regions in plant cells grown *in vitro*. In mouse cell cultures, lines can be selected that show enhanced resistance to the antifolate drug methotrexate, due to increased dihydrofolate reductase activity. The resistant cells achieve this by a selective amplification of the genes for dihydrofolate reductase (Alt *et al.* 1978). DNA amplification occurs in plants *in vivo* in the well documented example of the giant chromosomes of *Nicotiana* hybrids (Gerstel & Burns 1976). Claims of DNA amplification in plant cells grown *in vitro* have been made (see Buiatti 1977), but its phenotypic consequences have not been analysed; this is an important challenge for us. It is significant that in the mouse cells, DNA other than the gene sequences directly selected was amplified (Nunberg *et al.* 1978). If that also occurred in plant cells, then genes that could not themselves be directly selected in culture, but which were closely linked to those that could, might be amplified and the consequences examined.

The culture of tissues

The ability to culture organized tissues in the form of very small shoots or meristems has allowed a most valuable application of plant tissue culture. Meristem culture has long been used for the production of virus-free plants, while the culture of small shoots and meristems is being exploited for the rapid vegetative multiplication (micropropagation) of a range of horticultural and agri-

cultural plants (Holdgate 1977; Murashige 1978). In the context of plant breeding it is now also possible to cite many examples in which it is advantageous to exploit either the ability to propagate by tissue culture genotypes in which there is no natural or simple method of vegetative propagation, or the more rapid rates of multiplication that can be attained *in vitro*.
Such applications include the following.

(*a*) New varieties are often not available to the agricultural or horticultural industry for many years after the recognition of their value, simply because of the time taken to generate appropriate quantities for large-scale planting. Examples among vegetatively propagated horticultural crops are daffodils, freesia, gladioli and alstromeria, in all of which the natural rates of multiplication are low, and 10 or more years may elapse before a desired strain becomes available. In all of these micropropagation techniques are being used to speed up the release of new varieties to the industry. Another example in which the technique has been exploited is in the multiplication of new strains of rootstocks for top fruit.

(*b*) There is a need to maintain new varieties of vegetatively propagated plants in a disease-free condition for as long as possible during their period of multiplication before release. This means that micropropagation can be an attractive and economic alternative to conventional methods of multiplication even though a species may have a rapid rate of natural propagation. We may well find in the near future that strawberries and potatoes will be in this category.

(*c*) The breeding system of a crop plant can impose limitations on the multiplication of a genotype. In some such instances, micropropagation techniques can be a useful means of overcoming this, as the following examples illustrate.

(i) *Incompatibility systems*. In particular forms of *Brassica oleracea*, F_1 hybrids have a considerable commercial attraction; their production is dependent on the selection and maintenance of pairs of inbred parents. The sporophytic incompatibility system of these genotypes means that maintenance of the parents depends on bud pollination, which is both difficult and expensive; micropropagation offers an alternative method of maintenance and multiplication (Dunwell & Davies 1975).

(ii) *Male sterility*. Maintenance of male-sterile genotypes demands a continuous process of backcrossing, and again micropropagation can be an attractive alternative. For example, male-sterile onions can be readily propagated in tissue culture (Hussey 1978), and several male-sterile lines of wheat have been multiplied in this manner (G. Hussey, personal communication).

(iii) *Dioecious forms*. Tissue culture has been used for the clonal multiplication of selected genotypes of asparagus that are required as parental plants for the production of commercial quantities of seed (Dore 1975).

(iv) *Heterozygous genotypes*. The multiplication of large quantities of particular heterozygous genotypes to be used as parents in a seed production programme can now be achieved even though no, or only a slow, method of natural vegetative propagation exists. The onion crop again provides one example of such a species, and both diploid and tetraploid sugar beet (Hussey & Hepher 1978) may be other candidates for incorporating a micropropagation step into the breeding programme.

(*d*) Multiplication of existing superior genotypes. In some species the long life cycle or/and the heterozygosity of the plants renders conventional breeding methodology difficult; in addition, in some such instances there is no natural method of vegetative propagation. The availability of a micropropagation technique that would allow existing superior genotypes to be cloned could significantly improve the average level of performance of such a crop; a prime example in which

clonal plantings of superior genotypes will lead to an increase in productivity is the oil palm (Jones 1974).

(*e*) Correlation of seedling and mature plant responses. Selection for disease resistance is often facilitated if it can be based on the screening of seedlings. However, their responses need not be identical with those of mature plants, and the extent to which they are correlated is not easy to establish in many instances. In homozygous forms, sister plants can be used for this comparison, but with heterozygotes this is not possible. By exploiting micropropagation, my colleagues Matthews & Dunwell (1979) were able to overcome this problem in the carnation crop; from a given seedling, the tip was taken for culture and used to generate a clone of adult plants. The response of the remainder of the seedling to a given pathogen and of the adult plant derived from the shoot tips could then be compared.

(*f*) Manipulation of the genotype.

(i) *Production of polyploid forms*. Treatment of cultures with colchicine has allowed higher rates of production of polyploids than is usually possible by more conventional techniques. In one series of experiments with freesia, which involved placing the cultures in a colchicine solution for 24 h, 27 % of the plants regenerated from a treated diploid culture became tetraploid, whereas none were found in the control (Davies 1973). High yields of tetraploid carnations have been produced in a comparable manner (Dunwell & Cornish 1978).

(ii) *Manipulation of cytoplasmic male sterility*. It has been reported that cytoplasmic male sterility in sugar beet can be 'cured' by heat treatment (Lichter 1978) and that it is graft transmissible (Curtis 1967). Sugar beet is highly heterozygous and is not readily propagated by vegetative means, but the availability of clonal material would greatly facilitate such experimental approaches. Furthermore, heat treatment of cultured tissues can be readily undertaken, as well as the grafting of propagules *in vitro*. The feasibility of manipulating male sterility in this way is currently being examined by using tissue culture, and the provision of clonal material is also aiding the comparative analysis of mitochondrial DNA in cytoplasmic male sterile and in fertile genotypes (A. Powling, personal communication).

(iii) *Analysis of genotroph induction*. The production of genotrophs in flax (Durrant 1962) is accompanied by numerous changes in the genome (Cullis 1977). The study of one of these changes, that induced in the ribosomal genes, has been facilitated by the availability of a tissue culture system (Cullis & Charlton 1981). The terminal portion of the shoot of young flax seedlings was harvested at various times after initiating the treatments that induce the genotrophs, and the ribosomal DNA (rDNA) within them assayed; the remainder of the stem below this region was then cultured, and from each of the axillary meristems that were subsequently induced to develop, the rDNA was extracted and assayed. In this way Cullis & Charlton could determine when the changes occurred in the rDNA during the process of induction. They showed that the changes occurred rapidly and only in the terminal regions of the stem, an analysis that would otherwise be extremely difficult to achieve.

(iv) *Induction of mutations*. Adventitious meristems can develop from single cells; if these cells have been modified by exposure to mutagenic agents, wholly mutant meristems and plants may be immediately generated. In such meristems the competition that occurs between wild-type and mutant cells, which can result in the suppression of the latter, is avoided. The end result should be a higher rate of recovery of mutant plants. Adventitious buds are readily generated in culture, and as Broertjes *et al.* (1976) have shown with chrysanthemum, they can be a useful source of induced mutations.

(*g*) Storage of genotypes. The maintenance of selected heterozygous lines to be used as parents

in the production of commercial varieties can often be difficult. If they are maintained by seed multiplication, there is a danger of the occurrence of genetic drift. Storage of vegetatively propagated material can equally be difficult. Tissue cultures can, however, be stored for many months and even years in some instances, simply by keeping them on a nutrient agar medium at 4 °C and at low light intensities. This offers an easy, cheap and, furthermore, disease-free system of maintaining parental lines for variety production.

(*h*) Provision of disease-free material. Disease-free strains are required for an unbiased evaluation of potential new varieties and also for the selection of parents. Dale (1975) has suggested that the breeding of certain grass species in which virus infection rapidly leads to a marked reduction in plant vigour would be aided by the availability of disease-free forms.

These examples illustrate the opportunities that are already available to the plant breeder to exploit the culture of organized tissues, but a further expansion of the technology will undoubtedly occur as the culture of a wider range of species and genera becomes possible, and as plant breeders recognize the role it can play.

Organ culture

For the plant breeder it is the culture of embryos that is of primary interest in this context. The production of many interspecific hybrids has been possible as the result of the rescue of immature embryos by excision from the ovary and their subsequent culture *in vitro*. A recent review (Raghavan 1977) has summarized the applications of embryo culture and the range of hybrids produced in this manner. Included among them are intergeneric hybrids involving *Triticum* and related genera, and *Zea mays* and its relatives, as well as interspecific crosses involving *Triticum*, *Hordeum*, *Oryza*, *Sorghum*, *Nicotiana* and *Hordeum*, to list but a few of many important crop plants. While it is unlikely that such hybrids themselves or their allopolyploid derivatives will be useful as crop plants, they do offer a means of achieving an interspecific or intergeneric transfer of chromosomes or of chromosome fragments. For this latter purpose they can sometimes offer an easier alternative to the route offered by protoplast fusion. The interspecific and intergeneric embryos have the advantage that nuclear fusion, cell wall formation and initial cell divisions have already been achieved. The elimination of particular chromosomes in interspecific embryos can occur naturally, as in the hybrids of *Hordeum vulgare* and *H. bulbosum*, and of *Nicotiana plumbaginifolia* and *N. tabacum*, leading in these instances to the production of offspring in which only one parental genome is present; this has been the basis of the large-scale production by embryo culture of barley haploids (Kasha 1974). Until more effort is devoted to establishing whether the phenomenon of chromosome elimination occurs in other interspecific and intergeneric embryos, it is impossible to speculate on the wider applicability of this technology of producing haploids. A method of attaining an interspecific transfer of chromosome fragments has been developed by Pandey (1980), in which pollen is exposed to high doses of ionizing radiation to fragment the chromosomes, before its use for pollination. Fragments of the paternal chromosomes are then incorporated into, or attached to, the maternal chromosomes within the embryo. These experiments have not involved embryo culture, and few progeny incorporating alien chromosome fragments have been produced. My colleague, J. M. Dunwell, is attempting to modify Pandey's approach by inducing a proliferation of the cultured embryos produced after interspecific or intergeneric pollination with irradiated pollen; in this way he hopes to generate from each embryo a number of derivatives, each of which will have the maternal genome and also a different

paternal fragment(s). This may enable us to sample a greater range of the fragments that can be included in the embryos.

Few attempts have been made to culture plant embryos for experimental purposes other than the generation of hybrids. This is in marked contrast to animal embryology, which has a long tradition of experimental work. In many crop plants the embryo is the economically important component, yet we know singularly little about it in cellular, biochemical or molecular terms. Embryo culture could be useful in this respect and I shall describe some of our own work on peas (*Pisum sativum*) to illustrate this. Culture methods have improved to an extent that comparable growth rates can be achieved *in vivo* and *in vitro* in *Phaseolus vulgaris* (Thompson *et al.* 1977) and in peas (Stafford & Davies 1979), although in both species the period of growth over which these rates can be maintained is still relatively limited. Mature legume seed is composed almost entirely of the embryo, with the swollen cotyledons composing the storage tissue. The number and size of cells within the cotyledons are the determinants of seed size, and while genetic variation exists for both components (Davies 1975, 1977) we do not have at present much knowledge of their respective importance. The DNA in the cotyledon cells is highly endoreduplicated, the extent of DNA duplication being proportional to cell size (Davies 1977). By an appropriate culture technique we can trigger these cells, which normally remain in interphase, into a prophase stage, in which giant polytene-like chromosomes are seen (Marks & Davies 1979). Embryo culture could be used to examine the way in which factors influencing seed size affect the two components, cell size and cell number. It could also be used to study the control of storage product accumulation within the seed. We have shown that comparable amounts of protein and starch are synthesized *in vitro* and *in vivo* in peas (Stafford & Davies 1979). Beyond this we have examined the synthesis of legumin, one of the two main storage proteins in peas, in cultured embryos, and compared it with that occurring *in vivo*. Earlier work had suggested that legumin was synthesized at a fairly late stage of development of the embryo, when the greater proportion of the cells of the cotyledon were becoming endoreduplicated; secondly, it was believed that legumin synthesis could not be initiated in cultured embryos (Millerd *et al.* 1975). By using a more sensitive assay for legumin, an enzyme-linked immunoabsorbent assay (ELISA), which allows us to detect nanogram quantities of the protein (Domoney *et al.* 1980), we have shown that legumin is synthesized in much younger embryos than hitherto assumed, an observation to which I will return later. Secondly, with improved culture techniques we have demonstrated that legumin synthesis can be initiated *in vitro* (Domoney *et al.* 1980).

Returning to the observation of the presence of legumin in the cells of very young embryos, this implies either that there is a low rate of synthesis even in the diploid cells of the young cotyledon or that there are already a few endoreduplicated cells present, and it is these that are synthesizing the protein. Should the former be true, it is important to test whether other diploid cells within the plant, and even cells in culture, can synthesize legumin, albeit at a very low level, but levels that we might now detect with the ELISA technique. It has been suggested that callus cells derived from cultures of *Vicia* cotyledons can synthesize low levels of storage protein (Muntz, quoted in Boulter & Crocomo 1979). The possibility of selecting mutant cells in culture that can overproduce particular storage proteins is attractive, and the aim in peas would be to enhance the production of legumin, which has a higher proportion of sulphur amino acids than some of the other seed proteins.

Embryo culture is being used also for studying another aspect of storage product synthesis in peas. It has been recently shown (Davies 1980) that there are mutants in peas, somewhat akin to

those in maize and barley, in which both protein and carbohydrate composition is altered within the seed. In peas the two seed phenotypes, round and wrinkled, differ in starch quantity and quality, sugar content and storage protein composition, the proportion of legumin being higher in round seed (Davies 1980). The nature of the metabolic changes induced by the allelic alternatives at the r_a locus, which is involved in the determination of these two phenotypes, is not known, but we are using embryo culture to examine the proteins synthesized by these two genotypes when grown under various conditions to try to analyse the relation of carbohydrate and protein synthesis, and how it may be manipulated. We therefore have mutants in peas that affect storage product composition, we can culture the embryos in which these products are synthesized and stored, and we can define the ways in which we need to improve the phenotype. An important limitation, however, is the dearth of knowledge of the biochemistry and molecular biology of these important components of economic yield – the seed – and this needs to be remedied if we are to successfully manipulate and modify them by plant breeding.

Conclusion

A cautious optimism may be an appropriate conclusion; after all, plant breeders and geneticists took little interest in cell, tissue and organ culture until recently, but already the value of meristem and shoot culture is widely recognized. Other aspects of the subject will prove attractive as techniques improve and new applications are recognized, and if genetic engineering is to contribute to plant improvement, it will be mediated to a substantial extent through the manipulation of cells in culture.

References (Davies)

Alt, F. W., Kellems, R. E., Bertino, J. R. & Schimke, R. T. 1978 Selective multiplication of dihydrofolate reductase genes in methotrexate-resistant variants of cultured murine cells. *J. biol. Chem.* **253**, 1357–1370.

Behnke, M. 1980 General resistance to late blight of *Solanum tuberosum* plants regenerated from callus resistant to culture filtrates of *Phytophthora infestans*. *Theor. appl. Genet.* **56**, 151–152.

Boulter, D. & Grocomo, O. J. 1979 Plant cell culture implications: legumes. In *Plant cell and tissue culture* (ed. W. R. Sharp *et al.*), pp. 615–631. Ohio State University Press.

Bourgin, J.-P. 1978 Valine-resistant plants from *in vitro* selected tobacco cells. *Molec. gen. Genet.* **161**, 225–230.

Broertjes, C., Roest, S. & Bokelmann, G. S. 1976 Mutation breeding of *Chrysanthemum morifolium* Ram. using *in vivo* and *in vitro* adventitious bud techniques. *Euphytica* **25**, 11–19.

Buiatti, M. 1977 DNA amplification and tissue culture. In *Plant cell, tissue, and organ culture* (ed. J. Reinert & Y. P. S. Bajaj), pp. 358–374. Berlin: Springer-Verlag.

Carlson, P. S. 1973 Methionine sulfoximine-resistant mutants of tobacco. *Science, N.Y.* **180**, 1366–1368.

Chaleff, R. S. & Carlson, P. S. 1975 Higher plant cells as experimental organisms. In *Modification of the information content of plant cells* (*2nd John Innes Symposium*) (ed. R. Markham *et al.*), pp. 197–214, North Holland/American Elsevier.

Chaleff, R. S. & Parsons, M. F. 1978 Direct selection *in vitro* for herbicide-resistant mutants of *Nicotiana tabacum*. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5104–5107.

Collins, G. B. & Legg, P. D. 1979 Use of tissue and cell culture methods in tobacco improvement. In *Plant cell and tissue culture* (ed. W. R. Sharp *et al.*), pp. 585–614. Ohio State University Press.

Cullis, C. A. 1977 Molecular aspects of the environmental induction of heritable changes in flax. *Heredity, Lond.* **38**, 129–154.

Cullis, C. A. & Charlton, L. 1981 The induction of ribosomal DNA changes in flax. *Pl. Sci. Lett.* **20**, 213–217.

Curtis, G. 1967 Graft-transmission of male sterility in sugar beet (*Beta vulgaris* L.) *Euphytica* **16**, 419–424.

Dale, P. J. 1975 Tissue culture in plant breeding. *Rept Welsh Pl. Breed. Stn 1975*, pp. 101–115.

Davies, D. R. 1973 *In vitro* propagation of *Freesia*. *John Innes A. Rept*, pp. 67–68.

Davies, D. R. 1975 Studies of seed development in *Pisum sativum*. 1. Seed size in reciprocal crosses. *Planta* **124**, 297–302.

Davies, D. R. 1977 DNA contents and cell number in relation to seed size in the genus *Vicia*. *Heredity, Lond.* **39**, 153–163.

Davies, D. R. 1980 The r_a locus and legumin synthesis in *Pisum sativum*. *Biochem. Genet.* **18**, 167–179.

Domoney, C., Davies, D. R. & Casey, R. 1980 The initiation of legumin synthesis in immature embryos of *Pisum sativum* L. grown *in vivo* and *in vitro*. *Planta* **149**, 454–460.

Dore, C. 1975 La multiplication clonale de l'asperge (*Asparagus officinalis* L.) par culture *in vitro*: son utilization en selection. *Annls Amél. Pl.* **25**, 201–224.

Dunwell, J. M. & Cornish, M. 1978 Production of tetraploids in carnation. *John Innes A. Rept*, p. 42.

Dunwell, J. M. & Davies, D. R. 1975 Technique for propagating inbred brussel sprout strains by means of tissue culture. *Grower* **84**, 105–106.

Durrant, A. 1962 The environmental induction of heritable change in *Linum*. *Heredity, Lond.* **17**, 27–61.

Earle, E. D. 1978 Phytotoxin studies with plant cells and protoplasts. In *Frontiers of plant tissue culture* (ed. T. A. Thorpe), pp. 363–372. The International Association for Plant Tissue Culture.

Gengenbach, B. G., Green, C. F. & Donovan, C. M. 1977 Inheritance of selected pathotoxin resistance in maize plants regenerated from cell cultures. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5113–5117.

Gerstel, D. U. & Burns, J. A. 1976 Enlarged euchromatic chromosomes (megachromosomes) in hybrids between *Nicotiana tabacum* and *N. plumbaginifolia*. *Genetica* **46**, 139–153.

Helgeson, J. P., Haberlach, G. T. & Upper, C. D. 1976 A dominant gene conferring disease resistance to tobacco plants is expressed in tissue cultures. *Phytopathology* **66**, 91–96.

Hibberd, K. A., Walter, T., Green, C. E. & Gegenbach, B. C. 1980 Selection and characterization of a feedback-insensitive tissue culture of maize. *Planta* **148**, 183–187.

Holdgate, D. P. 1977 Propagation of ornamentals by tissue culture. In *Plant cell, tissue, and organ culture* (ed. J. Reinert & Y. P. S. Bajaj), pp. 18–43. Berlin: Springer-Verlag.

Hussey, G. 1978 *In vitro* propagation of the onion *Allium cepa* by axillary and adventitious shoot proliferation. *Scient. Hort.* **9**, 227–236.

Hussey, G. & Hepher, A. 1978 Clonal propagation of sugar beet plants and the formation of polyploids by tissue culture. *Ann. Bot.* **42**, 477–479.

Jones, L. H. 1974 Propagation of clonal oil palm by tissue culture. *Oil Palm News* **17**, 1–8.

Kasha, K. J. 1974 Haploids from somatic cells. In *Haploids in higher plants* (ed. K. J. Kasha), pp. 67–87. University of Guelph Press.

Kinnersley, A. M. & Dougall, D. K. 1980 Correlation between the nicotine content of tobacco plants and callus cultures. *Planta* **149**, 205–206.

Kohlenbach, H. W. 1965 Über organisierte Bilgungen aus *Macleaya cordata* Kallus. *Planta* **64**, 37–40.

Lichter, R. 1978 The restoration of male fertility in cytoplasmic male-sterile sugar beet by heat treatment and meristem culture. *Z. PflZücht.* **81**, 159–165.

McKusick, V. A. & Ruddle, F. H. 1977 The status of the gene map of the human chromosomes. *Science, N.Y.* **196**, 390–405.

Maliga, P. 1978 Resistance mutants and their use in genetic manipulation. *Frontiers of plant tissue culture* (ed. T. A. Thorpe), pp. 381–392. The International Association for Plant Tissue Culture.

Marks, G. E. & Davies, D. R. 1979 The cytology of cotyledon cells and the induction of giant polytene chromosomes in *Pisum sativum*. *Protoplasma* **101**, 73–80.

Márton, L. & Maliga, P. 1975 Control of resistance in tobacco cells to 5-bromodeoxyuridine by a simple mendelian factor. *Pl. Sci. Lett.* **5**, 77–81.

Matthews, P. & Dunwell, J. M. 1979 The relationship between the resistance of seedlings and cuttings to Fusarium wilt in the carnation. *John Innes A. Rept*, p. 48.

Millerd, A., Spencer, D., Dudman, W. F. & Stiller, M. 1975 Growth of immature pea cotyledons in culture. *Aust. J. Pl. Physiol.* **2**, 51–59.

Murashige, T. 1978 The impact of plant tissue culture on agriculture. In *Frontiers of plant tissue culture* (ed. T. A. Thorpe), pp. 15–26. The International Association for Plant Tissue Culture.

Narayanaswamy, S. 1977 Regeneration of plants from tissue culture. In *Plant cell, tissue, and organ culture* (ed. J. Reinert & Y. P. S. Bajaj), pp. 179–206. Berlin: Springer-Verlag.

Nickell, L. G. 1977 Crop improvement in sugarcane: studies using *in vitro* methods. *Crop Sci.* **27**, 717–719.

Nunberg, J. H., Kaufman, R. J., Schimke, R. T., Urlaub, G. & Chasin, L. A. 1978 Amplified dihydrofolate reductase genes are localized to a homogeneously staining region of a single chromosome in a methotrexate-resistant Chinese hamster ovary cell line. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5553–5556.

Pandey, K. K. 1980 Further evidence for egg transformation in Nicotiana. *Heredity, Lond.* **45**, 15–29.

Polacco, J. C. 1976 Nitrogen metabolism in soybean tissue culture. *Pl. Physiol.* **58**, 350–357.

Raghavan, V. 1977 Applied aspects of embryo culture. In *Applied and fundamental aspects of plant cell, tissue, and organ culture* (ed. J. Reinert & Y. P. S. Bajaj), pp. 374–397. Berlin: Springer-Verlag.

Stafford, A. & Davies, D. R. 1979 The culture of immature pea embryos. *Ann. Bot.* **44**, 315–321.

Steward, F. C., Mapes, M. O. & Mears, K. 1958 Growth and organized development of cultured cells. *Am. J. Bot.* **45**, 705–708.

Thompson, J. F., Madison, J. T. & Meunster, A.-M. E. 1977 *In vitro* culture of immature cotyledons of soya bean (*Glycine max* L. Merr.). *Ann. Bot.* **41**, 29–39.

Widholm, J. 1980 Differential expression of amino acid biosynthetic control iso-enzymes in plants and cultured cells. In *Plant cell cultures: results and perspectives* (ed. F. Sala *et al.*), pp. 157–159. Elsevier/North Holland.

Discussion

S. Bright (*Rothamsted Experimental Station, Harpenden, Herts, U.K.*). Some recent work on plant mutants that accumulate amino acids is relevant here after Professor Davies's paper in which he questioned whether accumulation would be expressed in seeds. Complex diploid tissue cultures of maize (Hibberd *et al.* 1980) or mature embryos of diploid barley have been used to select mutants resistant to lysine plus threonine (Bright *et al.* 1981). In both cases, threonine and methionine are accumulated in the growing tissues. One barley mutant, Rothamsted 2501, contains a single dominant gene for resistance (associated with recessive lethality). Normal seeds contain little soluble threonine (less than 1 % of total threonine) whereas seeds from resistant plants have 15 % of the total threonine in the soluble fraction. This is sufficient to change the total threonine content. There is evidence for increased total methionine also in the barley mutant described above: as soluble methionine is very low this must be in protein.

Reference

Bright, S. W. J., Miflin, B. J. & Rognes, S. E. 1981 Threonine accumulation in the seeds of a barley mutant with an altered aspartate kinase. (Manuscript in preparation.)

A SIMPLE METHOD FOR GROWING PLANTS

J. M. BRANNON

(Received for publication December 6, 1920)

In growing plants under sterile conditions, investigators have employed either agar cultures or some other substratum of solid or semi-solid character placed in culture tubes, or else they have used water or soil cultures. In the water or soil cultures the roots only are maintained under sterile conditions; the leaves and stems being exposed to an unconfined atmosphere. In the course of certain experiments which are to be reported at a future date, it was found that neither the agar-culture method nor the water-culture method was satisfactory for growing green plants in the dark.

In the course of investigations on the organic nutrition of plants, it was noted at various times that seeds would germinate and seedlings would grow even when entirely immersed in a liquid medium. As a result of these incidental observations, it was decided to test the possibility of using such liquid cultures for the investigations. Striking successes were obtained, and the superiority of this method for growing plants in the dark over the agar method or the water culture methods hitherto used was at once apparent.

In a flask or culture tube, the size depending upon the plants to be grown and upon the duration of the experiment, is placed the culture solution. The depth of the solution should not exceed six centimeters. The vessels are plugged with cotton and then autoclaved. The seeds to be sown are then sterilized and the desired number sown in the culture solution. In the work here reported, the seeds were sterilized by the calcium hypochlorite method of Wilson.[1] This method of growing plants has been used with flax, alfalfa, corn, pea, and timothy. These were all grown in the dark. The pea and alfalfa have been grown for nine months in the dark when supplied with sugar. The method may also be used for growing plants in the light.

Table 1 gives data from an experiment with timothy grown in the dark,

TABLE 1

Solution Used	Weight of Individual Plant Exposed in Gms.	Average Length of Plants in Cm.	No. of Leaves
Pfeffer's + sugar	0.0180	13.5	4
Pfeffer's + sugar	0.0172	15.	
Pfeffer's + sugar	0.0184	16.	2
Pfeffer's alone	0.0071	6.5	

[1] Wilson, J. K. Calcium hypochlorite as a seed sterilizer. Amer. Jour. Bot. 2: 420–427. 1915.

and in figure 1 is shown one of the cultures. The nutrient solution contained 2 percent sucrose.

The special advantage of this method is in the fact that the plants used will live and grow for a much longer period of time than by the other methods.

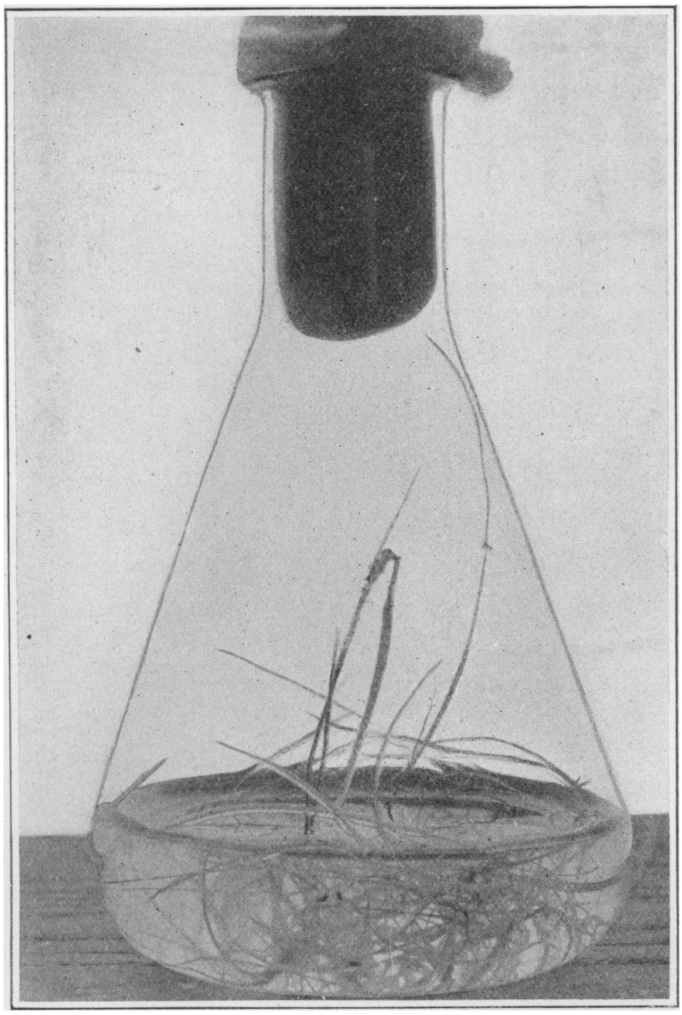

FIG. 1. Timothy grown for three weeks in the dark on Pfeffer's nutrient solution plus 2 percent sucrose.

It would seem, in the case of plants grown in the dark, that the sugars are either too slowly absorbed by the roots or that conduction of the sugars is too slow to satisfy the needs of the plant for organic matter. This idea has been suggested by Knudson and Lindstrom[2] in their work on albino corn. When a portion of the stem of the plant is also immersed, the stem probably absorbs sugars and so the needs of the plant are more nearly met.

Another advantage over the agar method is the greater ease of analyzing

[2] Knudson, L., and Lindstrom, E. W. Influence of sugars on the growth of albino plants. Amer. Jour. Bot. 6: 401–405. 1919.

the solution. In the agar method the agar must first be removed before the sugar determination can be made. Adsorption phenomena inadvertently play a part in the precipitation of agar, and thus another source of error is introduced.

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Germ-Resistant Plants

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ENTOMOLOGY

Grain Pest Season Here

➤ THE PEAK SEASON is here for devastation of stored grains by one of the most prolific and destructive insect pests in the country, the khapra beetle.

A U. S. Department of Agriculture expert told SCIENCE SERVICE he had seen a bin of barley, 100x40x14 feet, completely ruined by these tiny pests, which were only discovered in this country in 1953. In another bin, he saw khapra beetle larvae and cast-off skins one foot deep.

Since its successful eradication in New Mexico, the khapra beetle is believed to be bottled up in California and Arizona, where it continues to strike at stored barley and grain sorghum with disastrous results. Worried entomologists, however, are appealing to farmers and grain storage men to keep watching for the pest and to report its occurrence in an effort to keep it from spreading.

The khapra beetle, native of India, Ceylon and Malaya, has already spread from Japan, the Philippines, and Australia, to England, Europe and Africa. Although only found here in 1953, some astute detective work by the USDA indicates that it must have gotten into the Fresno area of California as early as 1946.

The insect belongs to the same family as carpet beetles and resembles them. It can be spotted in grain storage facilities by the presence of fuzzy larvae or cast-off skins, about one-eights of an inch long, in clusters around the corners of grain bins or in used sacks. Like rice and granary weevils, it attacks sound kernels of grain.

The only effective remedy for the khapra beetle is to cover infested bins with a tarpaulin and fumigate thoroughly. Fumigation will not hurt the grain for use as feed, the USDA said.

Anyone who thinks he has discovered khapra beetles in stored grains should send specimens to: Insect Identification and Parasite Introduction Section, U.S. Department of Agriculture, Beltsville, Md. Specimens should be placed in rubbing alcohol in a leakproof jar or vial. Do not send live specimens through the mail.

Science News Letter, August 27, 1955

AGRICULTURE

Germ-Resistant Plants

➤ MAN'S FIGHT against famine is netting startling victories, as new weapons pour forth from atomic laboratories and plant breeding stations that are putting resistance to diseases right into the hereditary make-up of his food crops.

Scientists from all over the country heard of the revolutionary progress in reports given to the American Society of Agronomy meeting in Davis, Calif.

Use of atomic particle radiation to induce hereditary changes in plants that leave them immune to certain diseases was reported by Dr. Calvin Konzak of the Brookhaven National Laboratory.

In his experiment, Dr. Konzak exposed oat seeds to radiation from an atomic reactor. The variety of oats he used was known to be highly susceptible to the fungus disease, helminthosporium blight.

He planted the seeds and, after they had sprouted, inoculated them with the destructive fungus.

Several of the plants from radiation-exposed seeds were resistant to the fungus disease. This resistance was found to be passed on to the offspring of the disease-immune oat plants, showing that the atomic radiation had done its beneficial work by changing the heredity of the plants.

In another experiment to breed disease-fighting power into plants, the high, inheritable resistance to leaf rust of a wild relative of domestic wheat has been transformed to the wheat plant itself, reported Dr. Ernest R. Sears, geneticist with the U.S. Department of Agriculture.

The wild "wheat" used was a member of the goatgrass family, Aegilops umbellulata, which is practically immune to leaf rust. Since it was impossible to cross the goatgrass directly with domestic wheat, Dr. Sears crossed it first with an intermediate plant, emmer wheat. The offspring of these two could then be crossed successfully with common wheat, with the disease resistance being passed on to the resulting hybrid.

The new rust-resistant wheat strains are still far from ready for commercial use, Dr. Sears said. The task of combining the rust resistance with other desirable qualities in a single variety of wheat still lies ahead for plant breeders.

While many plant breeders are looking for naturally-occuring or induced hereditary changes as a source of new and better varieties, Jack R. Harlan of the U.S. Department of Agriculture and Oklahoma A & M College told the A.S.A. meeting that more effort should be spent exploring in foreign countries for plants with the desired qualities already in existence.

Nearly all of the forage crops in the United States were imported here from somewhere else Mr. Harlan pointed out. In bringing them here we have brought only a very small and very restricted sample of the many variations that exist, he said.

As an example, orchard grass in the United States now is restricted in use because of lack of heat and drought tolerance. But in Mediterranean countries there are forms of this grass that prosper with less than eight inches of rainfall a year.

More progress in improving hereditary qualities might be made more quickly and at less expense by introducing these old-world forms than by conventional breeding programs based on current limited samples he said.

Science News Letter, August 27, 1955

BIOCHEMISTRY

Seek Clues to Inborn Errors in Body and Mind

➤ CERTAIN INBORN errors in body chemistry cause deviation from normal processes, leading to mental deficiency and other ailments is being studied by Dr. Max Dunn, University of California at Los Angeles biochemist, and others aided by grants from Swift and Company.

One such deviation results in inferior mental ability and is known as phenylketonuria. It is the result of a faulty processing of the amino acid phenylalanine.

Another instance in which body chemistry veers from the normal is a rare condition known as alcaptonuria, characterized by marked discoloration of excretory products. Key to the condition is homogentisic acid, which is being structurally explored by the group.

"It is only by scrutinizing these chemical errors in minute detail," Dr. Dunn pointed out, "that we can spot where body chemistry turned left when it should have turned right. From such information can be devised means of correcting harmful deviations in life processes."

Associated with Dr. Dunn in the research have been Howard Wolkowitz, Bernard Kaufman and Machio Yuchida.

Science News Letter, August 27, 1955

TECHNOLOGY

Flame-Proofing for Cotton Perfected

➤ A FLAME-PROOFING TREATMENT for cotton materials, much superior to previous methods, has been developed jointly by the U. S. Department of Agriculture scientists and the Army Quartermaster Corps' research branch.

A combination of two chemicals that up to now were used separately for flame-proofing cotton are employed in the new technique. In the treatment, one part of BAP, or bromoform-allyl-phosphate, is added to two parts of THPC-resin solution, or tetrakis (hydroxymethyl) phosphonium chloride, and applied to the cloth, which is then dried and heat-cured. The process increases the weight of the cloth about 18%, but shows little effect on other fabric qualities. The flame-proofing holds up well under both laundering and dry cleaning.

Science News Letter, August 27, 1955

Ozone: Selective Force in Plant Evolution?
Author(s): C. Simon
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satellites in space. Describing his agency's new posture before the Senate subcommittee on strategic and theater nuclear forces earlier this year, Undersecretary for Directed Energy Weapons Major General Donald Lamberson said DOD currently expects to spend $900 million for research on space lasers during the next five years, prior to beginning expensive demonstrations in orbit. Roughly $600 million will go for programs to investigate the technical feasibility and cost effectiveness of using lasers in space. Three programs directed by the Defense Advanced Research Projects Agency (DARPA) — ALPHA, LODE, and TALON GOLD — will dominate these efforts.

Lamberson says ALPHA is investigating the prospects for high-powered mid-infrared-wavelength devices, though some shorter-wavelength laser systems are being looked at too. LODE is examining the feasibility of producing very large, precision mirrors to direct laser beams at their targets. It is also focusing on the difficulties of directing these beams at high brightness levels. TALON GOLD is concentrating on problems associated with locking a laser beam onto a moving target from space — a target that will likely be moving five or more times faster than the Sidewinders encountered in the recent Air Force tests.

The Army's role in the Space Laser Program is more modest. Focusing on ballistic-missile defense, it is chiefly investigating the extent to which missiles can be "hardened" (protected) against laser radiation. The Army is also concentrating on short-wavelength lasers, the type expected to prove most useful in space operations. For its part, the Air Force is studying the hardening of aircraft, satellites and other potential targets for their survival under an attack by enemy weapons, including lasers.

Responding to a growing public concern over the further militarization of space, DARPA Director Robert Cooper told the Congress on March 23 of this year, "We are conducting research and planning related to space weaponry, but I emphasize that no commitment has been made to acquire space-based weapons. And, we will proceed only if our national security is so threatened." —*J. Raloff*

Ozone: Selective force in plant evolution?

Scientists, spurred by the prediction that in the next decade stratospheric ozone may be partially depleted, are trying to learn how such a decrease might affect not only human health, but plant health too. One such study has led researchers to suggest that plants that originated at tropical and temperate latitudes display different levels of sensitivity to solar ultraviolet-B (UV-B) radiation, much of which is absorbed by the ozone layer before reaching the earth.

Earlier research also has shown that plants now living at different latitudes vary widely in their tolerance to ultraviolet-B radiation. The amount of UV-B that reaches the earth is linked to the thickness of the ozone layer because stratospheric ozone absorbs most of the invisible light before it touches the planet. Plants growing in tropical latitudes, where natural levels of UV-B are the highest on earth, are more resistant to the radiation than plants in temperate latitudes, where most of the world's food crops are grown. Botanists Alan Teramura of the University of Maryland in College Park, and Martyn Caldwell of Utah State University in Logan, report that the degree of tolerance to UV-B is related to the level of the radiation at the time specific plants evolved. The ozone layer and its effect on UV-B, they say, may have been a selective factor in plant evolution.

In field studies and controlled experiments, 90 agricultural plant species were exposed to UV-B. At first the researchers could not identify a common factor within plant families that makes the plants more or less resistant to the radiation. But when the researchers considered where the plants originated, they found that three times as many crops that evolved in temperate latitudes in the Near East, Northern China and Mesoamerica (roughly north central North America to Nicaragua) were adversely affected by the same level of UV-B as crops that evolved in tropical latitudes in mid-Africa, Southeast Asia and South America. Teramura and Caldwell assert that naturally occurring UV-B has been an "important selective force in the evolutionary history of these agricultural species."

The plants with low resistance to UV-B are particularly vulnerable, the scientists found, because increases in UV-B radiation inhibit their photosynthesis, result in smaller plant size and smaller leaf area, and reduce yield and yield quality. Soybeans, for instance, fare poorly when UV-B levels are too great. The crop is cultivated because its seeds contain high proportions of oils and proteins. Some varieties, Teramura says, produce less oil and protein when exposed to levels of UV-B that are outside the tolerance ranges of the plants.

It is estimated that the protective layer of stratospheric ozone may be depleted from 5 to 9 percent in the next decade, primarily due to human use of chlorofluorocarbons in refrigerants and other industrial applications (SN: 4/10/82, p. 244). The increase in UV-B radiation would be disproportionately large at temperate latitudes, scientists say, with a 19 percent increase in the amount of UV-B radiation capable of affecting plant biology.

—*C. Simon*

Sex switch stimulated by size

In the lonely hearts club of coral reef fish, when the going gets tough, the tough change sex. Many fish are hermaphroditic, but most species change sex because they lack a nearby mate. For the first time, researchers have now found at least one species that bases its sex on the relative size, not the sex, of its neighbors.

Female saddleback wrasse *(Thalassoma duperrey)* can change to male. While smaller fish of either sex stimulate a female to switch, larger fish inhibit such a change. "Basically, if you put two of these fish together, only the bigger one will become a male," says Milton Diamond of the University of Hawaii in Honolulu.

As a result, the larger fish are usually male, either by birth or by subsequent sex change. On the reef, a relatively small female is likely to encounter males. But if the proportion of larger fish drops, a female would find more mates if she changed sex. "Since fish are considered to be fairly highly evolved, this brings up a number of philosophical implications," says Diamond. "The social situation of these animals determines their sexual physiology and behavior."

Like most fish, the dull green saddleback wrasse has no detectable sex chromosomes, yet when it reaches sexual maturity, it produces either sperm or eggs. "The initial sex is probably determined by multiple sites on different chromosomes," says Robert M. Ross, of the Hawaii Institute of Marine Biology in Kaneohe. "This makes the wrasse very sexually labile." Females can later stop producing eggs and start producing sperm. This protogynous (female first) sex change takes two to three months and is non-reversible. Since the wrasse cannot produce both sperm and eggs simultaneously, it cannot fertilize itself. Says Ross, "Virtually all females eventually become males, given the right social conditions."

To determine those conditions the Hawaii group studied isolated females, and females placed with one to three smaller, sexually mature fish. As reported in the Aug. 5 SCIENCE, the lone females continued to produce eggs, as did those in pens with larger saddleback wrasse or smaller fish of another species. But females that were the largest of their species changed sex even if the smaller fish were male.

In species that live in Harems, removing the dominant male prompts the largest female to switch sex. But the social structure of the promiscuous saddleback, which breeds in temporary pairs or swarms, is less clear-cut. "You can't usually tell a male from a female by color," says Ross. "The size ratio of nearby fish may be the best clue to sexual strategy."

—*S. Steinberg*

Race to find human stem cells ends in tie

Two research groups are reporting the isolation of seemingly immortal human cells that can give rise to any cell type in the body.

Researchers hope ultimately to use these cells, known as embryonic stem (ES) cells, to study human development, test drugs, and provide unlimited supplies of cells to replace tissues damaged by diseases or injuries. ES cells induced to form heart cells, for example, might help strengthen failing hearts. Or neurodegenerative illnesses, such as Parkinson's disease, might be treated with transplants of brain cells grown from ES cells.

Human ES cells are "potentially going to revolutionize medicine in the next century," says Austin G. Smith of the University of Edinburgh, Scotland, who has been searching for these mother cells.

Most human cells are specialists, forced during embryo development to choose a lifetime career as, say, muscle or liver cells. But until they make such a commitment, embryonic cells retain their ability to develop into any cell type. Recognizing the potential uses of these unrestricted cells, several research teams have braved the furor of working with human embryos and fetuses and have raced to isolate human ES cells.

At a meeting last summer, John D. Gearhart of Johns Hopkins Medical Institutions in Baltimore described his group's apparent success at finding these versatile cells by sifting through tissues, from aborted fetuses, normally destined to give rise to either sperm or egg cells (SN: 7/19/97, p. 36). Gearhart and his colleagues now detail their results in the Nov. 10 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

Researchers led by James A. Thomson of the University of Wisconsin-Madison have also unearthed human ES cells, but by following a strategy they employed to find monkey ES cells (SN: 8/26/95, p. 139). They plucked cells from the insides of human blastocysts, balls of about 100 cells at an early stage of embryonic growth. The blastocysts were originally created during in vitro fertilization efforts but went unused, says Thomson.

The blastocyst cells have proved able to replicate indefinitely; Thomson's group has kept some alive for 9 months. Moreover, in test-tube experiments, the cells show an ability to differentiate into specialized cells. When injected into mice, the putative ES cells form growths of human cells containing bone, muscle, nerve, and many other cell types, the researchers report in the Nov. 6 SCIENCE.

In addition to their medical potential, human ES cells should allow biologists to study areas of human development not well mirrored in animals such as mice. Thomson plans to examine how the cells differentiate into placental cells. "The placenta in mice and people are completely different," he notes.

Thomson suggests that human ES cells may also speed drug discovery. A firm wishing to test thousands of potential heart drugs might use ES cells to generate massive amounts of human heart cells. "You could screen 50,000 potential drugs and pick out the 3 that look promising," he says.

To provide desired cells for transplants or drug screening, investigators must still learn to convert ES cells into specialized cells. "You have to figure out how to teach cells which pathways to go down," explains David I. Gottlieb of Washington University in St. Louis. He and other researchers, for example, have already induced mouse ES cells to develop into neurons and other types of brain cells. That experience should carry over, predicts Gottlieb. "I'm very confident we will quickly go from human ES cells to human neurons," he says.

Some scientists hope to take a shortcut in that journey by using neural stem cells. While seemingly immortal, like ES cells, neural stem cells have already made a limited career choice. They can develop into the various cell types of the brain, but not into those of other tissues.

In the November NATURE BIOTECHNOLOGY, Evan Y. Snyder of Children's Hospital in Boston and his colleagues report for the first time the isolation of human neural stem cells. Derived from the brain tissue of an aborted fetus, these stem cells have been kept alive and healthy in the laboratory for more than 2 years. The researchers have also injected the neural stem cells into the brains of newborn mice and confirmed that the cells develop into neurons and glia, the two major classes of brain cells. Snyder's group can even add new genes to the stem cells, a skill that could prove useful in treating certain human brain disorders.

In a related NATURE BIOTECHNOLOGY paper in the same issue, Ronald D.G. McKay of the National Institute of Neurological Disorders and Stroke in Bethesda, Md., and his colleagues describe how they injected human fetal brain tissue into brains of embryonic rats. The human cells formed every kind of brain cell and integrated into all major regions of the rodents' brains. Creating such chimeric brains, notes McKay, should help scientists better understand how embryonic human brain cells develop, migrate, and form connections, issues almost impossible to investigate experimentally in people. —*J. Travis*

Radiation gives these plants the blues

With its chlorophyll extracted, this plant becomes a potential botanical Geiger counter by displaying some of its radiation-induced mutations as blue spots. These spots record the gene-altering threat of radioactive pollution, including fallout.

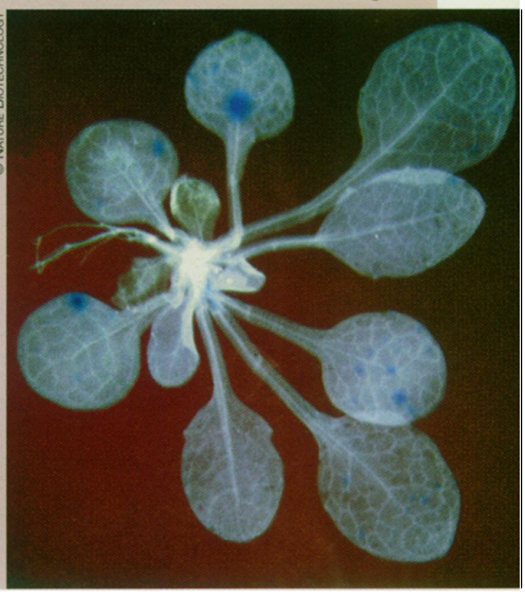
A Ukrainian-Swiss research team inserted inactive bacterial genes into thale cress (Arabidopsis thaliana). When mutated, these genes make an enzyme that accepts a standard, blue chemical stain.

Working both in a laboratory and at outdoor locations around Ukraine, the scientists exposed the plants for several weeks to soil tainted with fallout from the 1986 Chernobyl reactor accident.

The greater the radiation dose, the more plant tissue accepted the blue stain, the researchers report in the November NATURE BIOTECHNOLOGY. The increase in staining correlated with the genetic damage the researchers measured in chromosomes of onions exposed to similar levels of radiation.

The mutation rate fell, however, once radiation levels got too high (about 900 curies per square kilometer). At these exposures, the plants' cells began dying, explains Barbara Hohn of the Friedrich Miescher Institute in Basel, Switzerland, a study author. In practice, Hohn suspects, "Pots [of plants] would be put into contaminated areas for a week or two" and then treated to reveal any spots.

This is "a handy and useful tool," says geneticist Yuri E. Dubrova of the University of Leicester in England, who studies Chernobyl's effects. Until now, he notes, "it's required literally hours with a microscope and damaging one's eyes to [tally] chromosome aberrations" due to radiation. —J. Raloff



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'Welcome to the Atomic Park': American Nuclear Landscapes and the 'Unnaturally Natural'

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ABSTRACT

Atomic landscapes in the American West are typically understood as despoiled and irradiated territories. Nevada Test Site, with its grim medley of twisted military structures, bombed-out craters and radioactive desert, is an emblem of the nuclear age. By contrast, Yosemite National Park is a very different icon to hail from Western climes. Yosemite is legendary for its wild nature and monumental scenery. The two landscapes, Nevada Test Site and Yosemite National Park, have, on the surface, very little in common. However, in recent years, a number of nuclear and post-nuclear landscapes have been praised for attracting rare species of flora and fauna. A few nuclear sites have even become nature reserves. While aware that so-called atomic parks are hardly likely to become the Yellowstones and Yosemites of the late twenty-first century, this article explores a few of the unexpected links between two forms of landscape for so long considered extreme opposites.

KEY WORDS

Nuclear age, parks, American West, landscape

In 1962, Alfred Hitchcock filmed *The Birds* at Bodega Bay, a quiet fishing community fifty miles north of San Francisco. Hitchcock used the peaceful coastal village as a backdrop for a harrowing story of nature out of control. His depiction of a flock of seagulls terrorising small-town America won substantial acclaim as a natural disaster masterpiece. At the same time that Hitchcock faked an avian menace on the shores of Bodega, town residents rallied against a formidable nuclear presence. A major California electrical utility, Pacific Gas and Electric (PG&E), hoped to construct an atomic power plant on the wild

Environment and History **7** (2001): 449–72

reaches of Bodega Head peninsula. PG&E officials insisted that their nuclear project posed no threat to the region. A billboard on the perimeter of the inchoate construction site announced 'Welcome to Bodega Bay Atomic Park'.[1] The 'atomic park' promised an outlandish blend of high technology and primordial nature, public energy provision and coastal recreation. Yet some northern Californians remained unimpressed. Anti-nuclear campaigner David Pesonen distributed a pamphlet entitled 'A Visit to the Atomic Park' highlighting the less welcome features of PG&E's nuclear enterprise. According to Pesonen, Pacific Gas had misled citizens of Bodega as to the true nature of its project, with 'the use of the word "park" to describe a massive atomic complex' just one example of corporate unreasonableness.[2] A state park, rather than an atomic park, appeared the safer option for Bodega.

Competing visions of Bodega Head as an atomic park and a state park reflected the immense cultural symbolism attached to the park label in the latter half of the twentieth century. In post-1945 America, the 'park' emerged as a mass-produced icon of pleasure. Seeking a higher quality of life, US citizens found solace in the open spaces of city and state parks. Increased leisure time fuelled a boom in recreation, with the park promising redemption from the ills of congested, urban society.[3] Business magnates, recognising the cachet attached to the word 'park', renamed their manufacturing complexes 'industrial parks' and 'research parks'.[4] Walt Disney called his carnival-like fairgrounds 'theme parks'.[5] However, it was the 'national park' that most captivated the imagination of America in the 1950s and 1960s. In laden station wagons, middle-class Americans travelled to national parks on the weekends. The great outdoors attracted droves of vacationers. In 1965, Yellowstone National Park received two million visitors for the first time in its history.[6] The national park, with its rustic signposts and inviting picnic benches, represented the ultimate park – the archetypal outdoor recreational experience.

The atomic park was something else entirely. Both the atomic bomb and the national park were born in the American West. Yet the US park ideal, often celebrated as 'the best idea we ever had', shared little in common with dreams of artificial energy sources and unassailable military might.[7] National parks and nuclear sites represented disparate landforms and mindscapes. One represented the apogee of American conservationist thinking, the other highlighted the destructive potential of high technology. Test sites were treated as verbatim wastelands. While US citizens celebrated the national park as a repository of wilderness values, landscape gardening at nuclear plants conjured images of scientifically managed and modified plant life, artificial lawns in white, futuristic cities. At Bodega, PG&E employed the park motif in the hope of naturalising the atom, but failed to elaborate on the abstruse links between nuclear energy production and nature protection, of how the construction of reactor sites could practically service the preservation of wilderness. While initially receptive to claims of a clean, environmentally friendly energy source, conservationists grew

wary of atomic power, and became fearful of an accidental release of radiation into the biosphere. By the late 1970s, anti-nuclear activists had convinced the American public that there was nothing natural about atomic power.[8]

The axiomatic gulf between nuclear installations and nature preserves has traditionally barred any meaningful comparison between these two discrete forms of land use. However, exploring the history of the 'park' in its nuclear and preservationist incarnations suggests that apocalyptic and Edenic landscapes are not always polar opposites. The vigour with which nuclear lands have been derided, and nature parks exalted, owes more to entrenched social values than to any extensive consideration of the places involved. Nuclear landscapes have for too long been typecast as infertile no-mans-lands. Despite the irreverence of the comparison, the nature park offers a fresh perspective on atomic soil.

It is the intention of this article to explore the unexpected common ground between nature parks and nuclear landscapes. By considering how such lands were originally set aside, what practices (and attitudes) governed their early development, and what purpose they came to serve in the modern era, the 'atomic park' is intellectually set alongside more conventional park systems. Preservationist and military mandates are usefully compared. The term 'nature', employed in this essay to describe healthy biodiversity (usually due to a relative paucity of human impact), emerges as a complex, culturally laden, and idealistic reference point. In the light of what we know about radiation and its potential to cause genetic damage, it is hard not to think of atomic landscapes as 'unnatural'. In turn, the concept of the atomic park remains, at best, 'unnaturally natural'.

CHOOSING SUITABLE PARKLAND

In locating and appropriating land for atomic purposes, nuclear planners often followed rationales comparable to the motivations of early park stewards. This section considers how nuclear authorities searched for wild and remote regions for their projects, eventually coming into competition with the American conservation movement.

In 1864, Yosemite Park was set aside for 'public use, resort, and recreation'.[9] However, in contrast to city parks, Yosemite proved distant from white American communities and, at that time, inaccessible to all but the richest or hardiest travellers. Yosemite was located 'in nature'. The remoteness of the parkland, along with its unsuitability for settlement or farming, made public acquisition all the easier. Just as Yosemite was celebrated for its magnificent cliffs and waterfalls, preserved intact and 'inalienable for all time', it was also deemed 'worthless' by its marginal economic importance in terms of resource extraction.[10] Later parks, such as Yellowstone National Park (1872) and Death Valley National Monument (1933), were established according to a similar rationale. From the 1940s onwards, nuclear industrialists also laid claim to wild, remote,

and marginalised places. The desire for secrecy, allied to concerns over radiation, encouraged nuclear developers to search for territories on the periphery of mainstream American society. Nuclear projects were best situated on uninhabited and undeveloped land, far from major cities.

Both nature park planners and nuclear industrialists imagined the landscapes about them. Gathered around a campfire at Madison Junction in 1870, members of the Washburn expedition articulated a desire for 'a great National Park' at Yellowstone.[11] Proponents envisioned a museum of natural curiosities preserved for public use, insulated from the worst excesses of private capitalism by arbitrary straight-line boundaries. Yellowstone duly became a national treasure, with the Madison campfire immortalised in popular memory as the birthplace of the American park idea.[12] The idea encouraged Americans to see land as virtuous due to its untouched and unpeopled status. Western regions were re-conceptualised. Park planners and nature preservationists mythologised spectacular mountain climes and plunging desert canyons as the pristine American 'wilderness'.[13] Meanwhile, Native American residents had no place in the virginal park scene. Like so many Euro-American concepts, the nature park ran roughshod over indigenous rights and customs. Remnant Indian nations were evicted from their ancestral territories.[14] Rather than primeval nature frozen in time, the park wilderness was an inherently modern construction, with its own destructive logic.

In *Savage Dreams*, environmental writer Rebecca Solnit described the assembly of 'physicists in the wilderness' at Los Alamos, New Mexico, in 1942.[15] Like park planners at the campfire, atomic physicists played out future scenarios in their heads, anticipating how atomic fires would transform both material and political landscapes. The Manhattan Project had brought nuclear science to the West. Seeking secret, remote and uninhabited terrain, military authorities had appropriated vast tracts of 'wilderness' for the manufacture of the world's first atomic bomb. Stretches of New Mexico and Washington were regarded as barren, unpopulated and readily available for atomic purposes. Like national park planners, atomic engineers superimposed their desires for vacant spaces onto the physical landscape. Native American nations and recalcitrant ranchers lost their lands during the expansion of military projects at Los Alamos and Hanford Engineering Works (Washington) in the early 1940s, and Nevada Test Site in the early 1950s. Lecturer in American Studies Valerie Kuletz labelled the process 'nuclear colonialism'.[16] In their capacity to annex Indian territories, atomic pioneers resembled Euro-American frontiersmen. Nineteenth-century homesteaders, miners, town developers and national park planners had all imagined the West to be theirs for the taking. The atomic imagination fed off prior misconceptions of landscape and lingering forms of racial prejudice.

In their search for land, park boosters and nuclear developers rarely competed for the same sites. However, in the 1960s, both conservationists and atomic industrialists fervently pursued the expansion of their respective territories.

FIGURE 1. The American West (selected nuclear sites and nature parks)

Recognising public support for outdoor recreation, conservationists campaigned for more state and national parks.[17] Meanwhile, the nuclear industry launched an ambitious reactor construction programme, tied to Eisenhower's promotion of 'Atoms for Peace'. Most conservationists at that time supported nuclear power as a preferred alternative to dam building. The American conservation lobby vilified hydroelectric projects as concrete behemoths threatening large-scale disruption of river ecosystems, while welcoming talk of ecologically benign, self-contained atomic energy facilities. However, support for the peaceful atom wavered when atomic developers chose sites of specific interest to the conser-

vation lobby. A relatively small number of environmentalists, concerned at the loss of valuable coastal scenery and the chances of radioactive accident, had clashed with nuclear enthusiasts in the early 1960s at Bodega Head. In the mid 1960s, Pacific Gas and Electric announced plans for another nuclear plant on the California coast, on the Nipomo Dunes, 65 miles north of Santa Barbara.

As a potential site for a nuclear park, PG&E rated Nipomo as 'good' in terms of 'local topography', 'isolation', and 'physical features'.[18] Meanwhile, conservationists valued Nipomo for its rare sand formations and aesthetic beauty, and vowed to protect the region from industrial encroachment. Atomic aficionados and nature lovers converged on the same location. 'Another Bodega Head' loomed on the California coastline.[19] However, in an unexpected turn of events, PG&E representatives and directors of the Sierra Club, a national conservation organisation, agreed to a land deal in summer 1966. In order to free Nipomo for state park purchase, the Sierra Club endorsed an alternative site for PG&E's nuclear project. Leading members of the Club professed no antipathy towards atomic power, and merely pressed for the plant to be placed in a more convenient location. The nuclear park was relocated fifteen miles north along the coastline, to Diablo Canyon.

Separated from the nearest town by a line of steep hills, Diablo Canyon was a remote and secluded spot on an undeveloped promontory. PG&E engineers judged the canyon to be 'excellent' in terms of 'geology, seismology, and foundation'.[20] Diablo represented prime atomic material. Diablo also turned out to be a wild stretch of California coastline with potential as parkland. In the rush to save Nipomo, directors of the Sierra Club had mistakenly cast Diablo Canyon as a 'treeless slot' bereft of ecological significance.[21] However, on discovering that the 'real' Diablo featured unsullied tide pools and record-size coastal live oaks, a number of renegade Sierra Club members challenged the agreement with Pacific Gas. Director Fred Eissler drew attention to a favourable National Park Service survey of the headland in 1959.[22] Sympathetic Club stewards presented the Diablo lands as 'California's Last Unspoiled Pastoral Coastland'.[23] Fearing the collapse of the 1966 deal, defenders of Nipomo insisted that Diablo failed to meet state park standards. Local conservationist Kathy Jackson argued: 'Diablo Canyon has not been wilderness since 1832. It is an overgrazed oak woodland and chaparral canyon'.[24] Directors Ansel Adams and William Siri declared Diablo 'prophetically named', growing 'out of the moving sands and rare flora of Nipomo to sow doubt and dissension'.[25] The ensuing controversy almost split the Club.[26]

The same qualities that marked Diablo an ideal location for nuclear development also confirmed its potential as a nature reserve. Remoteness, wildness, and an absence of humanity appealed to conservationists and developers alike. 'Save-Diablo' Sierra Club members duly admonished PG&E for its inability to avoid wild and cherished landscapes in its quest to build a state-wide energy system. 'With its almost magnetic attraction for the untouched site, the clean

sand and the blue water, [PG&E] selects a hitherto inviolated [sic] area, applies the blade of the bulldozer to it and then come tumbling down the ferns, the glens, the trees, the valley', commented one California Public Utility Commission staff member sympathetic to the 'Save-Diablo' cause.[27] PG&E rejected any claims that it was in competition with the state park system or conservationists. While corporate officials admitted that the Nipomo Dunes represented attractive parkland, Diablo Canyon was another matter entirely. As an 'undistinguished' headland of 'ordinary nature', Diablo was presented as worthless to all but hardy nuclear industrialists.[28] Once used as an argument *for* national parks in the late nineteenth century, worthlessness appeared on the side of the nuclear park system in the late 1960s. PG&E also reminded Californians of their increasing energy needs. The energy sufficiency of the whole state depended on a nuclear landscape at Diablo Canyon. By contrast, a nature park at Diablo promised an unwelcome return to the electrical dark ages.

IMPLEMENTING THE DESIGN

Even wilderness regions such as Yosemite and Yellowstone are now acknowledged as (at least partial) constructions of the human psyche, with wood cabins and paved roads practical attestations of federal presence. Meanwhile, nuclear landscapes carry the physical scars of prolonged military tests and reactor building programmes. This section explores the making of two kinds of landscape, and reveals how themes of mastery over nature, outbreaks of fear, and national pride can bind places together, as well as separate them.

In implementing their design plans, both national park stewards and atomic authorities at times demonstrated reprehensible attitudes towards resident flora and fauna. In 1953, following a series of atomic explosions at Nevada Test Site, over 4,500 sheep died from radiation burns on surrounding ranch land.[29] Military personnel hid behind a cloak of secrecy and scientific jargon, insisting that the herbivores died of eating toxic plants or malnutrition. Ranchers had trouble believing what they were told. The sheep appeared neither thin nor diseased, nor did rifles or ravenous predators kill them. The military-atomic complex was the true culprit. Authorities apparently realised the cause of animal deaths in the locality, but failed to disclose such information to beleaguered ranchers. Such malversation helped ferment a popular understanding of nuclear landscapes as places of nefarious scheming and malign portent in subsequent decades. That flora, fauna, along with 'guinea pig' soldiers, emerged as victims of the atomic age gave credence to the idea of nuclear terrain as inherently destructive. Nuclear protesters came to associate the secret designs implemented at nuclear landscapes with the failure of responsible government.

National parks, as paragons of democracy and public accessibility, avoided such intense scrutiny. The National Park Service remained a highly respected

federal authority, with the public thankful for its transparent two-fold *raison d'être* of wilderness protection and recreational provision. While Americans expected the Nevada Test Site to have a woeful past owing to military exigencies, national parks were assumed to be in a pristine condition thanks to enlightened land stewardship goals practised by the Park Service. Yet, in a sense, national parks had their own secret past. Designs to protect 'nature' in early park systems (namely herds of local ungulates) entailed the premeditated killing of resident predators, with end results comparable to the radioactive sheep cull in Nevada in the 1950s. In national parks from the 1870s to the 1930s, hundreds of carnivores died from federal mismanagement. The United States Army assumed control of Yellowstone in 1886, and continued an anti-predator agenda inaugurated by early park stewards. Cavalry units also saw off any furtive enemies wandering Yosemite (1890), Sequoia (1890) and General Grant National Parks (1890). Sounds of gunshots and military patrols indicated that the first national parks began life as militarised zones. In 1916, the National Park Service, backed up by scientific dogma, institutionalised annual killing sprees. The grey wolf was one of the unfortunate species to be classified as a 'threat' to park ungulates and nature's balance. Just as likely to be killed inside as outside park borders, *Canis lupus* faced a torrent of prejudice. By the 1940s, the wolf had been extirpated from the continental United States.[30]

The burnt Nevada sheep and the castigated American wolves were the victims of large-scale human experiment. Military and park authorities relished exercising dominion over their respective territories. Federal officials sought absolute control of their surroundings. Destruction was tied to the creative process, with the laying of strychnine and the spread of plutonium part of the making of landscape.

Although at the time hidden from view, the scale of transformation that accompanied the nuclear age proved far-reaching. Manhattan Project engineers shaped vast expanses of the American West to match their World War and Cold War intentions. The Manhattan Project was huge in every way, from budgetary expenditure, to public deception, to the western lands appropriated for atomic testing. 'Secret' cities were constructed.[31] The wild western landscape was refashioned to meet an orderly military remit. Art historian Peter Hales located the Manhattan Engineering District as psychologically 'somewhere between an army base and a utopian social experiment'.[32] The Nevada Test Site, meanwhile, provided a 'massive outdoor laboratory' for the advancement of scientific knowledge.[33] Close to ground zero, army personnel packed beagles, mice, hogs and monkeys into wire cages to register the effects of atomic blasts, not realising that they too were 'experimental' animals. Nature incarnate represented the canary thrust into the mine as a meter of danger. In the 1950s, Project Plowshare took the nuclear experiment a stage further. Project proponent (and eminent nuclear physicist) Edward Teller insisted that atomic energy could be used to improve on nature's design. Grandiose plans included forging commercial ports,

melting polar ice caps, and transforming deserts into lush green paradises with the aid of nuclear explosives.[34] Whole ecosystems seemed ripe for redevelopment. Atomic energy promised the transformation of place on an unlimited scale, with the nuclear physicist assuming the al fresco role of landscape gardener.

Albeit on a far smaller scale, national park wardens similarly operated by an ethos of management, control and scientific advancement. Plant and animal populations were stringently monitored to meet park guidelines. Most wildlife biologists regarded intervention as necessary to keep nature in 'perfect' balance. Yet scientific knowledge of ecological systems proved far from flawless. In the early twentieth century, park officials encouraged ungulate numbers in excess of ecological capacity, with disastrous results.[35] Natural fire was artificially prevented in national parks until the 1970s.[36] Authorities, meanwhile, shaped their dominions to meet public expectations. At Yosemite in the 1920s, bears and mountain lions were kept in cages so that tourists could view nature 'red in tooth and claw' without having to stray from the safety of the park village.[37] Roads, railroads, hotels and stores were all initially welcomed into the 'wilderness'. State and national parks signified constructed landscapes.

Branching roads and animal culls aside, park authorities remained committed to the protection of wild nature in principle, if not always in practice. National parks denoted the crown jewels of the American homeland, majestic sequoias and rock formations cast as nature's cathedrals to rival European stone spires. Park staff defended such places from ruination, protecting America's natural heritage from unscrupulous developers. National pride inspired the safeguarding of natural assets.

Systematically exploding more than a thousand bombs on western soil, nuclear pioneers lacked such noble land stewardship goals. Nevertheless, the work of the nuclear establishment was still tied to the defence of American territory. In 1953, the *Las Vegas Review-Journal* declared, 'We like the AEC [Atomic Energy Commission]. We welcome them to Nevada for their tests because we, as patriotic Americans, believe we are contributing something, in our small way, to the protection of the land we love'.[38] Crater sites, irradiated atomic veterans, and burnt beagles were a small price to pay for national security. The military-industrial complex protected the whole of the United States, including state and national parks, from the 'red enemy'. Park authorities meanwhile experienced their own territorial skirmishes with Native Americans and industrial capitalists. In Glacier National Park (1910), Montana, park staff engaged in a perennial battle with the Blackfeet regarding indigenous user rights on the Eastern slopes of the preserve, while neighbouring oil and gas operations threatened the ecological integrity of the park.[39] Both atomic and park landscapes concerned the protection of 'America the beautiful'.

American pride proved integral to both institutionalised landscapes. Park and nuclear boosters rallied to win over the American public to their respective projects. Rail tracks and luxurious hotels attracted the rich and influential to

Yellowstone and Yosemite. The Atomic Energy Commission announced bomb blasts at Las Vegas hotels, inviting gamblers to temporarily leave behind the neon lights of their casinos for other bright sights across the desert. The *Nevada Highways and Parks* magazine for late 1953 used pictures of 'Doom Town' at Nevada Test Site to promote tourism, the beleaguered irradiated buildings offering a novel portrayal of state accommodation compared to the usual motel fare.[40]

Both the eruption of Old Faithful geyser at Yellowstone and the rise of giant mushroom clouds across Nevada drew outbursts of pride, wonder and horror from onlookers. Watching Yellowstone's Mud Volcano, Nathaniel Langford, member of the Washburn Party, wrote how 'The sensations inspired in me today, on again witnessing its convulsions, and the dense clouds of vapor expelled in rapid succession from its crater, amid the jarring of the earth, and the ominous intonations from belief, were those of mingled dread and wonder'. Yellowstone was deemed 'unnaturally natural'.[41] In *The Big Picture*, a 1950s military film, a chaplain described an atomic explosion: 'you look up and you see the fireball as it ascends into the heavens. It contains all of the rich colors of the rainbow, and then as it rises up into the atmosphere it assembles into the mushroom. It is a wonderful sight to behold'.[42] Observers claimed to have found god in the glow of ground zero and within the 'cathedrals' of Yosemite.[43] Nuclear tourism was never as explicit as nature tourism, but Americans were able to find divine beauty in both landscapes. The sublime inhabited both nuclear and natural domains.

What differentiated the nuclear park from the nature park was the level of fear assigned to it. Nature parks had successfully transformed the 'wilderness', once considered primeval by Euro-Americans, into a goodly and spiritual landscape. National parks were new Edens, providing honest pursuits for wholesome Christian families. By contrast, nuclear landscapes were insalubrious, malfeasant places, where invisible evils lurked. The nuclear priesthood readily sacrificed their lands in the pursuit of forbidden knowledge, the secrets of the atom. Meanwhile, atomic uses amplified, rather than wiped clean, lingering notions of the taboo and the unwelcome. Seeping radioactive barrels strengthened popular perceptions of arid lands in Nevada and California as desolate wastelands. The new nuclear wilderness had its roots in soil already deemed unfit for life.

For environmentalists, the barrenness of ground zero indicated the destructiveness of humanity and a fast approaching ecological doomsday. Nuclear landscapes signified tortuous practice grounds for a forthcoming holocaust. The spring 1971 edition of *The Living Wilderness* detailed 'The nuclear sword of Damocles', 'the greatest threat to the continuance of animal, vegetable and human existence', declaring 'not only the wilderness but the whole world is in peril'.[44] Released during the same year, saturnine science fiction movie *Silent Running* explored the possibility of life devoid of wilderness. With Planet Earth (and, more importantly, the United States) denatured to the point of supporting only the human species, American spaceships carried the 'last forests of our once

FIGURE 2. Old Faithful geyser, Yellowstone (US National Park Service photograph)

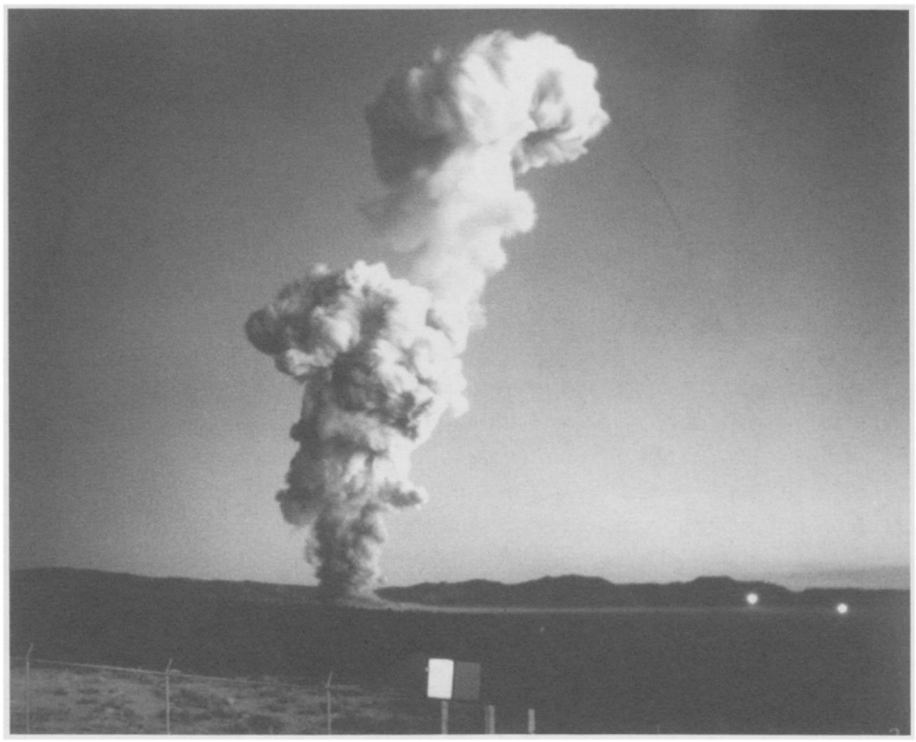

FIGURE 3. 'Nancy' tower shot, Nevada, 1953 (US Department of Energy photograph)

beautiful nation' in giant bio-domes, with the distant hope of re-establishing the 'parks and forest system'. However, budget cutbacks led to the abandonment of the space project. All but one of the domes was destroyed using nuclear explosives. The last forest survived thanks to an extrovert nature enthusiast disobeying orders. He then taught two friendly robots to look after the wilderness. *Silent Running* reflected popular concern over environmental collapse and nuclear destruction, and made an emotional plea for better land stewardship.[45]

Fearing a rise in public opposition, the nuclear industry attempted to reconnect atomic sites with natural landforms in the 1960s and 1970s. Corporations located nuclear plants amidst newly created 'nature reserves', hoping that local wildlife would freely congregate alongside reactors and thus show their support of the atom. One industry advert proclaimed 'Go Play in the Atomic Park', alleging that children could safely play in nuclear landscapes without fear of fallout.[46] A number of movies suggested that radioactive decay was not altogether bad for the world. Bizarre post-apocalyptic utopias were expected to rise from the ashes of nuclear Armageddon. Film historian Joyce Evans explained the 'attraction' of 'nuclear war' as 'like a cloth that wipes away the accumulated ravages of history and allows a clean, fresh world to be reborn'.[47] Movies such as *Genesis 2* (1973) predicted a return to the virgin wilderness, with 'man' as survivor, an atomic Daniel Boone, with his ragged clothes testament to the abandonment of former cultural excesses.[48] Meanwhile, radiation mutants, savage and predatory, replaced the bears and serpents of the original wilderness.

THE MODERN PARADOX: THE POST-ATOMIC PARK?

This final section details recent debates surrounding the setting aside of former nuclear lands as protected park areas. While atomic aficionados put great store by the abundance of species to be found at testing grounds and reactor sites in the American West, environmentalists struggle to make sense of unfolding events. The true meaning of the 'post-atomic park' remains open to interpretation.

In the 1990s, many nuclear projects were downscaled or decommissioned. Nuclear energy had proven itself uncompetitive in the marketplace, while the end of the Cold War abruptly halted the nuclear arms race. Attention gradually turned to the ecological costs of the atomic era. While the scale of radioactive spoilage defied public expectations, equally shocking was the survival of nature in atomic 'wastelands'. At ground zero, native vegetation had reclaimed Trinity. Ravens nested in the plugs of former underground nuclear tests.[49] The 'nuclear wilderness' of the 1990s was far less 'alien' than depicted in the movies. If there were any radioactive mutants, they were kept secret and well hidden.

Those responsible for cleaning up atomic sites welcomed signs of natural recovery. The presence of endangered species testified to a healthy rather than terminally polluted landscape. Wild flora and fauna also bolstered nuclear

FIGURE 4. Nevada test site
(US Department of Energy photograph)

tourism. Tour guides for Nevada Test Site stressed the natural legacy of the nuclear age. The Department of Energy proudly spoke of the 6000 acres surrounding Rocky Flats plutonium processing plant north-west of Denver, 'home to many species of animals and plants'.[50] The land had assumed a dual purpose, preventing nuclear contamination from reaching human settlements while protecting wild nature from increasing urbanisation and tourism. In May 1999, US Energy Secretary Bill Richardson announced the setting aside of 800 acres of Rocky Flats as Rock Creek Reserve, thus protecting 'a unique habitat that has been untouched by human development for 25 years'.[51]

Authorities stressed their commitment to preserving nuclear and post-nuclear wilderness. At Yucca Mountain, proposed site for high-level radioactive waste storage, and, as such, a nuclear landscape in the making, officials monitored the endangered desert tortoise and 'indicator species' such as the long-tailed pocket mouse for early warnings of environmental impact.[52] Just like national park rangers, nuclear authorities regretted their past record of land mismanagement, and vowed to make amends. Portland General Electric, as a gesture of 'responsible environmental stewardship' offered land occupied by Trojan nuclear plant to the state of Oregon for park use.[53] The atomic plant, dubbed 'Oregon's Trojan horse' due to its poor operating performance, was in the process of being decommissioned. Featuring 500 acres of woods and wetland, including 200 wildlife species and one concrete nuclear sarcophagus, the *Hanford News* commented, 'As far as parks go, it would indeed have a bit of everything'. The newspaper's headline read 'From nuclear to state park?'[54]

The gulf between the atomic park and the nature park appeared to be closing. Tennessee Valley Authority dams, along with other huge industrial adventures, had been accepted in the past for their accompanying picnic sites and boating lakes.[55] The atomic industry offered similar fringe benefits. The National Park Service assumed responsibility for a number of nuclear missile silos next to Badlands National Park as newly appointed national historic sites.[56] Park employees also restored the McDonald Ranch at Trinity Test Site, after rain (rather than atomic blasts) damaged its tin roof and mud brick construction.

Environmentalists, ranchers, farmers, real-estate developers and Native Americans all competed for stretches of the Hanford Engineering Works. Only five percent of the reservation had suffered plutonium contamination, leaving 530 acres of 'prime habitat'. In June 2000, Hanford Reach National Monument, home to bald eagles and peregrine falcons, was set up as a shrub-steppe reservation. Battelle-Northwest biologist Larry Caldwell elaborated on the importance of Hanford, explaining that 'in a state that is losing thousands of acres of wildlife habitat each year...We're sort of an island, sort of a last bastion of sagebrush-dependent species'.[57] With many more acres to be freed for purchase, environmental hopes centred on expanding the post-nuclear National Monument.

While nuclear landscapes received unexpected plaudits, national parks came under fire from wilderness purists. The vulnerable ecology of nature parks had been meddled with and trampled on for too long. Park authorities were encouraged to manage humans, not nature. While the National Park Service appeared receptive to environmentalist pleas, they struggled with a sizeable tourist problem. At Yellowstone, recreational vehicles roared across park landscapes in the summer months. Snowmobiles invaded in the winter. Yosemite village was famous for its neon shopping experience. The 'wilderness' experience appeared in danger of devolving into a vacuous retail industry.

Nuclear landscapes had yet to be tarnished by consumer capital. Trinity Test Site, open to the public twice a year, featured only a few gift sellers. Neither was

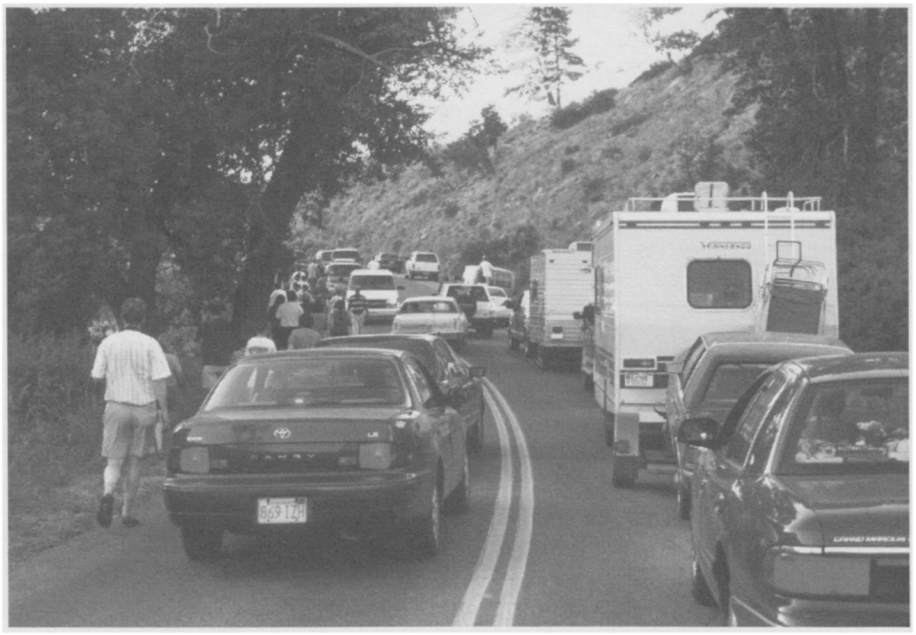

FIGURE 5. Traffic jam, Yellowstone (US National Park Service photograph)

overcrowding a problem. Rebecca Solnit found the unpopulated zones of Nevada Test Site preferable to the claustrophobic Yosemite, shocked to discover 'this country's national Eden so full of disturbing surprises and its Armageddon so comparatively pleasant'.[58] Solnit was not the only one to favourably compare nuclear lands with traditional park areas. One wildlife biologist claimed PG&E's Diablo property was in far better ecological condition than Montana de Oro State Park, its northerly neighbour.[59] Plans were put forward to protect Diablo Canyon following plant decommissioning.[60] While nature parks suffered from their own recreational success, nuclear lands, mostly off-limits to the nation, often resembled their pre-nuclear countenance. Buffer zones, as no-mans-land, had served as enigmatic wildlife refuges. Rather than national parks, nuclear parks boasted the human-less 'frozen' wilderness.

The nuclear wilderness nevertheless had its fair share of critics. Colorado environmentalists rejected claims of a 're-natured' Rocky Flats. The 'Rocky Flats Horror Picture Show', with over 170 contaminated hotspots, hardly qualified as wilderness.[61] Nor were its land stewards well-trusted nature lovers. One environmentalist described the Department of Energy as 'so focused on public image that they cast aside safety'.[62] The 'rebirth' of Denver's Rocky Mountain Arsenal (RMA), former chemical warfare site turned wildlife menagerie, was equally regarded with suspicion. According to the Army Corps of Engineers, the territory featured 'the most contaminated square mile on Earth'.[63] Reports of tumble mustard tree groves flourishing on Rocky Mountain soil seemed unlikely given the prodigious manufacture of mustard gas and other lethal concoctions. Attempting to bypass the issue of human access, unconscionable authorities had merely discovered 'a way to do less clean-up' by proposing wildlife reserves.[64] Even more suspect was a plan to make RMA part of a 'Central Park of the West'.[65] Both the Rocky Mountain Arsenal and Rocky Flats represented dubious additions to the US park system. Environmentalists fervently pushed their own 'toxic tours' of the sites surrounding Denver, showing a landscape connected by pollution, not protection.[66]

For several decades environmentalists had vilified atomic energy as an enemy of ecology. While clean-up authorities promoted stories of natural recovery and benign experimentation, anti-nuclear activists preferred to keep with their well-established narratives of environmental ruin. Along with cancer-suffering atomic veterans, nuclear and post-nuclear landscapes provided material proof of radiation damage. For vehement critics of the nuclear age, the landscape was itself a story of secret holocaust and the slow death of nature. 'Atomic photographers' in the late 1980s and early 1990s captured scenes of nuclear devastation in western territory. Carole Gallagher photographed brave but sickened residents of Utah and Nevada, and cloudy, contaminated landscapes.[67] Richard Misrach shot pictures of dead animal corpses and nuclear desolation in the desert.[68] The overwhelming image was one of needless human sacrifice and creeping ecocide.

Photographs situated the 'nuclear west' as a social creation, a landscape forged by atomic device. Unlike huge canvas paintings of national parks, or early portraits of the 'Great American desert', where humans were noticeably absent, the nuclear vista was an 'irrevocably social landscape' moulded by nefarious sapient endeavour.[69] To help magnify themes of poisoning, nature was often cast as a powerless victim of atomic 'progress' or a gloomy, deathly backdrop. Celluloid scenes of the nuclear landscape drew on deep-rooted fears of both atomic energy and harsh terrain. The tortured animal bones immortalised by Misrach resembled the buffalo skulls in classic paintings of the West by Charles Russell one hundred years earlier.[70] The myths of the American desert, 'wasteland' and 'wilderness', death and beauty, coincided. While tourists captured on film freakish geysers and the 'unnaturally natural' at Yellowstone, atomic photographers documented poisoned waterholes, misshapen military machinery, and the 'naturally unnatural' at Nevada Test Site. Nuclear industry pictures of healthy wildlife thriving in atomic spaces were fake and timid by comparison.

Environmentalists recognised that the 'nuclear park' ideal drew attention away from serious problems at atomic sites involving decontamination and waste storage issues. As well as supposed nature reservations, Rocky Flats and Hanford were also federal Superfund sites. Established by Congress in 1980, the Superfund program was designed to clean up the most polluted sites in the country, under the guidance of the Environmental Protection Agency. Peter Hales described 'the atomic spaces of the Manhattan Engineering District' as 'legendary deserts of toxic horror'.[71] Meanwhile, working uranium mines continued to spew toxins into the air and ruin neighbouring communities. The nuclear age was about pollution not preservation. Radioactive particles from more than a thousand nuclear tests had travelled the biosphere, tainting the Earth with poison. There was, in fact, no untouched wilderness thanks to atomic engineering. Even national parks fell victim to passing radiation clouds in the 1950s.[72] In the popular consciousness, nuclear landscapes remained the antithesis of the hallowed recreational paradises of Yosemite and Yellowstone. According to

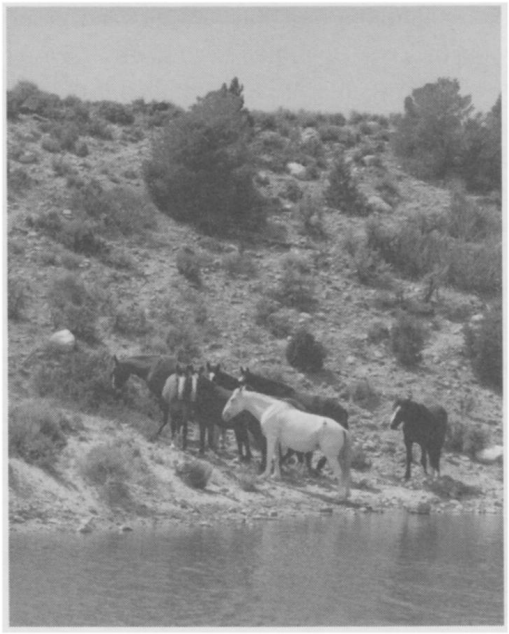

FIGURE 6. Wild horses at Nevada test site (US Department of Energy photograph)

the *New Atlas of the West*, nuclear landscapes were the quintessential 'ugly west', despoiled lands marked by 'atomic leftovers'.[73] While park landscapes testified to wholesome recreation and fondness for wild nature, nuclear and post-nuclear landscapes manifested destruction and deception. The most revealing 'nuclear park' was to be found just a half-mile from Lawrence Livermore National Laboratory, a nuclear weapons research centre east of Berkeley, California. To the shock of Livermore personnel, plutonium particles had been found at Big Trees Park, popular destination for local parents and children, not to mention birds and wildlife. The *San Francisco Examiner* renamed it the 'Plutonium Park'.[74]

REINTERPRETING ATOMIC SPACES

At Bodega in the early 1960s, any useful discussion of the atomic park had been cut short by the discovery of the San Andreas Fault directly beneath PG&E's groundbreaking plant. A natural, seismic threat put paid to any chances of a nuclear park on the headland. Pacific Gas was forced to withdraw its plans. The land set aside for nuclear status passed into state park ownership, with the shaft dug for the atomic plant (known by locals as 'the hole in the head') claimed by birds as a duck pond. The nature reserve gradually covered up all traces of PG&E's atomic aspirations. Nature had been saved, and the full ravages of the nuclear landscape avoided. The choice had been between an atomic park and a state park, industry and despoliation or nature and recreation. A journalist, recounting events at Bodega Head, declared 'It's a park alright, but not an atomic one'. The difference appeared self-evident.[75]

Over a period of fifty years, nuclear landscapes served as popular icons of danger and destruction. Hanford Engineering Works and Nevada Test Site represented sacrifice zones, Armageddon wastelands where humans experimented with deadly materials. Unlike US national parks, set aside to preserve wild scenery, lands appropriated for the nuclear cause were subject to exploding bombs and the annihilation of nature. In the 1990s, nuclear lands taken over for clean up or decommissioning were expected to bear testament to their deadly purpose. Decomposing waste barrels were the anticipated legacy of the nuclear era. However, a bunch of coyotes hanging out at ground zero told a slightly different story. Battered and irradiated, nature had survived the holocaust. Just as national park managers had partly crafted the 'virgin wilds', natural forces had maintained an influence on the man-made nuclear landscape.

Nature's survival was treated as something of an enigma. While bears wandering in Yosemite symbolised a wild American landscape cherished by its keepers, the presence of wildlife at Nevada Test Site hardly matched with the destructive mandate of military authorities. Puzzling over how to interpret the atomic park paradox, commentators turned to effete narratives of the nuclear era.

Pro-nuclear industrialists took credit for natural recovery, while environmentalists remained sceptical. Nuclear lands were inescapably tied to partisan interpretations of the nuclear age. In 1995, the Smithsonian revised a major exhibition on Enola Gay and the dropping of the atomic bomb to placate war veterans.[76] In 1994, New Mexico officials, fearing 'gatherings of peaceniks', rejected a request by thousands of US children for a peace park at Los Alamos, although a Missile Park at White Sands Missile Range Museum continued to attract its fair share of war technology enthusiasts.[77] The nuclear age, ended or not, had lost none of its controversy. American society and landscape still appeared gilded by their brush with atomic physics. Perhaps not the oxymoron that it first appears, the 'atomic park' is part of this contested territory. Just as US national parks remain fiercely controversial landscapes, subject to divergent interpretations, and imperfect monuments to America's past, nuclear parks are similarly contentious places.

Reaching a steadfast verdict on the ecological costs of the nuclear age is thus likely to remain out of reach until a scientific and intellectual common ground emerges. The advent of 'post-atomic parks' will need to be set alongside the trials encountered in burying mountains of nuclear waste. Despite a very different charter, Hanford Reach Monument shares its history with Yucca Mountain. Atomic landscapes need to be reinterpreted, and the nuclear story rewritten, to take into account themes of natural loss and recovery. This entails a greater role for environmental history in nuclear history, and perhaps a diminished role for studies based on Cold War mentalities.

Equally, nuclear issues have much to add to our understanding of environmental history, especially in regard to prominent terms such as 'nature' and 'park'. From this article, it is clear that much of the allure of the park rests on its wilderness imagery, of a landscape untouched by humanity, while nuclear landscapes are repugnant due to their overt military exigencies, and concomitant lack of naturalness. Situating nuclear landscapes and park territories as polar extremes reflects the influence of two important cultural paradigms, one asserting the nuclear age as intrinsically destructive, the other positing the conservation era as productive and praiseworthy. On a more profound level, nuclear landscapes are meant to symbolise the danger of human dominion and control, while parks embody idealistic notions of nature pure and unsullied by culture. However, the specific landscapes set aside as totems of cultural decay or biotic resurgence rarely conformed to their mantles. From abandoned, military vehicles to bustling concessionary stores, signs of human impact pepper both nuclear and national park landscapes. Meanwhile, nature (as a description of floral and fauna agents) fails to abide by the absolute definitions we foist on it. Endangered species rebound at nuclear wastelands, while grizzly bears struggle to maintain numbers in protected areas such as Yellowstone. Neat stereotypes disregard the complex interactions between nature and culture. Once a term used to describe the geologic curiosities of Yellowstone, today more appropriate to post-atomic wilderness, the 'unnaturally natural' remains not only a paradoxical phrase, but also leads to a sticky quagmire over how best to interpret the modern landscape.

NOTES

[1] A picture of the billboard can be found in Wellock 1992, 192.
[2] David Pesonen, 'A Visit to the Atomic Park'. The pamphlet reprinted articles published in the *Sebastopol Times* during autumn 1962. Held at the Bancroft Library, University of California, Berkeley.
[3] See Hays 1987, 23–4, 86–7.
[4] Eminent Western historian Richard White remarked how developers established 'park-like' industrial sites in Western states during the post-1945 era. Stanford Industrial Park, founded in 1951, was the first university-sponsored industrial park in the country. See White 1991, 547 and Findlay 1992, 117–59.
[5] For further insight into Disney landscapes, see Findlay 1992, 52–116.
[6] Yellowstone National Park received 2,062,476 visitors in 1965. Haines 1996 [1977], 480.
[7] Novelist Wallace Stegner is credited with having described the US national park system as 'the best idea we ever had' in 1983. Noted in Milstein 1996, 8.
[8] Throughout the 1970s, anti-nuclear protesters highlighted themes of radioactive contamination and even mutation, while offering solar power as a natural alternative energy source. Following the accident at Three Mile Island nuclear power plant, Pennsylvania, in 1979, mainstream American society adopted a critical stance towards atomic energy production, although nuclear weapons were still accepted as valuable 'peacekeepers' to counter the 'Soviet threat'.
[9] Yosemite Park Act, June 30 1864, U.S., *Statutes at Large*, 13 (1864), 325. Yosemite was expanded to become a National Park in 1890.
[10] Ibid.; See Alfred Runte's discussion of national parks as 'worthless lands' in Runte 1979, 48–64. California senator John Conness described the Yosemite bill as 'a grant of certain premises located in the Sierra Nevada mountains, in the State of California, that are for all public purposes worthless, but which constitute, perhaps, some of the greatest wonders of the world'. Runte, 48–9.
[11] Washburn expeditioner Cornelius Hedges is said to have first raised the idea of 'a great National Park'. See Milstein 1996, 39.
[12] Milstein 1996, 39. The origins of the park idea may alternatively be traced to events surrounding the establishment and operation of Yosemite Park (1864). See Runte 1990, 26–7, 33–5.
[13] For a study of the re-evaluation of wilderness in the late nineteenth century, see Nash 1982 [1967], 108–21.
[14] For park policy towards Indians, see Spence 1999 and Keller and Turek 1998.
[15] Solnit 1994, 136.
[16] Kuletz 1998, xiv. Solnit discusses Shoshone title to the Nevada Test Site in *Savage Dreams*, 28–30. For land issues at Hanford, see Ken Olsen, 'At Hanford, the real estate is hot', *High Country News*, 28/1, 22 Jan. 1996; for Los Alamos, Barbara Ferry, 'Homesteaders sue over ancestral land', *High Country News*, 32/6, 27 Mar. 2000.
[17] In 1956, the National Park Service also announced Mission 66, an extensive plan to expand the park system and attendant visitor services. Hays 1987, 117.
[18] PG&E, 'Summary Comparison of Sites for Nuclear Power Plant, South Coastal Area', Sierra Club Collection (henceforth SCC) 71/295c, box 189, file 30, Bancroft Library.
[19] In correspondence dated March 6, 1963, Sierra Club member Frederick Eissler suggested, 'There is every reason to believe that the Nipomo Dunes is another Bodega

Head', SCC 71/103c, box 78, file 13. The Bodega analogy was later applied to controversies surrounding a nuclear plant at Diablo Canyon. In early 1967, the *San Francisco Chronicle* detailed events at Diablo, commenting 'once again, as at Bodega, a good power plant site was also a good park site'. *San Francisco Chronicle*, 12 Feb. 1967.

[20] PG&E, 'Summary Comparison of Sites'.

[21] Sierra Club Board of Directors, Minutes of the Annual Organisation (May 7–8, 1966), 8, SCC 71/103c, box 4, file 5.

[22] For example, memorandum 'To Board of Directors from Fred Eissler', (September 8, 1966), SCC 71/103c, box 110, file 1. Eissler first referred to the Pacific Coast Recreation Area Survey (1959), published by the National Park Service, at the May 1966 Club meeting.

[23] 'The Diablo Canyon Area: *California's Last Unspoiled Pastoral Coastland*', signed by David Brower, Polly Dyer, Jules Eichorn, Fred Eissler, Martin Litton, Daniel Luten, David Pesonen, Eliot Porter, and Georg Treichel, *Sierra Club Bulletin*, 52/2 (February 1967), 7, author's personal copy.

[24] Kathy Jackson, 'Correction: John Muir Would Vote No', (February 1969), SCC 71/103c, box 123, file 11. The letter was part of a cantankerous battle between members regarding how John Muir (1838–1914), co-founder and 'patron saint' of the Club, would have voted on Diablo if alive in the 1960s.

[25] William Siri and Ansel Adams, 'In Defense of a Victory: The Nipomo Dunes', *Sierra Club Bulletin* (February 1967), 4.

[26] See Schrepfer 1992, 212–37 and Wellock 1998, 68–94.

[27] William Bennett quoted in *Ramparts*, February 15, 1968, SCC 71/103c, box 117, file 33.

[28] PG&E, 'Special Report of Diablo Canyon', *PG&E Life* (June 1967), 15, SCC 71/103c, box 113, file 40. In the Aleutians off the coast of Alaska, Atomic Energy Commission officials similarly downplayed the natural worth of Amchitka Island to bolster support for nuclear testing in 1971. See Coates 1996, 22, 33.

[29] Keith Schneider's foreword in Gallagher 1993, xvii. The incident is discussed more fully in Hacker 1998, 157–75.

[30] Wolves survived in Alaska. For an overview of National Park policy towards *Canis lupus*, see McIntyre 1993.

[31] For more on the construction of nuclear cities, see Abbott 1998, 90–115.

[32] Hales 1997, 2.

[33] Here I use the Department of Energy's description of Nevada Test Site as a 'massive outdoor laboratory,' at http://www.nv.doe.gov/nts.

[34] However, Project Plowshare promised far more than it could ever possibly (let alone safely) deliver. The American public remained wary of radiation side-effects, while the test grounds of Nevada and White Sands, marked by dusty craters and military ditches, were hardly the best indicators of what nuclear engineering offered. For insights into a few of the controversies surrounding Project Plowshare, see Coates 1989, 1–31, O'Neill 1994, and Krygier 1998, 311–22.

[35] In the 1910s and 1920s, the National Park Service killed predators in order to encourage huge elk herds. However, the herds overgrazed suitable range, and vast numbers died during harsh winters. This led to more protection for elk, and the cycle repeated itself until policy revisions in the 1930s. For a highly critical look at Yellowstone National Park management and elk overpopulation problems, consult Chase 1987, 19–24.

[36] Yosemite and Yellowstone park employees endorsed natural-burn policies for the first time in 1972: Chase 1987, 70 and Runte 1990, 216. The seminal work on the use of fire

through time remains Pyne 1982. On the 'creation' of national park landscapes, see McClelland 1998.

[37] Runte 1990, 133–4.

[38] *Las Vegas Review-Journal*, 21 May 1953. Cited in Fradkin 1989, 19.

[39] On the Blackfeet issue, see Warren 1997, 126–51 and Spence 1998, 29–49. On gas threats, see Buchholtz 1976, 78. On dangers to national parks in general, see Freemuth 1991.

[40] *Nevada Highways and Parks* magazine (June–December 1953). See Fradkin 1989, 103–4.

[41] Milstein 1996, 39.

[42] Gallagher 1993, xii.

[43] Upon witnessing the first atomic explosion at Trinity Test Site in July 1945, Los Alamos Laboratory director J. Robert Oppenheimer quoted a passage from the *Bhagavad Gita*, while the appropriately named 'Cathedral Rocks' and 'The Cathedral Spires' have been a source of inspiration for Yosemite visitors for decades.

[44] Lenore Marshall, 'The Nuclear Sword of Damocles', *The Living Wilderness* (Spring 1971), Papers of David Hartsough, American Friends Service Committee, San Francisco office.

[45] *Silent Running* (Universal Pictures, 1971).

[46] A copy of the advertisement can be found in Gofman and Tamplin 1973, 182–3.

[47] Evans 1998, 137.

[48] *Genesis 2* (TV movie, 1973) written and produced by Gene Roddenberry (of Star Trek fame), is brimming with atomic references. The post-nuclear war story (set in 2133) features a mutated race of humans (the Terranians) living underground, who depend on an arcane nuclear generator for their electricity. The surface has meanwhile become wild. Dylan, suspended by cryogenic experimentation in the 1970s, awakes into this bizarre world. While initially upset at losing his local highway and airport to wilderness, he soon comes to admire the beauty of blue skies and clean water, exclaiming, 'it's like the earth has been given a second chance'. On behalf of a remnant (and enslaved) human population, he destroys 'Terrania' with a nuclear missile left over from the Third World War. Other nuclear movies posted an anti-survivalist message, such as *Massive Retaliation* (Massive Productions, 1984).

[49] Journalist James Abarr related on a visit to Trinity how 'Ground zero at Trinity offers strong testimony to the recuperative powers of nature. Radiation levels are virtually nil, and the once-blackened and scorched land has fully recovered from the nuclear devastation of a half-century ago. Plants, grass, soil and wildlife have all returned…'. James Abarr, 'The Legacy of Trinity', *ABQ Journal.com*, 28 Oct. 1999. According to one Nevada Test Site tour guide, a raven annually nests atop the plug of a crater caused by Bilby, a 1963 atomic test. Bilby has become a 'drive through' crater on tours of the test site, a modern-day version of the drive-through redwood at Yosemite National Park. Solnit 1994, 208.

[50] Department of Energy, 'Rocky Flats Closure Project: Rocky Flats Overview', http://www.rfets.gov. The DOE similarly declared that land use restrictions at Nevada Test Site assured that 'biotic communities are in a relatively natural balance', in 'Nevada Test Site: National Environmental Research Park', http://www.nv.doe.gov/nts/researchpark.htm.

[51] Department of Energy, 'Energy Department – U.S. Fish and Wildlife Partnership Creates "Rock Creek Reserve"', press release, 17 May 1999, copy available at http://www.rfets.gov. The agreement was reached between the US Fish and Wildlife Service and the DOE.

[52] Such details are noted in the 'Environmental Program' posted at the Department of Energy's Yucca Mountain website, http://www.ymp.gov.
[53] 'From nuclear plant to state park?', *Hanford News/Tri-City Herald*, 15 Aug. 1999. The article is posted at http://www.hanfordnews.com/1999/aug25.html.
[54] Ibid.
[55] The Tennessee Valley Authority, established by Congress in 1933, is responsible for the economic (and, in turn, social) development of the Tennessee River drainage basin. Alongside huge industrial projects (including over 30 dams), the TVA has also created campgrounds, beaches and parks. For further insight into TVA's industrial and natural legacy, see Wilson 1992, 259–66.
[56] 'Strangelove park', *High Country News*, 26/13, 25 July 1994.
[57] John Stang, 'Hanford habitat key to survival', part of a series on Hanford, entitled 'A matter of habitat', *Tri-City Herald*, 25–28 Feb. 1996.
[58] Solnit 1994, 367.
[59] Conversation with Sue Benech, biologist, Diablo Canyon, 21 Aug. 1997.
[60] David Sneed, 'Water board working to preserve PG&E land', *The Tribune*, 17 Aug. 1999, and Sneed, 'PG&E supports Diablo preserve', *The Tribune*, 3 Oct. 1999. *The Tribune* was formerly the San Luis Obispo County *Telegram-Tribune*.
[61] Michael Fumento used the phrase 'Rocky Flats Horror Picture Show' in the as titled 'Rocky Flats Horror Picture Show: Rocky flats Plutonium-Processing Plant', *National Review*, 5 Nov. 1990.
[62] Sierra Club member Susan LeFever, quoted in Camille Colatosti, 'A "Toxic Tour" of Denver: Working for environmental justice at the grassroots', *The Witness* (July–August 2000). A copy of this document is available at http://thewitness.org/archive/julyaug00/toxictour.html.
[63] Cited in Wilson 1992, 281.
[64] Colastosti, 'A "Toxic Tour" of Denver...'
[65] Governor Roy Romer put forward the idea of a 'Central Park of the West'. See Mark Obmascik, 'Arsenal Billions Away from Being Picnic Site', *Denver Post*, 14 Feb. 1987, reprinted in Cronon 1995, 65. Maria Streshinsky included the RMA in a list of 'Five fabulous makeovers for Mother Earth', in 'From Blighted to Beautiful' *Via Online* magazine (November 1999), available at http://www.viamagazine.com/top_stories/articles/environment99.htm.
[66] The Colorado People's Environmental and Economic Network (COPEEN) offer toxic tours. See Colatosti, 'A "Toxic Tour" of Denver...'
[67] Gallagher 1993.
[68] Davis 1999, 341–5 briefly discusses the work of Richard Misrach. A useful article on pro-nuclear photography is Kirsch 1997, 227–55. Kirsch argues that AEC photographs were 'designed, quite literally, to take the place out of the landscape', (229) so that the public felt no attachment to areas used for testing.
[69] Davis 1999, 347.
[70] For a brief discussion of Russell's work, see Dippie 1994, 692–4.
[71] Hales 1997, 5.
[72] Downwind of the Nevada Test Site, Zion National Park (Utah), Bryce Canyon National Park (Utah) and Grand Canyon National Park (Arizona) inevitably received fallout from aboveground nuclear tests during the 1950s.
[73] Riebsame 1997, 134. Details of 'A Nuked Landscape' are located in a chapter looking at the so-called 'Ugly West'.

[74] Jane Kay and Erin McCormick, 'Bay's nuclear leftovers', *San Francisco Examiner*, 25 Nov. 1997.
[75] Simone Wilson, 'How Bodega Bay Nixed the Atomic Park', *Albion Monitor*, 3 Dec. 1995. A copy of this document is available at http://www.monitor.net/monitor. See also 'Bodega's Bird-Dogs Saved Town', *San Francisco Chronicle*, 23 Dec. 1997.
[76] On the controversies surrounding the Smithsonian exhibition on the Enola Gay, see Kai Bird's article 'Silencing History', *The Nation*, 20 Feb. 1995.
[77] 'Peace Gets No Chance', *High Country News*, 26 Dec. 1994. The peace park was planned as a 'sister memorial' to the Hiroshima Memorial Peace Park.

REFERENCES

Abbott, Carl 1998. 'Building the Atomic Cities: Richland, Los Alamos, and the American Planning Language'. In *The Atomic West*, ed. Bruce Hevly and John Findlay. Seattle: University of Washington Press.

Buchholtz, C.W. 1976. *Man in Glacier*. West Glacier: Glacier Natural History Association.

Chase, Alston 1987. *Playing God in Yellowstone: The Destruction of America's First National Park*. San Diego: Harcourt Brace.

Coates, Peter 1989. 'Project Chariot: Alaskan Roots of Environmentalism'. *Alaska History* 4/2 (Fall 1989): 1–31.

Coates, Peter 1996. 'Amchitka, Alaska: Toward the Bio-Biography of an Island'. *Environmental History* 1/4 (October 1996): 20–45.

Cronon, William (ed) 1995. *Uncommon Ground: Toward Reinventing Nature*. New York: Norton.

Davis, Mike 1999. 'Dead West: Ecocide in Marlboro Country'. In *Over the Edge: Remapping the American West*, ed. Valerie Matsumoto and Blake Allmendinger. Berkeley: University of California Press.

Dippie, Brian 1994. 'The Visual West'. In *The Oxford History of the American West*, ed. Clyde Milner II, Carol O'Connor and Martha Sandweiss. New York: Oxford University Press.

Evans, Joyce A. 1998. *Celluloid Mushroom Clouds: Hollywood and the Atomic Bomb*. Boulder: Westview Press.

Findlay, John M. 1992. *Magic Lands: Western Cityscapes and American Culture After 1940*. Berkeley: University of California Press.

Fradkin, Philip 1989. *Fallout: An American Nuclear Tragedy*. Tucson: The University of Arizona Press.

Freemuth, John C. 1991. *Islands Under Siege: National Parks and the Politics of External Threats*. Lawrence: University Press of Kansas.

Gallagher, Carole 1993. *American Ground Zero: The Secret Nuclear War*. New York: Random House.

Gofman, John and Tamplin, Arthur 1973. *Poisoned Power: The Case Against Nuclear Power Plants*. London: Chatto and Windus.

Hacker, Barton C. 1998. '"Hotter Than a $2 Pistol": Fallout, Sheep, and the Atomic Energy Commission, 1953–1986'. In *The Atomic West*, ed. Bruce Hevly and John Findlay. Seattle: University of Washington Press.

Haines, Aubrey L. 1996 [1977]. *The Yellowstone Story: A History of Our First National Park, Volume Two, Revised Edition*. Niwot, Colorado: University Press of Colorado.
Hales, Peter Bacon 1997. *Atomic Spaces: Living on the Manhattan Project*. Urbana: University of Illinois Press.
Hays, Samuel P. 1987. *Beauty, Health, and Permanence: Environmental Politics in the United States, 1955–1985*. Cambridge: Cambridge University Press.
Keller, Robert and Turek, Michael 1998. *American Indians and National Parks*. Tucson: University of Arizona Press.
Kirsch, Scott 1997. 'Watching the Bombs Go Off: Photography, Nuclear Landscapes, and Spectacular Democracy'. *Antipode* 29/3: 227–55.
Krygier, J.B. 1998. 'Project Ketch: Project Plowshare in Pennsylvania'. *Ecumene* 5/3 (Fall 1998): 311–22.
Kuletz, Valerie 1998. *The Tainted Desert: Environmental and Social Ruin in the American West*. New York: Routledge.
McClelland, Linda Flint 1998. *Building the National Parks: Historic Landscape Design and Construction*. Baltimore: The John Hopkins University Press.
McIntyre, Rick 1993. *A Society of Wolves: National Parks and the Battle Over the Wolf*. Stillwater, Minn.: Voyageur Press.
Milstein, Michael 1996. *Yellowstone: 125 Years of America's Best Idea*. Billings: The Billings Gazette.
Nash, Roderick 1982 [1967]. *Wilderness and the American Mind, Third Edition*. New Haven: Yale University Press.
O'Neill, Dan 1994. *The Firecracker Boys*. New York: St Martin's Press.
Pyne, Stephen 1982. *Fire in America*. Princeton: Princeton University Press.
Riebsame, William (ed) 1997. *Atlas of the New West: Portrait of a Changing Region*. New York: Norton.
Runte, Alfred 1979. *National Parks: The American Experience*. Lincoln: University of Nebraska Press.
Runte, Alfred 1990. *Yosemite: The Embattled Wilderness*. Lincoln: University of Nebraska Press.
Schrepfer, Susan 1992. 'The Nuclear Crucible: Diablo Canyon and the Transformation of the Sierra Club, 1965–1985'. *California History* 71/2 (Summer 1992): 212–37.
Solnit, Rebecca 1994. *Savage Dreams: A Journey into the Landscape Wars of the American West*. New York: Vintage Books.
Spence, Mark David 1998. 'Crown of the Continent, Backbone of the World: The American Wilderness Ideal and Blackfeet Exclusion from Glacier National Park'. *Environmental History* 1/3 (July 1998): 29–49.
Spence, Mark David 1999. *Dispossessing the Wilderness: Indian Removal and the Making of the National Parks*. New York: Oxford University Press.
Warren, Louis 1997. *The Hunter's Game: Poachers and Conservationists in Twentieth Century America*. New Haven: Yale University Press.
Wellock, Thomas 1992. 'The Battle for Bodega Bay: The Sierra Club and Nuclear Power, 1958–1964'. *California History* 71/2 (Summer 1992): 192–211.
Wellock, Thomas 1998. *Critical Masses: Opposition to Nuclear Power in California, 1958–1978*. Madison: The University of Wisconsin Press.
White, Richard 1991. *'It's Your Misfortune and None of My Own': A New History of the American West*. Norman: University of Oklahoma Press.
Wilson, Alexander 1992. *The Culture of Nature: North American Landscape from Disney to the Exxon Valdez*. Cambridge, Mass.: Blackwell.

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EXPERIMENTAL MUTATIONS IN PLANTS

The word mutation came into biological literature through its use by de Vries, of Amsterdam, about 60 years ago for abrupt inherited changes in an organism. He had observed such changes in the evening primrose and used the behaviour of this plant as the basis of a theory of evolution by mutation. Subsequent research has shown that the evening primrose is a curious kind of hybrid and that the mutations observed by de Vries were due to new combinations of genes rather than to changes in the genes themselves. The term mutation is now normally restricted to the latter phenomenon. The most characteristic feature of gene mutation is that the change is permanent, or in other words that the gene is inherited indefinitely in the changed form. A second feature is that mutations are usually of rare occurrence, the normal characteristic of genes being their great stability.

An outstanding development in the study of gene mutation was the discovery by Muller in 1927 that treatment with *x* rays much increased the frequency of mutation. It was subsequently found that mutations are induced by all types of ionizing radiations and also by ultra-violet light. With ionizing radiations such as *x* rays, the frequency of occurrence of mutations was linearly proportional to the dose in roentgen units and independent of other variables such as the wavelength of the radiation and the intensity of the dose (that is, the time of irradiation). Since roentgen units are measures of ionizations per given volume, it was suggested that mutation resulted from a single ionization event in the neighbourhood of the gene. Up to the time of the 1939–45 war hypotheses were favoured which attempted to explain mutation in such purely physical terms, although it was realized that the natural mutation rate of genes was considerably greater than could be accounted for by natural sources of suitable radiation, such as radioactive materials and cosmic rays.

An important development in the study of mutation occurred during the war, when Charlotte Auerbach and J. M. Robson[1] at Edinburgh discovered that mustard-gas will cause mutation. It is now known that many substances can do so. They range from inorganic to complex organic compounds, and so their mechanism of action must be diverse. Purely physical theories of mutation suffered further setbacks with the discovery that the mutagenic effects of ionizing radiations were much influenced by the chemical environment, such as the oxygen concentration, at the time of treatment. Also it was found that irradiating the culture medium prior to inoculating with fungal or bacterial cells caused mutations. It is not surprising therefore that hypotheses are now favoured which attempt to explain mutation primarily in terms of the liberation of organic peroxides or other active substances in the neighbourhood of the gene.

There have been several outstanding recent developments in the study of mutation. M. Demerec[2 3] and co-workers at the Carnegie Institution of Washington, New York, have made some remarkable discoveries with *Escherichia coli*. They have found that particular mutagens, whether chemical or physical, cause different mutation rates in specific genes over a wide range of frequencies. Thus, gene A was found to be particularly sensitive to manganous chloride, gene B to ultra-violet light, gene C to *x* rays, and so on. Indeed, some genes were found to be stable to some mutagens, or even to all the mutagens tested, although their capacity to mutate was evident, since they were observed to do so spontaneously. These findings were unexpected because it is known that the types of mutations caused by one mutagen are essentially similar to those caused by another. Demerec infers that at least the majority of these induced mutations arise through indirect action of the chemical or physical agent. The mutagen is thought to induce specific physiological changes in the treated cells, and these in turn to cause mutation of certain of the genes.

S. Benzer[4] at Purdue University, Indiana, and G. Streisinger and N. C. Franklin[5] at the California Institute of Technology have studied mutation in bacteriophages of *E. coli* and have obtained detailed information about the structure of particular genes. It appears that, just as in higher organisms genes are linearly arranged on the chromosomes, so in phage (and apparently in other organisms also) the gene itself is composed of linearly arranged units. Mutation of a particular gene results whenever a change occurs in any of its component units. Thus, if the

[1] Auerbach, C., and Robson, J. M., Report to Ministry of Supply, W3979, 1942.
[2] Demerec, M., Proc. 9th Internat. Cong. Genetics, *Caryologia*, 1954, Suppl. Vol., p. 201.
[3] —— *Amer. Nat.*, 1955, **89**, 1.
[4] Benzer, S., *Proc. nat. Acad. Sci. Wash.*, 1955, **41**, 344.
[5] Streisinger, G., and Franklin, N. C., *Cold Spring Harbor Symp. Quant. Biol.*, 1957, **21**, 103.
[6] McClintock, B., ibid., 1952, **16**, 13.
[7] —— ibid., 1957, **21**, 197.
[8] Shapiro, S., *Conference on Radioactive Isotopes in Agriculture*, 1956, 141, U.S. Atomic Energy Commission.

normal gene is represented by ABC . . . XYZ, one mutant might have b instead of B, another mutant of the same gene pqr instead of PQR, and so on. Some of the mutants are thought to be deletions of parts of the hereditary material, rather than substitutions.

Important contributions to the study of mutation have also been made with higher plants. In particular, Barbara McClintock, also working at the Carnegie Institution of Washington, New York, by a combined cytological and genetical study of maize, has revealed the existence of a mechanism for the biological control of the mutation rate of genes. That such biological control must exist has been known for some time, for mutation rate is known to be influenced by the genetic constitution of the organism, and in general to be adapted to the needs of the species. Thus mutation rates appear to be adapted to the generation time of the species, being lower per given time interval in long-lived organisms than in short. McClintock[6 7] finds evidence that certain structures in the chromosomes, to which she gives the name "controlling elements," influence profoundly the rate of mutation of numerous genes. The best known of these controlling elements, "Dotted," can cause one of the genes for anthocyanin pigmentation to mutate with high frequency. There is reason to believe that the controlling elements are situated in specialized parts of the chromosomes which are composed of heterochromatin. Hitherto heterochromatin has usually been regarded as genetically inert, and its biological function has not been understood.

From these recent studies, which are probably of wide application, it is evident that mutation is not a simple process. The great stability of the hereditary material, manifest from Demerec's discovery of mutagen-stable genes, has probably been achieved through specific adaptations in cellular organization, such as McClintock's controlling elements, which protect the genes to greater or lesser degree from chemical and physical mutagens. In view of this complexity, it is not surprising that the use of mutagenic agents in plant breeding must be on a largely empirical basis at present. (It is for the same reason that the magnitude of the mutagenic effects of atomic radiations on man is still largely unpredictable.) S. Shapiro[8] describes an experiment which is being conducted in the U.S.A. for the induction of mutations in plants by continual irradiation during growth by means of a cobalt-60 source. Although most mutations are harmful to the species, a small proportion may be desirable, and these can be selected by the plant breeder. The method has only recently been adopted, but shows promise of providing a valuable new source of hereditary variability in plants.

B.C.G. VACCINATION IN THE U.S.A.

The prevention of tuberculosis by B.C.G. vaccination is now an accepted public health measure in almost every country in the world. A striking exception is the United States of America, where B.C.G. has so far not been used on a national scale. This is surprising, for as long ago as 1949 the American Trudeau Society advocated the vaccination of contacts and others at special risk from tuberculosis; and the value of B.C.G. in such circumstances is also accepted by the United States Public Health Service. Medical opinion about B.C.G. may become more favourable in the U.S.A. now that a medical advisory committee has examined the validity of the objections raised to vaccination there.[1] Some of these objections are of long standing, and, although formerly advanced in other countries as well as the U.S.A., are now less seldom heard elsewhere. The committee, for example, examines and accepts the view that the safety of B.C.G. is undoubted. As to the efficacy of the vaccine, the committee's report discusses in some detail the early findings[2] of the Medical Research Council's clinical trial of tuberculosis vaccines at present being undertaken in this country, and concludes that the results "can leave no doubt in the mind of an unbiased observer that B.C.G. afforded a substantial protection against tuberculous disease." The contribution made to the prevention of tuberculosis by vaccination would in all likelihood more than compensate for the loss of the tuberculin test as a diagnostic measure.

Two less familiar objections to B.C.G. vaccination which have been heard only in recent years are also discussed by the committee. The first is that instead of vaccination chemotherapeutic drugs might be given to tuberculin-negative reactors as a precautionary measure. It is pointed out, however, that chemoprophylaxis is inferior to vaccination in that it does not raise immunity of itself, suppresses tubercle bacilli only during the period that the drug is being taken, is so far experimental, and requires continued cooperation from the patient for many years. For these reasons it seems unlikely that chemoprophylaxis could in the foreseeable future take the place of vaccination. The second recent objection to vaccination in the U.S.A.—that morbidity from tuberculosis is now so low that vaccination schemes are unnecessary—is one which is likely to be increasingly discussed in all countries as the mortality and incidence of tuberculosis

[1] Report of the Medical Advisory Committee of Research Foundation, Chicago. *J. Amer. med. Ass.*, 1957, **164**, 951.
[2] First (progress) report to the Medical Research Council by the Tuberculosis Vaccines Clinical Trials Committee, *Brit. med. J.*, 1956, **1**, 413.
[3] Palmer, C. E., and Shaw, L. W., *Amer. Rev. Tuberc.*, 1953, **68**, 462.
[4] Griffiths, M. I., and Gaisford, W., *Brit. med. J.*, 1956, **2**, 565.
[5] Pollock, T. M., ibid., 1957, **2**, 20.

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Radiobiology in the Atomic Age: Changing Research Practices and Policies in Comparative Perspective

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Abstract. This essay introduces a special collection of papers by Angela Creager, Soraya de Chadarevian, Karen Rader, Jean-Paul Gaudillière, and María Jesús Santesmases on the theme "Radiobiology in the Atomic Age."

Keywords: atomic energy, diplomacy, fallout, genetics, nuclear weapons, radiation, radiobiology, radioisotopes

Introduction

The emergence of several national atomic energy installations after World War II provided new contexts and opportunities for the development of biology. The contributions gathered here address these developments under the rubric used frequently at the time, *radiobiology*. In the 1920s, radiobiology referred to studies of the effects of X-rays on biological processes, exemplified by H. J. Muller's demonstration that X-rays induce mutations in *Drosophila*.[1] The term took on a new and charged valence in the wake of World War II, as it was associated with the development of nuclear reactors, artificial radioisotopes, and the health risks of radiation – just as these technological realities had become entangled with military capability and international relations.

[1] The Oxford English Dictionary, for instances, has as its earliest citation for radiobiology a 1919 abstract referring to the selective action of X-rays on biological materials. (Med. Sci. Abstr. & Rev. 1, p. 358). See also Muller, 1927.

Rather than seeing radiobiology as a simple application of physical instrumentation to biology or the influx of physicists themselves, we are interested in following the reciprocal patterns of exchange and collaboration between physicists and biologists. The development of the atomic bomb clearly changed the scale and range of such collaborations, as researchers in nearly every field sought to exploit tools associated with the new nuclear reactors, aided by civilian-oriented government policies for atomic energy. Yet the contributions here demonstrate that life scientists were already interested in methods and questions associated with radiobiology, and took advantage of national and international initiatives to advance their research interests.

By focusing on four national contexts (US, England, France and Spain), we have here a comparative perspective for seeing the growth in postwar radiobiology. Two features stand out in this collective picture. First is the central role that government agencies played in advocating and disseminating the scientific resources associated with atomic energy. The aims of new national atomic energy programs were not only domestic; science and technology became crucial tools of international diplomacy in the arena of atomic energy, as John Beatty has shown and more recently Ronald Doel and John Krige have stressed.[2] Biologists and physicians were well-positioned to benefit from attempts to develop the "humanitarian" applications of atomic energy, whether from their own national governments or from programs of international exchange. Second, the leading fields of postwar biomedical research – such as biochemistry, molecular genetics, endocrinology, and physiology – benefited directly from these atomic energy programs and the new tools they provided.[3] In this sense, the 'footprint' of radiobiology is both more extensive and less coherent than one might expect; the term signaled the bountiful experimental tools and funding associated with radiation and reactors more than any single scientific question or approach.[4]

In one respect or another, all of these essays respond to a historiography of atomic science that tends to be organized around physics.[5]

[2] Beatty, 1991; Doel, 1997; Krige, 2006.

[3] In addition to the essays in this collection, see Fragu, 2003.

[4] In the early 1960s, Alexander Hollaender argued that developments in biochemistry and molecular biology that had ushered in a more chemical view of the gene necessitated a broadening of the understanding of radiation biology beyond the reliance on physical approaches. Hollaender, 1963, p. vii. See also Creager, this issue.

[5] Two essays that frame the debate about the atomic bomb's legacy for physics research are Forman, 1987; Kevles, 1990. The centrality of physics to this historiography can be viewed as part of a broader trend; as David Kaiser has shown, until the 1980s, history of the physical sciences dominated history of science overall. See Kaiser, 2005.

Comparatively less attention has been paid to the consequences of atomic energy – and its legitimation – for biology, agriculture, and medicine. What scholarship exists provided an encouraging background to this collection. Evelyn Fox Keller, Nicolas Rasmussen, and Soraya de Chadarevian have addressed in various ways the significance of the bomb for the origins of molecular biology. Keller points to the symbolic continuities between a physics tainted by its secretive pursuit of a massive instrument of destruction and the physics-inspired pursuit of the secret of life by molecular biologists. In her view, molecular biology benefited from the high cultural authority of physics while providing it some vindication.[6] Moving the theme of redemptive biology to a more disciplinary level, Rasmussen argues that the infusion of funds and people into biophysics after the war – as American politicians and scientists attempted to find a "silver lining" in the mushroom cloud – seeded the subsequent emergence of molecular biology.[7] De Chadarevian analyzes the postwar British politics in similar terms, arguing that atomic energy-related funding for biophysics was crucial to the Unit for the Study of Molecular Structure of Biological Systems under William Bragg at Cambridge. In the 1950s this laboratory became the first institution to use the name Molecular Biology, even as the connections to atomic energy were progressively less visible.[8] Along similar lines, Bruno Strasser has shown how the growth of molecular biology in Europe (notably in Switzerland) drew support from atomic energy organizations, including EURATOM.[9] These accounts emphasize how scientists and politicians created and used initiatives in biology and medicine as a way to counteract ambivalence and fear about the atom bomb. Strikingly, however, the resulting scientific successes are seldom attributed to the atomic energy initiatives that enabled them.

Other arenas of research were more visibly connected to the opportunities and risks of the atomic age. Several historians of biology have traced how programs of research in biology and medicine of the US Atomic Energy Commission (AEC) – in some cases inherited from the Army or National Academy of Sciences – shaped the postwar direction of certain fields. John Beatty and Susan Lindee have examined the history of the Atomic Bomb Casualty Commission (ABCC), which investigated the radiation effects in survivors of the wartime atomic detonations in Japan.[10]

[6] Keller, 1990, 1992

[7] Rasmussen, 1997.

[8] de Chadarevian, 2002.

[9] Strasser, 2002.

[10] Beatty, 1991; Lindee, 1994.

The results were "negative," an outcome that the AEC tried to use to counteract public fear of radiation risks. Not that the agency succeeded; new casualties from exposure to peacetime tests (particularly the *Lucky Dragon* incident) reinforced public alarm.[11] The AEC sought to manage the consequences of agency-produced radiation in other ways. Stephen Bocking has shown how concerns about test-bomb fallout and radioactive contamination prompted the AEC to become the largest supporter of ecological research in the 1950s and 1960s.[12] Included among the AEC's activities in this area was a large-scale radioecology group at Oak Ridge National Laboratory that launched the growing emphasis there on environmental science. Timothy Lenoir and Marguerite Hays have examined how the AEC's programs for radioisotope distribution and clinical application drew on precedents from the Manhattan Project to shape the emergence of nuclear medicine.[13] These studies of AEC-sponsored research have probed the political context largely in terms of the reaction (both nationally and internationally) to the use of atomic weapons against the Japanese and the salience of atomic energy to the Cold War. Our contributions expand this framework by focusing on the explicitly civilian aspects of atomic energy, particularly the Atoms for Peace campaign. Indeed, our papers support the notion that even before President Eisenhower's initiative, biology, agriculture, and medicine served to represent the peaceful face of atomic energy, which the US viewed as increasingly strategic to the waging of the Cold War in non-military terms.[14]

The application of atomic energy to biology and medicine after World War II referred mainly to two resources: radiation sources and radioisotopes. In both cases, the resources were not novel, but the development of nuclear reactors changed the range and sheer quantity of radiant materials available for research and therapy. The shift to mass-production had political overtones as well in being identified with 'Americanisation,' as the French called it.[15] Government involvement with matters of atomic energy simultaneously encouraged and constrained radiation genetics and radioisotope usage. In the US – and slightly later in the UK and France – science policy for radiobiology was formulated in response to both the problem of radiation-induced mutations (particularly once public concern emerged over weapons

[11] See Hacker, 1994; Jolly, 2003.

[12] Bocking, 1995, 1997.

[13] Lenoir and Hays, 2000.

[14] Krige, 2006.

[15] See Gaudillière, this issue and 2002.

testing fallout) and the opportunities for radioisotope use in biology and medicine based on the existence of government-owned nuclear reactors. As in the case of the ABCC, creating the problem and promoting 'solutions' became faces of the same coin after the first bombs. How the aftermath of the atomic bomb changed laboratory instrumentation, biological knowledge, and medical practice comprises the core question behind this collection.

Our contributions feature government actions taken to promote research on genetics of radiation-induced mutations at the Oak Ridge Biology Division of the US AEC (Karen Rader), and at Harwell by the British Atomic Energy Authority in collaboration with the Medical Research Council (Soraya de Chadarevian). Both projects addressed the biological effects of low-level radiation on mammals. These studies were motivated by governmental responsibility for the occupational health of the thousands of workers in national atomic energy installations – as well as concern with civilian exposure to radiation from peacetime nuclear tests. On both sides of the Atlantic, the model organism of choice was the inbred mouse. Rader stresses the way in which mammalian genetics fit into a broader plan of radiobiological research at the national laboratories. There the life sciences suggested productive avenues through which "big science" and atomic energy could be deployed to promote health. This strategy was especially important at Manhattan Project facilities no longer needed for weapons production or nuclear physics. Alexander Hollaender's program oriented around studying the biological effects of radiation helped justify the continuation of atomic energy research at the US AEC's Oak Ridge site.

Hollaender's newly-hired mouse geneticists Bill and Liane Russell developed the "specific locus test" (SLT) as a highly efficient system for detecting radiation-induced mutations. The SLT mouse model, Rader argues, mediated both political and scientific concerns, providing an "acceptable middle ground, for both scientists and policy-makers, between experimental studies of flies and bacteria and the study of Japanese survivors."[16] De Chadarevian reminds us, however, that geneticists could not control the terms of public discourse about radiation hazards – in the 1950s British government studies of radiation risks gave the emerging anti-testing and disarmament advocates scientific justification. The British mouse genetics project started not in a national laboratory but at Edinburgh, under the direction of C. H. Waddington. In the mid-1950s the group moved to Harwell,

[16] Rader, this issue. For more on the SLT model, see Rader, 2004, chapter 6.

where the required 150,000 mice could be raised and analyzed. This also brought novel experimental resources into reach: geneticists had access to the reactors "Gleep" and "Bepo."

One cannot help but be struck that the American and British groups undertook similar massive breeding experiments with little coordination or communication. This was not necessarily due to constraints posed by military secrecy: As Rader emphasizes, Hollaender fostered an in-house research culture of openness and publication (in striking contrast to research done at Oak Ridge during the war). Rather the Harwell geneticists found the American group unwilling to share information on their experiments. Thus the politics of nuclear secrecy, which disrupted technical cooperation between American, Canadian, and British scientists after the war, was mirrored by scientific rivalries in genetics.[17] These parallel cases also help us understand the ways in which biologists managed to use atomic facilities to bring radiation genetics to a new scale of operation, nicely captured by reference to the "Mega-Mouse" project at Oak Ridge.

The application of radioisotopes also relied on the infrastructure of massive reactors, but these nuclear tools traveled beyond atomic energy facilities to thousands of laboratories, hospitals, and clinics. Before the end of the war, as Angela Creager's essay shows, leaders of the Manhattan Project decided to dedicate one of their facilities, the nuclear reactor at Oak Ridge, for mass-production and distribution of isotopes to outside users. Like Hollaender's radiobiology program, this plan helped justify continuing atomic energy operations at Oak Ridge, since the reactor there was no longer needed for plutonium production. The early uses of AEC-produced radioisotopes, following the precedent of cyclotron-produced isotopes 10 years earlier, demonstrate the strong historical link between biological research and clinical application. Particularly around the Berkeley cyclotron, therapeutic uses of radiophosphorus and radioiodine were developed in concert with biological tracer studies with these elements. The publicity surrounding the AEC's radioisotope program emphasized the medical dividends, at times explicitly contrasting the potential of atoms to heal with their destructive force in nuclear weapons. As health physicist Robley Evans asserted in 1946, "The sober truth is that through medical advances alone, atomic energy has already saved more lives than were snuffed out at Hiroshima and Nagasaki."[18]

[17] See Hewlett and Duncan, 1990.

[18] Evans, 1946, p. 68.

Beyond the links (rhetorical and experimental) with clinical uses of radioisotopes, the AEC's strategies built on and reinforced a trend in biology towards physical–chemical instrumentation, fostered in the 1930s through Warren Weaver's program in the Natural Sciences at the Rockefeller Foundation.[19]

The US AEC's decision in 1947 to expand their isotope distribution system to include foreign recipients affected the policy-making of many other countries regarding radiobiology. Policy-makers concerned themselves not only with access to atomic technologies, but with safety and regulation of the uses of radioactive sources by scientists and physicians. The papers by Jean-Paul Gaudillière and María Jesús Santesmases follow the radioisotopes from the atomic installations where they originated (in the US and then later in the UK and France) into European laboratories where biochemists and physiologists developed techniques for using them to visualizing life processes at the molecular level, such as metabolic pathways and hormone action. In Spain and in France, as well as in the US, the new availability of radioisotopes shaped the rapid growth of biochemistry even as its boundaries with other fields – physiology, endocrinology, and molecular biology – were being negotiated. Radioisotope usage continued to feature prominently in biomedical research until problems with nuclear waste disposal and concerns about worker safety prompted the development of alternative tracing technologies from the 1980s onwards.

American hegemony characterized the postwar development of atomic energy in Europe, but the US's atomic monopoly was shattered by the explosion of the first Soviet nuclear weapon in 1949.[20] In addition, the establishment of civilian reactors in Canada and Great Britain meant that users of radioactive materials often had several competing suppliers.[21] Scientists could work this multi-national supply system to their advantage; the competition put pressure on the US to lessen restrictions on their exports, and as Gaudillière shows, French researchers could turn to the American supply to circumvent institutional control over radioisotopes by the Institut National d'Hygiene and the France's Atomic Energy Commission. At the same time, the movement of nuclear materials was fraught with the politics of national security, which in the US was dominated by anti-Communism. Given this political background as well as the 1947 failure of the Baruch plan for the establishment of international control of atomic energy,

[19] See Abir-Am, 1982; Kohler, 1991; Kay, 1993.

[20] On American hegemony, see Krige, 2006.

[21] Gowing, 1974; Kraft, 2006.

scientists tended to view the actual transAtlantic circulation of non-fissionable nuclear materials positively – as the peaceful uses of atomic energy and international scientific cooperation – even though the patterns of material transmission were circumscribed by the emerging Cold War. Of course, European scientists were acutely aware that there was not a level playing field in postwar science; Americans possessed easier access to materials and instruments as well as funding. As a result, as Santesmases shows, scientists such as Margarita Salas and Eladio Viñuela strategically selected problems and materials for which they could corner the market, such as the working out of RNA and protein synthesis in *Bacillus subtilis* phage $\phi 29$ rather than in the more popular *E. coli* T-bacteriophages or λ phage.

These papers point to a greater range of resources and a more far-reaching set of policies than we usually associate with atomic energy. As names do matter, atomic energy was a captivating term that generated high-profile research projects. The industrial-scale production of mutant mice and radioisotopes reinforced the trend in biology towards reliance on standardized and commodified tools.[22] The effects of promoting these technologies were lasting; Daniel Kevles and Gerald Geison have referred to radioisotopes, for instance, as "sine qua non in molecular biological research."[23] In part, it is the apparent success of this set of practices in our present world that is at the basis of the questions the contributions pose and, in good part, answer. There is not a simple line for any atomic age story-telling, however. This is also shown by the diversity of paths taken by researchers and governmental agencies analyzed in each of the papers that contributes to this issue.

The excavation of atomic age-related biology, agriculture, and medicine is far from complete. In particular, the realm of agriculture is barely touched by these papers or the existing historiography, although the US AEC frequently touted the importance of atomic energy to improving plant science and agricultural practice.[24] At a different level, the collection should invite further international comparisons, particularly extending to countries in Eastern Europe and the Southern hemisphere. Here Gabrielle Hecht's recent work on uranium mining in Africa is particularly suggestive of how atomic science and technology (if not biology or medicine per se) played out at sites strategically important but geographically removed from the

[22] See Gaudillière and Löwy, 1998; Rader, 2004.

[23] Kevles and Geison, 1995, p. 101.

[24] See, for example, US Atomic Energy Commission, 1952.

centers of Cold War power.[25] And Paul Josephson's analysis of "atomic-powered communism" points to the system for disseminating radioisotopes and nuclear technology that emerged on the other side of the Iron Curtain, complete with "isotope stores" in cities such as Moscow and Kiev.[26]

Over the course of the three decades after Hiroshima, radiobiology served as an umbrella term for a variety of research interests supported by atomic science policy. At the policy level, radiobiology helped to legitimate previous developments on nuclear physics and to counteract the public fears associated with the use of atomic weapons. At the laboratory level, the research associated with radiobiology eventually contributed towards a molecular understanding of biological phenomena in an increasingly DNA-centered conceptualization of life, with its associated images and genetic orientation. While we do not want to suggest that the ramifications of atomic age science policy were restricted to research developments associated with radiation genetics and biochemistry (one thinks, for instance, of the significance of radioisotopic tracers to ecological investigation in the 1950s as well as the rapid growth of nuclear medicine), we hope that this collection will invite a fresh and more comparative assessment of the intellectual place and parameters of radiobiology in postwar life science.

References

Abir-Am, Pnina G. 1982. "The Discourse of Physical Power and Biological Knowledge in the 1930s: A Reappraisal of the Rockefeller Foundation's 'Policy' in Molecular Biology." *Social Studies of Science* 12: 341–382.

Beatty, John. 1991. "Genetics in the Atomic Age: The Atomic Bomb Casualty Commission, 1947–1956." Keith R. Benson, Jane Maienschein and Ronald Rainger (eds.), *The Expansion of American Biology*. New Brunswick, NJ: Rutgers University Press, pp. 284–324.

Bocking, Stephen. 1995. "Ecosystems, Ecologists, and the Atom: Environmental Research at Oak Ridge National Laboratory." *Journal of the History of Biology* 28: 1–4.

Bocking, Stephen 1997. *Ecologists and Environmental Politics: A History of Contemporary Ecology*. New Haven, CT: Yale University Press.

de Chadarevian, Soraya. 2002. *Designs for Life: Molecular Biology after World War II*. Cambridge: Cambridge University Press.

Doel, Ronald E. 1997. "Scientists as Policymakers, Advisors, and Intelligence Agents: Linking Contemporary Diplomatic History with the History of Contemporary Science." Thomas Söderqvist (ed.), *The Historiography of Contemporary Science and Technology*. Amsterdam: Harwood, pp. 215–244.

[25] Hecht, 2002.

[26] Josephson, 2000, p. 239.

Evans, Robley D. 1946. "The Medical Uses of Atomic Energy." *Atlantic* 177 (Jan.): 68–73.

Forman, Paul. 1987. "Behind Quantum Electronics: National Security as Basis for Physical Research in the United States, 1940–1960." *Historical Studies in the Physical and Biological Sciences* 18: 149–229.

Fragu, Philippe. 2003. "How the Field of Thyroid Endocrinology Developed in France after World War II." *Bulletin of the History of Medicine* 77: 393–414.

Gaudillière, Jean-Paul. 2002. *Inventer la biomédecine: La France, l'Amérique et la production des saviors du vivant (1945–1965)*. Paris: Éditions la Découverte.

Gaudillière, Jean-Paul and Löwy, Ilana, eds. 1998. *The Invisible Industrialist: Manufactures and the Production of Scientific Knowledge*. Macmillan: London.

Gowing, Margaret. 1974. *Independence and Deterrence: Britain and Atomic Energy, 1945–1952*. 2 vols. London: Macmillan.

Hacker, Barton C. 1994. *Elements of Controversy: The Atomic Energy Commission and Radiation Safety in Nuclear Weapons Testing, 1947–1974*. Berkeley, CA: University of California Press.

Hecht, Gabrielle. 2002. "Rupture-Talk in the Nuclear Age: Conjugating Colonial Power in Africa." *Social Studies of Science* 32: 691–727.

Hewlett, Richard G. and Duncan, Francis. 1990 (1969). *Atomic Shield: A History of the United States Atomic Energy Commission, Volume II 1947–1952*. Berkeley, CA: University of California Press; originally published: University Park, PA: Pennsylvania University Press.

Hollaender, Alexander. 1963. "Acceptance Speech." Michael Ebert and Alma Howard (eds.), *Radiation Effects in Physics, Chemistry and Biology*. Chicago: Year Book Medical Publishers, pp. vii–x.

Jolly, J. Christopher. 2003. "Thresholds of Uncertainty: Radiation and Responsibility in the Fallout Controversy." Ph.D. dissertation, Oregon State University.

Josephson, Paul. 2000. *Red Atom: Russia's Nuclear Power Program from Stalin to Today*. New York: W. H. Freeman.

Kaiser, David. 2005. "Training and the Generalist's Vision in the History of Science." *Isis* 96: 244–251.

Kay, Lily E. 1993. *The Molecular Vision of Life: Caltech, The Rockefeller Foundation, and the Rise of the New Biology*. New York: Oxford University Press.

Keller, Evelyn Fox. 1990. "Physics and the Emergence of Molecular Biology: A History of Cognitive and Political Synergy." *Journal of the History of Biology* 23: 389–409.

—— 1992. "From Secrets of Life to Secrets of Death." *Secrets of Life, Secrets of Death: Essays on Language, Gender, and Science*. New York: Routledge, pp. 39–55.

Kevles, Daniel J. 1990. "Cold War and Hot Physics: Science, Security, and the American State, 1945–56." *Historical Studies in the Physical and Biological Sciences* 20: 239–264.

Kevles, Daniel J. and Geison, Gerald L. 1995. "The Experimental Life Sciences in the Twentieth Century." *Osiris* 10: 97–121, 233–241.

Kohler, Robert E. 1991. *Partners in Science: Foundations and Natural Scientists, 1900–1945*. Chicago: University of Chicago Press.

Kraft, Alison. 2006. "Between Medicine and Industry: Medical Physics and the Rise of the Radioisotopes 1945–65." *Contemporary British History* 20: 1–35.

Krige, John. 2006. "Atoms for Peace, Scientific Internationalism, and Scientific Intelligence." *Osiris* 21: 161–181.

Lenoir, Timothy and Hays, Marguerite. 2000. "The Manhattan Project for Biomedicine." Philip R. Sloan (ed.), *Controlling Our Destinies: Historical, Philosophical, Ethical, and Theological Perspectives on the Human Genome Project*. Notre Dame, IN: University of Notre Dame Press, pp. 29–62.

Lindee, Susan M. 1994. *Suffering Made Real: American Science and the Survivors at Hiroshima*. Chicago: University of Chicago Press.

Muller, H.J. 1927. "Artificial Transmutation of the Gene." *Science* 66: 84–87.

Rader, Karen A. 2004. *Making Mice: Standardizing Animals for American Biomedical Research, 1900–1955*. Princeton, NJ: Princeton University Press.

Rasmussen, Nicolas. 1997. "The Mid-Century Biophysics Bubble: Hiroshima and the Biological Revolution in America, Revisited." *History of Science* 35: 245–293.

Strasser, Bruno J. 2002. "Les Sciences de la Vie a l'Age Atomique. Identités, pratiques et alliances dans la construction de la biologie moléculaire á Genève (1945–1970)," Ph.D. dissertation, Université de Genève, Université de Paris 7.

U.S. Atomic Energy Commission. 1952. *Some Applications of Atomic Energy in Plant Science*. Washington, D.C.: U.S. Government Printing Office.

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INDUCED MUTATIONS IN CROP PLANTS

The Fernhurst Lecture by

D. ROY DAVIES, B.Sc., Ph.D.,

of Wantage Radiation Laboratory, Atomic Energy Authority, delivered to the Society on Wednesday, 16th March, 1960, with F. R. Horne, C.B.E., M.A., N.D.A., Director, National Institute of Agricultural Botany, in the Chair

THE CHAIRMAN: The first time I think I heard of Dr. Davies was at Aberystwyth. This is a very pleasant sounding name to all those concerned with agriculture and with botany because of the outstanding plant breeding work which has been done there. Dr. Davies was also in America. I followed him round to some of the stations where pioneer work has been done on mutations in crop plants induced by artificial means. Dr. Davies is at present at the Wantage Radiation Laboratory, in what we know as the Technological Irradiation Group—the T.I.G. This, of course, is associated with the Atomic Energy Authority, and when I last saw their complicated and most impressive apparatus it recalled to me the story of the two ghosts. One asked the other how he came to be there, and the reply was, 'Well, I was in the car with my wife, and she said, "Be an angel and let me drive"; and I did, and I was'.

I have a very high admiration for those who are doing the pioneer work on atomic energy. I think it is one of the most remarkable phenomena of recent years that rarified nuclear physics should be combined with down-to-earth plant breeding, and I know something of the outstanding work which Dr. Davies has done to bring these together. His is a highly technical subject for the benefit of the agricultural industry, but it is one which has a very definite and close bearing on plant breeding work. I do not think it would be denied that of all the materials which go into the countryside and on to the farm, *seeds* are really the primary materials—the materials on which all production is based; and the difference between good seeds and not so good seeds rests very largely in the different heritage which they carry. That heritage is what Dr. Davies is going to tell us about.

I think we have to congratulate the Royal Society of Arts and the sponsors of the Fernhurst Lecture for having persuaded Dr. Davies to come, because at this time we hear a lot of the possibilities of inducing mutations and obtaining new varieties of crop plants in this way, and there have been a good many references in the agricultural and the national press. It is very valuable to have Dr. Davies' appraisal of it all.

We at the National Institute of Agricultural Botany, Cambridge, are in fact testing two new varieties, one a very promising new barley variety bred or induced in this particular way, which is going to claim the close attention of the people who have to decide about Recommended Varieties of barley. I won't attempt to forecast the future, but all this does indicate that now is the time when we want to have an authoritative assessment of the importance of this new technique in plant breeding and I have very much pleasure in asking Dr. Davies to deliver the 1960 Fernhurst Lecture on this important subject.

The following lecture, which was illustrated with lantern slides, was then delivered.

THE LECTURE

In 1927 two American scientists, Drs. Muller and Stadler, working respectively on *Drosophila* and barley, discovered that ionizing radiations could induce heritable changes or mutations in living organisms. Very soon after these initial studies the question arose as to whether such mutations could be usefully exploited in programmes of plant breeding, but many years elapsed before any concerted effort was made by plant breeders to elucidate the problem. Even to-day the answer is not readily available, though we are now in a position to evaluate the situation more critically. The early reluctance to enter this field of study was due in part to the fact that American geneticists especially were sceptical that any, other than deleterious, changes could be induced. Pioneering work by German and Swedish workers helped to overcome this early scepticism to some extent, but it was the post-war programme of atomic research and the greater availability of sources of ionizing radiations that gave a final impetus to programmes of research on the production and utilization of induced mutations in crop plants.

Since the biochemical nature of hereditary factors or genes is not known, the fundamental changes involved in the production of mutations must remain undefined. We can merely recognize genes as units of function, located in a particular segment of chromosome in the cell nucleus. After exposure to mutagenic agents such as ionizing, and even some non-ionizing, radiations, and a great variety of chemicals, the unit of function may be changed or lost, structural alterations may occur in the chromosomes and even changes in chromosome number may occur. The term mutation is often loosely defined to cover all these, but unless otherwise specified, in this lecture the term will be confined to gene mutations.

The various forms of radiations utilized produce mutations by transferring their energy through ionizations or excitations to sites within or near the genetic material, thereby increasing the chemical reactivity of those sites, whereas chemical mutagens interact directly or indirectly with the genetic material. Spontaneous mutations probably arise due to a variety of causes such as metabolic upsets, physical and chemical changes within or in the environment of the tissues, and natural radiations. The rate of occurrence of these spontaneous mutations has been variously estimated for different genes, tissues and organisms as being between 1 in 10^5 to 1 in 10^8 genes, but after exposure to mutagens this rate may be increased up to a thousandfold. However, most mutations, whether spontaneously or artificially induced, are deleterious and of no value to a plant breeder; in fact, it has been estimated that possibly only one out of every eight hundred induced mutations could be potentially of value. This rare occurrence of the event sought necessitates growing very large populations of plants, and in such large populations, a detection and recognition of the desirable change may be difficult. If mutants exhibiting such obvious changes as differences in height, colour or even disease resistance are sought, then the problem is minimized, but more subtle changes such as those in yield are not as easily found.

Another difficulty which arises when this technique is adopted is that induced chromosomal damage frequently leads to sterility or even an elimination of the cell bearing the damaged chromosomes, and both of these factors result in the non-recovery of mutant genes. Nevertheless in spite of these and other difficulties, useful mutations have been produced, detected and utilized in breeding programmes, and they have stimulated research into ways and means of minimizing the disadvantages of the technique. For example, it is now known that manipulation of various physical, chemical and biological factors within the organism and in its environment can lead to changes in radiosensitivity, amount of chromosome damage and the frequency and spectra of mutations, and these factors will now be considered briefly in relation to their effects on the response of plants to radiations. Since so little is known of chemical mutagens they will not be considered in this context.

Of the physical factors, the type of radiation utilized is of considerable importance. The various radiations which have been used include the low energy ultra-violet radiation, and the ionizing X- and γ-rays, α and β particles, and thermal and fast neutrons. Ultra-violet rays are of limited value for genetic work in higher plants because of their poor penetrating ability, and hence mutation studies with this agent have been confined to analysing the results obtained after treating pollen. It has been suggested that it produces less chromosome damage than the other radiations considered. The sparsely ionizing radiations X-rays and γ-rays have been most extensively utilized in plant breeding work, and they are of particular interest in that their biological effects can be modified very markedly by manipulating and controlling various factors within the organism and its environment. β particles are somewhat similar in action—the most common sources of this type of radiation being the radioisotopes P^{32} and S^{35}. These can be incorporated directly into a growing plant, but it is doubtful whether there is any distinct genetical advantage gained by treating biological material in this manner. There have been comparatively few studies of the effect of the very densely ionizing α particles, but they are of somewhat limited value because of their low penetrating ability. Finally, thermal and fast neutrons produce dense ionizations, but are not limited in terms of penetrating ability in biological material, and hence have been utilized extensively for inducing mutations. Because of the high ion density of their recoil protons, their effects cannot be modified as markedly as the γ- and X-rays. In practice the choice for the plant breeder usually lies between thermal and fast neutrons on the one hand, and X- and γ-rays on the other, and at present the relative merits of the two classes have not been defined clearly. Neutrons certainly have the high relative biological efficiency, that is, for a given amount of energy absorption they induce more damage. Swedish workers have stated that neutrons are fifty to one hundred times as efficient as X-rays in inducing mutations, and ten to twenty times in inducing chromosomal changes, whereas American scientists obtained a similar number of mutations with both when the frequency was measured on the basis of the numbers of chromosomal changes induced. Again, the latter have claimed no differences in the spectra of mutations

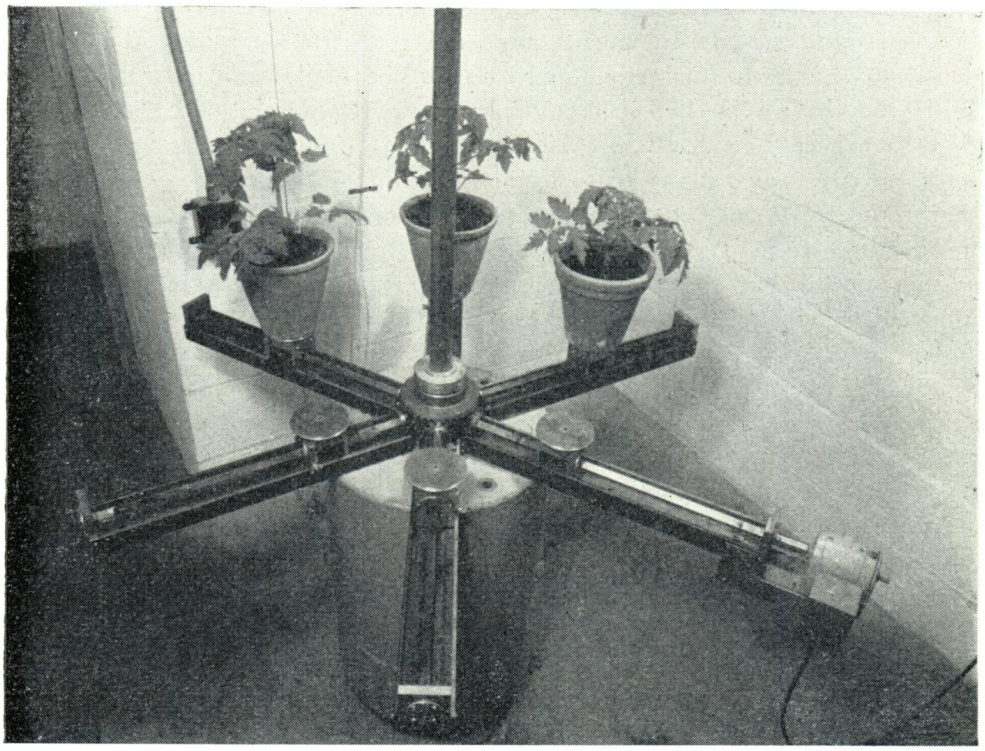

FIGURE 1. 120 *Curie* Co_{60} *installation at the Wantage Radiation Laboratory*

produced, whereas the former indicate that differences do exist. In the absence of more definite evidence there is at present little justification, apart from availability of sources and convenience, for a plant breeder stating a preference for one as opposed to another type of radiation. Figure 1 shows a Co_{60} gamma source at the Wantage Radiation Laboratory of the United Kingdom Atomic Energy Authority which has been specially designed for the irradiation of whole plants, seeds or pollen. The 120 curie Co_{60} source is stored in a lead 'coffin' sited on the floor of the cell and can be raised vertically by remote control through a guide tube to any height up to 100 cms. above the turntables on which the plants are located. Dose rates of 600 to 6,000 rads per hour are available by varying the distance of the turntables from the central guide tube; uniformity throughout the volume being irradiated is ensured by rotating the turntables at 1 r.p.m.

With the less densely ionizing radiations, and certain types of mutational event, a reduction in the dose rate or a fractionation of the dose into two or more parts, results in a lowering of the mutation rate. A few years ago it was suggested that low dose rate treatments or chronic irradiations would be advantageous, as they would allow an irradiated plant to suffer a minimum amount of physiological damage whilst permitting an accumulation of mutations. To allow such treatments to be undertaken, gamma fields were constructed, but as far as the

plant breeder is concerned, they now appear to have no advantage over installations permitting acute treatment only, and are certainly more expensive to construct. Environmental conditions can not be easily controlled in gamma fields; they have to be sited in remote places away from laboratories, and though they have been considered valuable for treating large specimens such as fruit trees, it has yet to be shown that chronic irradiation of these is any more efficient than acute treatments of cuttings which can later be rooted, or grafted on to unirradiated stocks. Again, let us assume that a plant is exposed for a month in a gamma field, but within the first week of treatment a desirable mutation is induced; then for the remaining three weeks that plant is exposed to physiological and genetic damage which may result in the non-recovery of that particular mutant.

The effect of temperature both during and pre- and post-treatment has been extensively investigated and shown to be important in determining the biological response of tissues to sparsely ionizing radiations. For example, seeds exposed at ca. −190°C. show much less damage than those irradiated at room temperature, and seeds immersed in water at +90°C. immediately post-irradiation are similarly protected. Germination at sub-optimum temperatures results in an enhancement of damage, presumably due to a lowered rate of metabolism and an inability to repair cell damage. Again, the water content of tissues is important in this respect; for example, dry barley seeds (ca. 6 per cent water) are more radiosensitive than wetter ones (ca. 16 per cent water). Finally, the injurious effects of X- or γ-radiation on dormant barley seeds increase with the length of time that the seeds are stored after irradiation and before hydration. This storage effect, as it is termed, is more marked in dry than wet seeds, and is enhanced in the presence of oxygen.

Of the chemical factors that can affect the response of biological material to X- and γ-rays, the effect of the atmosphere during irradiation has been investigated most thoroughly. Gases such as oxygen, carbon dioxide and carbon monoxide enhance the effect, whilst nitrogen and the inert gases protect tissues. Similarly a few chemicals such as ascorbic acid, cysteine and thiourea reduce, whilst others such as potassium cyanide and hydrogen peroxide enhance the effect of a given dose. The chemical content of tissues is also known to be important—those deficient in Calcium and Magnesium being more radiosensitive, whilst those deficient in Boron are more resistant.

There are numerous biological factors which are important in this context, including genotype, chromosome number, tissue, age, stage of cell division, and stage of development. Species vary greatly in their sensitivity, a dose which would kill 50 per cent of exposed plants in some *Pinus* species being about 4,000 rads, whilst for some *Brassica* species the value is near 100,000 rads. Little is known of the basic reason for these differences, but even two varieties or two sister plants differing very little in genotype can vary in sensitivity. If closely related species exhibit a range of chromosome numbers, it will be found in general that the higher chromosome number forms are more resistant. Tissue differences have not been investigated thoroughly—seeds are certainly more

resistant than whole plants or gametes as their cells are in a resting stage, but the more subtle differences, for example, between root cells, pollen mother cells and pollen grains have not been fully elucidated. As far as the plant breeder is concerned, the treatment of seeds has a number of obvious advantages—they are available in bulk, can be easily handled and can tolerate a great range of environmental conditions. Regarding the other factors—aged seeds are more sensitive than fresh seeds, whilst the stage of cell division, especially during meiotic stages, has been shown to be most important both in terms of the amount of chromosome damage and number of mutations induced. The importance of stage of development has been indicated by the work on pollen, where it has been shown that most mutations are produced if pollen is irradiated three to five days before anthesis, and by the fact that germinating seeds are more sensitive than resting seeds.

When all the information available from studies of these and other factors is considered, it is apparent that the response of a tissue to a given dose of sparsely, but not densely, ionizing radiations can be greatly modified. Modifications of practical value would include inducing as high a frequency of mutations as possible, but with a minimum amount of chromosomal aberrations and physiological injury. However, though the plant breeder can exercise some degree of control in this respect, it is still very far removed from the Utopian idea of a direction of the mutation process.

Chemical mutagens have not been as extensively investigated as ionizing radiations, but already some are proving of considerable interest to the plant breeder. For example, nebularine, a purine-9-d-riboside, is a mutagenic agent which does not produce any chromosomal breakage, and two other compounds—ethylene oxide and ethlene imine—have been shown by Swedish workers to produce up to four times as many viable mutations as X-rays, but with no increase in chromosomal aberrations.

In spite of the inefficiency of the techniques for inducing mutations, useful variation has been induced in many species of crop plants, some has been incorporated into breeding programmes and a few new varieties have resulted. In Sweden, barley, wheat, oat, pea, soya bean, flax and mustard mutants have been produced which vary in yield, straw strength, earliness and other characters, and new varieties have resulted in some instances. These include a higher yielding white mustard, oil-rape and pea, and a stiffer-strawed variety of barley. One interesting report from Sweden claims that they have produced barley mutants which flower earlier and hence can be grown in more northerly latitudes—such variants not being available to them previously. From Germany plant breeders have reported cereal variants having higher grain yield, up to eight days earlier flowering, stronger straw, higher protein content and improved disease resistance. The naked-kernel mutants of spring-barley reported by Scholz from Germany offer an instructive example of the use of radiation. A gene 'n' for nakedness can be introduced from non-adapted varieties of barley, but in spite of intensive work for many years, attempts to produce high-yielding naked lines were unsuccessful After irradiation, naked mutants were found having the same

yield as the parent lines. From that country there have also been reports of some very promising blackcurrant mutants, which vary in terms of size of bunches, taste, seed number, and earliness. These last results are especially encouraging as progress in the breeding of this crop by conventional methods had been extremely slow and unrewarding. In Britain a self-fertile form of cherry has been produced from the normally self-sterile type, that should be less dependent on insects for pollination and therefore give a better yield in colder regions and unfavourable seasons. A considerable number of cereal mutants have been produced at the Plant Breeding Institute at Cambridge, but none has proved very valuable as yet. American workers have reported variants similar to those mentioned previously, and in a great variety of crops, including horticultural species. Frequently the latter exhibit gross changes in form and colour which might be deleterious in agricultural crops but have considerable value as curiosities in horticulture. The most thorough studies in America of the potential value of mutation breeding have been undertaken by Gregory. In 1949 he instituted a programme for the improvement of groundnut varieties, and ten years later he had produced a new variety NC4X, by mutation breeding, which had a higher yield than the original variety (2,925 as opposed to 2,759 lb. per acre), improved disease resistance and a lower percentage of damaged and cracked pods. It is as well to note here that a few years ago, a great many claims were made by American workers regarding the induction of mutants which were resistant to various diseases, and their early optimism regarding the value of mutation breeding was to a large extent based on these claims. However, more recent analyses of these results, and a repetition of some of the experiments under more critical conditions has shown that most of these claims were not justified, and the disease-resistant forms were not in fact due to mutations, but to natural crossing of the irradiated plants with resistant varieties growing in the vicinity.

We can now make a preliminary evaluation of the status of mutation breeding, bearing in mind the techniques that are available at present, and the results that have been achieved.

Firstly it has yet to be shown conclusively that radiation can produce anything new, that is, a mutant not occurring naturally. There have been indications of this in the claims of American scientists regarding the production of winter hardy oats and a new source of stem rust resistance in the same species, in the production of different types of leaf marks in *Trifolium repens*, and in the production of early maturing forms of barley in Sweden, but conclusive proof of these claims is difficult, especially in view of the problems of exploring all naturally occurring genotypes.

Secondly, if the vast majority of induced mutations are merely duplications of those which occur naturally, then the relative merits of this technique and the more conventional methods of plant breeding must be considered in terms of economics—time, space and labour requirements. No valid comparisons of the two techniques in terms of these factors have been undertaken, and the potential amounts of variability which could be released by hybridization and

mutation has yet to be compared. Gregory's results with the breeding of peanuts are of interest in this connection. In 1949 he instituted two programmes of breeding for the improvement of groundnut varieties—one based on mutation and the other on hybridization. Ten years later he had succeeded in producing two new varieties—one by either method, both of which were very similar in terms of yield and general performance, and considerably superior to the original variety. Actual figures for yield in lb./acre were

Original variety NC4 — 2759
Mutant variety NC4X — 2925
Hybrid variety NC2 — 3059

That this technique offers no short cut to success is also indicated by the fact that Down & Anderson produced a mutant form of *Phaseolus vulgaris* in 1941, but seventeen years elapsed before it was suitable for release to the growers. Similarly Swedish workers produced a mutant form of barley in 1947 which is being released this year as a new variety—Pallas.

Thirdly, it has been suggested that this new method of breeding would be useful for adding a single characteristic to an otherwise well-adapted variety—in other words, one would not upset the genotype unduly by this method, whereas if one attempted to introduce a single desired character by hybridization, the background genotype would be considerably altered. There are a few examples of such situations; for example, Indian workers have succeeded in producing, from awnless forms of wheat, awned mutants which differ little, if at all, in other respects from the original variety. Attempts to introduce this character by backcrossing have already taken a considerable number of years, and the genotype is still different from the original awnless forms. A similar situation existed with the naked barley mutants reported by German workers and discussed earlier. In general, however, instances such as these two quoted are exceptions rather than the rule, and one finds that a mutation induced in one gene is almost invariably accompanied by numerous changes in the remainder of the genotype. It then follows that after the initial production of the desired mutation, a considerable amount of backcrossing has to be undertaken to restore the remainder of the genotype. In other words, this technique is not likely to result in the production of new varieties immediately, but is merely a method of inducing variability, and the conventional plant breeding techniques of selection and hybridization must follow.

Finally, there are certain situations where it is likely that induced mutations can make significant contributions to plant breeding. The exceptions mentioned previously must be included here, but there are other situations where, for example, a loss mutation is desirable, and a good example of this is the production of self-fertile cherries from the normally self-sterile forms. In the case of asexually propagated plants also, where due to the length of the life cycle and their genetical complexity, conventional methods of breeding are difficult, it is possible that artificially induced mutations could prove valuable, and the work on blackcurrants seems to support this contention.

Up till now, the value of radiations and mutagenic chemicals has been considered solely in terms of the production of useful variants in cultivated varieties of crop plants. However, it is conceivable that they could be valuable in other respects also. One particular application which we have investigated intensively at the Wantage Radiation Laboratory is the possibility of using ionizing radiations to overcome the barriers to crossing that frequently exist between our cultivated forms of plants and their closely related wild species. Frequently one finds that valuable characters, such as resistance to diseases, exist in these wild species, which it would be desirable to introduce into the cultivated forms, but this introduction is often precluded by the barriers to crossing between the two forms. The technique we have adopted in our work is to irradiate either male or female gametes at all stages of development from premeiotic cells to mature gametes with a range of doses of γ-radiation. These irradiated gametes of one species are then utilized on successive days as they mature, for crossing with unirradiated plants of the other species. In this way we have undertaken very comprehensive programmes, involving thousands of crosses, in a range of species. Of the series we have attempted, those between *Hordeum vulgare* and *Hordeum bulbosum*, and between *Antirrhinum majus* and *Antirrhinum orontium* were wholly unsuccessful. That between *Vicia faba* and *Vicia narbonensis* resulted in a significant increase of large aborted ovules over that obtained in the control series, and that involving *Lycopersicon esculentum* and *Lycopersicon peruvianum* resulted in the production of two hybrids, but in the latter instance the very rare production of a hybrid has been reported previously by other workers. The final series involved *Brassica oleracea*, *Brassica campestris* and *Brassica nigra*, and of the many possible combinations, the one involving *B. oleracea* x *B. nigra* proved extremely interesting. No hybrids were obtained in the control series but after irradiation a total of 35 hybrids were produced (Figure 2). Swaminathan has reported from India the production of hybrids between *Nicotiana tabacum* and *Nicotiana rustica* after irradiation, and from Japan there have been similar reports of new hybrids between *Avena strigosa* and *Avena barbata*, and between *Avena strigosa* and *Avena sativa*.

Though some new hybrids have been produced with the aid of mutagenic agents, we have once again to evaluate the potential usefulness of this technique. It is obvious that it is not going to succeed in overcoming many instances of interspecific incompatibility, and since there is such a diversity of types of barriers to crossing, it will probably be impossible to extrapolate from our present results and predict the results in any other cases. Again, even in those instances where it has succeeded, one has to consider whether the same end result would have been achieved more easily by screening naturally occurring genotypes in order to determine whether some more compatible forms exist, or whether in some instances other techniques such as the *in vitro* culture of hybrid embryos would have proved even more profitable.

Other specialized ways of exploiting ionizing radiations in plant breeding have involved utilizing induced chromosomal changes. In an earlier section chromosomal changes were referred to as undesirable features accompanying the process

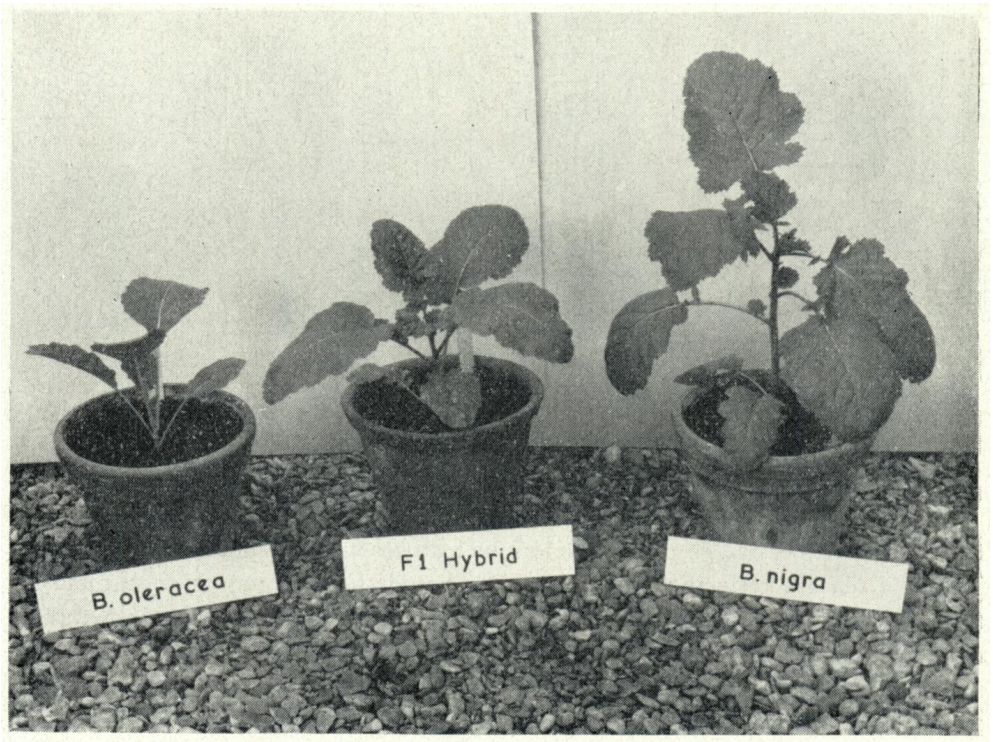

FIGURE 2. *Plants of* Brassica oleracea, B. nigra *and their interspecific hybrid*

of mutation, as they can often lead to sterility. Occasionally, however, chromosome breakage may have some value, and several examples may be quoted to illustrate this point. One involves the possibility of separating two genes or two parts of a single gene, one of which is useful and the other deleterious, but which are so closely linked together on the chromosome that it is difficult to separate them naturally. In oats there is a situation in which a dominant genetic locus conditions susceptibility to Victoria blight and resistance to several races of crown rust, and it will be of interest to follow the attempts being made to separate the two parts by radiation. We have shown that two parts of a single genetic locus in clover can be separated fairly easily by radiation, but are not separable in nature (Figure 3). Again, the changes produced by the breaking and rearranging of chromosomes are extremely valuable in locating the relative positions of genetic loci on chromosomes, but the outstanding use of the breakage phenomenon has been demonstrated by an American geneticist, Dr. Sears. One of the prime problems facing plant breeders in America is that of the leaf rust diseases which attack cultivated wheats. *Aegilops umbellulata*, a wild grass related to wheat, is resistant to some of these diseases, and moreover there is no effective barrier to introducing this resistance into cultivated wheats. However, progress in the utilization of hybrid plants has been impeded by the fact that wheat plants having the additional chromosome from *Aegilops* bearing the

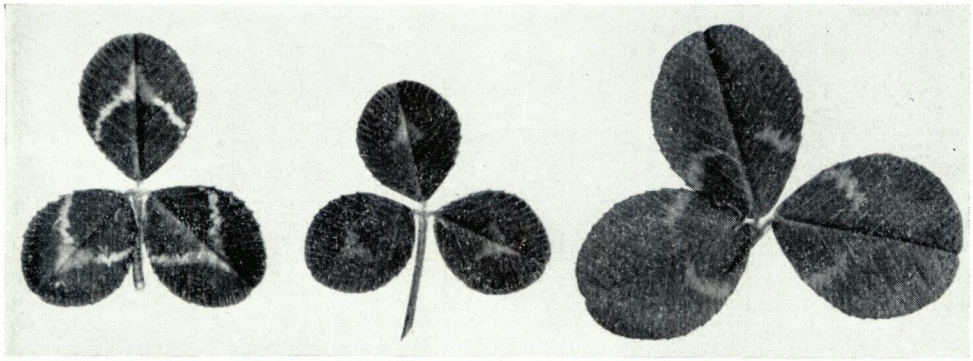

FIGURE 3. *Leaves of* Trifolium repens *showing on the extreme left a normal leaf, and to the right leaves in which the two component parts of the leaf mark have been separated*

disease resistance, had so many deleterious features which could not be separated from the disease resistance. Dr. Sears irradiated these plants prior to meiosis, and in this way broke the linkage of disease resistance and undesirable features, and translocated the segment of chromosome bearing the desirable gene on to one of the wheat chromosomes. The plants which he ultimately produced were distinguished from normal wheat only by their rust resistance and slightly later maturity. This work has been an outstanding achievement in the field of plant genetics, and is unlikely to have succeeded without the use of mutagens. Similar attempts are now being made at other research centres to transfer characters from one species to another, but it remains to be seen whether this technique can be exploited in many other instances.

In summarizing briefly the position of mutation breeding to-day, it is as well to remember that in Britain there is in general very little enthusiasm for the technique, and that in America the mood of tremendous optimism of a few years ago has changed to one of extreme caution. In Sweden and Germany, where during the last twenty to thirty years so much has been done to exploit the technique, there is much optimism that it can make a significant contribution to plant breeding. Elsewhere the tendency is for enthusiasm to be inversely related to knowledge and experience of the technique. As yet a total of only seven new varieties have been produced by mutation breeding, and even in their production conventional breeding techniques have still played a large part. It must be remembered, however, that a considerable proportion of the research effort on this topic has so far been confined to exploratory work concerned with techniques.

Future research work in this field will probably include studies of the effects of modifying factors, the relative merits of pollen, whole plant and seed irradiation, the sensitivities of different cell stages, the value of other radiations such as ultra-violet and α particles, the value of polygenic rather than major gene mutations, methods of modifying the competition of normal and mutant cells in

multicellular tissues, and the relative rôle of induced mutations in inbreeders and outbreeders. The more specialized investigations concerned with the use of chromosomal changes will be pursued further, and the field of chemical mutagenesis is still in its infancy and worthy of a considerable amount of research effort. It is in this last field that the greatest hope for this technique lies.

With the present status of mutation breeding and the inefficient techniques available to us to-day, there is little justification for a plant breeder seeking variability from irradiated material, if it is already available naturally. There are exceptions, but in general it has yet to be shown conclusively that the required variability can be produced more quickly, more economically, or with less utilization of space and labour by mutagens than by hybridization. The exceptions include some examples of loss mutations, mutations in asexually propagated plants, and those in horticultural crops where bizarre forms often have commercial value. Claims that mutation breeding is essential because we are running out of variability do not appear to be justified. It is true that in some instances where, for example, hybrid maize programmes are being introduced, gene pools may be restricted unless special precautions are taken to conserve old land varieties, but in general, the reservoirs of variability which are available in natural populations and which are being released continually by hybridization, are very great.

Future work, especially with chemical mutagens, may drastically modify our concepts regarding the status of mutation breeding, but it is obvious that much research work has to be undertaken before a full evaluation of its true potential is possible.

DISCUSSION

MR. P. K. SHAHANI: Could the lecturer tell us the reason why the biochemistry of the cells has not yet been found out?

THE LECTURER: The biochemistry of genes (which is what one is ultimately interested in) is a very specialized field which we are only starting to exploit, and we have very little understanding of the basic biochemical pathways of some of our genes.

DR. A. D. MCKELVIE (Plant Breeding Institute, Cambridge): I wonder if Dr. Davies could say anything about the future possibilities of producing different types of mutations after different treatments? At Cambridge at the moment we are doing a lot of work on mutations, trying to find out if mutagenic agents do in fact produce differences in mutation spectrum. So far we have not found any evidence of this but the programme is in its early stages and will continue for some time. Dr. Davies is probably aware of what is being done by the Fahmys at the Chester Beatty Institute with alkylating agents on *Drosophila*. They claim that some of these chemicals produce mutations not previously seen after radiation.

THE CHAIRMAN: Dr. McKelvie, before Dr. Davies answers would you care to say what mutagens you are using at Cambridge to cause the mutation?

DR. MCKELVIE: At Cambridge we are not working primarily on crop plants. We are using a small annual, *Arabidopsis thaliana*, which occurs naturally in this country. We use it because it flowers in three weeks from sowing the seed so that you can obtain a lot of generations in a year; and we are testing the effect of several mutagens on the mutation rate and spectrum of this plant. We are using X-rays as the sole

source of radiation because it is felt that the differences between types of radiation will be less important than between radiation and various chemicals. The chemicals we are using include some of the alkylating agents that Dr. Davies mentioned. We are also using nebularine, fluorouracil and several other chemicals which act upon the cell nucleus. Dr. Davies is aware that we do not know how these chemicals produce mutations, but at least they should have different modes of action in the cell.

THE LECTURER: I am delighted to have Dr. McKelvie's comments on this work. I suspect he knows a great deal more about it than I do. In relation to this particular question (the work of the Fahmys), I must apologize for not mentioning it and for having to rush unduly through this lecture. Chemicals are claimed to produce a different spectrum of mutations in *Drosophila*, which is the organism with which they are working. But as far as I am aware there has been no repetition of this work. At present I personally am not in a position to judge their results, but certainly there are indications that chemicals do produce a different mutation. I am very interested to hear of what Dr. McKelvie is doing at Cambridge and I think these chemical mutants he is using are the answer to many problems.

MR. R. A. CUMBERLAND: In octoploid strawberries, which already have a fairly wide variation, would there be any advantage in using mutagens?

THE LECTURER: Again, that is a very difficult question to answer. If you have variability available in your strawberry population, I would say every time, use it and do not seek the variability which is produced by mutation, because you are so liable to run up against the problem of sterility for one thing, and the chances of your finding a desirable mutation are so small. From an academic point of view these studies are always justified, but if I was a practical plant breeder, I think I would have to be very desperately in need of some variation before I could justify doing mutation breeding. I do not know what some of the other experts in the audience feel, but this is my own personal point of view.

DR. P. S. HUDSON (Commonwealth Bureau of Plant Breeding and Genetics, Cambridge): I think on this point it might be of interest to the speaker and the audience for me to mention a conversation I had with Dr. Lein, who it will be remembered was one of the earliest workers on mutation breeding in Germany—in fact, anywhere. Dr. Lein is now working for a commercial firm as a plant breeder, and when I asked him whether he was using mutation breeding, he said, 'Oh no, not any longer'.

DR. JOHN K. JONES (Department of Agricultural Botany, Reading): Whether you use mutation commercially seems to me to depend very much on whether you feel it is possible to change one thing at a time. I was surprised to hear that you regard this as so difficult that any attempt to induce mutations in individual genes is not worth while. Chromosome breaks have frequently been induced at one place, for example by Sears, and I suggest that it is not really so difficult to change only one gene at one time. The remaining problem is whether the frequency of desirable change is sufficiently high.

THE LECTURER: Yes, it is certainly true, you can change one gene at one time. In wheat, the suppressor gene for awn formation has been eliminated and awned varieties of wheat produced which are no different in any other respect from the awnless varieties. There are other instances—the naked-kernel mutants in barley is an example. But this is not the general case, and I would quote an eminent American worker in this respect; Dr. Frey of Iowa once stated at a symposium that you may well induce a single gene mutation and not affect the rest of the genes at all; but that in general, if you induce a mutation, and this I quote, 'You also introduce an awful lot of dirty genes'. What you say is perfectly true, it can be done in some instances;

you can just change the one character you want; but in general you will find that you have changed a tremendous number of other characters.

DR. KAMLA KANT PANDEY (John Innes Horticultural Institute): I should like to know how Dr. Davies produced these hybrids? At what stage did he overcome the barriers to crossing between species?

THE LECTURER: When I was trying to overcome the barriers to crossing between two species, at what stages did I irradiate the plants and what was my procedure? Well, let us take the two examples, *Brassica oleracea* and *Brassica nigra*. Our programme would be this: we would cross *Brassica oleracea* and *Brassica nigra*, as controls; we would do the reciprocal cross *Brassica oleracea* x *Brassica nigra*, again as controls; then the radiation programme would be this, first *Brassica oleracea* x *Brassica nigra* with the female irradiated. We would irradiate the female at all stages from the premeiotic divisions of the egg right up to maturity; we would thus be testing every successive stage, and every alternate day we would be crossing as successive mature eggs developed. That would be the first thing—irradiate the female at all stages. We would also irradiate with a range of doses, usually from about 50 rads to about 3,000 rads. The next cross would involve irradiating the male parent, which in this case would be the *Brassica nigra*. Again we would irradiate at all stages from meiosis right up to mature pollen again with a range of doses. That is the second cross. Then we would do the reciprocal, and take *Brassica nigra* as the female and irradiate that at all stages of development with a range of doses, and then the last cross would be *Brassica oleracea* as the male—irradiated at all stages of pollen development with a range of doses.

So in any one crossing programme there were thousands of crosses involved. All the female plants were emasculated every time. It involved a tremendous amount of work, and the returns for it were slight.

DR. D. J. GRIFFITHS (Welsh Plant Breeding Station, Aberystwyth): I should like to compliment Dr. Roy Davies on his very lucid and objective account of irradiation breeding, and I wish to thank him for presenting the pros and cons of the method as a technique of crop improvement. Speaking from experience of irradiation breeding studies conducted on winter oats over the last five years, I would also stress the need for a critical appraisal of the method; to be of use in a practical improvement programme, mutagenic techniques must be judged by comparison with other methods of achieving similar end results, as for example hybridization and selection. Comparisons between irradiation breeding and that by means of intervarietal hybridization and selection have led us to the conclusion that if the character sought does not exist in the collection of genetic material available to the breeder, the attempts to produce it *de novo* through irradiation are fully justified irrespective of the other limitations of the method, but I must state that it is worth while searching very hard indeed for the required variation in the wild before resorting to its artificial induction through ionizing radiations. When the desired variation is available, careful selection of parents for hybridization ensures a much higher probability that variation in the desired direction will be induced than by the irradiation of one of the parents.

I should like to ask Dr. Davies one point; if he were to initiate an irradiation programme with the cereals, would he irradiate seeds or pollen?

THE LECTURER: The answer to that, I hope, we shall be able to give in exactly one year's time. This is one of our main programmes at present: to compare the amount of polygenic variability which is induced when you irradiate inbred *Antirrhinums* (these have been inbred for about twenty years) as seed, and as pollen. We have undertaken preliminary programmes of pollen irradiation of several species. The first one involved *Melandrium album*, and we wished to determine exactly how much variability was induced when you irradiated mature pollen. We found there was a tremendous amount of variability induced for flowering time, in this particular

species. We were so encouraged by this result that we then went on to irradiate barley mature pollen in the hope that we would be equally successful. We undertook quite a big programme, and scored flowering time, height, tiller number, and ear length. After exposure to a range of doses ranging from quite low doses, 250 rads, up to the lethal dose, the net result of two years' work was that we found that we had induced no variability whatsoever in barley after irradiating pollen.

The next stage of this programme involves a comparison of the amount of quantitative variability produced when you irradiate the different stages of barley pollen development from premeiotic stages right up to maturity. We have taken this programme up through the first generation (x 1 generation) but in view of our disappointing results last year after irradiating mature pollen, I feel it is not worth pursuing this particular problem further.

If I was undertaking a practical programme, I think I would concentrate on seed irradiation until we have more evidence regarding pollen irradiation. Pollen irradiation can be difficult due to problems of handling, and the same quantity of x material cannot usually be produced as with seed irradiation.

PROFESSOR G. C. VARLEY (Hope Professor of Entomology, Oxford): The point you made that the tetraploids and octoploids were less affected than the diploid forms suggests that a reasonable proportion of the mutants that are produced are possibly recessives. To what extent is your technique directed to discovering recessive mutants?

THE LECTURER: I would say that 99.99 per cent of mutations are recessive. This is probably the reason why you get this reduced sensitivity in high polyploids—the recessives are just masked by the normal dominant alleles. Certainly we have never found dominant mutations and if anybody does find one his first thought should be one of suspicion, that it is a contaminant.

MR. WALKER (Messrs. Bees Ltd.): Is one more likely to get induced mutations in plants where one has a proportion of natural mutations? In roses, for example, where quite a lot of varieties are natural mutations?

THE LECTURER: In general if you have a gene which shows a high rate of spontaneous mutation the induced mutation rate is usually very high too. The cholorophyl mutations which I showed you have a fairly high spontaneous mutation rate, and the induced rate is very high. In the case of the roses—if you have no variability in your natural population I would say quite definitely you are justified in trying to produce a mutant type of rose. Horticultural plants are probably among the most promising for this technique because of the value of bizarre forms. In agricultural crops such mutants would have to be thrown out immediately.

MR. D. M. PITKETHLY (Charles Sharpe & Co. Limited, Seedsmen, Sleaford, Lincs): I should like to ask whether Dr. Davies thinks that the types of mutation coming from radiations are more frequently less beneficial than naturally occurring mutations?

It seems to me that in naturally occurring mutations there is a natural selection which will make many of them disappear before they are noticed, and only those giving an advantage become apparent; whereas in the artifically created ones we have all the effects, both good and bad, to choose from. Does Dr. Davies think that the artificially created ones are more lethal, should I say, than the naturally occurring mutations?

THE LECTURER: I would say that from the evidence in *Drosophila* the induced mutations which you get are almost exactly the same as the spontaneous mutations.

DR. L. A. DARBY (Glasshouse Crops Research Institute): Being associated with horticulture I am presumably classified as being bizarre, but I should like to comment about my experience with lettuce and chlorophyl mutants. A programme was initiated three years ago to attempt to produce a winter hearting lettuce, a lettuce which would

heart in short days, but of paler colour than existing types. Rather cautiously I decided to tackle this in both a radiation programme and also in an orthodox breeding programme. Over three years the proportion of the total material devoted to the radiation programme has fallen from 50 to 10, and then down to about 3 per cent. This is very much in keeping with what Dr. Davies has said.

THE LECTURER: I know of Dr. Darby's work and am very interested in it. I hope that I have not been too pessimistic, in this lecture and discussion. One tries to keep a sense of perspective; do not think I dismiss the technique completely out of hand. On the other hand, one must be very careful not to consider it as a panacea for all the plant breeder's ills. In some cases I feel sure the technique is going to be worth while, but one must have highly specialized knowledge and think very seriously before deciding to use it.

Though I have tried to present a balanced picture, my own feeling is that in general the mood should be one of pessimism rather than of optimism. But it must not, I repeat, be dismissed completely. It is worth while doing a lot more research before we finally make our decision as to what its contribution can be, and the sort of work which Dr. McKelvie is doing at Cambridge is most valuable.

THE CHAIRMAN: I should first like to say on behalf of us all how grateful we are, both to the Royal Society of Arts for arranging this meeting, and to the sponsors of the Fernhurst Lecture.

There are so many sides to the comprehensive survey we have heard, but I am sure you will all have noted with very much interest Dr. Davies' remark that induced mutations on the whole have not given a high proportion of really important and valuable improvements. I know there is a lot of interest at present in other countries as well as in Britain, in the encouragement of plant-breeding. Perhaps it is rather reassuring to the breeder who has spent perhaps fifteen years in hybridizing and testing his material, to know that there is not a very big chance that someone else can do just as well in two or three years simply by inducing mutations in the established varieties.

I am sure you will all agree with me that Dr. Davies has made a very important and difficult subject both intelligible and interesting, and on your behalf I should like to thank him most warmly for his lecture.

The vote of thanks to the Lecturer was carried with acclamation and, another having been accorded to the Chairman upon the proposal of Mr. A. R. N. Roberts, the meeting then ended.

Ion-beam irradiation, gene identification, and marker-assisted breeding in the development of low-cadmium rice

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Rice (*Oryza sativa* L.) grain is a major dietary source of cadmium (Cd), which is toxic to humans, but no practical technique exists to substantially reduce Cd contamination. Carbon ion-beam irradiation produced three rice mutants with <0.05 mg Cd·kg^{-1} in the grain compared with a mean of 1.73 mg Cd·kg^{-1} in the parent, Koshihikari. We identified the gene responsible for reduced Cd uptake and developed a strategy for marker-assisted selection of low-Cd cultivars. Sequence analysis revealed that these mutants have different mutations of the same gene (*OsNRAMP5*), which encodes a natural resistance-associated macrophage protein. Functional analysis revealed that the defective transporter protein encoded by the mutant *osnramp5* greatly decreases Cd uptake by roots, resulting in decreased Cd in the straw and grain. In addition, we developed DNA markers to facilitate marker-assisted selection of cultivars carrying *osnramp5*. When grown in Cd-contaminated paddy fields, the mutants have nearly undetectable Cd in their grains and exhibit no agriculturally or economically adverse traits. Because mutants produced by ion-beam radiation are not transgenic plants, they are likely to be accepted by consumers and thus represent a practical choice for rice production worldwide.

Cadmium (Cd), a contaminant that enters the food chain from multiple natural and industrial sources, is toxic to the kidneys, particularly to the proximal tubular cells, where it accumulates and leads to renal dysfunction (1). In Japan, *itai-itai* disease (renal osteomalacia), which is characterized by spinal and leg bone pain, is recognized as chronic toxicity caused by excess Cd in drinking water and crops (2). To reduce the risk of Cd poisoning, the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives established a provisional tolerable monthly Cd intake of 25 μg·kg^{-1} body weight (3), and the Codex Alimentarius Commission of the FAO/WHO established maximum Cd levels in food crops (4). The international maximum limit for rice is 0.4 mg Cd·kg^{-1} polished rice. Rice is a staple food for nearly half of the world's population, and global production and consumption of rice increased approximately threefold from 1960 to 2011 (5). The demand for rice continues to grow, so it is necessary to produce low-Cd rice to reduce the potential risk that Cd poses to human health.

There are substantial genotypic differences in Cd accumulation in rice (6, 7), concentrations generally being higher in *indica*-type cultivars than in *japonica*-type cultivars. Genetic loci determining genotypic differences in Cd accumulation have been identified by quantitative trait locus (QTL) analysis of several mapping populations (8, 9). Recently, genes involved in Cd uptake by the root (10–13), root vacuole sequestration (14, 15), root xylem loading (16, 17), and phloem transport in the node (18) have been found in rice, so the physiological and molecular processes of Cd transport in rice are increasingly well understood (19). Although regulation of Cd transport by transgenic techniques may enable us to reduce Cd accumulation in rice grain, commercial transgenic rice is not currently acceptable in many countries, such as Japan. Many consumers fear eating food produced by transgenic plants.

Energetic heavy-ion beams have been recently used to generate mutants in higher plants because they induce mutations with high frequency at a relatively low dose (i.e., at which virtually all plants survive), and they induce a broad spectrum of phenotypes without affecting other plant characteristics (20, 21). Using this technique, unique varieties of some flowers and trees have been commercialized, but this has not yet occurred in crop plants. Mutants produced by ion-beam radiation are not transgenic, so they are more likely to be accepted by consumers.

In the present study, we report (*i*) nontransgenic rice mutants with nearly cadmium-free grain produced by irradiation with heavy-ion beams and (*ii*) the development of a DNA marker for further breeding based on the identification of the gene (*OsNRAMP5*) responsible for low Cd uptake. Field studies show that these mutants have nearly nondetectable levels of Cd in the grain, even when cultivated in paddy fields contaminated with high levels of Cd.

Results

Isolation of Low-Cd-Accumulating Rice Mutants. We irradiated seeds of the most popular Japanese temperate *japonica* rice cultivar, Koshihikari, with accelerated carbon ions. Three low-Cd mutants (*lcd-kmt1*, *lcd-kmt2*, and *lcd-kmt3*) were identified in initial screening for grain Cd concentration from among 2,592 M_2 plants grown in Cd-polluted soil. The grain Cd concentration in the three mutants was <0.05 mg·kg^{-1}, compared with an average of 1.73 mg·kg^{-1} in the WT Koshihikari parent (Fig. 1*A*). The root and shoot Cd concentrations were significantly lower in all M_3 *lcd-kmt* mutants than in the WT (Fig. 1 *B* and *C*) when the seedlings were exposed to Cd in hydroponics. The concentrations of iron (Fe), zinc (Zn), and copper (Cu) in shoots and roots did not differ significantly between the *lcd-kmt* mutants and the WT (Table S1). However, the manganese (Mn) concentration in the shoots was significantly lower in the mutants (73.6–79.7 mg·kg^{-1}) than in the WT (1,004 mg·kg^{-1}). There was no difference in plant growth among the WT and *lcd-kmt1* or *lcd-kmt2* mutants, but the growth

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The authors declare no conflict of interest.

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Data deposition: The cDNA sequences reported in this paper have been deposited in the DNA Data Bank of Japan, http://ddbj.nig.ac.jp [accession nos. AB690551 (OsNRAMP5), AB690552 (osnramp5-1), and AB690553 (osnramp5-2)].

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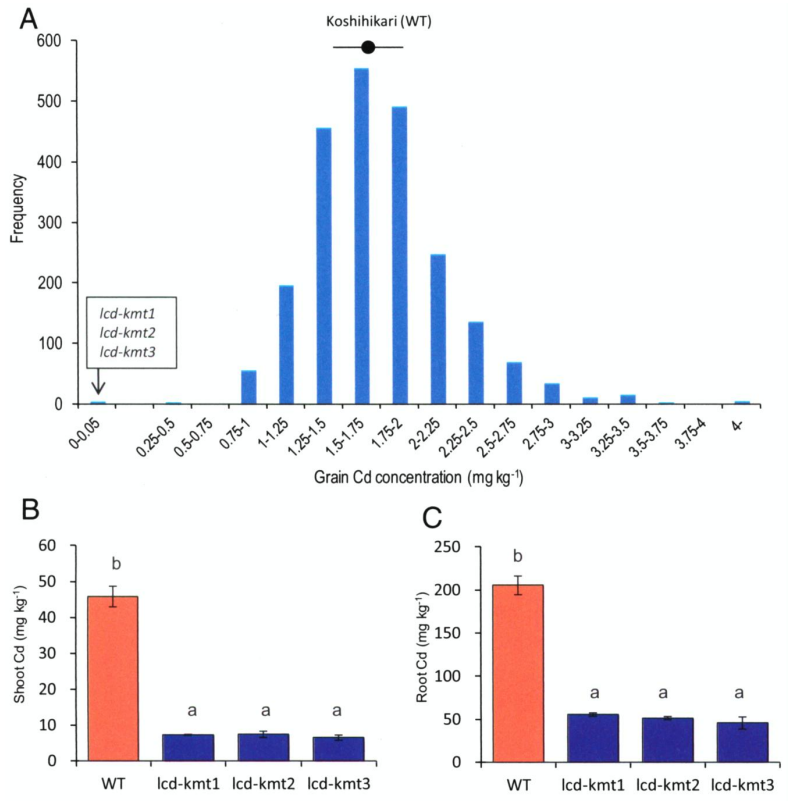

Fig. 1. (*A*) Frequency distribution of grain Cd concentration in rice mutants (2,592 M_2 plants) grown in pots filled with Cd-contaminated soil. The circle and range bar represent the mean and SD of grain Cd concentration in Koshihikari (288 plants). (*B* and *C*) Cd concentrations in the shoots and roots of WT Koshihikari and of three low-Cd Koshihikari mutants (*lcd-kmt1*, *lcd-kmt2*, and *lcd-kmt3*) grown in hydroponic culture containing 0.18 μM Cd. Bars labeled with different letters differ significantly ($P < 0.05$, ANOVA followed by Tukey's test).

of *lcd-kmt3* was reduced (Table S1) under the sufficient Mn level in hydroponics. These results suggest that Cd might be transported via the Mn pathway into the roots.

Metal Concentrations in Grain and Agronomic Traits of Field Grown *lcd-kmt* Mutants. The M_4 *lcd-kmt* mutants and WT were cultivated together in paddy fields to evaluate their metal concentrations and agronomic traits. There were no apparent differences in plant or grain morphologies between WT and *lcd-kmt1* (Fig. 2 *A* and *B*) or between WT and *lcd-kmt2* (Fig. S1 *A* and *C*). In addition, there were no significant differences in soil plant analysis development (SPAD) value for chlorophyll content (Fig. 2*C*), grain and straw yields (Fig. 2 *D* and *E*), or eating quality (Fig. 2*F*) between WT and *lcd-kmt1*. Similar results were found between WT and *lcd-kmt2* (Table S2). This was in contrast to the *lcd-kmt3* mutant, which had significantly earlier heading, smaller plant size, higher panicle number, and lower grain and straw yields than the WT (Fig. S1 *B* and *C* and Table S2). The concentration of Cd in the grain (unpolished rice) of *lcd-kmt1* was extremely low, near the limit of detection (<0.01 $mg \cdot kg^{-1}$), whereas the Cd concentration in the WT grain exceeded the maximum limit of 0.4 $mg \cdot kg^{-1}$ (Fig. 2*G*). Indeed, the Cd concentration in *lcd-kmt1* was <3% of that in the WT. The Cd concentration in the straw was also much lower in *lcd-kmt1* than in the WT (Fig. 2*H*). Similar results were observed for Cd in grain and straw of *lcd-kmt2* and *lcd-kmt3* (Table S3).

The Mn concentration in the grain of *lcd-kmt* mutants was approximately one-third that of the WT, and an even greater difference of nearly 30-fold was evident in the Mn concentration in the straw (Table S3). The concentrations of Cu, Fe, and Zn in grains of *lcd-kmt1* and *lcd-kmt2* were similar to those of the WT and slightly higher in *lcd-kmt3* than in the WT. This was presumably because of the smaller size of *lcd-kmt3* plants. There was no significant difference in Fe concentration in the straw between the WT and *lcd-kmt* mutants, whereas that of Zn was a little lower in *lcd-kmt1* and *lcd-kmt2*.

Gene Identification. We developed an F_2 population by crossing Kasalath, an *indica*-type rice cultivar, with *lcd-kmt1* and then performed positional cloning of the gene(s) responsible for reduced Cd uptake by *lcd-kmt1*. Among the 92 F_2 individuals, 22 plants were categorized as having a similarly low shoot Cd concentration to *lcd-kmt1*, whereas 70 plants showed a relatively high shoot Cd concentration (Fig. 3*A*). The segregation ratio was not significantly different from a 1:3 low:high ratio ($\chi^2 = 0.058$, $P = 0.810$), suggesting that the low-Cd trait of *lcd-kmt1* is controlled by a single recessive gene. The gene locus associated with shoot and root Cd and Mn concentrations was localized on the short arm of chromosome 7 (Table S4). Linkage analysis showed that the gene was localized in the interval defined by the simple sequence repeat markers RM8007 and RM3635 (Fig. 3*B*). The maximum logarithm of odds values for all four traits were found at RM3767 (Table S4), which was located 9.07 Mbp from the distal end of the short arm of chromosome 7.

We found two genes, *OsNRAMP5* (Os07g0257200) and *OsNRAMP1* (Os07g0258400), annotated as putative heavy-metal transporters around RM3767 in the Rice Annotation Project Database (http://rapdb.dna.affrc.go.jp/). The *OsNRAMP1* cDNA sequence was unchanged in the *lcd-kmt* mutants relative to the WT. On the other hand, the cDNA and genomic DNA sequences of *OsNRAMP5* revealed a single-nucleotide deletion in exon IX of *lcd-kmt2* and a 433-bp insertion in the exon X of *lcd-kmt1* (Fig. 3*C*). The latter replaced the terminal 32 bp in exon X of the WT with 50 bp in *lcd-kmt1*; the remaining 383 bp of the insertion in *lcd-kmt1* is expected to be spliced out with intron X. The inserted DNA sequence was identical to the sequence of *mPingA1*, a member of a class of miniature inverted-repeat transposable elements in rice (22). An ~227-kbp deletion that included all of

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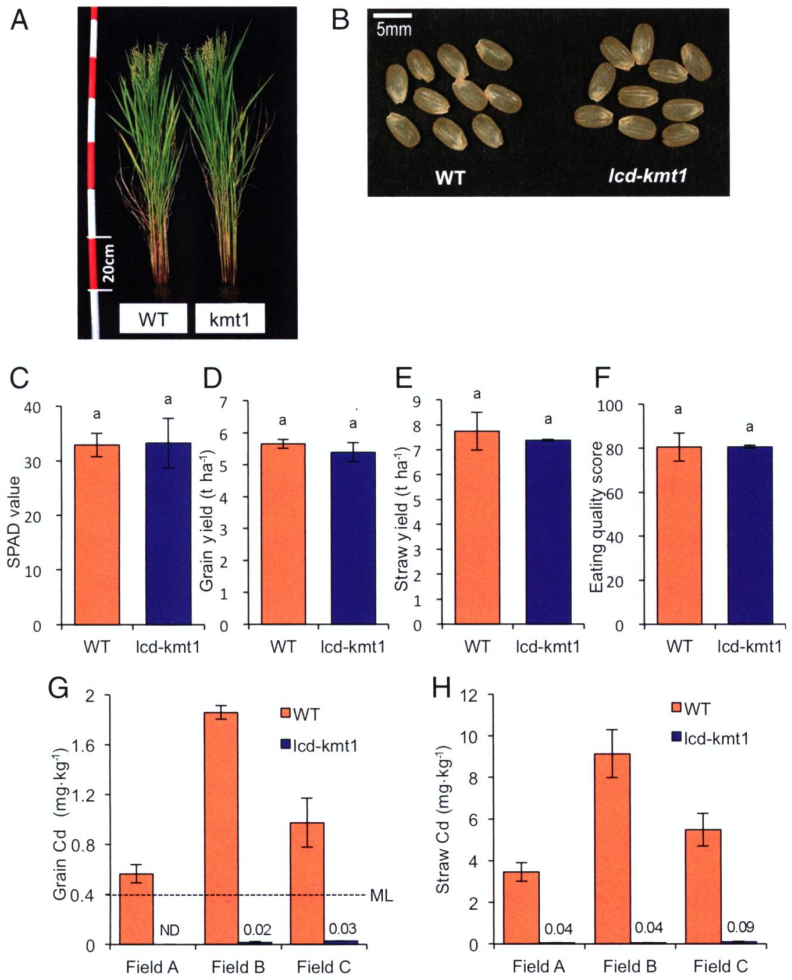

Fig. 2. Agronomic traits of Koshihikari and *lcd-kmt* mutants grown in the field. (*A*) Plant morphologies of WT Koshihikari and *lcd-kmt1*. (*B*) Morphologies of unpolished rice grains. (*C*) Chlorophyll content in the flag leaf determined using a SPAD meter. (*D*) Grain yield. (*E*) Straw yield. (*F*) Eating quality scores evaluated using a taste analyzer; values >80 are considered "good quality." No significant differences in agronomic traits or eating quality were observed between the WT and *lcd-kmt1* ($P > 0.05$, ANOVA followed by Tukey's test). (*G* and *H*) Cd concentration of unpolished rice (*G*) and straw (*H*). Plants were grown in Cd-polluted paddy fields in three regions of Japan. Data are presented as means ± SD ($n = 5$). ND, not detected; ML, maximum allowed Cd concentration for rice (Codex Alimentarius Commission).

OsNRAMP5 was found in *lcd-kmt3* (Fig. S2). On the basis of these results, we propose naming the mutant genes as *osnramp5-1* for *lcd-kmt1* (DNA Data Bank of Japan accession no. AB690552), *osnramp5-2* for *lcd-kmt2* (AB690553), and *osnramp5-3* for *lcd-kmt3*.

OsNRAMP5 (AB690551) from the WT Koshihikari is predicted to encode a 538-aa protein. The single base pair deletion in *osnramp5-2* results in aberrant translation of 53 aa before a new stop codon at amino acid 358 (Fig. S3). On the basis of the cDNA and genome sequencing data of *lcd-kmt1*, it is likely that an 11-aa region of the WT was replaced with 17 aa at the terminal position of exon X, resulting in a 544-aa protein in the osnramp5-1.

Microarray analysis showed a 2.5-fold increase in *OsNRAMP5* expression for *lcd-kmt1* compared with the WT (Table S5). The expression of other *OsNRAMP* genes did not change substantially. Moreover, marked changes in the expression of genes possibly involved in metal transport, such as *OsIRT*, *OsHMA*, and *OsLCT1*, were not found in the mutant. Rather, genes involved in the photosynthetic process were up-regulated considerably, and Fe-deficiency inducible genes were down-regulated in the *lcd-kmt1* mutant.

osnramp5-1 fused with GFP was observed at the periphery of the cells but not inside the cells (Fig. 4*A*), indicating the same localization as OsNRAMP5-GFP (10). This suggests that the mutation in osnramp5-1 did not alter the subcellular localization to the cell membrane. The growth of yeast cells expressing *osnramp5-1* was not affected by the Cd treatment (Fig. 4*B*), although the growth of transformed mutant yeast cells expressing *OsNRAMP5* was strongly impaired by Cd. This suggests that the osnramp5-1 could not transport Cd into yeast cells, whereas the WT OsNRAMP5 was able to do so. Furthermore, the mutant protein osnramp5-1 could not transport Mn and Fe, in addition to its inability to transport Cd.

Development of Genetic Markers for Breeding Low-Cd Rice. DNA markers that detect polymorphism in the region of *OsNRAMP5* would be useful for developing new cultivars with the low-Cd trait. Thus, we designed primer sets to amplify the mutated region. Different PCR fragment patterns could be readily detected between *lcd-kmt1* and WT, because there is a 433-bp insertion in *lcd-kmt1* (Fig. 4*C*). The F_1 heterozygous genotype derived from *lcd-kmt1* × WT appeared as two bands on the gel. In contrast, no differences in PCR fragment sizes were observed between the undigested PCR products of *lcd-kmt2* and WT (Fig. 4*D*). Although these two alleles differ in length by only 1 bp, the mutation created a unique FspI site in *lcd-kmt2*. FspI digestion cut the PCR product of *lcd-kmt2* (LK2) into two fragments of equal size, whereas the PCR product of WT was not cut by this enzyme. The alleles from both *lcd-kmt2* and WT could be detected in the F_1.

Using the developed genetic marker, we tested whether the mutant *osnramp5-1* allele significantly decreases Cd accumulation in F_2 plants derived from a cross between *lcd-kmt1* and Kasalath. All F_2 plants homozygous for the *osnramp5-1* allele of *lcd-kmt1* had significantly lower shoot Cd concentrations than

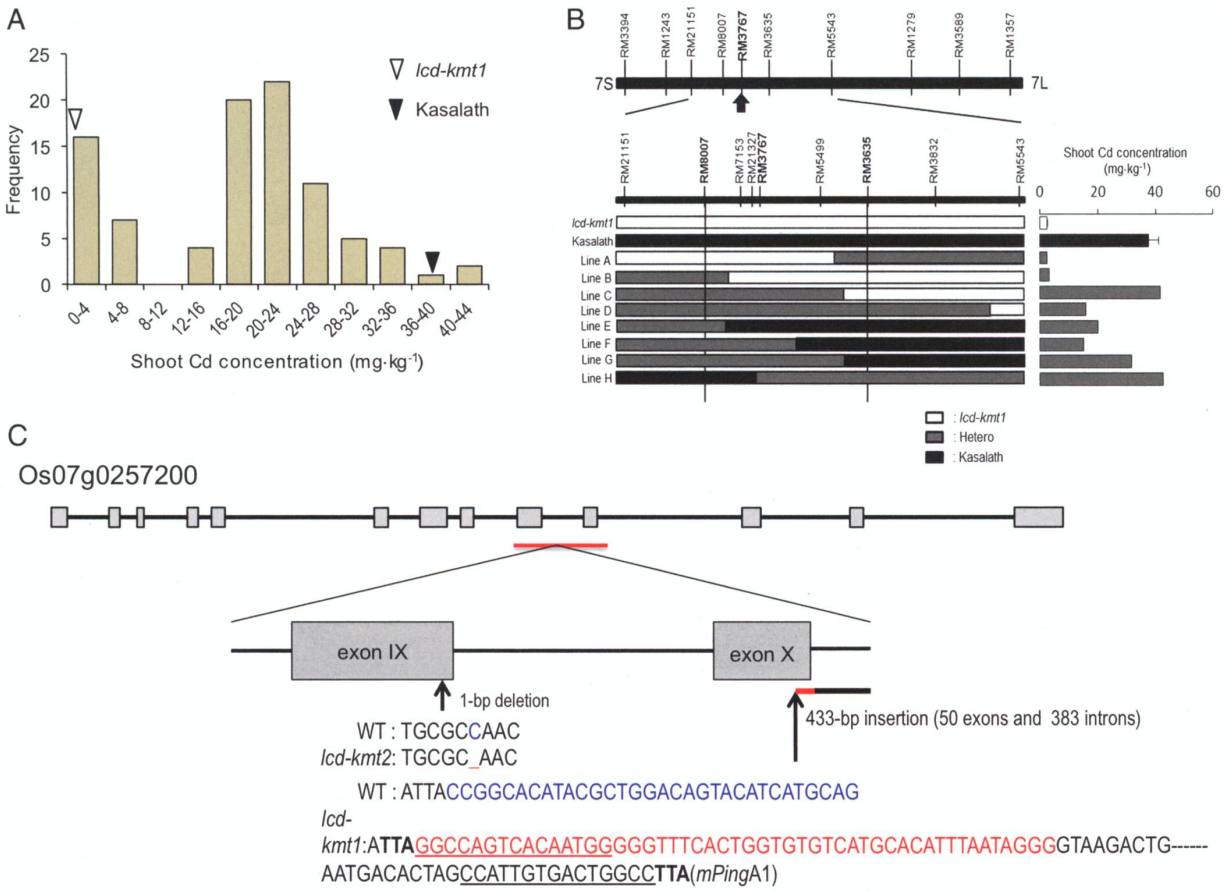

Fig. 3. Positional cloning of the gene. (*A*) Frequency distribution for shoot Cd concentration of 92 F_2 seedlings derived from a cross between *lcd-kmt1* and WT Kasalath, an *indica* cultivar. White and black triangles represent the mean shoot Cd concentration of *lcd-kmt1* and Kasalath, respectively. (*B*) Gene locus for low shoot Cd concentration on chromosome 7. Arrow indicates the peak logarithm of odds for the putative QTL gene. Graphical genotypes of F_2 plants having recombination in the candidate region (*Left*) and their shoot Cd concentrations (*Right*) are shown. White, black, and gray bars indicate regions homozygous for the *lcd-kmt1* allele, homozygous for the Kasalath allele, and heterozygous for the two alleles, respectively. (*C*) Structure of *OsNRAMP5* (Os07g0257200) and the mutation sites in *lcd-kmt1* and *lcd-kmt2*. Exons and introns are indicated by gray bars and black lines, respectively. The arrow below exon IX indicates the position of a 1-bp deletion in *lcd-kmt2* relative to the corresponding sequence in WT Koshihikari. The arrow below exon X indicates the position of a 433-bp insertion in *lcd-kmt1*. The blue WT nucleotides have been replaced by the red nucleotides in *lcd-kmt1*. The bold TTA sequences indicate 3-bp target-site duplications, and underlines indicate 15-bp terminal inverted repeats.

those homozygous for the *OsNRAMP5* allele of Kasalath and those that were heterozygous for the two alleles (Fig. 4*F*). This demonstrates that the allelic effect of *osnramp5-1* contributes to decreased Cd in rice plants. In addition, the plants homozygous for *lcd-kmt1* did not exhibit any significant decrease in their shoot dry weight (Fig. 4*E*), even if the genetic background was changed by crossing. These results indicate that introduction of the *osnramp5-1* allele into the other cultivars might not affect plant growth under the Mn-sufficient conditions.

Discussion

By using ion-beam mutagenesis, we succeeded first in producing nontransgenic rice mutants that accumulate very low Cd in grain of <3% that in Koshihikari, the most popular Japanese temperate *japonica* rice cultivar. Physiological studies in hydroponic culture demonstrated that decreased Cd uptake by roots leads to low levels of Cd in the shoot and grain of these mutants (Fig. 1). Our QTL analysis suggests that a Fe and Cd transporter gene, *OsNRAMP1*, on chromosome 7 was the most likely candidate gene, but the *OsNRAMP1* cDNA sequence was unchanged in the *lcd-kmt* mutants. Microarray analysis showed that the expressions of three other genes, *OsIRT1*, *OsIRT2*, and *OsHMA3*, previously related to Cd transport in rice, did not differ between the WT and *lcd-kmt1* mutant (Table S5). Instead, we found that the three mutant lines each had a different mutation [i.e., a transposon (*mPingA1*) insertion, a single-base pair deletion, and a large deletion], in the same gene *OsNRAMP5*, which is located near *OsNRAMP1* (Fig. 3*C* and Fig. S2). A *mPingA1* was probably activated by the ion beams (23), then transposed into a preferred insertion site (TTA) in an exon of OsNRAMP5 (22). It has been reported recently that OsNRAMP5 is involved in Mn, Fe, and Cd transport in rice roots (10, 13). Interestingly, in our previous study (10) the RNAi-induced silencing of *OsNRAMP5* in rice promoted Cd translocation to shoots, although root Cd uptake was decreased. In these *OsNRANP5*-RNAi plants, the expression of *OsNRANP5* was suppressed but the expressions of several Fe deficiency-inducible genes were up-regulated. In contrast, the expression of *osnramp5-1* present in the *lcd-kmt1* plant was increased but the expressions of Fe deficiency-inducible genes were down-regulated (Table S5). Therefore, the differential pattern in root-to-shoot Cd translocation between the RNAi-plants and *lcd-*

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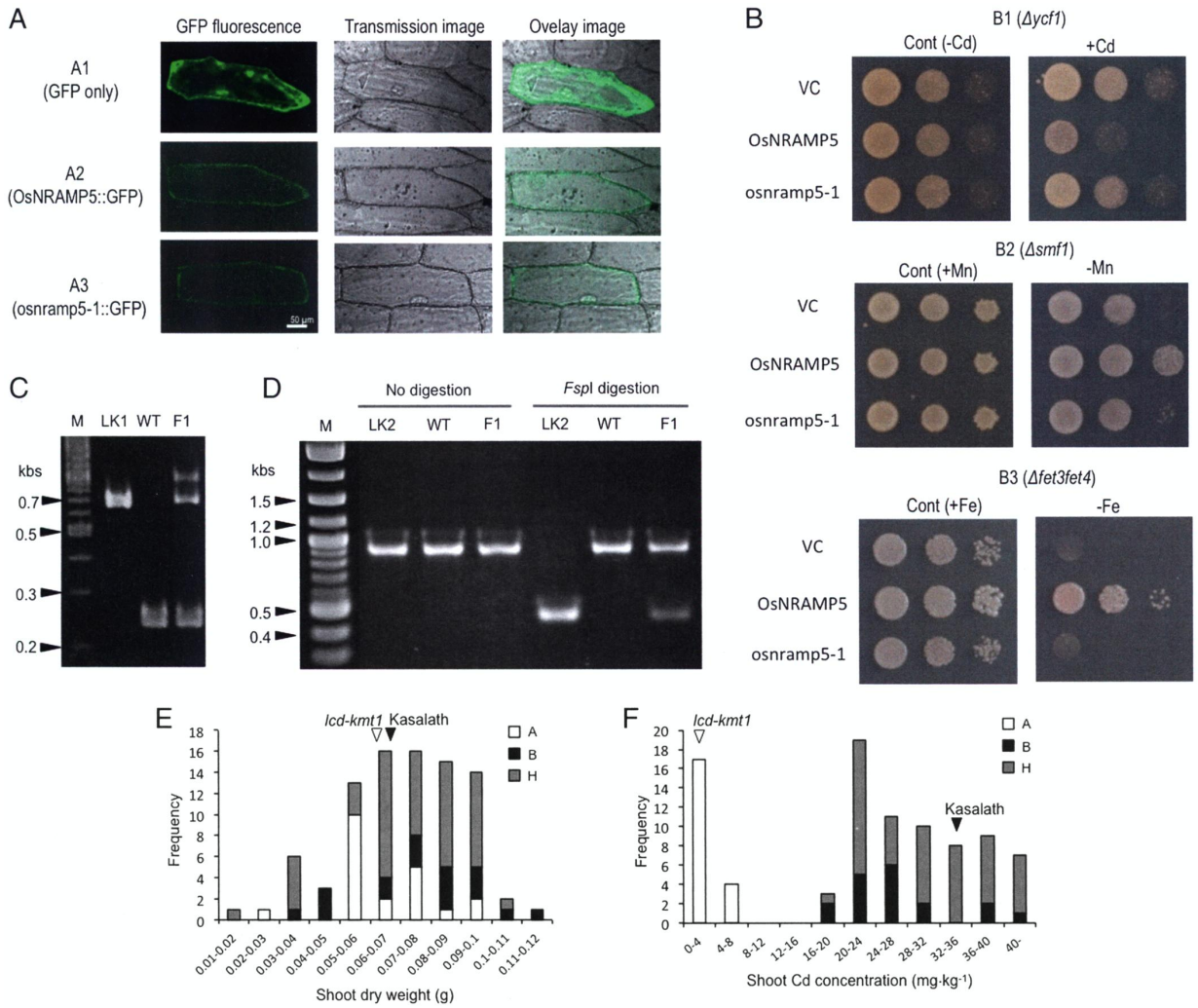

Fig. 4. (*A*) Subcellular localization of OsNRAMP5 and osnramp5-1 in transformed onion epidermal cells. (*A, 1*) GFP only; (*A, 2*) OsNRAMP5::GFP fusion protein; (*A, 3*) osnamp5-1::GFP fusion protein. (*B*) Growth of yeast cells transformed with the vector control (VC), *OsNRAMP5*, or *osnramp5-1*. Yeasts were spotted at three dilutions (optical densities at 600 nm of 0.1, 0.01, and 0.001, left to right). (*B, 1*) Growth of yeast *Δycf1* (Cd-sensitive mutant) cells; (*B, 2*) growth of *Δsmf1* (Mn-uptake mutant) yeast cells; (*B, 3*) growth of *Δfet3fet4* (Fe-uptake mutant) yeast cells. Cont, control, with (+) or without (–) the specified metal. (*C* and *D*) DNA fragments of the genomic region containing the mutation amplified by PCR. (*C*) M, size marker; LK1, *lcd-kmt1*; WT, wild-type Koshihikari; F1, F_1 progeny of *lcd-kmt1* × Koshihikari. (*D*) M, size marker; LK2, *lcd-kmt2*; WT, wild-type Koshihikari; F1, F_1 progeny of *lcd-kmt2* × Koshihikari. Where indicated, amplified samples were digested with FspI before electrophoresis. (*E*) Frequency distribution for shoot dry weight of F_2 plants derived from a cross between *lcd-kmt1* and Kasalath. Using the developed marker (*C*), the 88 F_2 plants were classified into three genotype classes: (A) those homozygous for the *osnramp5-1* allele of *lcd-kmt1*, (B) those homozygous for the *OsNRAMP5* allele of Kasalath, and (H) those that were heterozygous for the two alleles. (*F*) Frequency distribution for shoot Cd concentrations of F_2 plants used in *E*.

kmt mutants could be partly explained by the different expression of Fe deficiency-inducible genes.

Although the *osnramp5* mutant gene was expressed in the roots, the mutant transporter proteins failed to mediate uptake of Cd, Mn, and Fe in yeast (Fig. 4*B*), indicating loss of function of these metal transporters in the cell membrane. A highly conserved consensus transport motif (CTM) between transmembrane domains 8 and 9 was transformed into a hydrophobic segment in *osnramp5-1* and was truncated in *osnramp5-2* (Fig. S4). The CTM in NRAMP (natural resistance-associated macrophage protein) has been implicated in the interaction with ATP-coupling subunits and to be important for metal transport by these proteins (24). Within the CTM motif, the Gly-347 residue (based on position in OsNRAMP5) is absolutely conserved in all members of the NRAMP family. This residue could be especially important for metal transport activity because in mammalian NRAMP2, a mutant in which glycine is substituted with valine, lost NRAMP2 function in yeast (25). The Gly-347 residue is absent from the osnramp5 mutant proteins (Fig. S3). Therefore, such changes might affect Cd and Mn transport via the cell membrane in the roots.

The *lcd-kmt1* and *lcd-kmt2* mutants did not exhibit significant negative effects on plant or grain morphology, eating quality, grain yield, or straw yield (Fig. 2 and Table S2), indicating that a transposon insertion or a single base pair deletion on *OsNRAMP5* does not negatively affect agronomic traits. In contrast, *lcd-kmt3* had earlier heading and smaller plant size than the WT, presumably because of the large DNA deletions in this mutant

line (Fig. S2 and Table S2). These results indicate that *lcd-kmt1* and *lcd-kmt2* can be used directly in breeding programs.

Field trials showed that the *lcd-kmt* mutants have nearly undetectable Cd concentrations in their grain and staw (Fig. 2 *G* and *H*). Although root Cd concentrations were not measured in field conditions, root Cd uptake by *lcd-kmt* mutants is presumed to be substantially lower than in WT. If a small amount of Cd enters the root cells via other cell membrane metal transporters such as OsIRT1 (11) and OsNRAMP1 (12), a kind of "firewall" system might sequester Cd in the root vacuoles via a functional OsHMA3 transporter (14, 15). Therefore, a defective gene (*osnramp5*) working together with a functional gene (*OsHMA3*) may be responsible for the drastic decrease in grain and straw Cd concentrations in the *lcd-kmt* mutants.

Surprisingly, there were no differences in the leaf chlorophyll contents between WT and *lcd-kmt mutants* (Fig. 2*C* and Table S2), even though the shoot (straw) Mn concentration of the *lcd-kmt* mutants was markedly lower than that of the WT (Table S3), and several genes involved in the photosystem were up-regulated significantly (Table S5). Being adapted to the reducing conditions in paddy soils, rice accumulates high Mn in shoots of up to 2,000 $mg \cdot kg^{-1}$, an order of magnitude higher than that in soybean shoots (26), without damage (27). One T-DNA insertion line and RNAi lines of *OsNRANP5* in rice exhibited severe growth inhibition, although the Mn concentration in the straw was 100–200 $mg \cdot kg^{-1}$ in soil culture (13). In contrast, the *lcd-kmt* mutants did not show any adverse growth with <100 $mg \cdot kg^{-1}$ Mn in straw. Additionally, the F_2 plants harboring the *osnramp5-1* allele did not exhibit a significant decrease in shoot dry weight, even when their genetic background was changed by crossing. Our results indicate that rice may require less Mn for normal growth than is typically present in the shoot, and the introduction of *osnramp5-1* allele into other rice cultivars might not induce the growth inhibition under the Mn-sufficient conditions. Further investigation is needed on the effects of *osnramp5* alleles on plant growth and various agronomic traits under low-Mn conditions. The mutant *osnramp5* alleles did not significantly reduce Fe in grain and straw of the *lcd-kmt* mutants, indicating that other Fe transporters, such as OsIRT1 and OsIRT2, are more important than a functional OsNRAMP5 for Fe transport.

We have developed DNA markers that can be used to introduce the mutant *nramp5* alleles into various cultivars including *indica* type by means of marker-assisted selection. Indeed, breeding programs have been launched to transfer the low-Cd trait into other popular cultivars in Japan. These mutant alleles would also reduce the Cd concentration in rice straw being fed to livestock, thereby greatly reducing bioaccumulation of Cd in meat. We therefore believe that our findings provide an important tool for reducing the Cd levels in rice and that the risk of Cd exposure via the food chain will be greatly reduced.

Materials and Methods

Seeds of rice (*Oryza sativa* L. cv. Koshihikari) were irradiated with 320 MeV carbon ions ($^{12}C^{6+}$) at a dose of 40 Gy. Three low-Cd mutants (*lcd-kmt1*, *lcd-kmt2*, and *lcd-kmt3*) were identified according to the grain Cd concentration of 2,592 M_2 plants determined by inductively coupled plasma mass spectroscopy (ICP-MS). Positional cloning was conducted to identify the gene loci responsible for reduced Cd uptake by *lcd-kmt1*. The cDNA and genomic DNA of *OsNRAMP5* in the WT and *lcd-kmt* mutants were amplified by PCR and sequenced. Molecular methods were applied to observe the gene location in the rice plants and to analyze gene function. DNA markers were developed to assist breeding of low-Cd rice based on the sequences in the mutation regions for *lcd-kmt1* or *lcd-kmt2*. The cDNA sequences determined in this study have been submitted using the SAKURA nucleotide sequence data submission system through the Web server at the DNA Data Bank of Japan (DDBJ; http://sakura.ddbj.nig.ac.jp/) and are deposited in the DDBJ database under accession nos. AB690551 (*OsNRAMP5*), AB690552 (*osnramp5-1*), and AB690553 (*osnramp5-2*). Further details on the procedures used are available in *SI Materials and Methods*.

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1. European Food Safety Authority (2011) Scientific opinion on tolerable weekly intake for cadmium. *EFSA J* 9(2):1975.
2. Tsuchiya K (1976) Epidemiological studies on cadmium in the environment in Japan: Etiology of itai-itai disease. *Fed Proc* 35(12):2412–2418.
3. Food and Agriculture Organization/World Health Organization (2010) Joint FAO/WHO Expert Committee on Food Additives, Seventy-Third Meeting, Geneva, 8–17 June 2010. Summary and Conclusions. Available at: www.who.int/foodsafety/publications/chem/summary73.pdf. Accessed June 30, 2012.
4. Codex Alimentarius (2008) CODEX STAN 193-1995, Codex general standard for contaminants and toxins in foods and feed. Available at: www.codexalimentarius.net/download/standards/17/CXS_193e.pdf. Accessed June 30, 2012.
5. International Rice Research Institute (2011). World Production and Consumption of Domestic Milled Rice. Available at: http://ricestat.irri.org/vis/wrs_quickCharts.php. Accessed June 30, 2012.
6. Arao T, Ishikawa S (2006) Genotypic differences in cadmium concentration and distribution of soybean and rice. *Jpn Agric Res Q* 40:21–30.
7. Uraguchi S, et al. (2009) Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. *J Exp Bot* 60(9): 2677–2688.
8. Ishikawa S, et al. (2010) A major quantitative trait locus for increasing cadmium-specific concentration in rice grain is located on the short arm of chromosome 7. *J Exp Bot* 61(3):923–934.
9. Ishikawa S, Ae N, Yano M (2005) Chromosomal regions with quantitative trait loci controlling cadmium concentration in brown rice (*Oryza sativa*). *New Phytol* 168(2): 345–350.
10. Ishimaru Y, et al. (2012) Characterizing the role of rice NRAMP5 in manganese, iron and cadmium transport. *Sci Rep* 2:286.
11. Nakanishi H, Ogawa I, Ishimaru Y, Mori S, Nishizawa NK (2006) Iron deficiency enhances cadmium uptake and translocation mediated by the Fe^{2+} transporters OsIRT1 and OsIRT2 in rice. *Soil Sci Plant Nutr* 52:464–469.
12. Takahashi R, et al. (2011) The OsNRAMP1 iron transporter is involved in Cd accumulation in rice. *J Exp Bot* 62(14):4843–4850.
13. Sasaki A, Yamaji N, Yokosho K, Ma JF (2012) Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. *Plant Cell* 24(5):2155–2167.
14. Miyadate H, et al. (2011) OsHMA3, a P_{1B}-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. *New Phytol* 189(1): 190–199.
15. Ueno D, et al. (2010) Gene limiting cadmium accumulation in rice. *Proc Natl Acad Sci USA* 107(38):16500–16505.
16. Satoh-Nagasawa N, et al. (2012) Mutations in rice (*Oryza sativa*) heavy metal ATPase 2 (OsHMA2) restrict the translocation of zinc and cadmium. *Plant Cell Physiol* 53(1): 213–224.
17. Takahishi R, et al. (2012) The OsHMA2 transporter is involved in root-to-shoot translocation of Zn and Cd in rice. *Plant Cell Environ* 35(11):1948–1957.
18. Uraguchi S, et al. (2011) Low-affinity cation transporter (OsLCT1) regulates cadmium transport into rice grains. *Proc Natl Acad Sci USA* 108(52):20959–20964.
19. Uraguchi S, Fujiwara T (2012) Cadmium transport and tolerance in rice: Perspectives for reducing grain cadmium accumulation. *Rice* 5:5.
20. Kazama Y, et al. (2011) Characterization of highly efficient heavy-ion mutagenesis in *Arabidopsis thaliana*. *BMC Plant Biol* 11:161.
21. Tanaka A, Shikazono N, Hase Y (2010) Studies on biological effects of ion beams on lethality, molecular nature of mutation, mutation rate, and spectrum of mutation phenotype for mutation breeding in higher plants. *J Radiat Res (Tokyo)* 51(3): 223–233.
22. Kikuchi K, Terauchi K, Wada M, Hirano HY (2003) The plant MITE *mPing* is mobilized in anther culture. *Nature* 421(6919):167–170.
23. Maekawa M, Hase Y, Shikazono N, Tanaka A (2003) Induction of somatic instability in stable yellow leaf mutant of rice by ion beam irradiation. *Nucl Instrum Meth B* 206: 579–585.
24. Curie C, Alonso JM, Le Jean M, Ecker JR, Briat JF (2000) Involvement of NRAMP1 from *Arabidopsis thaliana* in iron transport. *Biochem J* 347(Pt 3):749–755.
25. Pinner E, Gruenheid S, Raymond M, Gros P (1997) Functional complementation of the yeast divalent cation transporter family SMF by NRAMP2, a member of the mammalian natural resistance-associated macrophage protein family. *J Biol Chem* 272(46): 28933–28938.
26. Ishikawa S, Ae N, Sugiyama M, Murakami M, Arao T (2005) Genotypic variation in shoot cadmium concentration in rice and soybean in soils with different levels of cadmium contamination. *Soil Sci Plant Nutr* 51:101–108.
27. Lidon FC, Barreiro MG, Ramalho JC (2004) Manganese accumulation in rice: Implications for photosynthetic functioning. *J Plant Physiol* 161(11):1235–1244.

Part I
Induced Mutations

The Handbook of Plant Mutation Screening. Edited by Günter Kahl and Khalid Meksem

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1
Physically Induced Mutation: Ion Beam Mutagenesis

Shimpei Magori, Atsushi Tanaka, and Masayoshi Kawaguchi

Abstract

Ion beams are novel physical mutagens that have been applied to a wide variety of plant species. Unlike other physical mutagens such as X-rays, γ-rays, and electrons, ion beams have high linear energy transfer, leading to high double-strand break yields and the resulting strong mutational effects. Takasaki Ion Accelerators for Advanced Radiation Application (TIARA) in Japan was established as the first ion beam irradiation facility for biological use. In this facility, positively charged ions are accelerated at a high speed and used to irradiate living materials, including plant seeds and tissue cultures. By utilizing this approach, several novel mutants have been successfully isolated even from *Arabidopsis*, in which thousands of mutants have already been obtained using different mutagens. This demonstrates that ion beams are a powerful alternative mutagen with a mutation spectrum different from other chemical, physical, and T-DNA-based mutagens. The application of such an alternative mutagen is of great importance not only to analyze any gene functions through novel mutant isolation, but also to improve global food situations by providing new crop varieties with beneficial traits. In this chapter, we describe the detailed methods of ion beam irradiation and discuss its applications in genetic research as well as plant breeding.

1.1
Introduction

Mutagenesis is one of the most critical steps for genetic studies as well as selective breeding. Successful mutant isolation largely relies on the use of efficient mutagens. In plant research, a chemical mutagen, ethylmethane sulfonate (EMS), has been commonly used for this purpose. Although this mutagen can be handled easily and applied to any plant, it primarily produces single base substitutions, but not drastic mutations such as large genomic deletions. Therefore, application of more powerful mutagens with different mutation spectra is of great significance in some cases. One good technology for this end is ion beam mutagenesis. The ion beam is a physical mutagen

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that has just recently come into use for plants. In this type of mutagenesis, positively charged ions are accelerated at a high speed (around 20–80% of the speed of light) and used to irradiate target cells. As a physical mutagen, ion beams are similar to other forms of radiation such as X-rays, γ-rays, and electrons, but it is different from them in that ion beams have much higher linear energy transfer (LET). This characteristic is important to understand the high biological effectiveness of ion beams.

1.1.1 LET

LET is the energy deposited to target material when an ionizing particle passes through it. Once an accelerated particle encounters any substance, it gradually loses its own energy (i.e., the same amount of energy is transferred to the substance causing "damage") and eventually stops at the point where the maximum energy loss is observed (Figure 1.1). LET is usually expressed in kiloelectronvolts per micrometer

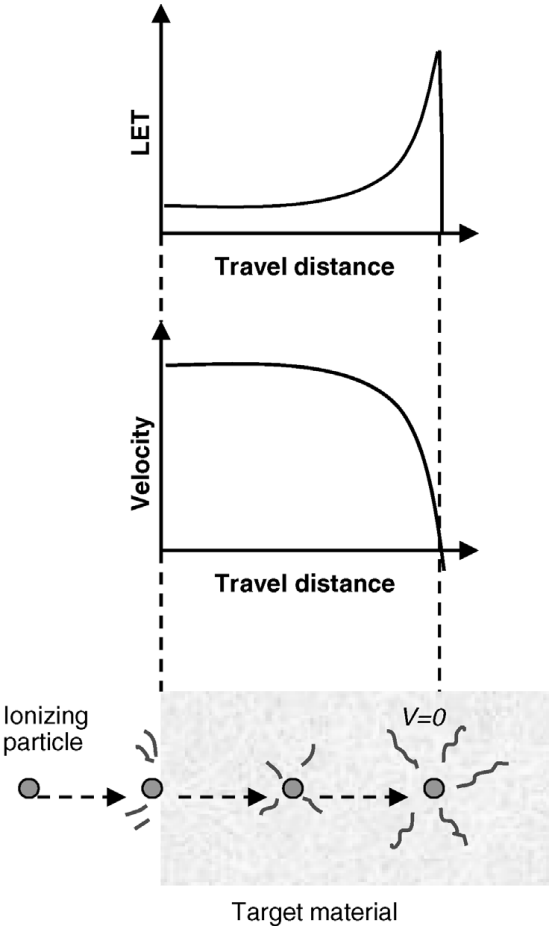

Figure 1.1 Conceptual diagram of LET. An ionizing particle gradually loses its own energy as it slows down in the target material. LET refers to this energy loss, which is deposited to the material. In this cartoon, LET is represented by wavy lines. LET reaches its maximum just before the ionizing particle stops. Immediately after this peak, LET plunges to zero.

(keV/μm), which represents the average amount of energy lost per unit distance. Ion beams have a relatively high LET (around 10–1000 keV/μm or higher), while X-rays, γ-rays, and electrons have low LETs (around 0.2 keV/μm). Therefore, ion beams are able to cause more severe damage to living cells than other forms radiation, resulting in the high relative biological effectiveness [1, 2].

1.1.2 Mutational Effects of Ion Beams on Plants

Biological effects of ion beams have been investigated not only in mammals, but also in plants. For example, studies using *Arabidopsis thaliana* and *Nicotiana tabacum* showed that ion beams were more efficient in decreasing the germination rate and the survival rate than low-LET radiation [3, 4]. More importantly, analysis focusing on *transparent testa* (*tt*) and *glabrous* (*gl*) loci revealed that 113-keV/μm carbon ions induced a 20-fold higher mutation rate per dose than 0.2-keV/μm electrons, thus demonstrating the power of ion beams as a mutagen [5, 6]. The detailed characterization of the carbon ion-induced mutations showed that ion beams can cause large DNA alterations (large deletions, inversions, and translocations) as well as small intragenic mutations and that ion beams frequently, but not always, produce deletions with variable sizes from 1 bp up to 230 kbp, compared to electrons (summarized in Table 1.1) [6]. Since such deletions possibly lead to frameshifts or total gene losses, mutants derived from ion beam mutagenesis can be considered as nulls in many cases. This is a significant difference from the conventional chemical mutagen EMS, which mostly generates point mutations resulting from GC → AT transitions.

These great mutational effects of ion beams are partly due to high double-strand break (DSB) yields induced by ions. The study using tobacco BY-2 protoplasts as a model system showed that initial DSB yields were positively correlated with LET, and that high-LET helium, carbon, and neon ions were more effective in causing DSBs

Table 1.1 Classification of mutations induced by carbon ions and electrons (modified from [6]).

Mutagen (LET)	Intragenic mutation		Large DNA alteration	
Carbon ions (113 keV/μm)	48%		52%	
	deletion	38%	inversion/translocation	21%
	base substitution	7%	total deletion	31%
	insertion	3%		
Electrons (0.2 keV/μm)	75%		25%	
	deletion	33%	inversion/translocation	25%
	base substitution	33%	total deletion	0%
	insertion	8%		

The distributions of the indicated mutation patterns were determined based on the sequence analysis with 29 and 12 mutant alleles produced by carbon ions and electrons, respectively [6]. Note that carbon ions induced large DNA alteration in the tested loci more frequently than electrons. Such large DNA alterations include total deletion, which refers to a complete loss of a gene locus.

than γ-rays [7]. Further, it was found that at least carbon and neon ions produced short DNA fragments more frequently than γ-rays, suggesting that ion particles can act densely and locally on target genomes [7].

It is plausible that DSBs are more difficult for cells to repair than single-strand breaks (i.e., DSB repair can be error-prone), which might partly explain the high mutation rates caused by ion beams. However, the molecular mechanism of ion-mediated mutation induction remains largely unknown. To address this issue, Shikazono *et al*. analyzed the DNA sequences flanking the breakpoints generated by carbon ions and showed that many of the tested sequences contained deletions (1–29 bp), whereas most of the electron-induced breakpoints were flanked by duplications (1–7 bp) [6]. Based on these findings, they hypothesize that unlike electrons, high-LET ions could induce not only DSBs, but also cause severe damage in the broken ends and that such damaged sequences might be eventually excised during the repair processes, resulting in deletion mutations (Figure 1.2) [6].

Although further analysis is necessary to elucidate its precise mode of action, ion beam mutagenesis appears to be a good alternative that can accomplish high mutational effects and a mutation spectrum presumably different from other mutagens such as EMS and low-LET radiation. To date, ion beam mutagenesis has

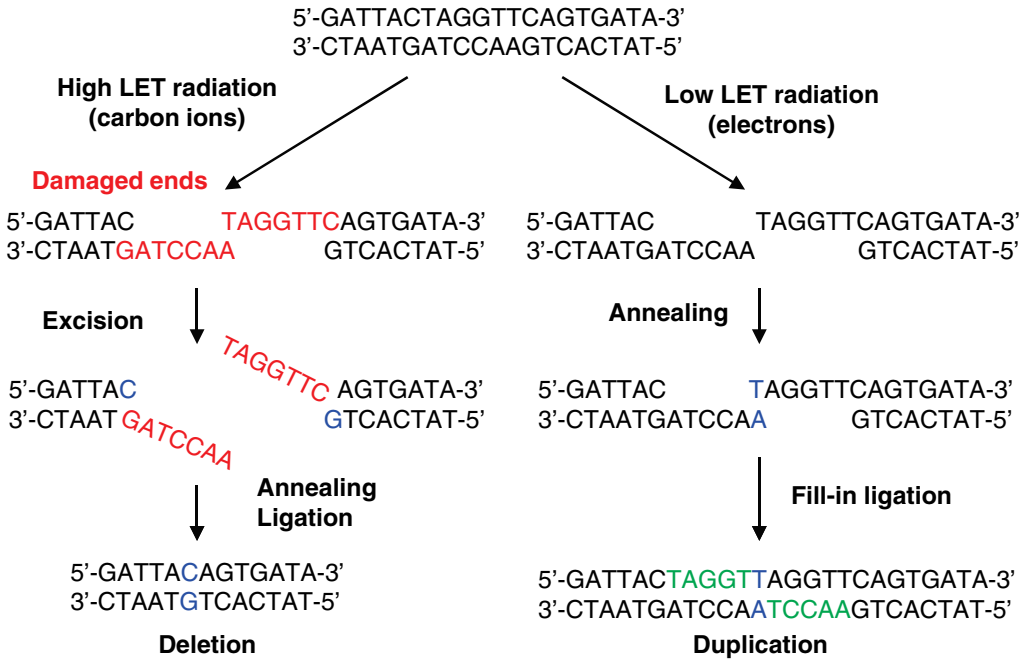

Figure 1.2 Model of mechanisms by which high-LET and low-LET radiation induce mutations (originally proposed by N. Shikazono). High-LET radiation such as carbon ions produce damaged ends of DSBs, which are excised before annealing and ligation of the broken fragments. On the other hand, low-LET radiation such as electrons cause intact ends, which are repaired without any removal of the end sequences. This difference in DSB repair leads to deletions and duplications generated by high-LET and low-LET radiation, respectively. Red letters: bases to be excised; blue letters: bases used for religation; green letters: bases filled in during DSB repair.

been applied to a wide variety of plant species, including *Arabidopsis thaliana*, *Lotus japonicus*, carnations, chrysanthemums, and so on. It is noteworthy that this approach has been successful in the isolation of novel mutants, making a great contribution to plant genetics and breeding (see Section 1.3).

1.2 Methods and Protocols

Currently, there are four facilities available for plant ion beam mutagenesis: Takasaki Ion Accelerators for Advanced Radiation Application (TIARA) of the Japan Atomic Energy Agency (JAEA), RIKEN RI Beam Factory (RIBF), the Wakasa Wan Energy Research Center Multi-purpose Accelerator with Synchrotron and Tandem (W-MAST), and the Heavy Ion Medical Accelerator in Chiba (HIMAC) of National Institute of Radiological Sciences (NIRS). Table 1.2 shows the physical properties of

Table 1.2 Ion beam irradiation facilities and the physical properties of the radiations [modified from the list in The Ion Beam Breeding Society web site (http://wwwsoc.nii.ac.jp/ibbs/)].

Facility	Radiation	Energy (MeV/u)	LET (keV/μm)	Range (mm)
TIARA, JAEA (http://www.taka.jaea.go.jp/index_e.html)	He	12.5	19	1.6
	He	25.0	9	6.2
	C	18.3	122	1.1
	C	26.7	86	2.2
	Ne	17.5	441	0.6
RIBF, RIKEN Nishina Center (http://www.rarf.riken.go.jp/Eng/index.html)	C	135	23	43
	N	135	31	37
	Ne	135	62	26
	Ar	95	280	9
	Fe	90	624	6
W-MAST, The Wakasa Wan Energy Research Center (http://www.werc.or.jp/english/index.htm)	H	200	0.5	256
	C	41.7	52	5.3
HIMAC, National Institute of Radiological Sciences (http://www.nirs.go.jp/ENG/index.html)	C	290	13	163
	Ne	400	30	165
	Si	490	54	163
	Ar	500	89	145
	Fe	500	185	97

Listed are representative ion radiations that have been used in each facility. The energy, LET, and effective range for each ion species are shown.

the ion beams frequently used in these facilities. Here, we describe the protocol of ion beam irradiation in TIARA, which was originally described elsewhere [3, 8].

1.2.1 Ion Beam Irradiation

In general, a variety of ion species, from protons to uranium ions, can be utilized for ion beam applications. In the case of carbon ions, they are produced by an electron cyclotron resonance ion source and accelerated by an azimuthally varying field (AVF) cyclotron to obtain 18.3 MeV/u $^{12}C^{5+}$ ions. At the target surface, the energy of the carbon ions slightly decreases to 17.4 MeV/u, resulting in the estimated 122 keV/μm mean LET in the target material (0.25 mm thick) as water equivalent. In this case, the effective range of the carbon ions is about 1.1 mm. These physical properties can be predicted by the ELOSSM code program [8]. ELOSSM requires the elemental composition and density of the specified substance to determine the potential LET of ion beams. As shown in Figure 1.3, ion beams scan a field of more than $60 \times 60\,mm^2$ in a vacuum chamber and exit it through a 30-μm titanium foil in the beam window. The samples to be irradiated are placed in the air at a distance of 10 cm below the beam window. In the case of *Arabidopsis* or tobacco seeds, for example, 100–3000 seeds are sandwiched between two Kapton films (7.5 μm in thickness; Toray-Dupont) to make a monolayer of seeds for homogeneous irradiation. As for rice or barley seeds, the embryo sides should be kept facing toward the beam window. On the other hand, when calli or explants cultured in a Petri dish need to be irradiated, the lid of the Petri dish should be replaced by a Kapton film cover to

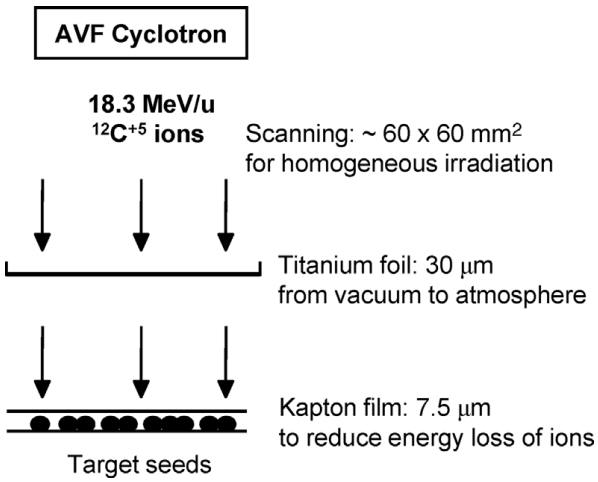

Figure 1.3 Schematic diagram of ion beam irradiation. Ion beams such as carbon ions accelerated by the AVF cyclotron first scan the irradiation field (greater than $60 \times 60\,mm^2$) in a vacuum chamber. Then, the accelerated ion beams exit through a titanium foil into the atmospheric conditions. Finally, the ion particles attack thinly prepared target samples. Here, plant seeds kept between two Kapton films are shown as an example of target biological materials.

minimize the energy loss of ion beams. The target samples are irradiated for less than 3 min for any dose.

1.2.2 Dose Determination for Ion Beam Irradiation

Determining an optimal irradiation dose of ion beams is the most important and laborious step before irradiating your samples. In principal, the ideal irradiation dose would be a dose at which ion beams show the highest mutation rate at any loci of interest; therefore, you might want to figure out your own favorite irradiation doses by testing different doses at a time and screening all of the resulting samples for your desired mutants. However, such an approach is not practical because plenty of time and effort need to be taken. Alternatively, survival rate, growth rate, chlorophyll mutation, and so on, can be the good indicators to determine appropriate doses for mutation induction.

Figure 1.4 shows the survival curves of *Arabidopsis* dry seeds against several ion beams in comparison with low-LET electrons. The effect of ion beams on the survival rate is higher than that of electrons, but it varies by energy and species of ions. Until now, 18.3 MeV/u carbon ions have been widely used, leading to high mutation rates and efficient novel mutant isolations. However, it has not been fully understood which kind of ions with how high energy would be the most effective for mutation induction. Supposedly, the optimal ion radiation might depend on plant species and materials as well as genome size, ploidy, water content, and also what kind of mutation a researcher wants to produce. Based on several results up to date, it has been suggested that the effectiveness of ion beams as a mutagen might not be

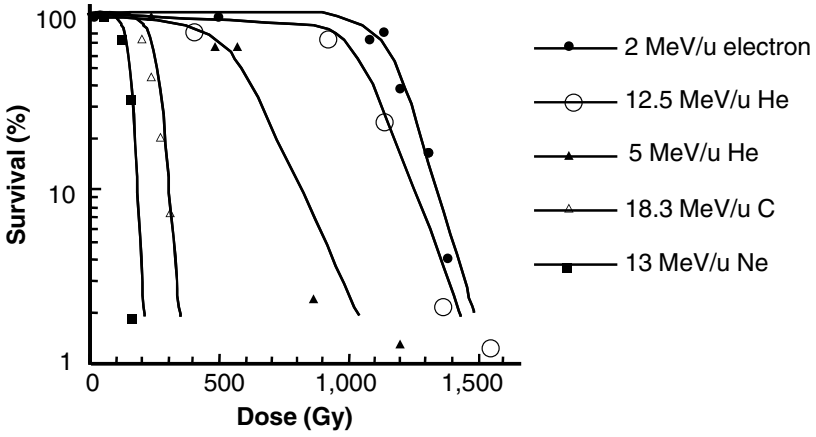

Figure 1.4 Survival curves of *Arabidopsis* dry seeds after irradiation of ion beams (modified from [3]). Dry seeds of the Columbia ecotype of *Arabidopsis* were irradiated with different kinds of ion beams as well as electrons for a low-LET radiation control. Survival responses are shown as a function of irradiation dose. A dose at the shoulder end of each survival curve (e.g., 200 Gy for carbon ions) or less than this dose is supposed to be the most efficient for mutation induction.

determined by the species of ions, but mostly by the LET of ions. So far, ion beams with LET of around 10–500 keV/u appear to be suitable.

As for doses, the median lethal dose (i.e., LD_{50}) has been thought to be the best dose for mutation induction using X-ray or γ-ray irradiation. Recent studies have shown that the dose at the shoulder end of the survival curves (200 and 1000 Gy for carbon ions and electrons, respectively, in Figure 1.4) or less than these doses is more efficient for ion beams as well as low-LET radiation (unpublished data). In fact, we are currently using 150 Gy with 18.3 MeV/u carbon ions for *Arabidopsis* dry seeds. In the case of plantlets, we usually irradiate ion beams at such doses that show 100–80% growth rate (around the shoulder end of the growth curve). Also, when tissue culture is concerned, we favor doses that lead to more than around 80% regeneration or growth rate of calli compared to unirradiated controls.

1.2.3 Plant Radiation Sensitivity

In order to determine irradiation doses, it is very useful to understand general radiation sensitivities of plants against radiation. Radiation sensitivities of plants differ greatly among not only plant species, but also plant materials (seeds, plantlets, tissues, etc.). Table 1.3 shows a comparison of the D_{50}s of representative plant materials. Basically, the radiation sensitivity of living cells depends on the genome size (i.e., the nuclear contents per cell). With increasing genome size of plant species,

Table 1.3 Effective irradiation dose on plant materials.

Plant material	Radiation		
	18.3 MeV/u C	12.5 MeV/u He	Low-LET radiations
(a) Dry seeds (genome size)			
Arabidopsis (130 Mb)	300	1100	1200 (electrons)
Rice (430 Mb)	40–50	200	350
Tomato (950 Mb)	70	240	—
Barley (4.8 Gb)	10–20	—	—
Wheat (16 Gb)	25	—	—
(b) Tissue culture			
Chrysanthemum var. Taihei	15	10–20	∼60–80
Chrysanthemum var. Jimba	3	2–3	∼10
Carnation	15	40	60

Listed are D_{50}s (Gy), the irradiation doses that lead to 50% lethality (a. dry seeds) or growth rate (b. tissue culture). D_{50} is a good indicator to know general sensitivity of plants against radiations. Here, carbon and helium ions with the indicated energy were used for high-LET radiation. For low-LET radiation comparison, γ-rays were used, unless otherwise noted. Note that D_{50} decreases as genome size increases (a) (i.e., plant species with larger genomes are more sensitive to radiation). In addition, even in the same species, D_{50} varies among different varieties (b). Data were extracted from experiments performed at TIARA, the electron beam facilities in JAEA, and the γ-ray irradiation facilities in Institute of Radiation Breeding (unpublished data).

the sensitivity against radiation increases. Occasionally, radiation sensitivities vary significantly even among different varieties of the same plant species. In the case of "Jimba," which is a major variety of chrysanthemum in Japan, its sensitivity is more than 5 times higher than that of a variety "Taihei," of which the sensitivity is considered as a standard level in chrysanthemum. Radiation sensitivities also differ among plant organs. This difference is thought to be due to DNA content, water content, and so on. Cells in S phase of the cell cycle are the most sensitive to radiation because in this stage, the DNA content increases and the chromosomal DNA molecules are unpacked, leading to a cell status that is readily attacked by radiation and the secondary radical products. Radicals such as hydroxyl radicals are a major cause of DNA damage. It is well known that these radicals are generated by reactions between water and radiation. Therefore, plant materials such as dry seeds, in which the water content is very low, tend to show high resistance to radiations.

In conclusion, irradiation dose should be carefully determined according to the kinds of ion species and energies, plant species, plant varieties, plant state of materials such as cell cycle, and water content.

1.2.4
Population Size of the M1 Generation

Apparently, it is preferable to prepare as much of the target samples as possible because mutations basically happen at random and therefore under the laws of probability. When the mutation frequency of a particular locus is known, the minimum size of irradiation treatment samples can be roughly estimated. In the case of 18.3 MeV/u carbon ions, the mutation rate at *tt* and *gl* loci is 1.9×10^{-6} per locus per dose [5]. As the irradiation was performed with a dose of 150 Gy, the mutation rate was about 2.85×10^{-4} (roughly 1/3500) per locus, indicating that about 3500 seeds are necessary on average to obtain at least one mutant for a certain locus.

In practice, the minimum population size to isolate one phenotypic mutation (not one gene) is likely to be around 2000–5000 M1 seeds for *Arabidopsis* [9–11], rice, and other crops (unpublished results). However, it is not fully understood how many seeds will be required for plants with different genome sizes, gene numbers, and ploidies. On the other hand, it seems that a smaller population size would be sufficient for mutation induction from explants or tissue cultures. Moreover, several phenotypes, such as flower colors and shapes, chlorophyll mutations, waxes, and so on, have been obtained even in the M1 generation, although the mutation mechanisms are still unclear [12–15].

1.3
Applications

Considering its high mutation rate and its mutation spectrum that potentially differs from other chemical and physical mutagens, ion beam mutagenesis can be a powerful and useful technique to induce novel mutants. In fact, ion beam muta-

genesis has been employed in many plant species and several novel mutants have been produced. Identification of such novel mutants will bring about a better understanding of any biological process of interest, and also a dramatic improvement in agriculture and horticulture. Here, we describe the effectiveness of ion beams by citing recent studies using ion beam radiation.

1.3.1 Ion Beams for Forward Genetics

In forward genetics, isolation of mutants is merely the first step, yet it is a very critical procedure that enables us to analyze any relevant gene functions and gain a new insight into any developmental/physiological event. The new technique of ion beam mutagenesis has contributed significantly to plant research in this respect. For example, a novel mutant, *antiauxin resistant1-1* (*aar1-1*), was identified by screening the M2 progeny of carbon-ion-irradiated *Arabidopsis* seeds for plants resistant to *p*-chlorophenoxyisobutyric acid – a chemical that inhibits the auxin signaling pathway [16]. Further characterization of *aar1-1* showed that this mutant exhibits attenuated response specifically to a synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D), but not to the native auxin, indole-3-acetic acid (IAA) [16]. This finding is quite surprising because it has been believed that 2,4-D and IAA have similar effects on auxin signaling despite differences in their stability. It was revealed that the *aar1-1* mutation is a 44-kb deletion encompassing eight annotated genes [16]. Among them, a gene encoding a small acidic protein (SMAP1) was shown to be solely responsible for the *aar1* phenotype [16]. Further molecular analysis of SMAP1 is necessary to dissect the previously underestimated 2,4-D-specific auxin signaling pathway.

Ion beam mutagenesis has also been applied to the model legume *Lotus japonicus*. Leguminous plants develop symbiotic root nodules to confine soil bacteria called rhizobia, which provide the host plants with ammonia produced through bacterial nitrogen fixation. Since this organogenesis is energetically expensive, the host plants should tightly regulate the development and number of nodules. For this purpose, legumes have evolved a long-distance signaling pathway that inhibits unfavorable overproduction of nodules. This systemic regulation requires at least a *CLAVATA1*-like receptor kinase gene and the mutations of this gene lead to the hypernodulation phenotype [17–20]. However, the precise molecular mechanism have been unclear, partly due to the absence of any other hypernodulating mutants, in spite of many attempts to isolate such plants from *L. japonicus* using EMS or T-DNA mutagenesis ([18, 21, 22] and N. Suganuma, personal communication). To circumvent this problem, helium ions were utilized as an alternative mutagen and a novel *Lotus* hypernodulating mutant, *klavier* (*klv*), was readily produced [23]. Grafting experiment using *klv* mutants showed that *KLV* is necessary in the shoots rather than in the roots, indicating that *KLV*, together with a *CLV1*-like receptor kinase gene, constitutes a long-distance signaling control of the nodule number control [23]. This successful identification of the *klv* mutant indicates that ion beams can be a relatively efficient mutagen, possibly having a different mutation spectrum from EMS and T-DNA.

1.3.2
Ion Beams for Plant Breeding

The problem of food shortages is one of the most crucial global challenges that we have ever faced. For this concern, production of new crop varieties with beneficial traits such as drought tolerance is important to fulfill a stable food supply. Moreover, industrialization of these induced varieties could have a great economical impact on societies.

Kirin Agribio in collaboration with the JAEA has generated many varieties of ornamental plants including carnations, chrysanthemums, and petunias by utilizing ion beams [12, 24, 25]. In the case of carnations, the parental leaf tissues were irradiated with carbon ions and then the plants were regenerated from them [24]. Using this approach, a great number of flower mutants including unprecedented round-petal carnations were obtained and some of the new varieties have been commercialized as "Ion Series" varieties (Figure 1.5) [12, 25].

1.3.3
Limitations of Ion Beams

We have shown that ion beam mutagenesis has been applied to a wide variety of plant species in many research fields and it has been successful for novel mutant production. The effectiveness of ion beams can be attributed largely to their high-LET characteristics, which lead to high DSB yields, strong mutational effects, and a

Figure 1.5 Carnation varieties codeveloped by Kirin Agribio and the JAEA using ion beams. The flower on the upper-left corner is the parent carnation flower (var. "Vital") and the others are mutant flowers produced by carbon ions. Note that ion beams successfully induced many flower color and shape mutants.

unique mutation spectrum compared to other chemical and physical mutagens. However, some limitations of ion beams also need to be taken into consideration. For example, ion beam-induced mutations are mostly deletions that can cause frame-shifts or total gene losses; therefore, ion beams may not be favorable for hypomorphic mutant isolation. In addition, ion beam irradiation results in various kinds of mutations such as small intragenic deletions, large deletions (greater than 100 kb), translocations, inversions, and chromosomal aberrations. Although this broad mutational effect of ion beams is advantageous with respect to novel mutant induction, the unpredictability of the mutation patterns could potentially hinder the subsequent molecular cloning of the relevant genes in some cases.

1.4 Perspectives

A mutagenesis technique – ion beam irradiation – has been exerting a huge impact on plant basic and applied research. Given that only a small fraction of the annotated genes have been analyzed for their functions even in *Arabidopsis*, the presence of such an alternative mutagen will become increasingly important. Further, application of ion beams in plant biotechnology will be more and more valuable to tackle global issues like food and environmental problems. However, some improvements are still necessary to make this mutagen a more reliable tool. For example, at present, the size of deletions generated by ion beams is variable from 1 bp to over 6 Mbp [26]. In this regard, development of techniques that enable us to control the deletion size will provide us with more efficient gene knockout approaches that can delete only a single gene at a time or sometimes tandem-duplicated multiple genes altogether if necessary. To achieve such an improvement, the precise molecular mechanism by which ion beams induce mutations needs to be elucidated.

Acknowledgments

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References

1 Blakely, E.A. (1992) Cell inactivation by heavy charged particles. *Radiat. Environ. Biophys.*, **31**, 181–196.

2 Lett, J.T. (1992) Damage to cellular DNA from particulate radiations, the efficacy of its processing and the radiosensitivity of mammalian cells. Emphasis on DNA double strand breaks and chromatin breaks. *Radiat. Environ. Biophys.*, **31**, 257–277.

3 Tanaka, A., Shikazono, N., Yokota, Y., Watanabe, H., and Tano, S. (1997) Effects of heavy ions on the germination and survival of *Arabidopsis thaliana*. *Int. J. Radiat. Biol.*, **72**, 121–127.

4 Hase, Y., Shimono, K., Inoue, M., Tanaka, A., and Watanabe, H. (1999) Biological effects of ion beams in *Nicotiana tabacum L.* *Radiat. Environ. Biophys.*, **38**, 111–115.

5 Shikazono, N., Yokota, Y., Kitamura, S., Suzuki, C., Watanabe, H., Tano, S., and Tanaka, A. (2003) Mutation rate and novel *tt* mutants of *Arabidopsis thaliana* induced by carbon ions. *Genetics*, **163**, 1449–1455.

6 Shikazono, N., Suzuki, C., Kitamura, S., Watanabe, H., Tano, S., and Tanaka, A. (2005) Analysis of mutations induced by carbon ions in *Arabidopsis thaliana*. *J. Exp. Bot.*, **56**, 587–596.

7 Yokota, Y., Yamada, S., Hase, Y., Shikazono, N., Narumi, I., Tanaka, A., and Inoue, M. (2007) Initial yields of DNA double-strand breaks and DNA Fragmentation patterns depend on linear energy transfer in tobacco BY-2 protoplasts irradiated with helium, carbon and neon ions. *Radiat. Res.*, **167**, 94–101.

8 Fukuda, M., Itoh, H., Ohshima, T., Saidoh, M., and Tanaka, A. (1995) New applications of ion beams to material, space, and biological science and engineering, in *Charged Particle and Photon Interactions with Matter: Chemical, Physiochemical, and Biological Consequences with Applications* (eds A. Mozumder and Y. Hatano), Marcel Dekker, New York.

9 Tanaka, A., Sakamoto, A., Ishigaki, Y., Nikaido, O., Sun, G., Hase, Y., Shikazono, N., Tano, S., and Watanabe, H. (2002) An ultraviolet-B-resistant mutant with enhanced DNA repair in *Arabidopsis*. *Plant Physiol.*, **129**, 64–71.

10 Sakamoto, A., Lan, V.T., Hase, Y., Shikazono, N., Matsunaga, T., and Tanaka, A. (2003) Disruption of the *AtREV3* gene causes hypersensitivity to ultraviolet B light and gamma-rays in *Arabidopsis*: implication of the presence of a translation synthesis mechanism in plants. *Plant Cell*, **15**, 2042–2057.

11 Hase, Y., Tanaka, A., Baba, T., and Watanabe, H. (2000) *FRL1* is required for petal and sepal development in *Arabidopsis*. *Plant J.*, **24**, 21–32.

12 Okamura, M., Yasuno, N., Ohtsuka, M., Tanaka, A., Shikazono, N., and Hase, Y. (2003) Wide variety of flower-color and -shape mutants regenerated from leaf cultures irradiated with ion beams. *Nucl. Instrum. Methods B.*, **206**, 574–578.

13 Nagatomi, S., Tanaka, A., Kato, A., Watanabe, H., and Tano, S. (1996) Mutation induction on chrysanthemum plants regenerated from *in vitro* cultured explants irradiated with C ion beam. *TIARA Annu. Rep.*, **5**, 50–52.

14 Ishii, K., Yamada, Y., Hase, Y., Shikazono, N., and Tanaka, A. (2003) RAPD analysis of mutants obtained by ion beam irradiation to Hinoki cypress shoot primordial. *Nucl. Instrum. Methods B*, **206**, 570–573.

15 Takahashi, M., Kohama, S., Kondo, K., Hakata, M., Hase, Y., Shikazono, N., Tanaka, A., and Morikawa, H. (2005) Effects of ion beam irradiation on the regeneration and morphology of *Ficus thunbergii* Maxim. *Plant Biotechnol.*, **22**, 63–67.

16 Rahman, A., Nakasone, A., Chhun, T., Ooura, C., Biswas, K.K., Uchimiya, H., Tsurumi, S., Baskin, T.I., Tanaka, A., and Oono, Y. (2006) A small acidic protein 1 (SMAP1) mediates responses of the *Arabidopsis* root to the synthetic auxin 2,4-dichlorophenoxyacetic acid. *Plant J.*, **47**, 788–801.

17 Krusell, L., Madsen, L.H., Sato, S., Aubert, G., Genua, A., Szczyglowski, K., Duc, G., Kaneko, T., Tabata, S., de Bruijn, F., Pajuelo, E., Sandal, N., and Stougaard, J. (2002) Shoot control of root development and nodulation is mediated by a receptor-like kinase. *Nature*, **420**, 422–426.

18 Nishimura, R., Hayashi, M., Wu, G.J., Kouchi, H., Imaizumi-Anraku, H., Murakami, Y., Kawasaki, S., Akao, S., Ohmori, M., Nagasawa, M., Harada, K., and Kawaguchi, M. (2002) *HAR1* mediates systemic regulation of symbiotic organ development. *Nature*, **420**, 426–429.

19 Searle, I.R., Men, A.E., Laniya, T.S., Buzas, D.M., Iturbe-Ormaetxe, I., Carroll, B.J., and Gresshoff, P.M. (2003) Long-distance signaling in nodulation directed by a

CLAVATA1-like receptor kinase. *Science*, **299**, 109–112.

20 Schnabel, E., Journet, E.P., de Carvalho-Niebel, F., Duc, G., and Frugoli, J. (2005) The *Medicago truncatula SUNN* gene encodes a *CLV1*-like leucine-rich repeat receptor kinase that regulates nodule number and root length. *Plant Mol. Biol.*, **58**, 809–822.

21 Schauser, L., Handberg, K., Sandal, N., Stiller, J., Thykjaer, T., Pajuelo, E., Nielsen, A., and Stougaard, J. (1998) Symbiotic mutants deficient in nodule establishment identified after T-DNA transformation of *Lotus japonicus*. *Mol. Gen. Genet.*, **259**, 414–423.

22 Szczyglowski, K., Shaw, R.S., Wopereis, J., Copeland, S., Hamburger, D., Kasiborski, B., Dazzo, F.B., and de Bruijn, F.J. (1998) Nodule organogenesis and symbiotic mutants of the model legume *Lotus japonicus*. *Mol. Plant-Microbe. Interact.*, **11**, 684–697.

23 Oka-Kira, E., Tateno, K., Miura, K., Haga, T., Hayashi, M., Harada, K., Sato, S., Tabata, S., Shikazono, N., Tanaka, A., Watanabe, Y., Fukuhara, I., Nagata, T., and Kawaguchi, M. (2005) *klavier* (*klv*), a novel hypernodulation mutant of *Lotus japonicus* affected in vascular tissue organization and floral induction. *Plant J.*, **44**, 505–515.

24 Okamura, M., Ohtsuka, M., Yasuno, N., Hirosawa, T., Tanaka, A., Shikazono, N., Hase, Y., and Tanase, M. (2001) Mutation generation in carnation plants regenerated from *in vitro* leaf cultures irradiated with ion beams. *JAERI Rev.*, **39**, 52–54.

25 Okamura, M., Tanaka, A., Momose, M., Umemoto, N., Teixeira da Silva, J.A., and Toguri, T. (2006) Advances of mutagenesis in flowers and their industrialization, in *Floriculture, Ornamental and Plant Biotechnology*, vol. I (ed. J.A. Teixeira da Silva), Global Science Books, Isleworth.

26 Naito, K., Kusaba, M., Shikazono, N., Takano, T., Tanaka, A., Tanisaka, T., and Nishimura, M. (2005) Molecular analysis of radiation-induced DNA lesions in *Arabidopsis* genome by the pollen irradiation system. *Genetics*, **169**, 881–889.

Plant breeding: Induced mutation technology for crop improvement

Scientists at the IAEA's Seibersdorf Laboratories are helping breeders to develop crops having more desirable traits

by F.J. Novak and H. Brunner

All present forms of life are the product of three factors:

- mutation, the fundamental source of heritable variation,
- environmental factors, which influence the selection of those mutations that survive and reproduce, and
- time, during which the genotype and environment constantly interact and evolutionary change is realized.

Genetic variation found in nature does not represent the original spectrum of spontaneous mutations. Rather, this is the result of genotypes recombining in populations and continuously interacting with environmental forces.

Green plants are the ultimate source of resources required for human life, food, clothing, and energy requirements. Prehistoric people, who depended on their skills as hunters, drew upon abundant natural vegetation to collect nutritious and nonpoisonous fruits, seeds, tubers, and other foods. As human populations increased, greater and safer supplies of food had to be found, and gradually production systems based on plant domestication were developed.

The domestication of crops historically has been influenced by ecological and agricultural conditions, as well as by food gathering preferences. Genotypes that have adapted to a wide range of climatic and edaphic conditions typically have been selected for cultivation. The achievement of higher yielding crops facilitated population growth, sedentary settlements, and further development. Which crops were domesticated depended not only on the number of seeds or the size of fruits, but also on taste, palatability, and other factors.

Only a small fraction of the world's approximately 200 000 plant species have been found suitable for domestication; humans have used about 3000 of these for food, fibre, spices, etc., with 200 ultimately domesticated as crops. Today, only 15-20 of these are food crops of major importance.

The means of developing new plant varieties for cultivation and use by humans has come to be called plant breeding. Early on, it primarily involved selection, the choice between good and bad plants. People learned not to eat all the "best fruit" but to plant the seed from some of them.

Genetics became a fundamental science of plant breeding after the Moravian monk J.G. Mendel discovered the laws of heredity in the mid-19th century. Plant breeding further advanced when the methodology of hybridization was developed. Its aim was to combine various desirable properties of many plants in one plant, instead of just choosing between good and bad plants. This method, often supplemented by germplasm derived from induced mutation, has become the most common one for breeding plants through sexual reproduction.

However, some crops—including bananas, apples, cassava, and sugar cane—reproduce vegetatively, especially those that are fully sterile without seeds. For this important group, alternative approaches had to be developed, namely techniques of manipulation with somatic tissue: mutation breeding and biotechnology.

Mutation breeding

Plant breeding requires genetic variation of useful traits for crop improvement. Often, however, desired variation is lacking. Mutagenic agents, such as radiation and certain chemicals, then can be used to induce mutations and generate genetic variations from which desired mutants may be selected.

Mutation induction has become a proven way of creating variation within a crop variety.

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One natural evolutionary product of genetic variation: a mutant of dwarf coconut palm.

It offers the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evolution. When no gene, or genes, for resistance to a particular disease, or for tolerance to stress, can be found in the available gene pool, plant breeders have no obvious alternative but to attempt mutation induction.

Treatment with mutagens alters genes or breaks chromosomes. Gene mutations occur naturally as errors in deoxyribonucleic acid (DNA) replication. Most of these errors are repaired, but some may pass the next cell division to become established in the plant offspring as spontaneous mutations.

Although mutations observed in a particular gene are rare, there are probably 100 000 genes in a cell of a higher plant. This means that every plant may carry one or more spontaneous mutations into the next generation. Gene mutations without phenotypic (visible) expressions are usually not recognized. Consequently, genetic variation appears rather limited, and scientists have to resort to mutation induction. There are no other economic ways of altering genes, except to wait a long time for spontaneous mutations to occur.

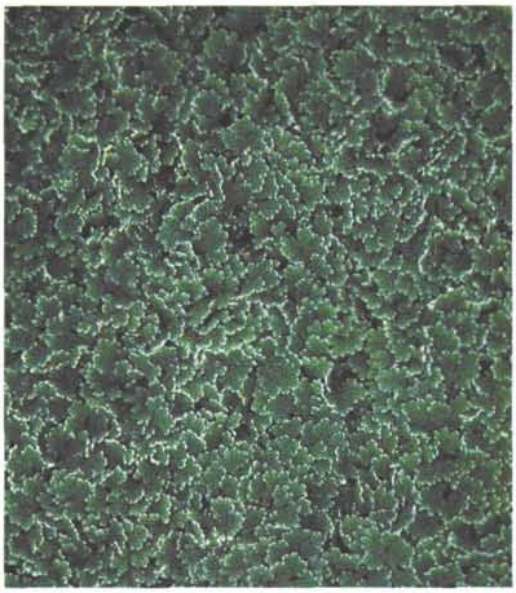
Artificial induction of mutations by ionizing radiation dates back to the beginning of the 20th century. But it took about 30 years to prove that such changes could be used in plant breeding. Initial attempts to induce mutations in plants mostly used X-rays; later, at the dawn of the "Atomic Age", gamma and neutron radiation were employed as these types of ionizing radiations became readily available from newly established nuclear research centres.

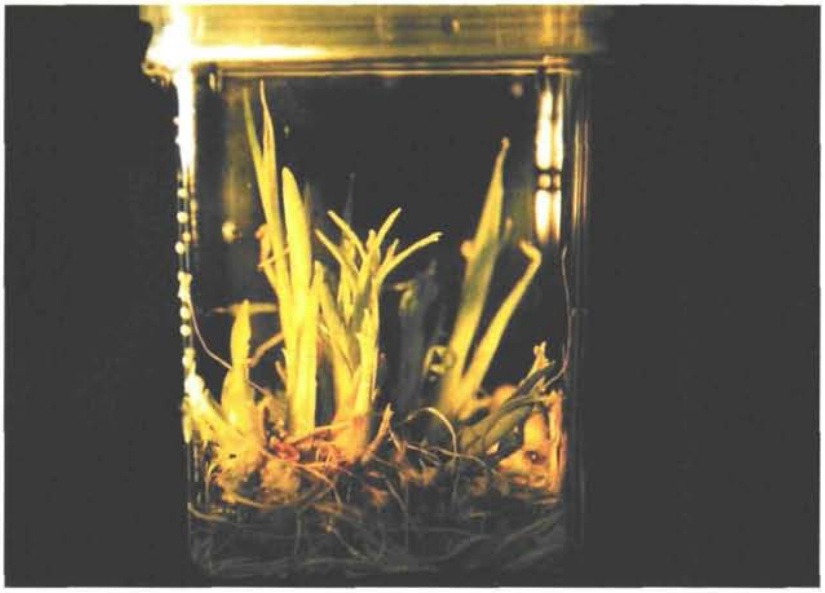
Major efforts were devoted during this initial phase of mutation induction to define optimal treatment conditions for reproducibility. Research focused on changing "random" mutation induction into a more directed mutagenesis to obtain more desirable and economically useful mutations. However, it did not lead to the desired alterations in the mutant spectrum. Limitations were the concomitant increase of plant injury with increasing radiation dose and the low frequency of economically useful mutations. This led scientists to search for potentially better mutagens. As a result, new methods of radiation treatment, as well as chemical agents with mutagenic properties, were found.

Plant biotechnology

Breeding for improved plant cultivars is based on two principles: genetic variation and selection. The process is extremely labourious and time consuming with high inputs of intellectual and manual work. *(See box.)* However, the development of plant cell and tissue culture over the last 20 years has made it possible to transfer part of the breeding work from field to laboratory conditions.

Extensive research has resulted in new areas of plant breeding, namely "plant biotechnology" and "genetic engineering". They are based on cellular totipotency, or the ability to regenerate whole, flowering plants from isolated organs (meristems), pieces of tissue, individual cells, and protoplasts. The isolated plant parts are aseptically grown in test tubes on artificial media





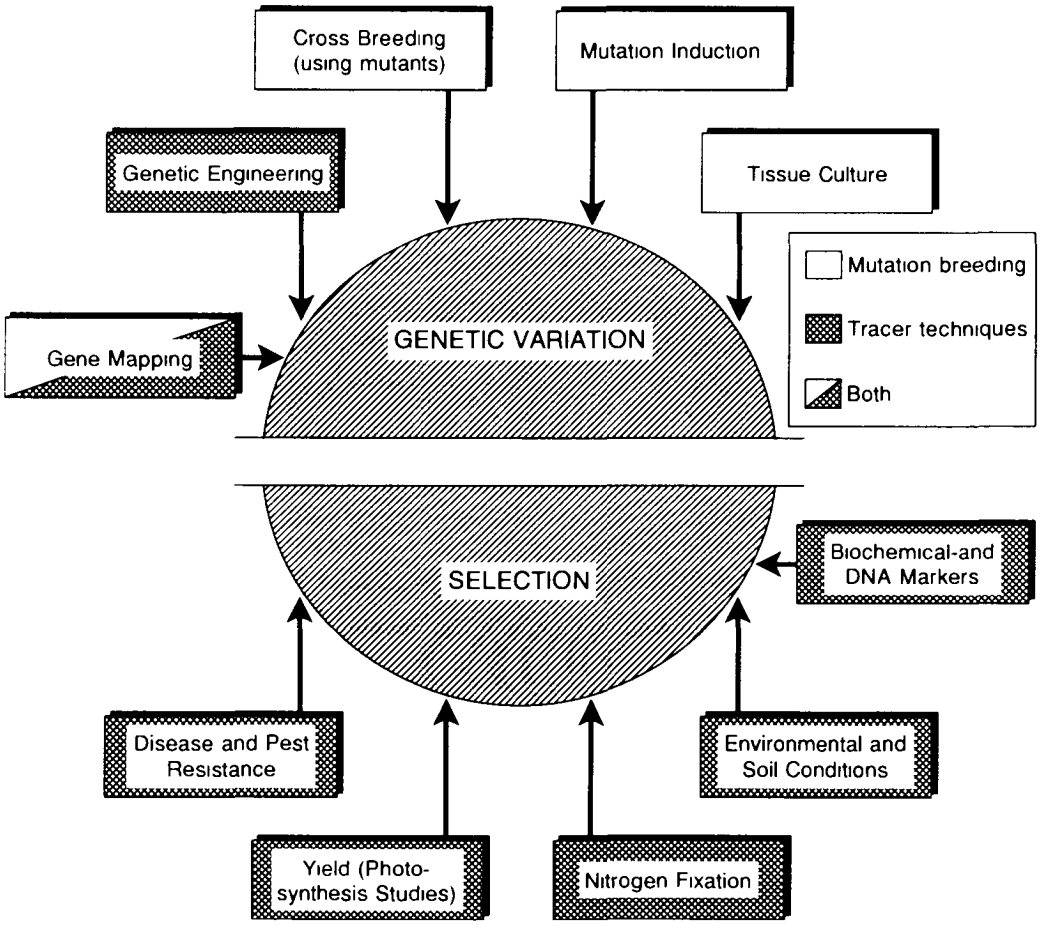
Some tools and products of plant breeding *(clockwise from top left)*: a mutant of paddy rice induced by ionizing radiation; yams and other root and tuber crops can be genetically improved by mutation breeding; tissue culture and in vitro mutagenesis are basic methods of biotechnology for improving crops; "Golden Haidegg", an apple mutant with improved market value, was induced at the Seibersdorf Laboratories by irradiation of cuttings from "Golden Delicious" apples; mutation breeding has improved the tolerance to environmental stress of Azolla, a water fern used as biofertilizer in rice paddies.

General scheme of mutation breeding

Breeding a new variety of crop takes anywhere from 12 to 15 years of intensive effort The steps include:

Generation	Characterization
	Seeds, pollen, vegetative parts, or tissue cultures treated by physical (radiation) or chemical mutagens.
$M_1(M_1V_1)$	Plants grown from treated seeds (M_1) or vegetative propagula (M_1V_1).
$M_2(M_1V_2)$	Population of plants grown from seeds (M_2) or vegetative parts (M_1V_2) harvested from M_1 or M_1V_1 respectively. Selection of desired mutants may start in this generation or later.
M_3 - M_8 (M_1V_3 - M_1V_8)	Continuing selection, genetic confirmation, mulitplication and stabilization of field performance of mutant lines.
Next 2 - 3 generations	Comparative analyses of mutant lines during different years and in different locations.
Next 2 - 3 generations	Official testing before release as a new variety.

Applications of nuclear techniques in plant breeding



Crop improvement is based on two basic principles: genetic variation and selection. Serving as invaluable tools are mutagenic irradiation and isotope tracer techniques, which are incorporated into the various breeding methods.

of known chemical composition (*in vitro* culture). Under strictly controlled conditions, they form plantlets that subsequently can be transferred to soil where they grow to maturity.

Tissue culture has been exploited commercially for micropropagation of disease-free stocks of horticultural plants (strawberry, potato, and ornamentals, for example). *In vitro* techniques also are useful in various steps of the breeding process, such as germplasm preservation, clonal propagation, and distant hybridization.

Radiation mutation breeding and isotope techniques, combined with tissue culture, have made a significant contribution to plant breeding. They have introduced new techniques for inducing genetic variation, by improving selection technology, and by accelerating breeding time. *(See box.)*

Other methods, known as anther, or pollen culture, make it possible to regenerate plants from male gametes with half the number of chromosomes—haploids. Compared to plants with full chromosomal content (diploids), the use of haploids in mutation breeding is advantageous since it allows detection of mutations immediately after their induction. Haploid methods have proven to significantly speed up the breeding of new varieties of rice, barley, and vegetables, for instance.

Genetic engineering procedures allow the transfer of genetic material (DNA) from the cell of one species to that of another genetically unrelated organism. For example, a piece of DNA from a bacterial cell may be integrated into the genome of a plant cell to form a transgenic plant. The new DNA (gene) expresses itself in the plant phenotype regenerated from the transgenic cell. Nuclear techniques, based on nucleic acid bases labelled by isotopes, are employed in genetic engineering, to identify and isolate suitable genes for transfer; as delivery systems to introduce genes into recipient cells; and to detect new genetic material in recipient organisms.

Genetic engineering already has resulted in the production of plants with new desirable traits, such as insect resistance, virus disease resistance, and better ripening properties. However, early enthusiasm is being tempered by the growing discussion of the potential hazards of releasing transgenic plants to the environment.

Another issue that has emerged concerns the commercialization of this technology, and the access of developing countries to it. Recent developments in plant biotechnology have resulted in enormous capital investments and in a concentration of highly qualified human resources into the commercial sector of many industrial countries. In the process, scientific knowledge and its technological applications increasingly are becoming the subject of commercial legislation, involving patents, industrial secrecy, and licensing policy. As a result, developing countries have experienced difficulties in gaining access to biotechnological results and their applications in national programmes.

In this context, specialized agencies of the United Nations—including the Food and Agriculture Organization (FAO), United Nations Educational, Scientific and Cultural Organization (UNESCO), United Nations Industrial Development Organization (UNIDO), and IAEA—are playing an important role. Programmes have been set in place whose main concerns are to identify and transfer appropriate biotechnologies, and to train personnel, in developing countries. In this way, national capabilities in research and development are being strengthened in this field.

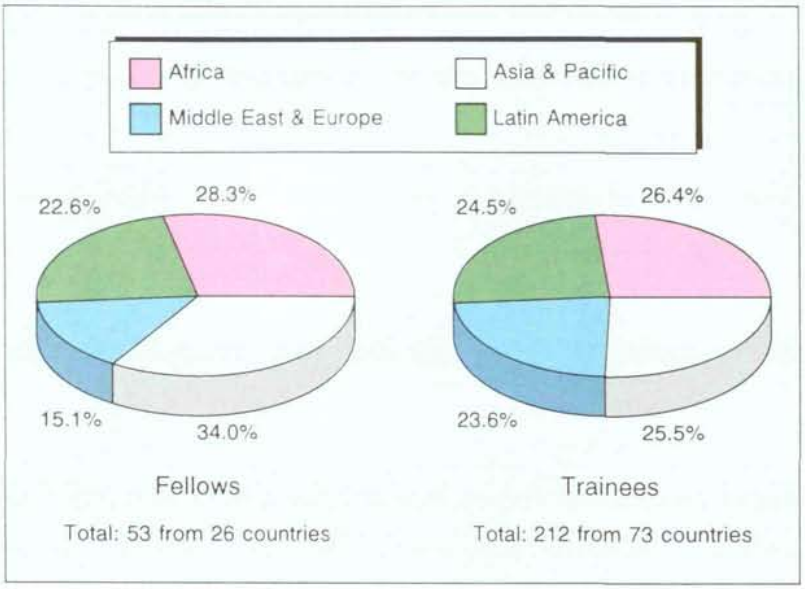
Plant breeding for sustainable agriculture

Plant breeding and biotechnology contribute greatly to environmentally friendly agriculture. The focal point is the establishment of a comprehensive gene pool for crop improvement.

New technologies have been developed to stop the loss of biodiversity of plant species. Tissue culture technology in cassava, banana and plantain, potato, yam, sweet potato, and coconut, for example, is becoming the preferred method for germplasm preservation and for the international exchange of clonal material. Molecular marker techniques are applied for the classification and genetic analysis of cultivars and related wild species. Plant breeding methods such as cross breeding, *in vivo*, and *in vitro* mutation breeding very often include nuclear components. They are employed for the enhancement of genetic resources by mutation induction, recombination, and selection.

Most breeding projects are directed towards developing new plant cultivars that exhibit greater disease resistance and tolerance to pests than the original plant. Such varieties decrease dependence on agro-chemicals, which is a basic feature affecting sustainable agricultural development in industrialized and developing countries alike. Resistance breeding may help to avoid the epidemic situation in plantation crops. These include the swollen-shoot virus disease epidemic on cocoa in Ghana, and Panama disease on banana in several tropical and subtropical areas.

Sustainable plant breeding today faces some major challenges. They specifically relate to breeding plants for improved nitrogen fixation and plants with better capacities to utilize nutrients. Integrated research involving both soil



Training activities in the Plant Breeding Unit of the Seibersdorf Laboratories, 1982-92

scientists and plant breeders already has identified desirable genotypes in grain legumes (soybean, garden bean) and other plant species, including trees.

For many developing countries, breeding crops for tolerance to salinity and acidity in soils is of high priority. Current breeding strategies (including mutation induction and *in vitro* selection) have clearly been successful in incorporating degrees of tolerance in different species. The use of genetic engineering for creating environmental stress-resistant plants will depend on the identification of specific genes which contribute to the adaptation to specific stress environments.

In tropical countries, agriculture practices have maintained the yield level of different crops through "intercropping" instead of by increased monocrop cultivation. Breeding crops for multiple functions—such as biomass production, improved soil and water practice, and composting —is a desirable support of sustainable agriculture in developing countries. The mixed planting of a main crop with specific cover crops (e.g. forage legumes or grasses) minimizes the use of herbicides.

Radiation service statistics, 1967-92

Treated samples	20 329
Treated species	217
Treated cultivars	1 134
Recipient Member States	108
Seed samples	17 872
Vegetatively propagated plants	1 046
Cobalt-60 gamma treatments	14 382
Fast neutron treatments	5 416
Other mutagen treatments	531

Note: Examples of major plant species treated include: cereals (rice, wheat, barley, tricilale, millet, tef); legumes (soybean, peanut, common bean, cowpea, mungbean); root and tuber crops (cassava, yam, cocoyam, potato); fruits (citrus, apple, apricot, peach, grape vine); ornamentals (chrysanthemum, antirrhinum, achimenes, tulip); and others (rape, sesame, amaranth, quinoa, niger).

Role of the Seibersorf Laboratories

The Plant Breeding Unit of the IAEA Laboratories at Seibersdorf was set up in the mid-1960s to support the Joint FAO/IAEA Division's programme of genetic crop improvement. Nuclear techniques in plant breeding are developed and transferred to countries by research and development in mutation breeding and related biotechnological techniques, training scientists from developing countries, and providing irradiation services and technical advice.

Initial research in the Plant Breeding Unit focused on the development of mutation induction methods with ionizing radiation and chemical mutagens. The aim was to achieve high mutagenic efficiency, i.e., a high frequency of desirable mutations at minimal plant injury and the highest possible reproducibility. This required a definition of radiation source characteristics in terms of dose homogeneity and precise assessment of absorbed dose in biological targets by appropriate dosimetry. Irradiation of seeds with gamma rays and neutrons was commonly done, given the ease of handling, the simple standardization of factors which modify radiation sensitivity, and good reproducibility. The establishment of methods for controlling oxygen-dependent effects in the radiobiological response to electromagnetic radiation was a major achievement. The Laboratory actively contributed to standardizing neutron irradiation of seeds in nuclear reactors by developing special facilities for this purpose. These were known as SNIF, for Standard Neutron Irradiation Facility for swimming-pool-type reactors; and as USIF, for Uranium Shielded Irradiation Facility for Triga-type reactors.

This research was the basis for the IAEA Laboratories' worldwide seed irradiation service using fast and thermal neutrons at a high-dose precision and reproducibility of induced effects. Moreover, efficient and accurate treatments of seeds with chemical mutagens, mostly alkylating agents and azides, were developed with the aid of isotope-labelled compounds and compared with mutation induction by ionizing radiation. The Unit has undertaken supportive research on mutation breeding in cereals, pulse crops, industrial crops, and vegetatively propagated crops.

As each crop species has a variable reproductive capacity (number of progenies per plant) and a specific system of reproduction (self- or cross-pollinated sexual reproduction or asexual propagation), a universal breeding approach cannot be developed and species-specific procedures have to be applied. Most vegetatively or asexually propagated species are difficult to improve genetically by conventional cross- and mutation breeding methods. These breeding problems can be more easily resolved by using biotechnology in combination with mutation induction, and the Unit initiated *in vitro* mutation breeding activities during the mid-1980s. Several tropical food crops of great importance to the food security of developing countries were chosen as the main focus of R&D and training activities in biotechnological plant breeding at the IAEA Laboratories.

Research and development activities

The Unit provides focused support to the FAO/IAEA's co-ordinated research and technical co-operation programmes. Assistance is provided to numerous projects in terms of expertise for building facilities for plant tissue culture and mutagenic treatment, for quality control of dosimetry of mutagenic irradiation, and for the development and transfer of nuclear technologies for plant improvement.

Ongoing R&D includes the application of nuclear methods and associated advanced techniques, such as *in vitro* culture and molecular genetics, to improve the production of a wide range of crops through mutation breeding. The development of biotechnological methods for breeding vegetatively propagated crop plants of major importance in developing countries has a high priority.

Currently, the following R&D areas are being pursued:

● ***Somaclonal and mutagen induced variation.*** Systematic studies are being conducted to compare the genetic variation caused by tissue culture (somaclonal) variation with that induced by irradiation and chemical agents. Genetic variation is being studied among maize plants derived from *in vitro* cultured material via somatic embryogenesis. This is being done to assess the nature of somaclonal and induced variation and its potential for use in practical breeding.

● ***Mutation induction and breeding technology for banana and plantain.*** Low genetic variation and sterility handicap genetic improvement of banana and plantains *(Musa spp.)* by conventional breeding techniques. Shoot-tip culture and *in vitro* plant regeneration are being investigated for use in mutation induction and mutant selection. Somatic embryogenesis and plant regeneration from cell suspensions of *Musa* are used to develop somatic cell manipulation procedures for banana and plantain breeding. Methods of screening such plants for resistance to Panama disease are studied in tissue culture, and biochemical markers (peroxidase) are applied for the identification of tolerant genotypes. DNA markers are used for identifying mutants and characterizing cultivars and species of *Musa*. Mutant clones identified at the Seibersdorf Laboratories are tested in the field in tropical countries.

● ***Mutation breeding to improve the tolerance to environmental stress of Azolla.*** *Azolla* is a small aquatic fern that lives in symbiotic relationship with the nitrogen-fixing cyanobacterium *Anabaena*. Under suitable field conditions *Azolla* can double its weight every 3-5 days. The *Azolla-Anabaena* symbiotic system provides green manure for flooded crops, particularly rice. Induced mutagenesis has produced *Azolla* variants tolerant to high salinity, toxic aluminium levels, and/or to herbicides. Tolerant plants are being investigated under field conditions to confirm that heritable changes cause the increased tolerance to environmental stress.

● ***Methods of mutation induction and breeding of tropical root and tuber crops (cassava and yam).*** Cassava and yam are among the most important staple food crops of the lowland tropics. Mutation breeding technology is being developed to increase variation in plant stature, cyanide content, disease, and pest resistance. *In vitro* techniques are used for the propagation of healthy plants and improved clones. Somatic embryogenesis is being developed for cassava and yam improvement through *in vitro* mutagenesis and later on by somatic cell manipulation. Mutant and polyploid clones are prepared for field testing in Member States.

● ***Tissue culture in cocoa as a system for more efficient mutation breeding.*** Attempts to breed cocoa for disease resistance have yielded very limited success. A major constraint is that little variation exists in currently available cultivars. Somatic embryogenesis is being developed for propagation of desirable genotypes and, through *in vitro* mutagenesis and pollen mutagenesis, is being applied for induction of virus-resistant cocoa trees in Ghana.

Plant breeding research at the Seibersdorf Laboratories is directly problem- and client-oriented. Many positive results of scientific work have been achieved by junior scientists from developing countries during their assignments

under the IAEA's fellowship training programme. Local cultivars and genetic material from tropical countries are brought to the Seibersdorf Laboratories, transferred to tissue culture conditions and used for experimental work. Protocols and techniques that are specifically developed for a crop and a particular genotype are then directly used in national programmes. Additionally, breeding material originating from mutant lines and clones which are ready for field testing are dispatched from Seibersdorf to developing Member States in support of their breeding programmes.

Training of plant breeders

Training in plant breeding represents the most active component of technology transfer at the Seibersdorf Laboratories. For 20 years the Plant Breeding Unit has supported the Agency's fellowship programme and organized interregional training courses. Training activities are closely connected with R&D efforts on crop plant improvement and the application of nuclear techniques in breeding. *(See graphs.)* During a period of three to twelve months, fellows usually work with radiation or chemical induced mutagenesis in plant species cultivated in their home countries. Whenever possible, training of small groups of two-to-five fellows is organized for solving common problems. The experiments are individually designed to assure that laboratory techniques and results will be directly applicable upon return to the home institute.

As a result of their work, fellows have produced numerous scientific publications in internationally recognized journals and symposia proceedings. Very often, as continuation of a fellowship in Seibersdorf, fellows participate in co-ordinated research and technical co-operation projects of the IAEA.

The FAO/IAEA Interregional Training Course on "Induction and Use of Mutations in Plant Breeding" has been held at the Seibersdorf Laboratories since 1982. Twenty participants from different Member States of FAO and IAEA are admitted annually to this intensive training course that usually lasts 6 to 8 weeks. Through lectures, laboratory exercises, field experiment evaluations, seminars, and excursions, participants are made aware of the latest advanced mutation techniques and biotechnological and molecular biology methods for crop improvement. Special training is given in the safe handling of radiation sources, radioisotopes, and particularly hazardous mutagenic chemicals. At the end of each course, participants are able to discuss and evaluate the potential role of induced mutations and advanced biotechnologies in their national breeding programmes for specific crop improvement of cereals, legumes, oil crops, forages, vegetables, fruits, root and tuber crops, palms, rubber, and other plants.

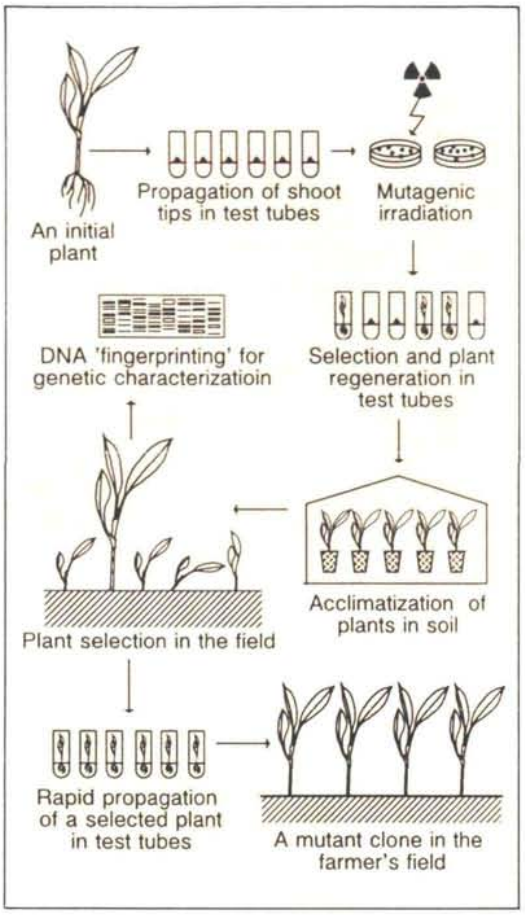
Support for national programmes

A radiation treatment service is provided at no cost to FAO and IAEA Member States to foster the application of nuclear techniques in crop improvement programmes and to render direct support to plant breeders in developing countries. Mutagenic treatment is applied to seeds, corms, tubers, scions, cuttings, and tissue cultures ("*in vitro* materials") with precise doses of gamma and fast neutron radiation. The doses are carefully calibrated to assure reproducible effects. Users of the service are requested to report on the objectives of the applied mutation breeding project and to provide an adequate material (population size) to ensure a high probability for mutation induction of desired characters. Moreover, a prior radiosensitivity test in a greenhouse is frequently performed to assess useful radiation doses for the great variety of biological samples in mutation breeding. The treated materials are dispatched with a detailed irradiation protocol and with the request to report on the induced radiation effects in the first and second mutation generation. This feedback is required to improve radiosensitivity estimates of species and cultivars from different environments.

Over the last 25 years, the Unit has provided radiation services on more than 20 000 samples from the majority of Member States from the FAO and IAEA. *(See table.)* Most of these were seed samples which were irradiated with cobalt-60 gamma rays.

Recently, however, requests for mutagen treatment of *in vitro* materials and for fast neutrons have become more frequent. This reflects the increasing importance of biotechnology and molecular genetics in plant improvement programmes.

Less than 80 mutant varieties were officially released before the start of irradiation services. Over the past quarter century, more than 1500 cultivars of crop plants and ornamentals with significantly improved attributes — increased yield, improved quality, higher market value, disease resistance, and/or stress tolerance — have been released. Some of these mutant varieties were derived from radiation services provided by the Seibersdorf Laboratory. ❐



A banana plant developed by mutation breeding using ionizing radiation. *At left*: The schematic represents a banana mutation breeding system.

Breeding hardier bananas

Bananas, plaintains, and cooking bananas are different cultivars and species belonging to the botanical genus *Musa*. Banana "trees" are actually big herbs which produce fruit that are one of the most important foods for hundreds of millions of people in developing countries. The world's production is more than 70 million tonnes per year and about 90% of the total harvest is used as food for domestic consumption. The banana industry generates an income of about US $1.7 billion annually for exporting developing countries.

The cultivation of bananas and plantains is seriously threatened by several diseases caused by pathogenic fungi, bacteria, viruses, and nematodes. Some of them may be controlled by pesticides; however, the most epidemic pathogen, Fusarium, is a soil borne fungus which causes Panama disease. There is no effective chemical control against the spread of this fungus in infested soil. Panama disease has devastated several hundred thousand hectares of banana plantations in Central America and created serious problems in Africa where many people are dependent on plantains and cooking bananas as part of their staple diet. The only way to resolve this problem is to breed varieties having disease resistance.

The world's production of bananas is based on a very limited number of genetically unimproved clones that were selected and domesticated from nature. Although cross breeding has contributed a little to banana breeding, the most important varieties are entirely sterile and therefore impossible to improve by conventional breeding techniques.

Research on the induction of mutations in bananas by exposing them to radiation and supporting tissue culture techniques was initiated at the Seibersdorf Laboratories in 1985. Shoot tips were isolated from several economically important banana and plantain cultivars and micropropagated on artificial media in test tubes. Several types of mutagenic irradiation (gamma rays and fast neutrons) were applied on actively growing cells of apical shoot tips which were regenerated into plants. This research resulted in the development of mutant clones of the most important cultivar of the dessert banana, "Grand Nain". These varieties are now being tested in several countries for agronomic performance such as yield, quality of fruit, and earlier harvest.

The Seibersdorf Laboratory supports a co-ordinated research programme on breeding for improvement of *Musa* crops and assists several technical co-operation projects in establishing national breeding programmes in Colombia, Panama, Costa Rica, Cuba, Ghana, Malaysia, and Thailand.

Recent developments in molecular biology have made it possible to characterize plant genomes and to identify markers for practical use in plant breeding. Genetic "fingerprinting" of banana cultivars and mutants opens new perspectives for breeding these genetically "recalcitrant" crops which are of such vital importance to people in developing countries.

Atomic Agriculture: Policymaking, Food Production, and Nuclear Technologies in the United States, 1945–1960

NEIL OATSVALL

While most stories of splitting the atom—from Chernobyl to Fukushima and Hiroshima to the Marshall Islands—revolve around images of pure destruction and human misery, the truth is that a much more complicated relationship has existed between nuclear technologies and human existence. This article focuses on agriculture to explore how executive branch policymakers in the United States implemented nuclear technologies in the budding nuclear age. Part of that tale involves how nuclear technologies, especially radioactive isotope tracers, helped improve agricultural science and knowledge. The other side of the story is that agriculture also proved important to the development of nuclear technologies because it provided a clearly peaceful output for atomic research. Atomic agriculture thus frequently assumed a place of prominence for explaining how splitting the atom was a gift to the world and not the red horse rider of the apocalypse.

> *A growing plant is a chemical factory, of course. Scientists have known this for years—but haven't known exactly what went on in that factory. They didn't know and couldn't find out how chemicals entered the plant, what the chemicals did, how they accomplished their work. So, agriculture has had to depend on trial-and-error in*

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producing vital food.
Now agricultural science has perfected a way for studying and following plant chemicals from the time they leave the soil until they are finally deposited in the various parts of the plant. By mixing small quantities of radioactive isotopes with the soil, the scientist, with his Geiger counter, can now follow the movement of important chemicals through the whole cycle of plant life. . . .
Food production, therefore, is passing from trial-and-error to certainty.[1]

THE LOVEABLE CARTOON CHARACTER DAGWOOD BUMSTEAD is best known for eating impossibly large sandwiches, napping, and, of course, his beautiful wife Blondie. During the Cold War, however, the United States enlisted the good patriot Dagwood to help teach the nation about nuclear science. In *Learn How Dagwood Splits the Atom!* the magician Mandrake shrank our animated protagonist and his family to the size of molecules, and in their diminutive states, the Bumsteads learned about the composition of atoms and how nuclear chain reactions work. The booklet not only sent Dagwood on his miniaturized journey but also acted as a booster for the nuclear industry.

In this mission as a booster, Dagwood also promoted the benefits of harnessing the atom to improve agriculture. Completely outside the tiny Dagwood story arc, several pages at the end of the comic were single-page snapshots of how atomic energy had benefitted, and would continue to benefit in the future, medical science, industry, and agriculture. Atomic tracers could track "plant chemicals from the time they leave the soil until they are finally deposited in the various parts of the plant" and seemed to be a miracle technology that would transform growing food from "trial-and-error to certainty." Such statements held a clear implication: if researchers could only understand the exact biological processes that govern how plants grow and produce food, those scientists would be able to help farmers feed the nation in a failsafe fashion. As an Atomic Energy Commission (AEC) report to Congress in the same year of the Dagwood cartoon's publication claimed, "The story of the Garden of Eden and the myth of Promethean fire find uncanny parallels in the huge responsibilities of the Atomic Energy Commission to control the unprecedented forces of atomic energy for the welfare of man." And yet, with atomic energy and its lessons, US policymakers hoped to turn the country's agricultural lands into a modern-day Garden of Eden, albeit with less devastating apples.[2]

The Dagwood cartoon is important for the attitudes and mindsets it repre-

sents. The cartoon is emblematic of how, during the Truman and Eisenhower administrations, policymakers paid careful consideration to the ways growing nuclear scientific understandings might be applied outside of improving war-making capabilities. General Leslie Groves, former head of the Manhattan Project, wrote of *Learn How Dagwood Splits the Atom!*, "This book will reassure the fearful that the future can be made bright." Historian Joel B. Hagen has described how many people, specialists and not, quickly realized that atomic energy could pose incredible dangers to both human health and the environment. Hagen explained that, "In response, professional ecologists effectively used concerns over atomic energy as a convincing justification for ecosystem studies." He further elucidated that for postwar professional ecologists (or in our case, agronomists), nuclear energy became "a kind of double-edged sword" that could wreak havoc on the natural world, but also unlock "many of nature's secrets for human benefit." In the same way, research into atomic agriculture allowed US policymakers simultaneously to increase research into atomic energy and nuclear technologies and learn more about how these affected the natural world without necessarily creating bombs that could cause incredible harm to both human and natural systems.[3]

During the 1940s and 1950s, executive policymakers, especially in the AEC, wanted to improve the nation's agriculture. They believed that doing so would represent their commitment to more than creating weapons of greater and greater mass destruction. Indeed agricultural research could be used as a way to mitigate the effects of any potential nuclear attack. In short, atomic agriculture represented an attempt by policymakers to repurpose atomic energy research as a peaceful entity. Those decision-makers intended atomic agriculture to create hope for the future and held an optimistic belief that technology and greater control of nuclear energy could create a better nation. Moreover, they realized that, if the AEC improved agriculture, agriculture could help the AEC better develop atomic energy and cast its research into a much more publicly palatable form. The August 4, 1947 issue of *Time* magazine depicted AEC Chairman David Lilienthal in front of a fiery red horse and asked, "Is there any way out of the Atomic wilderness?" Agriculture, however, frequently assumed a place of prominence for explaining how splitting the atom was a gift to the world and not the red horse rider of the apocalypse.[4]

In order to understand this interplay between the atom and agriculture, however, atomic agriculture must be placed in several different contexts—nuclear weapons research, the modernization of agriculture, and the Green Rev-

olution. The first atomic weapon exploded over Alamogordo, New Mexico, during the summer of 1945, but a system of research networks existed for years before that. The detonation of an atomic bomb over Hiroshima on August 6, 1945 announced nuclear research to the world and immediately conjured mixed emotions among the world's peoples. It signaled that the world had changed a great deal and would continue to do so in the coming years, with that change being felt even by rural residents as the US government attempted to inculcate them into civil defense networks. And though an emphasis on using nuclear technologies for peaceful endeavors began early in Harry Truman's presidential term, President Dwight Eisenhower's "Atoms for Peace" speech at the United Nations in 1953 called for an international focus on turning the atomic sword into a plowshare, calling the application of some nuclear technologies to agriculture, among other industries, "one of the bright spots in the atomic energy program." While these efforts provided a necessary jolt to atomic agriculture, some of the patterns of change had been set in motion long before.[5]

Agriculture in the United States began its journey to modernized farm production in the nineteenth century, but after the First World War that action accelerated. Farmers adopted more machines, particularly machines powered by hydrocarbon fuels like gasoline, and these mechanical aids let farmers do their jobs easier, quicker, and with less human labor. With those machines came a rising industrial logic, as the transformation also had an ideological component. After World War II agriculture took off in an even more spectacular way, as it "underwent a revolution in productivity spurred by machines, chemicals, and improved plant and animal breeds." Continued use of machines combined with a budding US chemical industry, as tractors went hand in hand with fertilizers, herbicides, and pesticides. The process did not happen seamlessly, however, and farmers made many individual decisions along the way as the process advanced. In the end, farming became more of a business, leading to the current state of trucking cheaply produced agricultural products across the country to feed a nation that eats better and at less expense than any before it in history.[6]

Once the United States reached that situation, however, a series of decisions to share the methods to such agricultural productivity coalesced into what is commonly called the Green Revolution. Previous world hunger, such as the El Niño–exacerbated fin-de-siècle drought famines that killed tens of millions, had elicited little attention from the United States and especially the US gov-

ernment. After World War II the world political climate changed enough that feeding the world and eradicating hunger became an important political goal. In addition to mechanical and chemical advances, improved plant breeding also played a crucial role in getting food to mouths. In particular, improved cereal grain crops like highly productive dwarf wheat and rice strains, combined with chemical fertilizers and petro-fuels, meant producing incredible amounts of food could be done easier than ever before. Also crucial was a reconceptualization that foreign nations needed the United States' help to feed their peoples. In addition to that new philosophical approach to foreign aid, US Public Law 480 (also called Food for Peace) was enacted during the Eisenhower administration with the general goal of using agricultural surpluses—surpluses that would only increase with the use of nuclear technologies to advance agriculture—in the United States to feed poor people across the world and open up new markets for US agricultural products. The Green Revolution did not deliver on everything it promised, however. Though intended as a foreign aid solution that would put the Third World into the United States' camp, the Green Revolution did nothing to change existing social imbalances. And a host of unexpected outcomes, like pesticides damaging both the environment and human health, meant that even its successes came with distinct failures. In short, the Green Revolution was no perfect solution and perhaps what US planners considered to be the problem (lack of food) was more a symptom of uneven development than the problem itself. No matter the problem, though, atomic agriculture did play a key role in increasing agricultural production.[7]

Fundamentally, though not exclusively, agricultural research with atomic energy began with the use of radioisotope tracer atoms. A June 1946 press release by President Truman declared, "The first peacetime applications of the results of wartime atomic research becomes immediately possible with announcement today of availability of radioisotopes for biological and medical research." Although less than a year had passed since the bombings of Hiroshima and Nagasaki and the United States represented the only nuclear power in the world, Truman's administration had begun to promote atomic energy as a peaceful entity. Produced from the "atom pile," radioisotopes offered scientists the ability to use "tagged" atoms—radioactive versions of common elements—to track how these atoms moved through biological processes, ecosystems, or anything else through which elements moved. Applying the tracers to agriculture seemed logical and, as Truman expressed, would revolutionize biological research. The results from radioisotope re-

search caused policymakers to champion the atom as a boon to agriculture.[8]

Lilienthal embraced the president's message when he gave an address in 1947 at the American Farm Bureau Federation's annual meeting. In that speech, he explicitly connected the atom to agriculture and showed the AEC's commitment to improving agriculture. He offered the Chicago-gathered crowd advice as to why they should care about atomic energy. He explained as his number one point, "No one in this country has a greater stake in the vigorous development of atomic energy, and the consequent increase in knowledge of the fundamental laws of Nature, than you who day after day work most closely with nature—the farmers of America." Since he thought farmers had such a high stake in atomic development, Lilienthal's second point followed closely when he contended that farmers needed to stay informed of atomic energy discoveries and peaceful uses of the atom.[9]

Lilienthal further claimed, "the farmer and the farm family have a very special stake in the wise and vigorous development of the science of the nucleus of the atom, for peaceful purposes." He even compared the incredible stores of atomic energy to farm energy, saying, "the energies that produce great poems, that build churches and homes, the energies from which spring such noble ideas as our Constitution and Bill of Rights. That energy has been stored up in the plants of the field, and in the tissues of the animals that feed on your pastures; thence it comes to men." Farms had produced food from the atomic energy of the sun for millennia, and farmers represented "the trustee and steward of that never-ending miracle by which the atomic energy of the sun becomes chemical energy and then human energy." With this reasoning, farmers held an important stake in the development of atomic energy and its application in peaceful endeavors. Farmers made possible all the United States' great history and ideas by nourishing the bodies that produced these marvels, and the country needed them to help continue this great legacy. Moreover, the AEC needed farmers, the trustees and stewards of the sun's atomic forces, to help support its atomic energy research agenda.[10]

To Lilienthal, the difference between "a modern American farm and a backward poverty-stricken farm" was knowledge, and "In this country the farmer has seen that the scientist is his partner, his companion and friend." Lilienthal's message held a clear implication—if providence (or the AEC) gave farmers, the "custodian of the sun's energy and the forces of growth," the opportunity to do something like develop nuclear power they surely would. The AEC chairman gave the example of phosphorous to help explain why the wise

farmer would want atomic science developed. He elaborated that, even though it cost a great amount, US scientists could produce radioactive phosphorous. Phosphorous, like many other elements, is taken up by plants during the growth process, but at the time agricultural knowledge had not advanced enough to know exactly what the plant did with that phosphorous after the chemical's uptake. By using tagged radio-phosphorous, scientists could help "in a way never before possible chart the changes that occur in matter in the process of plant life and growth. In your behalf, the researcher can gain new and important knowledge of how plants convert the sun's energy into life energy on this planet." Clearly this represented the farmer's "big stake" in nuclear development. Since, according to Lilienthal, scientists worked on behalf of farmers, it seemed only logical that farmers would support their efforts, as supporting scientists truly was, in effect, supporting themselves.[11]

Near the end of his speech, Lilienthal brushed aside any concerns his audience might have had over exactly what the uses of the atom in agriculture might be, anticipating that the breakthroughs would be significant. He reminded them that many prominent scientists, like Gregor Mendel, had been unsure of what their research might mean when they began, though that research eventually proved fundamental to farmers. Lilienthal noted that harnessing the atom might also improve agriculture through pest control, pointing to an upcoming conference on the subject at Alabama Polytechnic Institute at Auburn (today Auburn University). And while radiation might not be useful directly as fertilizer or in foods (though research would continue on this subject), agricultural improvement remained "one of the glorious promises of atomic science. It well may help to solve one of the most vexing problems of humanity—how to keep food production in pace with the growth of the world's population." With this flourish Lilienthal ended his speech. He claimed, "Trained as are no other group of men in the discipline of understanding and working with and through natural forces, endowed by the very nature of your calling with both persistence and patience, you American farmers are uniquely qualified to play a leading part in realizing the beneficial possibilities of this new force." Thus farmers, using atomic agriculture, would play a pivotal part in US foreign aid plans in the future as the United States reconceived its world role as helping poor, underdeveloped, and hungry countries become modern, fed, and prosperous nations.[12]

Beyond rousing speeches by its leaders, the AEC's technological optimism about the role of nuclear energy in agriculture also showed in its reports to

Congress. The January 1949 report showed that the commission had an emphasis on both using radioactive tracer atoms to follow life processes and also on studying how living creatures absorb radiation. That report asked, "Does Radiation Stimulate Plant Growth?" and demonstrated an AEC commitment to harnessing the atom on a very blunt force level to improve agriculture. Even though Lilienthal warned farmers in 1947 that radiation would not be useful as fertilizer, during the 1948 growing season, the AEC supported experiments in fourteen states on nineteen different crops to see if radiation could be used to boost plant growth. Unsurprisingly, the experiments were not successful, but nevertheless the AEC planned more for 1949. The report clearly stated, however, that such experiments were "quite separate and distinct" from others using radioactive isotopes to better understand plant growth—experiments on "the rate and volume of movement of various fertilizer materials in the soil, their absorption into the plants, and their accumulation in plant parts." The commission expected such studies would "solve practical problems of fertilizer application which are of direct dollars-and-cents interest to farmers, fertilizer producers, and farm machinery manufacturers." Even if radiation did not work as a fertilizer itself, research using radioactive isotopes could make existing fertilizers work better and unequivocally save farmers, and through them the rest of the nation, money.[13]

The July 1949 report further explained the AEC's research plans regarding agriculture and portrayed improving agriculture as one of the commission's goals. A section on "Radiation and Life," described all of the ways that humans had learned about radiation, peaceful and violent, helpful and harmful. The report explained, "Radiation attacks, disrupts, and destroys the delicate electro-chemical balance in the atoms, molecules, and protein combinations within the bodies of living things. As a result, it damages and kills the cells of which atoms and molecules are a part. If enough cells are destroyed, the whole organism—plant, animal, man—is severely injured or dies." In spite of this statement, though, the AEC continued its program on radioactive fertilizers. It is unclear where the logical disconnect occurred. Clearly knowledge existed that radiation harmed living things, but somehow this fact did not manifest itself into the cognizance that radiation might not be successful as a fertilizer. Researchers tested the same crops as in 1948 and still found no beneficial effects. The AEC also studied cattle exposed to radioactive fallout dust, looked at how fertilizers feed into plants, and ran many other smaller programs on photosynthesis, mineral nutrition, and improving fungicides and herbicides.[14]

Despite knowledge of radiation's dangers, and even though Lilienthal had stated publicly that hopes for radioactive fertilizers were pointless, such research continued, demonstrating a deep commitment to atomic agriculture and a desire to prove that radiation could be beneficial to life forms. In 1950 the commission reported that too much radiation could slow tomato plant growth. Its studies found that if tomatoes received twenty thousand roentgens total at a rate of one hundred fifty an hour, the plants would suffer ill effects. The next AEC report clarified the seemingly commonsense (even then) position that radiation would hurt plants: "Experiments gave no indication that radiation could improve growth rate or yield, but in large doses caused marked damage to both." Fortunately for taxpayers, not all AEC-supported research proved so fruitless.[15]

Use of radioisotope tracers continued to form a crucial component of the commission's research and helped it show how radiation could be beneficial. For example, research delved into how cattle interacted with their environment, particularly how the ruminants broke down feed and converted that to milk. Other investigations used radioiodine to study plant growth regulators and also looked into mealybugs and their effect on pineapple plants, using radioisotopes to study the salivary secretions of the pests. Research even tested radioactive weed killers to determine how plants interacted with the chemicals. Further studies used radioisotopes to look at how plants absorb nutrients into their roots, transport them throughout the plants themselves, and then deposit those nutrients in the various plant structures. Radioisotope research proved diverse and robust, and the AEC continued its research programs in 1951. That year agriculture and animal husbandry research advanced especially on the subjects of the metabolism of cows, fertilizers, and plant nutrition.[16]

The January 1952 AEC report to Congress contained the largest section yet on the atom and agriculture, with dozens of pages under the heading, "Atomic Energy and Its Applications in Plant Science" and helped explain the AEC's research program and its goals. Important for understanding the commission's motivations, the report claimed that there were two broad objectives in supporting research in plant science, one related to radiation safety and the other to directly improve agriculture as an industry. The first meant determining "the effects of radiation and radioactive products upon plants in order to broaden scientific understanding and to aid manufacturers and users of atomic energy in adopting measures to safeguard life and property." In short, the AEC wanted to help protect "crops and other property" from the damages radiation

might present, as research "is necessary to cope with circumstances that may follow atomic explosions." Focused on protecting the United States during an atomic bomb attack, knowing how plants and animals reacted to radiation exposure would be vital to the nation's long-term survival. The second reason for studying the atom and agriculture was to "help in the application of atomic energy products and techniques to fundamental and applied research with plants" for the benefit of the United States' people and industries.[17]

The first research listed in that January 1952 AEC report focused on "Intense Radiation and Plant Development" and provided an endpoint to previous research. Different from past investigations, though, the AEC did not present this inquiry as any sort of fertilizer program. Instead, pertaining to objective one of the plant science research program, the research focused only on how radiation affected plant growth so that the commission would know how plants might react after a nuclear blast. In general, the experiment produced mixed results. For example, on tested potatoes, some grew malformed, but others failed to sprout at all. Interestingly, these latter potatoes did not rot in the ground—irradiating the potatoes seemed to preserve them. This information would be important in the future. Fungi tended to handle radiation better than plants, so using radiation as a fungus control seemed impractical—dosing the undesired fungus with enough radiation to kill it would do more harm to the plants to be protected than to the attacking fungus.[18]

Finally clearing up previous investigations into radiation being used as a fertilizer, the January 1952 report stated, "Claims that radioactive fertilizers would increase crop yields have been discredited by repeated tests." The USDA had claimed back in 1914 that radioactive fertilizers did not work. Even with such a judgment, agricultural scientists considered radioactive fertilizers anew after the Hiroshima and Nagasaki bombings because observers claimed in the aftermath there had been "greatly increased crop yields" near the cities. In hindsight, though, it became clear that something else caused those bountiful harvests. In short, findings showed that if radiation had any effects on plant growth, those effects would be negative, either killing the plant or stopping it from growing (or never growing in the first place).[19]

At this point, the notion of radiation as a fertilizer seemed officially dead, but the fact that it held sway for as long as it did in research programs is important for what it says about the AEC. Whether it was for political reasons, merely to satisfy their own consciences, or due to blind technological optimism, the idea that splitting the atom could and should be used for more than

making war wove a common thread through both the Truman and Eisenhower presidencies. Agriculture represented an easy way to show the benign effects of nuclear research and demonstrating that radiation was not a wholly bad entity seemed important in promoting the peaceful uses of atomic energy. Radiation, especially at high levels, breaks down living tissues. Thus while it may be put toward useful ends (such as in x-rays or for use as a radioactive tracer), it does not benefit biological life. The US public knew that radiation represented a real threat to human health, especially after John Hersey's *New Yorker* articles (which ultimately became the book *Hiroshima*) described in vivid detail the devastation wrought by the first atomic blast in Japan. Clearly, then, research into ways that radiation, with no qualifications, might be a good and useful thing would have been important for policymakers. If they could show that radiation had benefits or even could be healthy for some organisms in certain contexts, the moral position of creating radiation (such as in a nuclear blast) would change dramatically.[20]

On the other hand, the AEC chronicled radiation's harmful effects quite clearly, which reinforced its need to find peaceful and helpful aspects of atomic energy. Beyond its obvious effects on living tissues, radiation also seemed either to kill soil microorganisms, including those around plant roots that help fix nitrogen, or make these less effective. Also, the January 1952 report to Congress recognized strontium 90 (Sr^{90}), an isotope produced as fallout from nuclear explosions, as "potentially the most biologically hazardous of the fission products." In biological processes, Sr^{90} mimics calcium, and therefore, plants readily draw in the fallout product. Once Sr^{90} enters plant tissues it then bioaccumulates as it works up the food chain and eventually is passed onto humans, especially in cow's milk (the largest source of calcium in the human diet). In contrast to Sr^{90}, reactor-cooling water seemed safe, as even though it has some radioactivity, most of that is either short lived or diluted, even if some might be absorbed by plants or algae.[21]

Research into how fallout radiation might harm US agriculture also reflected a deep understanding of new geopolitical realities. In August 1949 the Soviet Union detonated its first nuclear weapon, meaning the United States suddenly had to contend with the possibility of another country unleashing an atomic blast upon it. With this new reality came a desire to know exactly how the nation might be affected. Thus experimentation into what might happen to US agriculture after nuclear attack—discerning how fallout and other radiation affected plants—became an even more important part of the US atomic

energy program.

Studies on genetics and radiation also spoke to the AEC's mission to better understand living beings through radiation. For example, inquiries found that corn exposed to less than five roentgens of radiation exhibited no appreciable effects, but exposure between five to fifty-five roentgens caused mutations proportional to the radiation dose. Mutations can occur naturally, and, although some are beneficial, an overwhelming majority end up being negative (at least from the perspective of the individual organism). If radiation in a controlled laboratory setting could speed up the rate at which mutations occurred, beneficial mutations could be created, discovered, and isolated much more quickly than if humans left nature to its own devices. Radioisotopes also helped make possible research into tree and crop diseases, insecticides, herbicides, and photosynthesis.[22]

As the Eisenhower presidency began, no great changes in agricultural research occurred from the AEC perspective, although a focus on peaceful uses of the atom increased. Developing atomic energy into a true industry formed an important part of Eisenhower's 1952 presidential campaign platform. Citing the need to both "improve the atomic arsenal" and continue "to probe the frontier of knowledge," soon-to-be President Eisenhower cautioned against being afraid of advancing nuclear technology and instead explained that policymakers needed to be prescient and support the development of this new technology, atomic energy, and all its beneficial advances. From this perspective, properly developing atomic energy certainly would create great developments in many fields, including agriculture.[23]

These campaign speeches made sense in the context of an Eisenhower administration that tried to base agriculture much more on free market ideals than had his Democrat predecessors. As one example of this emphasis, Eisenhower selected Ezra Taft Benson for secretary of agriculture, representing a conservative shift in policy. Benson was a well-known conservative who believed that agricultural problems of the 1950s stemmed from overproduction by farmers in previous decades. Benson's policies, especially cutting holdover price floors from the 1930s, combined with other modernizing impulses in US agriculture and led to over half of the country's 5.8 million farms failing. Edward and Frederick Schapsmeier claimed this happened due to "business failure, particularly among the small, inefficient operators." In hindsight, it is clear this occurred as part of a trend toward larger industrial farms and away from family farming. Decisions during the Eisenhower era represented notions

that agriculture should be considered a business, and atomic energy could improve agricultural technology.[24]

With the nuclear industry and the threat of nuclear war in mind, AEC-sponsored investigations continued into how plants dealt with radiation. Those experiments studied how plants grew in soil containing concentrations of "fission products" (such as Sr^{90}) equal to the maximum fallout observed at nuclear blast sites. Growing radishes, barley, oats, cowpeas, and ryegrass, researchers found that strontium was indeed the radioactive element most likely to be absorbed by plants, but this occurred at a lower rate in soils rich with calcium. When cattle ate plants that contained radioactive fallout, they absorbed 25–30 percent of ingested Sr^{90}, with about 25 percent of that reaching the bone. Researchers said this bone contamination would only be a hazard to humans if they ingested the bone splinters that might be present in the meat. Other experiments measured how radiation sickness affected animals and used radioisotopes as tracers to study how tropical crops absorbed potassium.[25]

In addition to previous research, intentionally induced plant mutations continued to function as another example of the usefulness of atomic energy to agriculture. Even though radiation from space, "the so-called cosmic rays," produce natural mutations, these do not occur very often, and breeders frequently wish they could speed up these mutations—radioactivity could help speed up this process. The Gamma Field, located on Long Island near Brookhaven, represented the best example of this. There, radioactive cobalt was lowered into the ground by remote control when needed, and then researchers planted crops in concentric circles around the cobalt. Researchers studied the resulting crops, and "occasionally" one of the resulting mutations from exposure to the radioactive cobalt proved beneficial. For example, experiments produced what appeared in 1954 to be one promising crop, "a mutant of oats," that "seems to have resistance to one of the most destructive diseases which attack this important crop." In conjunction with ideas that increased food production could be important to both national security and peace, atomic research helped transition agriculture from merely being how the nation fed itself into a way that the United States could support geopolitical stability and set itself up as the leader of that new world order. The combination of such research endeavors represented the idea that improving agriculture with the atom meant more than enhancing food production—it meant a policy decision about the security of the nation.[26]

The combination of technological optimism and boosterism of atomic en-

ergy in relation to agriculture coalesced in 1956 with the "Report of the Panel on the Impact of the Peaceful Uses of Atomic Energy to the Joint Committee on Atomic Energy." The panel devoted chapter five of its report entirely to agriculture and argued, "Peaceful uses of atomic energy in the field of agriculture are a significant addition to the many other modern methods of improving farm technology." Not only did atomic agriculture mean "increased productivity and lower costs for individual farmers," but the report also argued that improved agriculture gave the United States a "dramatic opportunity to lead underdeveloped, undernourished nations to higher living standards." Only by sharing food production techniques with impoverished nations, by cultivating the Green Revolution, could US planners safeguard the Third World from communist influence and keep those nations secure from destabilizing influences. Hence, atomic agriculture could play a significant role in defining the United States' place in the world.[27]

The panel held up plant breeding as a dramatic expression of how radiation could be a good thing for living beings just as the AEC had hoped earlier experiments into radiation fertilizers would. Scientists could use atomic energy "to speed the evolution process." This implied that radiation mutations were not unnatural, but instead merely helping nature work a little faster than it might on its own. Exposing plants, animals, or insects to radiation made it possible to create new varietals more quickly and replace natural selection with human choices. The report further explained that only a small percentage of the new "variations" would be good, and scientists still had to winnow these from the unhelpful ones so they could be "put to work on the farm." The report closed the section by boldly claiming, "At least on a laboratory scale, the day of the tailor-made plant seems close at hand."[28]

Other parts of the panel's report seem like science fiction, even in today's world. The report claimed that researchers could duplicate many of the steps involved in photosynthesis, meaning that a time was "within the realm of possibility" that humans would not depend on plants "to produce edible energy in the form of starches, sugars, fats and proteins," but this could instead be done chemically on a commercial scale. And if other boosterish claims were not so far-fetched, they still presumed a great deal. The report made claims about how atomic energy would help produce more food on fewer acres at a lower cost. Since a "principal fact of the American way of life is that it is based on abundance," creating even more abundance with food would only enhance the lives of the nation's citizenry, as surely low food prices would

stay low (that such production might hurt farmers went unmentioned). Again, the greatest problem the United States ran into with this line of reasoning was how to deal with all the agricultural surpluses that such research would surely help produce. Historian Shane Hamilton has interpreted these surpluses as aggressively undermining New Deal liberalism with free market solutions. Whether this is true or not, policymakers did believe that if excessive production meant consumer prosperity then even more food would lead to ever-lower prices on the shelves and improve lives of the nation's citizenry.[29]

This report also explicitly insisted this new knowledge and technology could help the United States feed the world, emphasizing a perception that the United States' role in the geopolitical realm had changed. It stated bluntly that the United States "can help the undernourished peoples of the world have more to eat" so long as more research, education, and work occurred, as there would be "no miracles" without these. The report finished with three recommendations: the United States needed to keep researching; those dealing with the farm surplus problem should take into account that atomic developments will exacerbate the problem; and an exploration of the humanitarian benefits that could result should begin immediately. The third point held particular importance, as "Only in this way can the United States bring to bear atomic contributions to agriculture, so as to demonstrate our historic sense of international humanitarian leadership." This particular sentiment likely proved particularly important as the United States sought to establish its place as a world leader in contrast to the Soviet Union. If the United States could help feed the world it would have a significant bargaining chip in the Cold War court of world public opinion. Thus agriculture, and by extension atomic agriculture, became fundamentally tied to a US global imperative.[30]

One new avenue of research pursued by the AEC in the late 1950s centered on irradiating seeds and crops to produce positive effects and continued the theme of searching for benefits of radiation. Just as earlier research had accidentally discovered with potatoes, irradiating, if done at proper levels, could significantly improve the storage of agricultural products. In contrast to early efforts at using radiation as a fertilizer, irradiating foods and seeds at precise levels did seem to have real benefits and at the end of the decade occupied much of the ink received by atomic agriculture. This is not to say older sorts of experiments (such as using radioisotope tracers to track how nutrients travel through plants' leaves, stems, and fruits) disappeared entirely, but irradiating plants became much more important. Previous experiments had used radiation

to create beneficial mutations, and the AEC reported, "beneficial mutations are being found in sufficiently high numbers to justify continuing efforts."[31]

It is no surprise that the AEC moved toward development of irradiated agriculture because such a program well fit its goals of benefitting agriculture and the nation by using atomic energy. On February 25, 1960 the commissioners of the AEC met and discussed the establishment of a radiation-processed food program. The Interdepartmental Committee on Radiation Preservation had proposed a conservative investigation into the potential of irradiated foods building upon a similar Army study from 1953. At that time, the Army had performed experiments on twenty-six types of food, particularly focused on unrefrigerated preservation for up to a year. It found that only certain meats—beef, pork, poultry, and ham—fit the desired specifications. Thus while atomic agriculture could serve the national security mission by feeding a hungry world, it also could enable the US military to conduct even longer troop deployments than previously.[32]

Though the Army program found some success, there had been no testing on civilian foods. The commissioners decided that civilian food should be tested. More than seeking to fill a hole in a research program, though, the AEC thought the food irradiation program fit its mission of finding peaceful applications of atomic energy and also made sense for the AEC to pursue because of its "unique knowledge and competence" concerning the involved technology. Eventually John McCone, the AEC chairman, declared that the program "held promise for revolutionary developments for the food industries of the world." The commission then approved $115,000 in their budget for research in fiscal year 1960, with $500,000 planned for the 1961 fiscal year. In the end, the Joint Committee wanted to push the program "because preservation of food by radiation was a dramatic program easily understood by the public." The commissioners agreed, and their only concern was how the program might appear to a public that had been promised rapid results—results that might be hard to deliver so quickly.[33]

After Eisenhower's term, significant research into the applications of atomic energy in agriculture continued, particularly by the United Nations' Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA). In many ways modeled after the United States' AEC, the IAEA developed after Eisenhower's 1953 "Atoms for Peace" speech and in 1964 even teamed up with the FAO to create a special FAO/IAEA Joint Division. Historian Jacob Darwin Hamblin chronicled this tale and showed a

confluence of modernizing principles, science, technology, international politics, and agriculture. In his estimation, the IAEA "succeeded in reshaping the UN toward a particular technological path of modernity," often at the expense of the FAO and the scant resources of developing countries, all the while brushing aside any significant critiques of its activities. As Hamblin described, the IAEA's "*raison d'être* [was] to promote a particular set of technologies"—promoting peaceful uses for nuclear technology—and not necessarily foster agricultural development. Hamblin said specifically, "To abandon food and agriculture would have been to undermine a crucial component of 'Atoms for Peace' that specifically targeted the developing world." Thus a story that began with research sponsored by the AEC in the mid-1940s had a continued history long after Eisenhower left office.[34]

In the end, using atomic energy and its products to improve agriculture showed several things about the United States. First and most obviously, it functioned as a way to improve the nation's agriculture and agricultural production, even though by the 1950s one of the most serious problems the nation's agriculturalists faced was how to deal with the incredible surpluses of food they already had created. Yet atomic energy helped scientists uncover new ways to farm and raise livestock, and this achievement proved important to policymakers. Even though helping the nation better produce greater amounts of food might have seemed inconsequential or even harmful, those in power repurposed overproduction as a way for the nation to feed a world that policymakers conceived of as being filled with hungry people in need of US aid (for both their own good and that of the United States). Particularly with radioisotope tracers that helped unlock many biological mysteries, US agriculture harnessed the atom quite successfully. And yet using atomic energy did more than nobly ensure that food production passed "from trial-and-error to certainty" as the Dagwood cartoon claimed.

Perhaps even more important than its obvious purpose of improving farming, atomic agriculture functioned as a way to show how splitting the atom could do more than unleash death and destruction. By emphasizing the nonviolent possibilities, programs that attempted to improve agriculture allowed policymakers to say to the public, with good reason, that they desired peaceful applications of nuclear energy. Clearly the first worldwide uses of atomic energy had been horrific. Showing that using atomic energy could be peaceful dramatically changed the AEC's mission and transformed the organization from death dealer to life bringer. In this way, research into agriculture using

atomic energy was just as useful to the AEC as it was to fields and farms.

Studying atomic agriculture also opens a window into the perceived place of agriculture in both the United States and the world at the time. Agricultural modernization with mechanization and chemicals found its logical next step in atomic agriculture, as the atom represented the newest technology that could be put to work for the good of farming. This let US farmers produce food more cheaply and efficiently, which meant that US citizens got more bang for their buck in grocery stores, all while supporting the rise of agribusiness. Internationally, anxieties about feeding the world (necessitating increased food supplies) also meant that the United States could manufacture a new place for itself as world food supplier and as a distributor of knowledge. Both of these facets of food production—at home and abroad—aided atomic agriculture in bolstering nuclear technologies and furthering their development, which created a sort of feedback loop between the atom and agriculture. Supporting atomic research thus meant furthering agricultural modernization and the Green Revolution, and frequently the inverse of that held true as well.

NOTES

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Joe Musial, *Learn How Dagwood Splits the Atom!* (New York: King Features Syndicate, 1949), 31.

2. US Atomic Energy Commission, *Sixth Semiannual Report of the Atomic Energy Commission: July 1949* (Washington, DC: GPO, 1949), 16.

3. Musial, *Learn,* introduction; Joel B. Hagen, *An Entangled Bank: The Origins of Ecosystem Ecology* (New Brunswick: Rutgers University Press, 1992), 100–101.

4. *Time* 50 (Aug. 4, 1947): cover image. In the Christian holy texts, the red horse rider of the Apocalypse represents war. Revelation 6:3–4.

5. Paul Boyer, *By the Bomb's Early Light: American Thought and Culture at the Dawn of the Atomic Age* (New York: Pantheon, 1985), xviii. For more on the US atomic program, see, for example, Ferenc M. Szasz, *The Day the Sun Rose Twice: The Story of the Trinity Site Nuclear Explosion, July 16, 1945* (Albuquerque: University of New Mexico Press, 1984); J. Samuel Walker, *Prompt and Utter Destruction: Truman and the Use of Atomic Bombs Against Japan* (Chapel Hill: University of North Carolina Press, 1997); Kai Bird and Martin J. Sherwin, *American Prometheus: The Triumph and Tragedy of J. Robert Oppenheimer* (New York: Alfred A. Knopf, 2005); Jenny Barker-Devine, "'Mightier Than Missiles': The Rhetoric of Civil Defense for Rural American Families, 1950–1970," *Agricultural History* 80 (Fall 2006): 415–35. "Material prepared for hearings by the Congressional Joint Committee on Atomic Energy," Feb.

1957, p. 23, official file, folder OF 108-F Atoms For Peace (5), box 453, White House Central Files, Dwight D. Eisenhower Presidential Library, Abilene, Kans. (hereafter DDEL).

6. Deborah Fitzgerald, *Every Farm a Factory: The Industrial Ideal in American Agriculture* (New Haven: Yale University Press, 2003), 4; Edmund Russell, *War and Nature: Fighting Humans and Insects with Chemicals from World War I to* Silent Spring (Cambridge: Cambridge University Press, 2001); J. L. Anderson, *Industrializing the Corn Belt: Agriculture, Technology, and Environment, 1945–1972* (DeKalb: Northern Illinois University Press, 2009), 5–12, 192; John Fraser Hart, *The Land That Feeds Us* (New York: W. W. Norton, 1991); Shane Hamilton, *Trucking Country: The Road to America's Wal-Mart Economy* (Princeton: Princeton University Press, 2008), 5.

7. Mike Davis, *Late Victorian Holocausts: El Niño Famines and the Making of the Third World* (New York: Verso, 2001), 6, 12; John Perkins, *Geopolitics and the Green Revolution: Wheat, Genes, and the Cold War* (New York: Oxford University Press, 1997), vi, 4; Nick Cullather, *The Hungry World: America's Cold War Battle Against Poverty in Asia* (Cambridge: Harvard University Press, 2010), ix, 7; Kristin L. Ahlberg, *Transplanting the Great Society: Lyndon Johnson and Food for Peace* (Columbia: University of Missouri Press, 2008), 4, 13–20; Angus Wright, *The Death of Ramón González: The Modern Agricultural Dilemma* (Austin: University of Texas Press, 1990), xi, xiv–xv.

8. Press Release "First Peacetime Application of Atomic Research Becomes Immediately Possible under Army Program," June 14, 1946, Press Releases [2 of 3], folder Atomic Bomb, box 174, President's Secretary's Files, Papers of Truman, Harry S. Truman Presidential Library, Independence, Mo. (hereafter HSTL).

9. "Atomic Energy and the American Farmer," Dec. 16, 1947, p. 1, folder Atomic Energy—Lilienthal, box 1, subject file 1945–1954, Papers of Clark Clifford, HSTL.

10. Ibid., 2, 5–6.

11. Ibid., 6, 9, 11, 12.

12. Ibid., 13–16; Cullather, *Hungry World,* 4.

13. US Atomic Energy Commission, *Fifth Semiannual Report of the Atomic Energy Commission: January 1949* (Washington, DC: GPO, 1949), 90–91.

14. *Sixth Semiannual Report,* 18, 21, 101, 104.

15. In general, the AEC tried to downplay any potential dangers of nuclear energy, especially radiation. See, for example, "Atomic Bomb, Survival Under Atomic Attack," Oct. 29, 1950, folder OF 692-A, box 1527, official file, Papers of Truman, HSTL. US Atomic Energy Commission, *Eighth Semiannual Report of the Atomic Energy Commission: July 1950* (Washington, DC: GPO, 1950), 172; US Atomic Energy Commission, *Ninth Semiannual Report of the Atomic Energy Commission: January 1951* (Washington, DC: GPO, 1951), 24.

16. *Ninth Semiannual Report,* 24–25, 28; US Atomic Energy Commission, *Tenth Semiannual Report of the Atomic Energy Commission: July 1951* (Washington, DC: GPO, 1951), 42–44.

17. US Atomic Energy Commission, *Eleventh Semiannual Report of the Atomic Energy Commission: January 1952* (Washington, DC: GPO, 1952), 69, 71, 75.

18. Ibid., 75, 81.

19. Ibid., 82–83.

20. John Hersey, *Hiroshima* (1946; repr., New York: Alfred A. Knopf, 1981), 46.

21. *Eleventh Semiannual Report,* 86–89, 92.

22. Ibid., 93, 95, 99, 101, 123–26.

23. "General Principles Regarding Atomic Energy Development," pp. 1–3, folder Atomic Energy, box 6, Campaign Series, Ann Whitman File, Dwight D. Eisenhower Papers as President, DDEL.

24. Edward L. Schapsmeier and Frederick H. Schapsmeier, "Eisenhower and Agricultural Reform: Ike's Farm Policy Legacy Appraised," *American Journal of Economics and Sociology* 51 (Apr. 1992): 153.

25. US Atomic Energy Commission, *Thirteenth Semiannual Report of the Atomic Energy Commission: January 1953* (Washington, DC: GPO, 1953), 120; US Atomic Energy Commission, *Fifteenth Semiannual Report of the Atomic Energy Commission: January 1954* (Washington, DC: GPO, 1954), 46.

26. "Atomic Energy and the Improvement of Agriculture," Jan. 12, 1954, pp. 6–7, folder 1, box 449, official file, White House Central Files, DDEL.

27. "Report of the Panel on the Impact of the Peaceful Uses of Atomic Energy to the Joint Committee on Atomic Energy," vol. 1, Jan. 1956, p. 61, folder Operation "Candor" (1), box 9, Administrative Series, Ann Whitman File, Dwight D. Eisenhower Papers as President, DDEL; Cullather, *Hungry World,* 3–4.

28. "Report of the Panel on the Impact of the Peaceful Uses of Atomic Energy," 64.

29. Ibid., 65–67; Hamilton, *Trucking Country,* 7.

30. "Report of the Panel on the Impact of the Peaceful Uses of Atomic Energy," 67–68.

31. US Atomic Energy Commission, *Twenty-second Semiannual Report of the Atomic Energy Commission: July 1957* (Washington, DC: GPO, 1957), 116–17; US Atomic Energy Commission, *Twenty-third Semiannual Report of the Atomic Energy Commission: January 1958* (Washington, DC: GPO, 1958), 64–67.

32. Meeting No. 1595, Feb. 25, 1960, pp. 152–53, Entry A1 19, Minutes of the Meetings of the AEC, box 13, RG 326, Records of the Atomic Energy Commission, National Archives and Records Administration II, College Park, Md. (hereafter NARA II).

33. McCone's sentiments were paraphrased in the notes, and therefore it is unlikely that the wording is a direct quotation of his. Ibid. Meeting No. 1603, Apr. 1, 1960, pp. 221–23, Entry A1 19, Minutes of the Meetings of the AEC, box 13, RG 326, Records of the Atomic Energy Commission, NARA II.

34. Jacob Hamblin, "Let There be Light and Bread: The United Nations, the Developing World, and Atomic Energy's Green Revolution," *History and Technology* 25:1 (2009): 27, 40, 42.

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Agronomic Mutations in Oats Induced by X-Ray Treatment[1]

Kenneth J. Frey[2]

THERE has developed in the United States, largely as a result of Swedish publications, a renewal of interest in "mutation breeding" in the cereal crops. From 1930, when Stadler (8) reported upon his experiments with irradiation of cereal grains, until 1950, there was a conspicuous absence of the use of induced mutations in plant breeding in the United States. Meanwhile, plant breeders in Sweden and Germany (1, 5) succeeded in inducing and isolating mutations with agronomic value from X-ray treated barley. Gustafsson (4, 5) published upon several induced beneficial agronomic mutations in barley including a stiff strawed strain called "erectoides," and two or three mutant lines which produced very high yields. The best of these yielded 10% more grain than the parental variety, Gull, and one line showed improved malting quality.

Shebeski and Lawrence (7) have reported a mutant barley strain from irradiated Montcalm variety which is equal to Montcalm in grain production and malting quality, but has shorter and stiffer straw. MacKey (6) obtained a number of the mutant strains from irradiated oats which were earlier and produced higher yields than the parental varieties. Similar results were obtained with wheat.

This paper is a more complete report of an earlier publication by Frey (2) in which beneficial mutations selected from irradiated oats were briefly described. The data presented herein are from only a few of the 61 mutant lines tested. The families of lines shown were selected to illustrate the various agronomic mutations obtained. Only the agronomic mutations will be discussed since a companion paper (3) will deal with the induction of disease resistance mutations in the same materials.

[1] Contribution from the Iowa Agr. Exp. Sta., Ames, Iowa. Journal paper No. J-2657. Project 1176. Rec. for publication Dec. 8, 1954.

[2] Associate Professor of Farm Crops; Agronomy Department, Iowa State College, Ames, Iowa.

METHODS AND MATERIALS

Four hundred primary seeds of Huron variety of oats containing 9.5% moisture were irradiated with 25,000 r units of X-ray and planted in the field in 1950. Mature X_1 plants were produced from 45% of the irradiated seeds. Each X_1 plant was harvested and threshed separately and in 1951 one row containing 25 spaced plants was planted from each X_1 progeny, resulting in approximately 4,500 X_2 plants which were observed for mutations. Because of the confounding influence of environment on the single plants, it was necessary to save all plants that deviated, even slightly, from the parental variety. The X_3 progenies were sown in plant rows in 1952 and 61 mutant strains which appeared to breed true were grown in yield tests at Ames, Iowa in 1953 and 1954. Plot size was 4 rows wide and 8 feet long with measurements being taken on the 2 center rows. Coefficients of variability for yield in these experiments were 5.0 and 3.5% respectively, in 1953 and 1954. In each year a rather severe epiphytotic of oat stem rust, predominantly race 7, developed resulting in a confounding of the yielding ability and stem rust reaction of the mutant strains.

EXPERIMENTAL RESULTS

The most common mutations found in the irradiated material were fatuoids and vine-type plants. The fatuoids were discarded in the X_2 generation because they were common

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MUTATION BREEDING OF CHRYSANTHEMUMS

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With 13 figures

Received 17 Jan. 1966

ABSTRACT

Rooted cuttings of the pot-grown Chrysanthemum variety "Hortensien Rose" were irradiated with X-rays, fast neutrons, thermal neutrons and electrons. As soon as the plants grew they were topped to stimulate side-shoot formation, often resulting in complete periclinal chimeras (sports). In addition mutation frequency in a given number of plants is enlarged by such a method.

Electrons proved to be ineffective, producing only 6–10% mutated plants. The optimum dose X-rays is 1500 Rads. Both fast and thermal neutrons showed a marked higher mutation frequency, the best dose resulting in both cases in c. 28% of mutated plants.

The mutation spectra, e.g. the type of mutations induced, showed some variation after the various treatments. But the number of plants irradiated, as well as the number of mutations induced, do not justify the conclusion that a certain treatment results in a specific mutation spectrum, although there was a tendency towards greater variability after neutron irradiation.

The mutation spectrum as well as the frequency greatly depends on the genetic constitution of the treated material. Irradiation of the pink-flowering "Hortensien Rose", with the maximum number of dominant genes for flower colour, resulted in a great number of different flower colours, as was expected, as well as a number of mutations of flower shape and size. Other pink-flowering varieties showed a similar spectrum, although in some cases the frequency was low or even zero. Chrysanthemum varieties with other flower colours showed a lower mutation rate, most of the flower colour mutations being based on a lower number of dominant genes.

Finally, the practical importance and ways of application were discussed.

INTRODUCTION

Mutations induced by radiation treatments are being increasingly applied by Dutch plant breeders, especially those dealing with asexually propagated ornamentals. Most of such plants are highly heterozygous, so that mutations, in most cases from dominant to recessive, can be detected in the irradiated material itself. Moreover the selection of ornamentals is very easy when such visible characters, as flower colour, form and size, or leaf form and growth habit are concerned. A mutated shoot can usually be readily propagated by means of cuttings, grafts or buds and the subsequent clones can finally be compared with each other and with existing cultivars. This may eventually lead to one or more new varieties.

Various authors have already pointed out these possibilities [4, 7, 10] and in Chrysanthemum a number of authors report flower-colour and other mutations [1, 6, 8, 11, 12, 13, 14].

The experiments described were carried out in 1963, 1964 and 1965 for the purpose

of investigating the possibilities of mutation breeding in Chrysanthemum and determining which irradiation and what treatment gives optimal results.

The irradiations were carried out with the Institute's radiation facilities, e.g. a 250/25 Philips deep therapy X-ray machine, a 1.5 MeV van de Graaff electron generator and the 100 kW swimming pool type reactor, the B.A.R.N. (Biological Agricultural Reactor Netherlands), which can be used as a fast or slow (thermal) neutron facility.

MATERIAL AND METHODS

Choice of material

The genetic constitution of the irradiated material determines the possibilities of mutation breeding. As stated earlier, a mutation practically always changes a dominant gene into a recessive one. Obviously, therefore, it is useless to irradiate a variety which is recessive for the characters we wish to mutate. This should be borne in mind when choosing the material. However, it is often unknown on what kind and what number of genes the characters in which we are interested are based. Spontaneous mutations may offer the only indication of the possibility of mutation induction.

Spontaneous mutations are common in Chrysanthemum cultivars and many of the existing ones are bud mutations (sports). As stated above, flower colour mutations can easily be induced by irradiation. We also know that the pink flower colour is based on the maximum number of dominant genes. This means that theoretically all other flower colours based on a lower number of dominant genes can be induced by mutation. Statistical analysis and experimental mutation induction has shown that the flower colour range in the hexaploid *Chrysanthemum indicum*-cultivars runs from pink, via white, bronze, red, purple, yellow, salmon, gold-orange, yellow-bronze, yellow with red and brown to greenish [9].

In our experiments therefore, we worked exclusively with pink-flowering cultivars of which few if any sports were known, e.g. "Asta Lee", "Breitner", and "Jacob Maris" and with the cultivars "Juweeltje" and "Dr. Wasscher" in co-operation with private breeders. Most of the results, however, were obtained with the pink-flowering "Hortensien Rose", a pot-grown variety that takes up comparatively little space and of which a number of flower colour sports might be a welcome addition to the fairly meagre range of pot chrysanthemums.

Irradiation facilities

The following types of radiation were applied:

1) X-rays from a Philips deep therapy X-ray machine, operating at 250 kV and 15 mA, without an additional filter. In most cases a dose-rate, of from 20 to 80 Rads per minute, was used. The total dosages applied ranged from 1500 to 2500 Rads.

2) Electrons from a 1.5 MeV van de Graaf electron generator, using a 85 mgr. absorber. The exposure time was 12 seconds, whereas the voltages were varied from 2.05 to 10.2mV in order to obtain the desired range of total dosages.

3) *Thermal neutrons* were administered in the thermal column of the BARN, the flux being 5.57 $N_{th}/cm^2sec.$ and the γ-contamination being 125–150 Rads/hour when operating at 100 kW. A range of different exposure times was employed in order to establish the optimum treatment.

4) *Fast neutrons* were administered in the irradiation room of the BARN, the flux being approximately $10^8 N_F/cm^2 sec$. and the γ-contamination approx. 120 Rads/hour when operating at 100 kW. We used 30 and 60 minutes at 20 kW, comparable to 20% and 40% respectively of the total dose given for 30 minutes at 100 kW. Higher doses were lethal.

In all cases rooted cuttings were exposed to radiation, the roots being protected whenever possible.

Post-irradiaton treatment of the material

Another decisive factor for optimal results is whether measures can be taken to increase the chance of a mutated cell participating as fully as possible in the formation of a shoot. A mutation is a one-cell event and the mutated cell, which is exposed to diplontic selection (competition between normal and mutated cells) has to grow into a group of cells, a sector and finally, via axillary buds to a complete shoot. Since the apex of most of our crops consists of a number of autonomous layers, the mutation is limited to the layer in which it originates. Hence the final result will nearly always be a periclinal chimera [2, 3, 5, 7].

As soon as the irradiated plants have started to grow they are therefore pruned to promote side-shoot formation. This also increases the mutation frequency. This year a plant was found with as many as four shoots each of a different flower colour.

Selection on flower colour, shape and size is done during the flowering period, interesting looking shoots being propagated by cuttings. As it is difficult to root such cuttings, which sometimes are woody, a plant hormone (2% indolylic acid) is applied to promote rooting. To prevent flower bud formation induced by the natural short-day conditions, it is advisable to grow both plants (as soon as they flower) and cuttings under long-day conditions. When this was done 80% of the cuttings produced normal plants. These can afterwards be propagated in the usual way, e.g. rooting of very young, soft shoots.

Results

Various radiations were compared to find out which one gives the best *mutation frequency* and or *mutation spectrum.*

X-rays showed a good dose relationship, as can be seen in Table 1.

Table 1. Mutation frequencies in "Hortensien Rose" after various X-ray dosages

Dose	No. of plants irradiated	Survival percentage	No. of mutated plants	Mutation frequency in %
1500 Rads	230	100	34	15
2000 Rads	421	100	85	22
2500 Rads	23	100	6	26
control	90	100	0	0

The mutation rate increases almost linearly with a increasing dose. In spite of the lower mutation rate the optimal dose proved to be 1500 Rads. Plant growth at the higher doses decreases so much that propagation of the barely developed (mutated) shoots becomes questionable.

COLOUR PICTURES

Number 1, 2, 4, 5, 8, 11 and 13 resulted from an X-ray treatment (1.5–2.5 Krads), 3, 7, 10 and 12 from a thermal neutron irradiation in the thermal column of the BARN and the no's 6 and 9 from a fast neutron treatment in the irradiation room of the BARN.

Figs. 1–6.

first row (top to bottom):

1. Chrysanthemum "Hortensien Rose" with a dark yellow sector
2. left control; right pale pink mutant
3. left control; right light orange mutant

second row:

4. left control; right (almost) white mutant
5. dark yellow mutant
6. left control; right brown-orange mutant

Figs. 7–12.

first row:

7. left control; right light orange-brown mutant with flat (non-tubular) petals
8. mutant with long, narrow, tubular petals
9. mutant with a greater number of gracefully curled petals

second row:

10. left control; right red mutant
11. dark pink mutant with graceful petals
12. left control; center half of the flower mutated towards pale pink; right compact flower (greater number of curled petals). Both mutations in one plant.

Fig. 13.

13. Red flowering sport of "Hortensien Rose".

Electrons turned out to be fairly ineffective; there was no clear dose-relationship, and the mutation frequencies were low, as can be seen in Table 2.

TABLE 2. MUTATION FREQUENCIES IN "HORTENSIEN ROSE" AFTER VARIOUS ELECTRON DOSAGES

Dose	No. of. plants irradiated	Survival percentage	No. of mutated plants	Mutation frequency in %
c. 500 Rads	50	94	4	8.5
c. 1000 Rads	50	96	5	10.5
c. 1500 Rads	50	98	3	6
c. 2000 Rads	50	90	3	6.5
c. 2500 Rads	50	100	0	0
control	50	100	0	0

With *fast neutrons* there is a marked increase in mutation frequency with increasing dose (see Table 3), the survival percentage for the dosages applied being 100%.

TABLE 3. MUTATION FREQUENCIES IN "HORTENSIEN ROSE" AFTER VARIOUS FAST NEUTRON DOSAGES

Dose (Integrated flux/cm²)	No. of plants irradiated	No. of mutated plants	Mutation frequency in %
$3.6 \times 10^{10} N_F$	50	3	6
$7.2 \times 10^{10} N_F$	50	9	18
$18 \times 10^{10} N_F$	50	14	28
control	50	0	0

The optimum dose seems to be 30 minutes in the irradiation room of the BARN, operating at 100 kW, giving $18 \times 10^{10} N_F/cm^2$ and in addition a γ-contamination of only c. 60 Rads total dose. The growth of the plants, compared to the control was satisfactory.

With *thermal neutrons* the dose response curve proved to be irregular (see Table 4).

TABLE 4. MUTATION FREQUENCIES IN "HORTENSIEN ROSE" AFTER VARIOUS THERMAL NEUTRON DOSAGES

Dose (Integrated flux/cm²)	γ-contamination (Total dose)	No. of plants irradiated	Survival percentage	No. of mutated plants	Mutation frequency in %
$1.6 \times 10^{12} N_{th}$	1 KR	50	94	4	8.5
$3.2 \times 10^{12} N_{th}$	2 KR	50	100	9	18
$4.8 \times 10^{12} N_{th}$	3 KR	50	100	10	20
$6.4 \times 10^{12} N_{th}$	4 KR	50	86	6	14
$8.0 \times 10^{12} N_{th}$	5 KR	100	90	21	23
$9.6 \times 10^{12} N_{th}$	6 KR	50	100	13	26
$11.2 \times 10^{12} N_{th}$	7 KR	50	90	14	31
$12.8 \times 10^{12} N_{th}$	8 KR	50	90	4	9
control	–	50	100	0	0

The optimum dose lies around $10 \times 10^{12} N_{th}/cm^2$ resulting in some 28% mutated plants. But the γ-contamination in the thermal column was considerable, namely 125–150 Rads per hour. From these data it is impossible to calculate the possible contri-

bution of the γ-rays since the dose-rate effect is not precisely known. Table 4 shows that there is a considerable dose-rate effect: after 8 Krads γ-rays (in addition to $12.8 \times 10^{12} N_{th}/cm^2$) during 64 hours 90% of the irradiated plants survive. An acute irradiation with 3–4 Krads X-rays is lethal. A dose-rate effect after (semi-) chronic irradiation has been reported in many plants; in Chrysanthemum it has been found by SHAPIRO and BROERTJES (unpublished) and reported by WEAVER [14].

When we compare the mutation frequencies of various kinds of radiation we see a striking difference between thermal and fast neutrons, both having a frequency of 28% after the optimum dose, as compared to 1500 Rads X-rays (15% mutations) whereas electrons come far behind with 6–10%.

The *mutation spectrum*, e.g. the types of mutations induced is important. This decides whether a certain mutation can be induced and if so, which radiation is optimum. The flower colour and flower form mutations are grouped in Table 5. For each type of radiation used the mutations found are expressed as a percentage of the total number of mutations per radiation type.

TABLE 5. FREQUENCY OF FLOWER COLOUR AND FLOWER FORM MUTATIONS IN "HORTENSIEN ROSE" EXPRESSED AS A PERCENTAGE OF THE TOTAL NUMBER PER RADIATION TYPE

	X 1963	X 1964	X 1965	N_F 1965	N_{th} 1965	E1 1965
paler or darker pink	41	44	20	31	25	21
bronze and yellow	32	42	26	20	27	36
brown-red-orange-bronze	15	2	39	34	30	43
(almost) white	–	–	1	–	–	–
smaller flower	–	2	–	–	1	–
larger flower (often with flat petals)	–	–	–	7	8	–
fewer petals	9	5	3	–	–	–
more petals (sometimes more graceful)	–	–	11	8	8	–
other mutations	3	3	–	–	1	–

It clearly shows that most of the mutations are found in the first three groups, the totals being 88, 88, 85, 85, 82 and 100% respectively. In addition a number of flower size and flower form mutations were found, spread over the various radiation types.

The data suggest a greater variability after neutron irradiation compared to X-rays. Although such a tendency has been found in other crops as well (ITAL: African violet) the total number of mutations found after each radiation type, however, was too small (see Table 1) for a definite statement in this matter.

It was also difficult to classify the flower coulours owing to the vast enormous colour variation which was found (see colour-pictures).

Other Chrysanthemum varieties have been irradiated, in most cases in co-operation with private plant breeders. In Table 6 the results of a number of these are listed; a plus sign indicates that a certain flower colour mutation has been found.

TABLE 6. INDUCED MUTATIONS IN A NUMBER OF CHRYSANTHEMUM VARIETIES

Variety	Original flower colour	pink	white	bronze	red	yellow	orange	brown
Asta Lee	pink	+		+		+		
Breitner	pink	+	+	+		+		
Dr. Wasscher	pink		+	+	+	+		
Jacob Maris	pink							
Juweeltje	pink	+		+	+	+		
Koens Elite	pink	+		+	+	+		
Silver Rose	pink				+			+
Diplomaat*	purple pink	+					+	
Tiptop*	purple	+	+		+			
Roland	salmon pink	+		+	+	+		
Wim Lange	salmon	+		+				+
Lilian Hoek	orange-bronze	+			+			+
Market Grower	id.				+			
Cotswold Flame	orange red				+	+		
Alec Bedger	creamy					+		
Yellow Spoon	yellow				+			

* Plus a number of similar tints

This table shows that irradiation of pink-flowering varieties results in a greater flower colour variability as compared to varieties with other colours. (An exception is "Jacob Maris" which did not mutate at all). Although this would appear logical it is not an absolute law as a colour mutation is sometimes found which is classified higher according to the JANK listing [9]. His list, however, is partly the result of statistical analysis and it is clear that a certain genetical constitution of a variety may differ from the average. There are more explanations for a different reaction, such as uncovering or rearrangement of periclinal chimeras, induction of dominant mutations, whereas chromosome aberrations also may result in unpredictable effects.

PRACTICAL IMPORTANCE

As has been shown, mutation breeding can be successfully applied to the Chrysanthemum. Actually, a number of Dutch plant breeders, in co-operation with ITAL, have been employing the method for some years, but more time is needed to obtain practical results.

For mutation breeding one should only use varieties which:

a. are "leaders", in a certain group of the range as regards health, quality, response to special growing methods (year-round culture, for instance) and the like;
b. preferably have a flower colour listed as high as possible;
c. are new and consequently with few if any sports.

A very refined method would be to induce flower colour sports of a new variety, even *before* this variety is put on the market. As soon as the variety turns out to be a very good one, the winner of the variety can progressively provide the market with

good (flower colour) sports, before they have been induced elsewhere by spontaneous mutation.

As mentioned above, mutation breeding in Chrysanthemum had not yet resulted in commercial varieties. But it will be a matter of only a few years before a number of private plant breeders will put some sports on the market, whereas a number of the artificial sports, induced in "Hortensien Rose" undoubtedly deserve serious consideration for marketing. The most promising ones are being propagated at the Research Station for Floriculture at Aalsmeer and will be compared with each other and with the existing range of pot-grown chrysanthemums (see colour-pictures).

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REFERENCES

1. BOWEN, H. J. e.a., The induction of sports in chrysanthemum by gamma irradiation. Rad. Bot. **1** (1962) 4: 297–305.
2. BROERTJES, C., Ioniserende stralen als hulpmiddel bij het onderzoek van periklinaal chimaeren. Genen en Phaenen **5** (1960) 5: 53–56.
3. ———, Chimaeren en mutatieveredeling. Meded. Dir. Tuinb. **24** (1960) 5: 291–295.
4. ———, Mutatieveredeling bij vegetatief voortgeplante gewassen. Meded. Dir. Tuinb. **26** (1963) 12: 736–743.
5. DOMMERGUES, P., La destinée de la cellule mutée: conséquences dans le cas des plantes à multiplication végétative et dans le cas des plantes à réproduction sexuée. Eucarpia, 3e Congrès. Paris 1962, pp. 115–143.
6. FUJII, T. and T. MABUCHI, Irradiation experiments with Chrysanthemum. Seiken Zihô **12** (1961): 40–44.
7. HESLOT, H., L'induction expérimentale de mutations chez les plantes florales. Acad. d'agriculture de France, Séance 16 Déc. 1964, pp. 1281–1308.
8. JAIN, H. K. e.a., Mutation studies in annual chrysanthemum. I. Radiation-induced variation in flower form. Ind. J. Genet. Plant Breed. 1961, **21**, p. 68–74.
9. JANK, H., Experimentelle Mutationsauslösung durch Röntgenstrahlen bei Chrysanthemum indicum. Züchter **27** (1957) 5: 223–231.
10. NYBOM, N., The use of induced mutations for the improvement of vegetatively propagated plants. Mutation and Plant Breeding, 1960, pp. 252–294.
11. RANA, R. S., A radiation induced useful mutant of annual chrysanthemum. Current Sci. **34** (1965) Jan. 20, p. 58.
12. SHCHERBAKOV, V., Chimères végétales. Tsvetovodstvo **3** (1965), p. 18–20.
13. SHIMOTSUMA, M., and N. SAKURAI, Irradiation experiments with chrysanthemums. Seiken Zihô **14** (1962), pp. 106–110.
14. WEAVER, G. M., The effect of cesium 137 gamma radiation on plant-growth and flower color of greenhouse Chrysanthemum varieties. Canad. J. Genet. Cytol. **5** (1963): 73–82.

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PRESENT ASPECTS OF INDUCED MUTATIONS IN PLANT BREEDING *

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ABSTRACT

The present status of the utilization of induced mutations in plant breeding is briefly reviewed. It is concluded that with induced mutations in principle successes can be expected similar to those with the conventional breeding methods. Owing to the relatively small yield of progressive mutations the efficiency of mutation breeding, however, is rather poor at present. Greater efficiency may be expected with increased knowledge of both control of original mutation production and of selection.

Control of mutation production seems to be possible in at least three ways, (a) by raising the total mutation frequency (b) by changing the relative frequency of chromosome versus point mutations and (c) by altering the spectrum of point mutations. The possibilities of such control through the diverse action of different radiations given with or without modifying agents is reviewed and various mutagenic chemicals are mentioned.

Control of mutation selection may be achieved in two ways, (a) by a better understanding of diplontic selection (intrasomatic selection) of mutated cells and (b) by developing appropriate screening methods. A working hypothesis concerning the diplontic selection is briefly outlined. The "one initial cell theory" means that the greatest efficiency of mutation production can be expected after radiation of primordia or young buds with only one or a few initial cells which will form the tissue of interest. It is hoped that by this the intercellular competition is restricted and a reduced elimination of mutated cells will result.

Procedures for selection of mutants, at least in barley, can already start with M_1-spikes. It was shown that completely fertile M_1-spikes possess the same frequency of point mutations (chlorophyll mutations) as those with disturbed fertility. Selection of fertile M_1-spikes should, therefore, eliminate to a large extent the undesirable chromosome mutations and in this way increase the efficiency of screening for progressive mutations. Maximum mutation frequencies of fertile M_1-spikes can, however, only be achieved if the tillering is reduced.

It is suggested that more emphasis be put on screening of small mutations, which may generally be expected to have a greater importance for practical purposes than drastic deviations. Usually screening of micro vital- mutations will be advisable in the M_3-generation. Indicator characters may be found through mass selection methods which by their pleiotropic gene action also effect properties eventually of breeding value.

*) (Lecture delivered at the organizational meeting of the section "Mutation and Polyploidy" of EUCARPIA in Lund and Svalöf, July 9–11, 1958).

1. General survey

The idea to induce mutations for practical breeding purposes is more than 30 years old. It is closely connected with the discovery in 1927 of the mutagenic action of X-rays by H. J. Muller (58) who himself considered the practical importance of induced mutations.

Historically, the pioneer work of Gustafsson and the Swedish research group deserves great credit,[1] though also in other countries the practical importance of induced mutations was early recognized, particularly in Germany (Stubbe, 80; Freisleben and Lein, 17) and in Russia (Delaunay, 8; Sapehin, 65). It may be mentioned that in the USA early scepticism (Stadler, 77) recently has given way to a more optimistic view on the part of many research workers. For several years in that country the significance of mutations for plant breeding has been under test on a large scale (Shapiro, 71, 72; Shapiro and Sagawa, 73; Osborne, 64).

Present situation

In recent years a number of reviews dealing with the induction of useful mutations have been published (Gustafsson and v. Wettstein, 37; Gaul, 21, 26; Mac Key, 57; Singleton, 74; Sparrow, 76; Konzak, 49; Smith, 75).

Most of these reviews are more or less complete, and there is therefore no need for another at this time. The progress made in the past 30 years is great and fascinating. However, we still seem to be at the very beginning of this mysterious field of research when we consider such questions as the nature of mutations and how they should be controlled and utilized.

Today there is little doubt that all the genes involved in the world collections of our cultivated plants can be reproduced by induced mutations. Particularly in *Drosophila* (Muller, 59), *Hordeum* and *Antirrhinum* evidence for such a conclusion has been accumulated. But also in all the other numerous organisms investigated there is no argument against the assumption that every spontaneous mutation can also be induced if the material is comprehensive enough and if the mutation is thoroughly searched for with appropriate methods. Among the characters of economic importance which have been repeatedly induced in cultivated plants are earliness, stiff straw, dense spikes, large kernels, higher protein and oil content, disease resistance, etc. It is important to note that even in highly productive varieties the kernel yield can be increased, as has been shown most extensively for barley by Hoffmann (39), Fröier (18) and Scholz (67), for wheat and oats by Mac Key (55) and for peanuts by Gregory (30). Moreover, apart from such gene mutations it is possible to rearrange the chromosome structure and by this to create new caryotypes.

Utilization of induced mutations therefore is a matter of fact in plant breeding today. Unknown, however, is the future extent and the relative importance of this method as compared with the conventional ones. Though according to our present knowledge it seems that in principle most of the progress made by the traditional breeding methods can also be gained through induced mutations, the question of the efficiency of what can be called mutation breeding is still open. Until now the frequency of progressive mutations obtained has been rather low, necessitating

[1]) see survey in "Mutation Research in Plants", Acta Agr. Scand. **4**, 3, pp. 359–642 (1954).

much labour and the expenditure of large sums of money for their detection. It is, however, only this low frequency of progressive mutations which we get with our present methods that makes the practicability of mutation breeding questionable, not the new tool itself.

Mutation frequency and micro vital-mutations

There exists an estimate of the frequency of progressive mutations in barley. In this crop we have the most experience concerning practical aspects of induced mutations. With suitable radiation dosages the frequency of mutants easy to detect is of the order of one or two per 100 M_2-plants [1]) (GAUL, 21). Among these mutants the frequency of those which are superior in yield has been estimated to be one or two per thousand (GUSTAFSSON, 34). It should however be emphasized that this estimate is based mainly on drastic mutations, the most common type selected in the past barley mutation work. One of the main intentions of the present paper is to direct attention towards small mutations, the screening of which is considered to be an important task of the future. The frequency of progressive micro-mutations may be expected to be higher than that of macro-mutations. It may be mentioned that from a genetical point of view a classification into these two groups of mutations is arbitrary. Its practical value is, however, obvious. Micro-mutations are difficult to detect in a single plant but easier in a group of plants. They often change the physiological behaviour of the plant without any pronounced morphological effect. The significance of small mutations ("Kleinmutationen") in the course of evolution was early recognized by BAUR (2) and is emphasized again in most of the modern conceptions (STEBBINS, 78). The problem of utilizing micro-mutations for practical purposes lies in the difficulty of detecting them. Questions concerning relevant screening methods will be considered at the end of this paper. Here it will only be emphasized that, assuming the efforts of selecting micro-mutations will be successful, at present we actually possess no adequate estimate of the frequency of induced progressive mutations. GREGORY (31) in his extensive work with peanuts takes more notice of small mutations than was previously done in any crop. He indicates that the frequency of mutants which are superior in yield may be of the order of 1 among 500–5,000 M_2-population plants, which is a remarkable difference from the estimate mentioned above.

Without doubt there is a need for increasing the yield of progressive mutations. This is one of the most important problems in the practical application of induced mutation. The question of whether or not mutation breeding will become more popular depends on its solution. The present status of this problem and prospects for the future will be discussed in the following parts of this paper. There is a demand for increasing the total frequency of induced mutations and/or a control of the different types, i.e. for intentionally raising the number of more desirable types obtained. This can be done by both a control of the original production of mutations and by selection methods. These two possibilities will be considered separately.

[1]) M_1, M_2 etc. refers as a non-specific term of mutagenic treatment to the first, second, etc. generation after seed treatment (cf. KONZAK, 48).

2. Control of mutation production

A control of the mutation process seems to be possible in at least three ways, (a) by increasing the total mutation frequency, (b) by changing the relative frequency of chromosome versus point mutations and (c) by altering the spectrum of point mutations.

Raising of mutation frequencies

The problem of increasing the total frequency of induced mutations is closely connected with the killing effect of radiations as well as of mutagenic chemicals. It has been well known for several years, however, that densely ionizing radiations, e.g. neutrons, produce appreciably less lethality than X-rays; the same percentage of surviving M_1-plants gives a higher mutation frequency with neutrons than with X-rays (Mac Key, 54; Ehrenberg and Nybom, 10; Caldecott, Beard and Gardner, 6). Therefore, at least for the production of chromosome mutations, neutrons are more efficient. The question of whether neutrons are also superior in the production of vital mutations needs further investigation (Ehrenberg and Nybom, 10; Nybom, 63).

Another possibility of increasing the mutation frequency per surviving M_1-plant is the application of chemical or physical treatments given in addition to radiation. These secondary factors may be present before, during and/or after the radiation. Some treatments are known in barley which have led to an increase of the surviving capacity of M_1-plants but after which the frequency of point mutations (chlorophyll mutations) has remained the same (Nilan, 61) or has even been enlarged (Gaul, 22). Recently it was shown e.g. that a sublethal heat treatment given just after X-raying increased the survival from 54% to 64%, and also increased the frequency of point mutations from 8% to 14%. A combined treatment of CO_2 and heat given in addition to X-rays increased the surviving capacity by nearly 50% without any marked effect on the frequency of point mutations (Gaul l.c.).

As concerns the efficiency of chemical mutagens in producing mutations, noteworthy progress has been made. Judging from previous experience in plants (particularly cereals), mutagenic chemicals generally have a pronounced toxic effect accompanying the purely genetic action. The relatively high killing effect prevents raising either the concentration or the duration of application of the mutagenic chemicals beyond certain threshold values. Consequently mutation frequencies often remain far behind those achieved by radiations. With chemical mutagenesis we are however even more at the very beginning than with radiation. Indeed, recently there are exciting indications that in barley, with ethylene oxide (Ehrenberg and Gustafsson, 12) and with ethyl sulfate (Heslot and Ferrary, 38), frequencies of chlorophyll mutations can be obtained which are quite comparable to those resulting from high X-ray dosages.

Chromosome versus point mutations

There are good reasons to assume that chromosome and point mutations originate independently though at about the same time (Gaul, 27). The relative frequency of both these mutation types can be altered either by means of physical or chemical treatments in addition to radiation or by the use of different mutagenic agents, particularly chemicals.

It has been shown repeatedly in barley in recent years that the relative frequency of chromosome mutations can be either decreased or increased as compared with point (chlorophyll) mutations through the use of appropriate agents in combination with X-rays (KAPLAN, 44; CALDECOTT and SMITH, 5; NILAN, 61; GAUL, 22). Furthermore, there are chemicals, like nebularin, which produce no M_1-sterility at all but give rise to point mutations. Also the reverse type of action has been detected; 8-ethoxycaffeine induces an obvious M_1-sterility without any increase of the rate of point mutations beyond the spontaneous level (EHRENBERG, GUSTAFSSON and v. WETTSTEIN, 11).

Since knowledge of treatments which increase the yield of either point or chromosome mutations has considerable practical bearing, future work in this direction is extremely important. Mostly the breeder will be interested in obtaining only point mutations. This holds particularly true for diploid species. In polyploids like wheat and oats the relative importance of chromosome versus point mutations is not yet fully understood. Because of the duplicated condition of many genes, polyploids seem to have greater tolerance against chromosome mutations. This leads apparently to higher mutation frequencies as compared with the related diploids, especially as more minor and delicate deviations are concerned (MAC KEY, 54, 55, 57). Deficiencies and gene inactivations need not necessarily be deleterious, since through them new gene interactions may be balanced or a permanent heterosis established (cf. GAUL, 21). Such an assumption seems not to be unreasonable since it has been found that for instance certain chlorophyll mutations which are lethal in the homozygous condition, surpass on the heterozygous level the yield of the non-mutated parent (GUSTAFSSON, NYBOM and v. WETTSTEIN, 36; GUSTAFSSON, 34, cf. also HOLM, 41).

High frequencies of chromosome mutations are extremely desirable in those modern breeding methods which combine species and genus hybridization with induced translocations. There, gene mutations are actually without interest. Where there is in distant hybrids no or little pairing between the chromosomes of interest, the transfer of the desired genes by induced translocations has indeed proved a worthy tool. With an elegant method SEARS (68) succeeded in transferring the leaf rust resistance from *Aegilops umbellulata* to common wheat. He started with X-raying prior to meiosis, aneuploids having the resistant *umbellulata*-chromosome arm as an iso-chromosome in addition to the 21 pairs of wheat. The pollen of the radiated plants was used for crossing with untreated normal wheat. Cytogenetic analysis led SEARS (l.c.) to the conclusion that the practically most interesting type recovered among the resistant F_1-plants with 42 chromosomes had an intercalary translocation from *Ae. umbellulata*. This substitution line was cytologically entirely regular and morphologically indistinguishable from the common wheat parent except for its resistance and slightly later maturity. Also the transfer of resistant genes from *Agropyron elongatum* (ELLIOTT, 13, 14) to *Triticum aestivum* by means of induced translocation has been successful, resulting in cytologically stable types with 21 pairs of chromosomes. Similar positive results were indicated when *Agropyron intermedium* was used as the source of resistance for common wheat (WIENHUES-OHLENDORF, personal communication). From a comparison of X-rays, thermal neutrons and radioactive phosphorus and sulphur, LARTER and ELLIOTT (50) inferred that neutrons were most efficient, since this radiation source yielded the most translocations on the basis of surviving M_1-plants.

Both chromosome and gene mutations are of interest in recurrent radiation pro-

grams of artificially induced autoploids. The procedure of "diploidization of autoploids" presumably involves something more than creation of structural differentiation of the two homologous chromosome complements involved, though this goal may be considered as the main part of the project. There is, besides, ample evidence that the amount of bivalent and multivalent formation in polyploids also is genically controlled (GAUL, 19, p. 535, 20). Our present knowledge concerning the actual physiological background of this gene control is insufficient, but the assumption seems to be not unrealistic that bivalent formation in polyploids is a result of a diverse balance between structural differentiation, asynaptic gene action and chromosomal interference (the action of the last factor was inferred by LINNERT, 52, 53). Moreover, it is well-known that there is not necessarily a correlation between the degree of multivalent formation and fertility in terms of seed setting (cf. MÜNTZING, 60). Despite abundant multivalents in natural polyploids there may be high fertility (cf. also LEVAN, 51), sometimes caused by a pronouncedly regular zig-zag orientation of the quadrivalents in the meiotic metaphase combined with a high degree of terminalization (e.g. v. BERG, 3). Nevertheless, in programs trying to change the physiological behaviour of an artificial autoploid into one similar to that of an old established natural polyploid, chromosomal reorganization may be considered as the main part, as pointed out above. That reduction in quadrivalent formation can be achieved by radiation is already indicated in tetraploid *Dactylis* (STEBBINS, 79). The structural differentiation can be induced on the tetraploid or on the diploid level, in the latter case followed by chromosome doubling (MAC KEY, 57). In either case it can be expected that additional hybridization, not only of selected plants of the same variety, but also plants of different varieties will accelerate the accumulation of structural and genic diversity. Results concerning most such projects seem to be still unpublished. To the knowledge of the author (cf. also MAC KEY, l.c.) they are running on the tetraploid level with red clover (JULÈN, personal communication), vetches (NORDENSKIOLD, 62), *Linum*, rye (HAGBERG, personal communication) and barley (MAC KEY, 57; HAGBERG, personal communication, GAUL, unpublished). The author's program with 4n barley is advanced to the fourth radiation cycle, which is growing this year in the nursery. On the diploid level experiments with barley are being conducted by CALDECOTT (personal communication) and SHEBESKI (personal communication).

Mutation spectrum

Returning to the question of controlling mutation production, evidence is accumulating that more than the relative frequency of chromosome versus point mutations can be changed. The possibility of altering the proportion of different point mutations and inducing by this a sort of "group mutability" was considered seriously by GUSTAFSSON (32, 33) in barley. Also in *Antirrhinum* there were early indications (KNAPP und KAPLAN, 47; KAPLAN, 43, see also KAPLAN, 42).

Recently in barley new evidence of different spectra of chlorophyll mutations has been obtained by a comparison of the effect of X-rays alone with the effect of certain chemical and physical treatments combined with X-rays (D'AMATO and GUSTAFSSON, 7 GUSTAFSSON and NYBOM, 35; GAUL, 22) and through a comparison of different radiation sources with mutagenic chemicals (MAC KEY, 56, EHRENBERG, GUSTAFSSON and v. WETTSTEIN, 11; HESLOT and FERRARY, 38). Colchicine treatment for instance, given

in addition to X-rays produced nearly three times as many *viridis* mutations as did X-rays alone, whereas the frequency of *albinas* remained about equal (GAUL, l.c.). Also the fraction of *erectoides* among the total of viable mutations was reported to be different with X-rays than with neutrons (EHRENBERG and NYBOM, 10; NYBOM, 63). Furthermore there is increasing evidence for selective mutability in microorganisms (see reviews of KAPLAN, 45, 46; DEMEREC, 9) and some evidence in *Drosophila* (FAHMY and FAHMY, 15). Most striking in barley are the cases of nebularin and β-propiolactone which produce no *albina* mutations but in the one case only *viridis* and in the other only *xantha* plus a small fraction of *viridis* mutations (EHRENBERG, GUSTAFSSON and v. WETTSTEIN, 11; HESLOT and FERRARY, 38).

Though for various reasons, not all the data published on this subject are entirely convincing, considering the total evidence there can be little doubt at present that group mutability is a real phenomenon. In higher plants our knowledge of experimentally directing the creation of mutation spectra is so far almost completely restricted to genes controlling various chlorophyll deficiencies in barley. There is, however, no argument against the assumption that similarly the spectrum of viable mutations is alterable. Future work along this line will be extremely important. The fact that appropriate mutation spectra for viable mutations have not yet been worked out is a result of the fact that until now too few mutations have been produced and/or screened.

3. CONTROL OF SELECTION

The aim of selection methods for induced mutations is quantitative and qualitative control of the final output. There are two ways of selection control which may be considered here. First the problem of "diplontic selection" (intrasomatic selection) will be discussed and then some ideas concerning screening methods of progressive mutations will be propounded.

Diplontic selection

Most of the applied work with mutations has been done with radiation of seeds or of buds of shoots. Both of these consist of many cells. After the mutagenic treatment there is, therefore, a competition between cells preserving their full vitality and those more or less damaged. This competition leads to drastic elimination of mutated cells (cf. GAUL, 23), which was formerly called intrasomatic or intra-individual selection (KAPLAN, 44). Recently, for various reasons the term diplontic selection of mutated cells was proposed (GAUL, 28). Between the treatment of seeds and the screening of mutations in M_2 there is not only the diplontic elimination of mutated cells but also the filter of haplontic and zygotic selection. Only the diplontic selection of the M_1-generation will be regarded in the following.

In plants propagated sexually the primary intention of controlled diplontic selection should be to obtain high mutation rates of the germ lines. In crops propagated vegetatively one is interested in getting high mutation frequencies of isolated shoots or other parts of the plants not necessarily including the germ line. Any plan to get insight into diplontic selection must start with the histologic structure as well as the histogenesis which is characteristic of the material treated. Unfortunately in plants our present knowledge of the developmental mechanics is limited.

In barley a working hypothesis concerning the course of diplontic selection was advanced recently (GAUL, 28). In it the ontogenetical data available from the literature are considered and it is in agreement with preliminary experimental results gathered by cytogenetic and genetic as well as statistical means.

There is evidence that the generative tissue of a single spike is generally derived from one or two cells of the embryo after radiation of seeds. Therefore, not infrequently individual spikes are chimeras. However, this situation presumably holds true, only for about the first five tillers. The axillary buds or primordia of these are already present in the dormant embryo. With strong tillering two or even more spikes may have a single embryo cell in common. This situation seems to be established fairly safely. Furthermore there is some evidence that the mutation frequency of the later formed tillers is smaller than that of about the first five.[1]) This refers to chromosome as well as to point mutations (chlorophyll mutations). The influence of tillering on the mutation frequency is shown in table 1. After X-raying, the seeds were divided into

TABLE 1. EFFECT OF CLOSENESS OF PLANTING OF M_1-PLANTS ON TILLERING, FERTILITY AND FREQUENCY OF CHLOROPHYLL MUTATIONS

Year and spacing	Number of M_1-plants	Number of spikes per plant	% fertility	Number of M_2-seeds sown	% mutants
1953					
Close	650	2.9	64.7	15,640	0.6
Wide	116	8.3	68.5	9,000	0.4
1954					
Close	319	6.2	62.0	25,096	0.9
Wide	320	8.1	64.5	34,481	0.7

two portions, the one part being planted with small space and the other with large, which led to considerable differences in tillering. From results of two years it may be inferred that there is a dilution-effect of mutations among the later formed tillers: the more tillers per plant the lower the frequency of chromosome mutations (in terms of M_1-sterility) and of point mutations (chlorophyll mutations). Thus the working hypothesis makes the assumption that intercellular competition takes place extensively only among the later formed tillers of the irradiated embryo. Consequently there is a pronounced loss of mutations. It is, however, assumed that with about the first five tillers the situation is entirely different. In agreement with the fact that about five primordia are detectable in the dormant embryo, it is supposed that the corpus initial cells which will form the generative tissue of the first five spikes are already differentiated. There are only one or a few (surviving) initial cells in the corpus of each axillary bud, at least after radiation with commonly used dosages. If these initials are somewhat disturbed through mutations there are generally no cells in the neighbourhood which compete with or replace those already more or less determined to form the generative tissues of the spikes. Consequently these axillary buds will carry on the genetic error. Mutated cells will be eliminated only if the damage is so heavy that it surpasses a certain thres-

[1]) The enumeration means the order in which the axillary buds were originally formed, independent of the fact that many of them are supposed to be killed. Thus, e.g. the fourth growing tiller can really be the seventh formed.

hold value, leading to death of the initial cell and, therefore, presumably also to death in most cases of the whole bud or primordia. In other words, since there is only a restricted competition among the initial cells forming the generative tissue of about the first five spikes, there is relatively little elimination of mutations. The real field of intercellular competition lies within the later formed tillers not yet represented by definite initial cells, and results there in a tremendous loss of mutations.

The bearing of this hypothesis was generally extended to all other plants (GAUL, 28). Its basic idea is that the highest mutation frequencies can be obtained through radiation of primordia or very young buds having only one or a few initial cells which will form the tissue of interest. On the other hand, pronounced elimination of mutations can be expected by selection of those shoots etc. which during the time of radiation were represented by large growing points with many initial cells and layers. Here a pronounced elimination of mutated cells takes place, whereas in the first case the intercellular competition is restricted. According to this "one initial cell theory" of high mutation frequencies as a result of diplontic selection, in practice the theoretical ideal may be approached either by removing all visible buds before irradiation or by selection after radiation of those shoots etc. which during the time of mutagenic treatment were represented by a simple histological structure of relatively few cells.

With this hypothesis also the remarkable results of BAUER (1) with *Ribes nigrum* can reasonably be explained. Many efforts had been made for a long time in several parts of the world to get induced mutations in fruit trees and shrubs. The fact that the results were poor can be explained by the great efficiency of the diplontic selection which eliminated all the originally induced mutations within the large growing points irradiated. BAUER (l.c.), however, in black currants, obtained the highest mutation frequencies – and they were surprisingly high – from shoots which regenerated at the base of the irradiated buds, after the primary shoot was cut back (root stock selection). These shoots are developed from primordia or young dormant buds which at the time of irradiation had, presumably, only a structure of relatively few cells. It may be that besides the limited competition of the respective initials, the "root pressure" forced these cells to form shoots even though they may sometimes have carried a mutation which reduced the vitality. ZWINTZSCHER (83), applying a similar method to pome and stone fruits, obtained encouraging results, too.

Thus raising the total mutation frequency by a certain control of the diplontic selection seems to be a realistic goal of the future, as is indicated by the results with barley and black currants mentioned above. The question how eventually to utilize the selection phenomena within the plant to get rid of detrimental mutations is, of course, completely open.

Selection of mutated plants

Selection of mutated plants can already start in the M_1-generation. It was recently suggested that if in barley one is interested in getting a high fraction of point mutations and a low frequency of chromosomal aberrations, one should select fertile M_1-spikes and grow only these in M_2 (GAUL, 21). This was proposed because the frequencies of point mutations (chlorophyll mutations) were found to be no smaller in the progenies of fertile M_1-spikes than in those with disturbed fertility (GAUL, 27). Table 2 shows the

relation of seed-setting in M_1-spikes and the M_2-mutation frequencies of their progenies. The M_1-spikes were put into six fertility classes and the mutation frequencies of each class determined in M_2. Table 2 presents the pooled results of two experiments carried out in two different years. Each experiment consists of a number of series with different dosages or treatments. The table is based on the fertilities and mutation frequencies of 26,587 M_1-spikes giving rise to 1,434 mutated spikes with 3,655 mutants. It is obvious that the frequency of point mutations is essentually independent of the sterility, which is mainly a consequence of chromosome aberrations. Even completely fertile spikes, which were grouped separately in table 2, have no smaller mutation rate. This situation is the same with low and heavy dosages, since each series was carefully analyzed separately preceding the pooling of the total material.

TABLE 2. FERTILITY CLASSES OF M_1-SPIKES AND THE RESPECTIVE FREQUENCIES OF CHLOROPHYLL MUTATIONS, EXPRESSED AS PERCENT OF MUTATED SEEDS (= MUTATED M_2-PLANTS) ON THE M_1-SPIKES.[1]) REDUCED TILLERING (4.6 SPIKES PER PLANT)

Fertility classes	0 – 20	– 40	– 60	– 80	– <100	100%
% mutated kernels	1.1	1.2	1.1	1.1	1.1	1.2

[1]) based on 26,587 total spikes, 1,434 mutated spikes, 3,655 mutants.

Through selection of fertile M_1-spikes, therefore, one can expect to eliminate many of the undesired chromosomal aberrations and by this to increase the efficiency of screening for progressive mutations.

The equal mutation frequency of fertile spikes, however, is only approached when the amount of tillering in the M_1-generation is reduced. This was intentionally done in the experiments of table 2 by sowing the radiated seeds extremely close together and late (photoperiodic influence). There is, however a series which was sown with abundant space for the sake of comparison. Whereas the other material had an average of 4.6 tillers per plant, this series had 8.3 and showed a decrease in mutation frequency with increasing M_1-fertility, as is shown in table 3. This high tillering series is the same as in the 1954 experiment of table 1, which had a lower total mutation frequency. Again, because of the dilution effect on mutations in later formed tillers, these spikes fell into the more fertile classes of table 3, having concomitantly fewer point mutations.

TABLE 3. FERTILITY CLASSES OF M_1-SPIKES AND THE RESPECTIVE FREQUENCIES OF CHLOROPHYLL MUTATIONS, EXPRESSED AS PERCENT OF MUTATED SEEDS (= MUTATED M_2-PLANTS) ON THE M_1-SPIKES[1]). INCREASED TILLERING (8.3 SPIKES PER PLANT).

Fertility classes	0 – 40	– 70	– <100	100%
% mutated kernels	1.0	0.8	0.6	0.4

[1]) based on 2,597 total spikes, 91 mutated spikes, 239 mutants.

The discrepancy of these investigations with those published previously by Swedish and German authors seems to be a consequence of both the intentional reduction of tillering and the use of a measuring method of the mutation frequency which is free of the statistical bias inherent in the method applied formerly (GAUL, 24, 25).

Regarding now questions of screening methods in M_2 and advanced generations, the

emphasis should be put again on micro vital-mutations, as was already indicated in the beginning of this paper. Induced small mutations in plants (*Antirrhinum*) were already described by STUBBE (81) in 1934. That physiological mutations of economic importance can also be induced and detected if appropriate screening methods are applied was early shown by FREISLEBEN and LEIN (16), who found a mildew-resistant mutant in barley. Since then selection of many diverse resistant mutations has been reported in various crops (for literature see reviews cited at the beginning of this paper). Though it appears now that not all of these resistant genes are really a consequence of mutations, being sometimes rather the result of outcrossing,[1]) considering the whole evidence there can be little doubt that resistance can be induced. How abundant resistant mutations are is, however, still an open question because there are considerable discrepancies with different investigators. Another indication for the assumption that physiological mutations are, as a whole, more frequent than drastic ones is given by the character of earliness. Because of the ease of detecting earliness mutations, they have been found frequently in all crops. There have been all gradations of earliness ranging from one day to several weeks. In wheat HOFFMANN (40) screened among progenies of morphologically unchanged M_2-plants for baking quality and found some "physiological mutants" which were distinctly superior to the mother strain. Recently most convincing evidence of the abundance of small mutations was presented by GREGORY (29, 30, 31) in his extensive and most careful investigations of X-rayed peanuts. After random selection of normal appearing M_2-plants he found in progeny tests in the M_3 a striking rise of the genetic variance of a quantitative character (yield), the increase being fourfold as compared with the controls.

If the hypothesis of the frequent occurrence of micro vital-mutations is correct, efforts will have to be made to develop mass selection methods for detecting them. Concerning characters as resistance or earliness such methods are already easily available. Another classical example where screening for a specific character was succesful, though with spontaneous mutations, was early given by v. SENGBUSCH (69, 70) who discovered by a simple chemical reaction sweet lupines in a tremendous material of bitter ones. Recently, in *Melilotus albus*, SCHEIBE and HÜLSMANN (66) screened successfully for plants with low content of cumarin after treatment with mutagenic chemicals. Mass selection methods should have to focus on more or less specific characters. It was however suggested recently (GAUL, 21) that the characters chosen for screening need not necessarily be themselves of breeding value. From overwhelming evidence, particularly in *Drosophila*, *Zea Mays*, *Antirrhinum* and *Hordeum*, it is clear that almost every mutation has a pronounced pleiotropic effect. It is only a matter of investigating thoroughly enough to detect it. Sometimes a drastic change in the environment where the plants grow makes it easier to detect the manifold effects of a single gene mutation, as was e.g. shown in *Antirrhinum* by BRÜCHER (4) and STUBBE (82). It was, therefore, suggested (GAUL l.c.) to attempt, as a first step, screening for "indicator mutations"

[1]) Owing to the pronounced male sterility in M_1, flowers often remain open for a longer period of time than normally, and are thus more subject to cross-pollination. Presumably appropriate isolation of the M_1-generation has not been made in all of the previous experiments, so that the mutagenic nature of selected plants of agronomic value reported may sometimes be doubtful. This question was subject to a serious discussion during the Golden Anniversary Meetings of the American Society of Agronomy, Division VII, November 18–22, 1957 (cf. also KONZAK, 48).

characterized by small morphological or physiological deviations. Then, as a second step, these mutants would have be to tested as to whether they also effect by their pleiotropic gene action characters of breeding value like yield etc. There is, at least in diploid plants, the hope that among these mutations the fraction of progressive ones is much higher than among either drastic deviations or a pure random sample. It can be expected that, following such a program, selection in M_3 will usually be more advisable than in M_2. Since in the M_3-generation not only single plants but a group of mutants is available having the same genotype, it seems easier to search for small mutations. This may be done either by purely morphological methods with plants grown normally in the field, using possibly ruler and scale, or by testing reactions of seeds or seedlings under various artificial and extremely changed laboratory conditions.

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SAMENVATTING

De aspecten van kunstmatige mutaties voor de plantenveredeling

Na een kort overzicht van de perspectieven voor toepassing van kunstmatige mutatie bij de plantenveredeling wordt nader ingegaan op enige problemen die thans de aandacht hebben. Voor het beheersen van de kunstmatige mutaties is het van veel belang de frequentie van mutatie te kunnen vergroten en te kunnen bevorderen dat relatief minder chromosoom- en meer gen-mutaties tot stand komen. Vooral van de micromutaties verwacht schrijver vooruitzichten voor de plantenveredeling. Het is echter thans nog zeer moeilijk daarop te selecteren.

REFERENCES

1. BAUER, R., The induction of vegetative mutations in *Ribes nigrum*. Hereditas **43** (1957): 323–337.
2. BAUR, E., Untersuchungen über das Wesen, die Entstehung und die Vererbung von Rassenunterschieden bei *Antirrhinum majus*. Bibliotheca Genetica **4** (1924): 1–170.
3. BERG, K. H. v., Autotetraploidie bei *Hordeum bulbosum* L. Züchter **8** (1936): 151–158.
4. BRÜCHER, H., Experimentelle Untersuchungen über den Selektionswert künstlich erzeugter Mutanten von *Antirrhinum majus*. Z. f. Botanik **39** (1943): 1–47.
5. CALDECOTT, R. S. and L. SMITH, The influence of heat treatments on the injury and cytogenetic effects of X-rays on barley. Genetics **37** (1952): 136–157.
6. CALDECOTT, R. S., B. H. BEARD and C. O. GARDNER, Cytogenetic effects of X-ray and thermal neutron irradiation on seeds of barley. Genetics **39** (1954): 240–259.
7. D'AMATO, F. and Å. GUSTAFSSON, Studies on the experimental control of the mutation process. Hereditas **34** (1948): 181–192.
8. DELAUNAY, L. N., Resultate eines dreijährigen Röntgenversuches mit Weizen. Züchter **3** (1931): 129–137.
9. DEMEREC, M , What is a gene? – Twenty years later. The Amer. Naturalist **89** (1955): 5–20.
10. EHRENBERG, L. and N. NYBOM, Ion density and biological effectiveness of radiations. Acta Agr. Scand. **4** (1954): 396–418.
11. EHRENBERG, L., Å. GUSTAFSSON and D. v. WETTSTEIN, Studies on the mutation process in plants-regularities and intentional control. "Conference on Chromosomes", W. E. J. Tjeenk Willink, Zwolle, the Netherlands, 1956: 131–159.

12. EHRENBERG, L. and Å. GUSTAFSSON, On the mutagenic action of ethylene oxide and diepoxybutane in barley. Hereditas **43** (1957): 595–602.
13. ELLIOTT, F. C., X-ray induced translocation of *Agropyron* stem rust resistance to common wheat. Journ. of Heredity **48** (1957): 77–81.
14. ELLIOTT, F. C., Induced translocations in wheat. Wheat Information Service **5** (1957): 4.
15. FAHMY, O. G. and MYRTLE J. FAHMY, Further evidence for differential effects of mutagens in *Drosophila melanogaster*. J. of Genetics **55** (1957): 280–287.
16. FREISLEBEN, R. und A. LEIN, Über die Auffindung einer mehltauresistenten Mutante nach Röntgenbestrahlung einer anfälligen reinen Linie von Sommergeste. Naturwiss. **30** (1942): 608.
17. FREISLEBEN, R. und A. LEIN, Möglichkeiten und praktische Durchführung der Mutationszüchtung. Kühn-Archiv **60** (1943): 211–225.
18. FRÖIER, K., Aspects of the agricultural value of certain barley X-ray mutations produced and tested at the Swedish Seed Association, Svalöf, and its branch stations. Acta Agr. Scand. **4** (1954): 515–542.
19. GAUL, H., Genomanalytische Untersuchungen bei *Triticum* × *Agropyrum intermedium* unter Berücksichtigung von *Secale cereale* × *A. intermedium*. Z. ind. Abst. Vererbgsl. **85** (1953): 505–546.
20. GAUL, H., Asynapsis und ihre Bedeutung für die Genomanalyse. Z. ind. Abst. Vererbgsl. **86**, (1954): 69–100.
21. GAUL, H., Stand der Mutationsforschung und ihre Bedeutung für die praktische Pflanzenzüchtung. Arbeiten der DLG **44** (1956): 54–71.
22. GAUL, H., Die Wirkung von Röntgenstrahlen in Verbindung mit CO_2, Colchicin und Hitze auf Gerste. Z. f. Pflanzenzücht. **38** (1957): 397–429.
23. GAUL, H., Zur Frage der ontogenetischen Elimination mutierter Zellen nach Röntgenbestrahlung von Samen. Naturwiss. **21** (1957): 566.
24. GAUL, H., Die verschiedenen Bezugssysteme der Mutationshäufigkeit bei Pflanzen, angewendet auf Dosis-Effektkurven. Z. f. Pflanzenzücht. **38** (1957): 63–76.
25. GAUL, H., Die Bestimmung der Frequenz von Punktmutationen bei Pflanzen. Z. f. Naturforschg. **12** (1957): 557–559.
26. GAUL, H., Entstehung von pflanzlichen Mutationen durch Bestrahlung. Z. Landw. Forschg. **11**. Sonderheft (1957): 110–116.
27. GAUL, H., Über die gegenseitige Unabhängigkeit der Chromosomen- und Punktmutationen. Z. f. Pflanzenzücht. **40** (1958): 151–188.
28. GAUL, H., Über die Chimärenbildung in Gerstenpflanzen nach Röntgenbestrahlungvon Samen. Flora **147** (1959): in the press.
29. GREGORY, W. C., X-ray breeding of peanuts (*Arachis hypogaea* L.). Agron. Journ. **47** (1955): 396–399.
30. GREGORY, W. C., Induction of useful mutations in the peanut. Brookhaven Symp. in Biol. **9** (1956): 177–190.
31. GREGORY, W. C., Progress in establishing the effectiveness of radiation in breeding peanuts. Proc. Ninth Oak Ridge Regional Symp. Jan, 14–16, (1957): 36–48.
32. GUSTAFSSON, Å., Studies on the genetic basis of chlorophyll formation and the mechanism of induced mutating. Hereditas **24** (1938): 33–93.
33. GUSTAFSSON, Å., The mutation system of the chlorophyll apparatus. Lunds Univ. Årsskrift. N.F. **36** (1940): 1–40.
34. GUSTAFSSON, Å., Mutations, viability, and population structure. Acta Agr. Scand. **4** (1954): 601–632.
35. GUSTAFSSON, Å. and N. NYBOM, Colchicine, X-rays and the mutation process. Hereditas **35** (1949): 280–284.
36. GUSTAFSSON, Å., N. NYBOM, and U. v. WETTSTEIN, Chlorophyll factors and heterosis in barley. Hereditas **36** (1950): 383–392.
37. GUSTAFSSON, Å. and D. v. WETTSTEIN, Mutationen und Mutationszüchtung. Handb. der Pflanzenzücht. **1** (1956): 612–699.
38. HESLOT, H. et R. FERRARY, Action génétique comparée des radiations et de quelques mutagènes sur l'orge. Ann. de l'Institut Nat. Agronom., Paris **44** (1958): 2–20.
39. HOFFMANN, W., Ergebnisse der Mutationszüchtung. Vorträge über Pflanzenzücht., Land- und Forstwirtschl. Forschungsrat e.V., Bonn (1951): 36–53.

40. HOFFMANN, W., Aussichten der Mutationszüchtung zur Verbesserung der Qualität des Weizens. Die Qualitätszüchtung von Brotgetreide. Bericht über die Tagung der Arbeitsgemeinschaft für Getreideforschung e.V., Detmold, 9.–10. 2. 1954, 46–54.
41. HOLM, G., Chlorophyll mutations in barley. Acta Agr. Scand. **4** (1954): 457–470.
42. KAPLAN, R. W., Über die Häufigkeiten phänotypisch abweichender Pflanzen in der F_1-Generation aus verschieden gequollenem und bestrahltem Pollen von *Antirrhinum majus*. Z. ind. Abst. Vererbgsl. 77 (1939): 568–579.
43. KAPLAN, R. W., Über die Häufigkeit von Faktormutationen durch Röntgenbestrahlung des Pollens von *Antirrhinum majus* in verschiedenen Quellungszuständen. Naturwiss. **33** (1946): 348–349.
44. KAPLAN, R. W., Chromosomen- und Faktormutationsraten in Gerstenkörnern bei verschiedenartigen Quellungsbehandlungen oder Kälte während oder nach der Röntgenbestrahlung sowie bei Dosisfraktionierung. Z. ind. Abst. Vererbgsl. **83** (1951): 347–382.
45. KAPLAN, R. W., Genetics of microorganisms. Ann. Rev. Micr. **6** (1952): 49–76.
46. KAPLAN, R. W., Gegenwärtige Probleme der Strahlengenetik. Ärztl. Mittlg. **41** (1956): 1–23.
47. KNAPP, E. und R. KAPLAN, Beeinflussung der Mutationsauslösung und anderer Wirkungen der Röntgenstrahlen bei *Antirrhinum majus* durch Veränderung des Quellungszustandes der zu bestrahlenden Samen. Z. ind. Abst. Vererbgsl. **80** (1942): 501–550.
48. KONZAK, C. F., Some procedural problems associated with mutation. Studies in crop plants. Work Conference on Radiation Induced Mutations. Biol. Dept. Brookhaven, Nat. Lab. Upton, L.I., New York (1956): 132–151.
49. KONZAK, C. F., Genetic effects of radiation on higher plants. Quarterl. Rev. Biol. **32** (1957): 27–45.
50. LARTER, E. N. and F. C. ELLIOTT, An evaluation of different ionizing radiations for possible use in the genetic transfer of bunt resistance from *Agropyron* to wheat. Canad. Journ. of Bot. **34** (1956): 817–823.
51. LEVAN, A., Aktuelle Probleme der Polyploidiezüchtung. Archiv der Julius Klaus-Stiftung für Vererbungsforschung, Sozialanthropologie und Rassenyhgiene **20** (1945): 142–152.
52. LINNERT, G., Untersuchungen über die Cytologie polyploider Pflanzen. I. Chromosoma **3** (1950): 328–356.
53. LINNERT G., Untersuchungen über die Cytologie polyploider Pflanzen. II. Chromosoma **3** (1950): 399–417.
54. MAC KEY, J., Neutron and X-ray experiments in wheat and a revision of the speltoid problem. Hereditas **40** (1954): 65–180.
55. MAC KEY, J., Mutation breeding in polyploid cereals. Acta Agr. Scand. **4** (1954): 549–557.
56. MAC KEY, J., The biological action of mustards on dormant seeds of barley and wheat. Acta Agr. Scand. **4** (1954): 419–429.
57. MAC KEY, J., Mutation breeding in Europe. Brookhaven Symp. in Biol. **9** (1956): 141–156.
58. MULLER, H. J., Artificial transmutation of the gene. Science **66** (1927): 84–87.
59. MULLER, H. J., On the relation between chromosome changes and gene mutations. Brookhaven Symp. in Biol. **8** (1955): 126–147.
60. MÜNTZING, A., Polyploidiezüchtung. Handb. der Pflanzenzücht. **1** (1957): 700–731.
61. NILAN, R. A., Relation of carbon dioxide, oxygen and low temperature to the injury and cytogenetic effects of X-rays in barley. Genetics **39** (1954): 943–953.
62. NORDENSKIÖLD, H., An investigation into two tetraploid strains of vetches (*Vicia sativa*) and their hybrid products. Kungl. Lantbrukshögskolans Ann. **19** (1953): 209–225.
63. NYBOM, N., On the differential action of mutagenic agents. Hereditas **42** (1956): 211–217.
64. OSBORNE, T. S., Past, present and potential uses of radiation in southern plant breeding. Proc. Ninth Oak Ridge Regional Symp., Jan. 14–16, (1957): 5–10.
65. SAPEHIN, A. A., X-ray mutants in soft wheat. Bull. Appl. Bot. Gen. and Plant Breed., Ser. II. **9** (1936): 3–37.
66. SCHEIBE, A. und G. HÜLSMANN, Mutationsauslösung beim Steinklee (*Melilotus albus*). Z. f. Pflanzenzücht. **39** (1958): 299–324.
67. SCHOLZ, F., Mutationsversuche an Kulturpflanzen. VII. Untersuchungen über den züchterischen Wert röntgeninduzierter Mutanten verschiedener Merkmalsgruppen bei Sommer- und Wintergerste. Z. f. Pflanzenzücht. **38** (1957): 181–220, 225–274.

68. SEARS, E. R., The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Symp. in Biol. **9** (1956): 1–22.
69. SENGBUSCH, R. v., Bitterstoffarme Lupinen. Züchter **2** (1930): 1–2.
70. SENGBUSCH, R. v., Süsslupinen und Öllupinen. Landw. Jahrb. **91** (1942): 723–880.
71. SHAPIRO S., The Brookhaven radiations mutation program. Atomic Energy Commission Report Number TID–7512, a Conference on Radioactive Isotopes in Agriculture (1956): 141–150.
72. SHAPIRO, S., The Brookhaven radiations mutation program. Work Conference on Radiation Induced Mutations. Biol. Dept. Brookhaven Nat. Lab. Upton, L.I. New York (1956): 1–21.
73. SHAPIRO, S. and Y. SAGAWA, The Brookhaven cooperative program for radiation mutation research. Proc. Ninth Oak Ridge Regional Symp., Jan. 14–16, (1957): 11–35.
74. SINGLETON, W. R., The use of radiation in plant breeding. Atomic Energy and Agriculture (1956): 183–194.
75. SMITH, H. H., Radiation in the production of useful mutations. Bot. Rev. **24** (1958): 1–24.
76. SPARROW, A. H., Cytological changes induced by ionizing radiations and their possible relation to the production of useful mutations in plants. Work Conference on Radiation Induced Mutations. Biol. Dept. Brookhaven Nat. Lab. Upton, L.I., New York (1956): 76–113.
77. STADLER, I . J., Some genetic effects of X-rays in plants. Journ. of Heredity **21** (1930): 2–19.
78. STEBBINS, G. L., Variation and evolution in plants. Columbia Univ. Press, New York. 1950: 1–643.
79. STEBBINS, G. L., Artificial polyploidy as a tool in plant breeding. Brookhaven Symp. in Biol. **9** (1956): 37–52.
80. STUBBE, H., Über die Möglichkeiten der experimentellen Erzeugung neuer Pflanzenrassen durch künstliche Auslösung von Mutationen. Züchter **1** (1929): 6–11.
81. STUBBE, H., Einige Kleinmutationen von *Antirrhinum majus* L. Züchter **6** (1934): 299–303.
82. STUBBE, H., Über den Selektionswert von Mutanten. Sitzungsberichte Dt. Akad. d. Wiss. Berlin. Kl. f. Landw. Wiss. **1** (1950): 42 pp.
83. ZWINTZSCHER, M., Die Auslösung von Mutationen als Methode der Obstzüchtung. I. Die Isolierung von Mutanten in Anlehnung an primäre Veränderungen. Züchter **25** (1955): 290–302.

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MUTATION BREEDING OF *ALSTROEMERIA*

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SUMMARY

Actively growing young rhizomes of various *Alstroemeria* cultivars, most sterile hybrids, were treated with X-rays. The optimum dose was about 400 rad for diploid cultivars and 500–600 rad for triploid ones.

Although the buds on the rhizomes most certainly have multicellular apices, no X-ray mutant showed any sign of chimerism. Hence only solid(-looking) mutants were obtained. This phenomenon, an unforeseen but advantageous circumstance, could not be explained.

Among the rather large number of mutants, several proved to be improvements and have been released to the trade such as cvs. Canaria Stagula, Yellow Tiger Stavero, White Wings Staretto, Harmony Stabroza and Rosita Stareza.

INTRODUCTION

The main advantage of mutation breeding is the ability to induce one or a few favourable mutations of an outstanding cultivar without altering the remaining genotype. Furthermore induction of mutations is the only means of introducing genetic variation in sterile cultivars or species of vegetatively propagated plants.

The possibilities of mutation breeding in vegetatively propagated species are favourable, in general, for various reasons, such as the usually large heterozygosity of the material which allows direct detection of mutations in the irradiated material. In vegetatively propagated ornamentals, in which the intention is often improvement in visible characteristics, selection of potentially useful mutations is generally easy (BROERTJES, 1968).

One of the main stumbling-blocks in vegetatively propagated species is chimera formation, after irradiation of multicellular apices, as well as 'diplontic selection' to which the mutated cell is exposed. They can be avoided and restricted by the adventitious bud technique (BROERTJES et al., 1968), which has already proved itself in various crops, such as *Streptocarpus* (BROERTJES, 1969), *Achimenes* (BROERTJES, 1972) and *Kalanchoë* (BROERTJES & LEFFRING, 1972).

This technique, however, cannot be applied in the case of *Alstroemeria* and the only available method seemed therefore to be the irradiation of actively growing rhizomes of young plants (Fig. 1). These steadily growing rhizomes carry tiny buds, in various stages of development, ultimately growing out into flowering shoots. The irradiation of shoots is useless since no method of propagation for them is yet available.

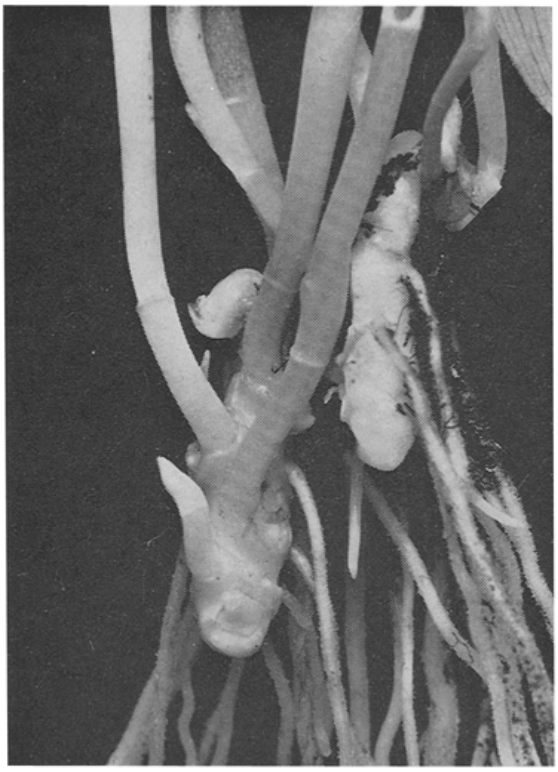

Fig. 1. Actively growing rhizomes of a young *Alstroemeria* plant with buds in various stages of development.

MATERIALS AND METHODS

The genus *Alstroemeria*, a herb with leafy stems, is a member of the monocotyledonous family Amaryllidaceae. It is named after a Swedish botanist, Baron Klas von Alstroemer, who brought the first seeds of *A. pelegrina* from South America to Europe. Most, if not all, species originate from S. America, mainly from central Chile and central Brazil, but also from Bolivia, Paraguay and Argentina (KOORNNEEF, 1972; VAN RAALTE, 1971).

The species *A. aurantiaca*, *A. ligtu* and *A. pelegrina* are probably progenitors of the modern hybrids, which produce larger flowers and are extremely lasting as a cut-flower (OOSTHOEK, 1967).

Many of the recent hybrids are developed by GOEMANS (1962) (Parigo Horticultural Co., Spalding, England). By crossing a sport of an unpublished species with a second species and subsequently with *A. aurantiaca* he obtained the so-called 'Parigo hybrids', such as the pink-flowered 'Ballerina', 'Pink Attraction' and 'Pink Perfection', the red-flowered 'Carmen', 'Marina' and 'Pimpernel', the yellow-flowered 'Sussex Gold' and the pinky-yellow flowered cultivar 'Parigo's Charm', to mention only a few. A somewhat different type, *Alstroemeria* cv. Orchid f1 (syn. cv. Walter Fleming), a white and yellow flowered cultivar, originated much earlier, probably as a spontaneous interspecific hybrid. The cultivars Beauty, Regina and others are recent products of van Staaveren's breeding programme.

The majority of these cultivars are sterile, probably either through interspecific hybrid sterility with the diploid chromosome number ($2n = 16$) (cvs. Beauty and

Plate 1. Rhizomes of *Alstroemeria* cv. Orchid fl.

Plate 2. *Alstroemeria* cv. Orchid fl (control).

Plate 3. Mutant of 'Orchid fl' (larger flowers).

Plate 4. *Alstroemeria* cv. White Wings.

Plate 5. *Alstroemeria* cv. Yellow Tiger.

Plate 6. *Alstroemeria* cv. Canaria.

Plate 7. Mutant of a mutant of 'Orchid fl' (more pronounced striping).

Plate 8. Mutant of 'Orchid fl' (pronounced striping).

Plate 9. Pink-flowered mutant of *Alstroemeria* cv. Starosa.

Plate 10. *Alstroemeria* cv. Regina (control).

Plate 11. *Alstroemeria* cv. Rosita.

Plate 12. *Alstroemeria* cv. Harmony.

Orchid f1) or because one of the parents (*A. aurantiaca*?) was an unknown spontaneous tetraploid, resulting in triploid sterile seedlings (most of the Parigo hybrids). Triploids such as cv. Regina are also reported after crossing diploids, most likely because one of the gametes had an unreduced chromosome number. (We have not checked the chromosome numbers but triploidy seems to be confirmed by (small) differences in radiosensitivity between the diploid and triploid categories.)

All these sterile hybrids are among the best of present-day *Alstroemeria* cultivars. They are attracting a fast increasing interest among cutflower growers because of their flower form, the increasing choice of flower colours, the favourable lasting quality and also because of the increasing knowledge of cropping methods (ANONYMOUS, 1969; VAN DORDT, 1969; LELIEVELD, 1972a, 1972b; VAN RAALTE, 1971, p. 48–51; VERBOOM, 1972).

The area on which *Altroemeria* is grown increases steadily by about 20% per year. In the Netherlands it amounted to 7 ha in 1972. The total annual value of production is now estimated at 2.5–3.0 million guilders and it is therefore not surprising that breeders are looking for methods of improving the modern assortment by, for instance, enlarging the range of flower colours of the best cultivars. These could be the cultivars with the highest quantity or quality of flowers, or types that react most favourably to the cropping methods practised or types that tend to be year-round.

In the Netherlands, the main flowering period is May. Over a rather short period, 50–70 flowering shoots per plant are cut. About six weeks earlier, from mid March – mid April, the rhizomes are actively growing, forming shoots and secondary rhizomes. Probably as the result of long day and higher temperatures the plant stops growing until August when regrowth starts and gives rise to a second crop in October-November.

To our knowledge nothing has been published about the genetics of *Alstroemeria*. Neither the number nor the nature of flower colour genes, for example, was known so that its suitability for mutation breeding had to be investigated by trial and error. The same was true for characters like flower size, form, plant height and number or quality of the flowers. Spontaneous mutations occur but seldom.

The only available method of vegetative propagation, at present, is to divide plants and to make use of the fairly large number of fast-growing rhizomes that continue to grow and regularly develop shoot apices which then become flower-bearing shoots.

Since a mutated cell has its largest chance when induced in the youngest possible developmental stage of a meristem or apex, the best material to be irradiated seemed to be the actively growing rhizomes of young plants. Our experience was that the best time for irradiation was during March-April. First selections could often be made in May or June and promising mutants were isolated by cutting them off the rhizome. A second selection was made during the next flowering period. If the irradiation was too heavy, the first selection could be made only in spring of the next year. Plants irradiated during reduced vegetative growth (May through August; November through February) generally die.

The irradiation was carried out with a Philips 250/25 deep therapy apparatus, usually operating at 250 kV and 15 mA, without an extra filter. The dose rate ranged from 50–150 rad/min. X-ray doses were determined with a Philips Universal Dosimeter connected to a hose-shaped intracavity ionization chamber, placed among the material at a representative position.

RESULTS

The first irradiations were intended to estimate the radiosensitivity of the material. The dose ranged from 50 rad to several kilorads. *Alstroemeria* cultivars and, more specifically, actively growing rhizomes proved to be very radiosensitive. For cv. Orchid f1 (syn. cv. Walter Fleming) and other diploid cultivars the optimum dose of X-rays lays between 350 and 400 rad. A good choice is either 400 rad for all material or, even better, a choice of three or four doses ranging from 350 to 500 rad. By this method, the risk of small fluctuations in radiosensitivity or inaccuracies in the dose was less. Cultivars reported as being triploids were less sensitive, as would be expected. Their optimum dose was between 500 and 700 rad.

A selection of cultivars was irradiated during about 10 years of experiments (Table 1), being a cooperative project between BV Handelskwekerij van Staaveren and the Association Euratom-ITAL. Similar projects have also been initiated with other

Table 1. Various data of mutation breeding experiments with *Alstroemeria* cultivars.

Cultivar irradiated	Optimum dose (rad)	Total number of plants irradiated[1]			Mutation frequency[2] (%)	Mutation spectrum (potentially favourable mutations)
		until 1969	1969 + 1970	1971 + 1972		
Various[3]	about 500	50	–	–		
Orchid f1 (syn. cv. Walter Fleming) (2n = 16)	400–450	530	500	90	±	flower: more white, more yellow, pinkish; increased striping; larger or smaller plant: heavier growth (generally less productive) quality: better (more 1st grade flower stalks
Regina (2n = 24)	500–600	160	–	–	+	flower: champagne to light pink colours plant: reduced height
Beauty (2n = 16)	about 400	–	90	90	– (so far	
Edison (2n = 16)	about 400	–	40	100	– (so far)	
Starosa (2n = 16)	about 400	–	–	90	±	flower: pink colour plant: better growth
Various numbers (seedlings) or mutants (2n = 16) (2n = 24)	300–600	50	–	650	from – to +	flower: better pink, heavier striping plant: better growth (sometimes less productive)

[1] The average number of rhizomes per plant was about 5. The number of apices per rhizome is also probably about 5.

[2] + more than 5; ± less than 1; – zero.

[3] Various preliminary small-scale experiments on method of propagation, material to be irradiated, and radiosensitivity were carried out between 1962 and 1964 with *A. aurantiaca*, *A. ligtu* and *A. pelegrina*.

private plant breeding firms, but, since these started later, no commercial results can yet be reported. Since such proiects are set up on a very practical basis, no extensive data are to be expected, such as precise number of mutants per dose or an exact description of the mutation spectrum. Only absolutely necessary observations were carried out and only those mutants were collected, multiplied and observed that were likely to succeed in the trade.

As can be seen from Table 1, most experience was obtained with cv. Orchid fl (syn. cv. Walter Fleming) which was also the first one to be irradiated on a relatively large scale. Although the mutation frequency was low (<1 %), a few hundred mutants were obtained, which demonstrated that all kinds of characteristic could be induced to mutate (last column of Table 1). Selections were made for flower colour, different degrees of striping of the inner petals (especially the two upper ones), flower size, but differences were also found in plant height, production and quality.

An advantageous circumstance was the fact that all mutants for directly visible characteristics seemed to be solid (non-chimeral) mutants. Whether they were true solid mutants or periclinal chimeras was not investigated. The former, however, seems more probable since various radiation-induced cultivars of *Alstroemeria* have been grown on a large scale without a single case of 'backsporting', (in other words spontaneous (partial) uncovering of a periclinal chimera). During all the years of mutation breeding only one sectorial chimera was observed.

This phenomenon of mutants being solid or resembling solid mutants could be explained on the basis of a unicellular top meristem of the rhizome. But this seems unlikely. Another explanation would be that the shoot apices on the rhizomes develop from one or a restricted number of epidermal cells similar to adventitious buds on petiole basis of detached leaves, which originate from one cell (BROERTJES et al., 1968).

Whatever the explanation, this advantageous circumstance facilitates (early) selection as well as further clonal propagation of a promising mutant.

So far, five mutants have been introduced to the trade, mainly from material irradiated before 1970. They are:

cv. Canaria Stagula: mutant of cv. Orchid fl; yellow outer petals (colour code RHS 8 A) and darker orange-yellow inner petals (RHS 17B and C);

cv. Yellow Tiger Stavero: mutant of cv. Orchid fl; darker yellow outer petals with more pronounced reddish-brown striping of inner petals;

cv. White Wings Staretto: mutant of cv. Orchid fl; white outer petals and white central inner petals;

cv. Harmony Stabroza; mutant of cv. Regina, the outer petals being mutated from RHS 62C to RHS 26C, with the inner petals more yellow;

cv. Rosita Stareza: mutant of cv. Regina. The outer petals being RHS 54C, the inner petals having a more pronounced reddish-brown striping. The growth in the length of cv. Rosita is about 20 % less than of cv. Regina.

Since 1970, many more rhizomes of various cultivars have been irradiated. They have produced a number of mutants, which are now being propagated and selected. Among the cultivars irradiated are also several mutants which, in turn, have produced mutants after irradiation. From cv. Canaria, for instance, a mutant has been obtained with more pronounced striping.

Also in cooperation with other plant breeding firms, hundreds of plants of several

different cultivars have been irradiated, in some cases already with first results. It is therefore to be expected that the number of commercial mutants will increase rapidly during the next 5–10 years.

CONCLUSION

Apart from the fact that mutation breeding is the only way of inducing variation in sterile cultivars of vegetatively propagated crops, as in many of the best *Alstroemeria* hybrids, it has been demonstrated that commercial mutants can be produced fairly easily and rapidly by this method. This is, to some extent, due to the circumstance that (almost) exclusively solid (looking) mutants are obtained when actively growing rhizomes of young plants are being irradiated. The heterozygosity, which was not expected to be large since the modern assortment derives from only a few ancestral species, turned out to be large enough to induce and obtain variability for a number of important (ornamental or commercial) characteristics. Thus, within a few years (bulk irradiation started in 1967), five commercial mutants and an even greater number of promising mutants were obtained. Many more are to be expected in the near future, as *Alstroemeria* becomes increasingly popular as a cut flower.

REFERENCES

ANONYMOUS, 1969. These new hybrids could be winners under glass. Commerc. Grower 3832: 877–878.

BROERTJES, C., 1968. Mutation breeding of vegetatively propagated crops. Proc. 5th Eucarpia Congr. (Milan, Italy): 139–165.

BROERTJES, C., 1969. Mutation breeding of *Streptocarpus*. Euphytica 18: 333–339.

BROERTJES, C., 1972. Mutation breeding of *Achimenes*. Eyphytica 21: 48–63.

BROERTJES, C., B. HACCIUS & S. WEIDLICH, 1968. Adventitious bud formation on isolated leaves and its significance for mutation breeding. Euphytica 17: 321–344.

BROERTJES, C. & L. LEFFRING, 1972. Mutation breeding of *Kalanchoë*. Euphytica 21: 415–423.

DORDT, H. W. VAN, 1969. Nieuwe *Alstroemeria*. Vakbl. Bloemisterij 24(23): 846–847.

GOEMANS, J. A. M., 1962. Breeding of *Alstroemeria*. J. R. hort. Soc. 87 (6): 282–284.

KOORNNEEF, M., 1972. Karyotype analyse bij *Alstroemeria*. Verslag van het ingenieurs onderzoek voor Plantensystematiek, p. 3–8.

LELIEVELD, H. P. J., 1972a. De teelt van *Alstroemeria* (1). Vakbl. Bloemist. 27 (27): 8–9.

LELIEVELD, H. P. J., 1972b. De teelt van *Alstroemeria* (2). Vakbl. Bloemist. 27 (28): 8–9.

OOSTHOEK, H., 1967. *Alstroemeria*-hybriden aantrekkelijk in meer dan één opzicht. Vakbl. Bloemist. 22: 1572–1573.

RAALTE, D. VAN, 1971. Handboek voor de bloemisterij, Deel 5: Snijbloemen, 4e druk, p. 48–51, Born, Assen.

VERBOOM, H., 1972. Het gebruik van kunstlicht bij de teelt van Vasa-*Alstroemeria*. Vakbl. Bloemist. 27 (44): 20–21.

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Selection of a mutant from adventitious shoots formed in X ray treated cherry leaves and differentiation of standard and mutant with RAPDs

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Key words: adventitious shoots, cherry, chimeras, mutation breeding, *Prunus cerasus*, RAPD marker, X ray

Summary

An obstacle when using scions or *in vitro* shoots for mutation induction is the occurrence of chimeras. When adventitious shoots are formed from irradiated material these usually are derived from single cells, this leading to homohistont mutants. Since *Prunus avium* regenerates adventitious shoots from leaves at a low rate only (Yang & Schmidt, 1992), leaves of the interspecific cherry rootstock '209/1' (*P. cerasus* × *P. canescens*) were irradiated. '209/1' regenerates adventitious shoots readily. Dosages applied were 5, 10, 20 and 40 Gy. Shoot production following 5 Gy irradiation was similar to the control. The application of 40 Gy resulted in strong damage with only few leaves regenerating. Among the adventitious shoots from leaves irradiated with 20 Gy one shoot was evident already *in vitro* with thicker and smaller leaves having a serrate margin. It was cloned as '209/1-20m'. The clone stayed stable since 1990 *in vitro*, in the greenhouse and the field. Compared with standard '209/1' the mutant is very dwarf.

Research to differentiate between standard '209/1' and '209/1-20m' was done using RAPDs. Among the decamer primer kits D, J, and T from Operon Technologies, Calif. Only primer OPJ05 (5'CTCCATGGGG3') differentiated between '209/1' and '209/1-20m'. Rootstock '209/1' showed one band of 2 kb additionally. This band is missing in the mutant.

Introduction

For mutation breeding the production of non-chimeric mutants is highly desirable. Unstable mericlinal chimeras automatically occur when plant parts with multi-cellular apices are irradiated. Moreover, the so called intrasomatic selection in a multi-cellular apex often prevents the appearance of mutated cells. Chimeras can be converted into stable solid mutants by repeated pruning. Vegetative propagation is often necessary.

The possibility to regenerate plants from single cells would automatically result in a high percentage of solid mutants. The adventitious bud technique has successfully been used for mutation breeding of vegetatively propagated horticultural crops (Broertjes & van Harten, 1988). This method involves irradiation of, and adventitious shoot regeneration from detached leaves. The basis for the method is the origin of adventitious shoots from single cells.

Since adventitious shoots could be regenerated from *in vitro* leaves of cherry rootstock '209/1' (Yang et al., 1991), this genotype was used as model plant to investigate the potential significance of the adventitious shoot technique for mutation breeding of woody fruit plants. At first attention was paid to assay the radiosensitivity of *in vitro* leaves, because such informations are prerequisites for a mutation induction experiment. An induced mutant with changed leaves can be identified during the stages of *in vitro* shoot regeneration. This mutant was investigated morphologically and by means of PCR techniques.

Table 1. Adventitious shoot regeneration from leaves of '209/1' in relation to irradiation dosages, n = 60

Dose (Gy)	% of leaves forming shoots	Number of adventitious shoots/leaf
0	52a	2,0a
5	55a	1,9a
10	32b	1,1b
20	28b	0,6b
40	2	0

Values having different letters are significantly different at $\alpha = 5\%$.

Material and methods

The cherry rootstock '209/1', an interspecific hybrid derived from the crossing between *Prunus cerasus* × *P. canescens*, was used in all experiments.

Adventitious shoot regeneration from detached leaves
All leaves used are derived from *in vitro* propagated shoots. The media and environmental conditions previously described (Yang, 1992) were used.

Irradiation procedure
Immediately after plating, the detached leaves were irradiated with doses between 5, 10, 20 and 40 Gy, using an X-ray machine. The technical data were: 12 mA, 150 kV, 1.7 mm Al-filter; dose rate at a distance of 55 cm from the focus to the petridishes: 0.9 Gy min^{-1}. For the measurement of the radiosensitivity of the *in vitro* leaves the mean number of adventitious shoots per explant and the percentages of leaves forming shoots were estimated.

Identification of the mutant with RAPDs
DNA was prepared from fresh leaves by using the isolation method described by Colosi and Schaal (1993). The samples were assayed in comparison with a standard after gel electrophoresis and contained approximately 20 ng/μl DNA. Two microliter DNA sample containing app. 40 ng of genomic DNA were used for the PCR. The PCR procedure described by Williams et al. (1990) was followed with minor modifications (Yang & Krueger, 1993). The decamer primers of Kits D, J and T from Operon Technologies, Calif. were screened.

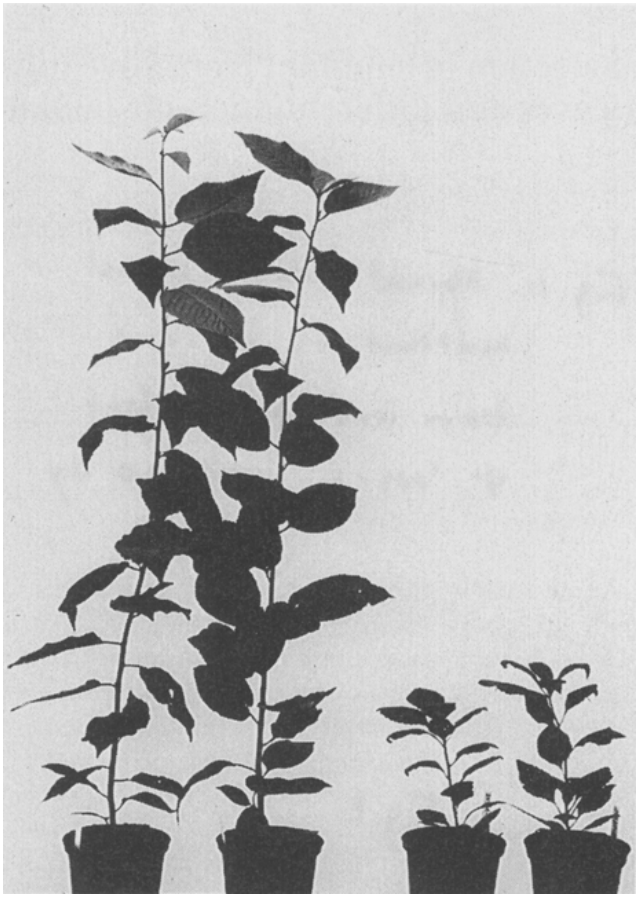

Fig. 1. Normal plant (l.) and mutant (r.) derived from irradiated leaf of '209/1' using 20 Gy.

Results and discussion

Radiosensitivity of in vitro *leaves*

X-ray treated leaves were cultivated under optimal conditions for adventitious shoot regeneration. Differences were observed four weeks after the irradiation with regard to the mean number of shoots per explant and the percentage of leaves forming shoots (Table 1). The treatment by 5 Gy does not show significant irradiation damages, the treated leaves produced as many shoots as does the control. LD50 was calculated as nearly 20 Gy. Since only one out of fifty leaves treated with 40 Gy formed shoots this dose is regarded too high for *in vitro* leaves of '209/1'.

Mutant selection and identification with RAPDs

Among the adventitious shoots from leaves irradiated with 20 Gy one shoot was evident already *in vitro*

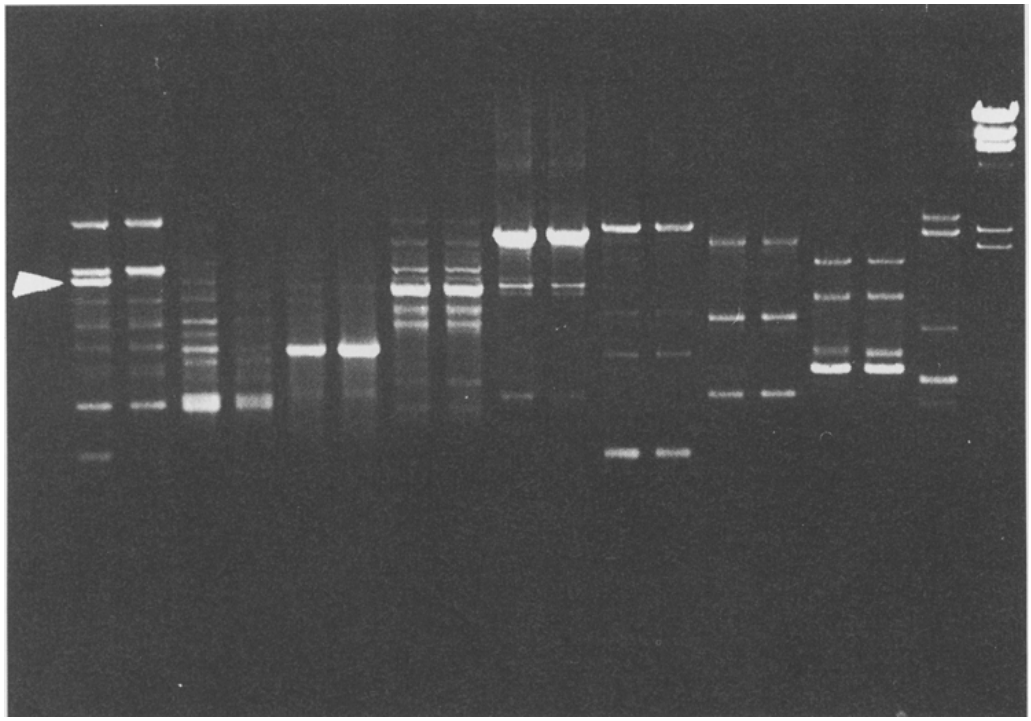

Fig. 2. Comparison of RAPD fragment patterns of '209/1' (l.) and its X ray mutant '209/1-20m' (r.), produced by different decamer primers, the lane 1 and 2 were amplified using primer OPJ05.

with thicker and smaller leaves having a serrate margin. Compared with standard '209/1' the mutant is very dwarf (Fig. 1). The mutant was cloned *in vitro*; several hundred propagated plants proved to be identical, indicating that '209/1-20m' is a homohistont mutant.

The screening of 60 decamer primers of Kits D, J and T resulted in the selection of primer OPJ05 which differentiated between '209/1' and '209/1-20m' as is shown in Fig. 2. The standard has one band of 2 kb additionally; this band is missing in the mutant, indicating that '209/1-20m' is a solid mutant. The band of two kb must be also present in the mutant when being a chimera.

A prerequisite for the induction of solid mutants using the adventitious bud technique is the single cell origin of shoots. Because regeneration from leaves is an unpredictable event, it was quite difficult to observe the patterns of shoot formation. The results presented in this paper give an indirect evidence for the origin of the adventitious shoot from single cells.

If it is possible to achieve a reasonably high number of leaves regenerating the adventitious bud technique could be of great potential value for mutation breeding of woody fruit plants as was shown in the herbacious plants (Broertjes et al., 1976; Broertjes, 1982).

References

Broertjes, C., 1982. Significance of *in vitro* adventitious bud techniques for mutation breeding of vegetatively propagated crops. In: IAEA, Induced Mutations in Vegetatively Propagated Plants II. Vienna.

Broertjes, C. & A.M. van Harten, 1988. Fruit Crops: In: C. Broertjes & A.M. van Harten (Eds) Applied mutation breeding for vegetatively propagated crops. Elsevier Scientific Publ. Co. Amsterdam, Oxford, New York, Tokyo.

Broertjes, C., S. Roest & G.S. Bokelmann, 1976. Mutation breeding in *Chrysanthemum morifolium* Ram. using *in vivo* and *in vitro* adventitious bud techniques. Euphytica 25(1): 11–19.

Colosi, J.C. & B.A. Schaal, 1993. Tissue grinding with ball bearing and vortex mixer for DNA extraction. Nucleic Acids Research 21(4): 1051–1052.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, A. Rafalsky & S.V. Tingey, 1990. DNA polymorphisms amplified by arbitary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531–6535.

Yang, H.Y., 1992. Untersuchungen zur *in vitro*-Regeneration, *in vitro*-Mutationsinduktion und Selektion bei Süßkirschen (*Prunus avium* L.), Ph. D. thesis, University of Hannover.

Yang, H.Y. & J. Krueger, 1993. Identification of a RAPD Marker linked to the *Vf* gene for scab resistance in apples, Plant Breeding, in press.

Yang, H.Y. & H. Schmidt, 1992. Untersuchungen zur Adventivsproß-regeneration *in vitro* bei Kirschen, II. Adventivsproßbildung an *in vitro*-Blättern verschiedener *Prunus avium*-Idiotypen. Gartenbauwiss. 57(1): 7–10.

Yang, H.Y., H. Schmidt & U. Kett, 1991. Untersuchungen zur Adventivsproßbildung *in vitro* bei Kirschen, I. Adventivsproßbildung an *in vitro* Blättern der Kirschunterlage 209/1. Gartenbauwiss. 56(5): 210–213.

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RADIATION IN THE PRODUCTION OF USEFUL MUTATIONS[1]

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INTRODUCTION

In contrast to the indirect use of radiation in tracer techniques or the direct cause and effect phenomena that characterize its use in sterlilization and food preservation, the induction by radiation and usage of beneficial genetic variants are in a different category of complexity. Here the event of an ionizing radiation causing a change in the fundamental hereditary material is only the beginning of a chain of circumstances. The change is first at a molecular level; it is then taken up by the self-reproducing chromosome units; there is recombination with the residual genotype; and the reaction of the genotype with the environment becomes involved. Then the influence of man is felt in selecting those genotypes which meet his particular needs and there is the multiplication of these individuals and populations which carry the desirable inherent qualities at the expense of others. Thus a whole series of events occurs, not predictable in terms of the physical or chemical properties of the initial response, so that artificial alteration of a single locus, in a single chromosome, in a single organism could conceivably result in such a divergent phenomenon as the growing of a new variety of plant throughout a large area.

It is commonly stated that the use of radiation in agriculture to improve crop plants represents a new departure from conventional methods. A more meaningful comparison than that of "mutation breeding" vs. "conventional breeding" may be made by considering the relation of both methods to experimental evolution—defined in this context as the experimental modification of gene frequencies in populations. There are four evolutionary forces that influence gene frequency. They are mutation, gene migration or the consequences of hybridization, selection, and chance. In conventional breeding methods the store of natural variability, either present in the sample population initially or introduced through hybridization, is subjected to recombination and selection, and the frequency of favorable combinations of genes is thereby increased and fixed. The initial variability is, however, provided only through spontaneous mutation. Spontaneous mutations occur at such low frequencies that it is not usually considered practical to await the appearance of desirable ones; so the conventional methods of plant or animal improvement stress selection and, where necessary,

hybridization in order to accomplish a favorable shift in gene frequency.

So-called "mutation breeding" differs in that there is initial emphasis on induction of desired hereditary changes, rather than on utilization of existing variability. After variability has been produced artifically, as with radiation, the techniques of selection and testing are no different from those employed in conventional methods.

It is the experience of genetics that when an organism is studied extensively enough genetically, as, e.g., maize and *Drosophila,* the conclusion appears justified that all parts and processes of the organism are under the control of genes. It is further the experience of genetics that all genes are subject to mutation. If these two statements are correct the inevitable conclusion is that all parts and processes of an organism are capable of being altered by mutation.

Although the naturally occurring or spontaneous mutations have in the past provided the basis for improvement of all cultivated plants and domesticated animals, we are not satisfied with the accomplishment. We are not only dissatisfied, but are also impatient. We seek to control more effectively and to speed up appreciably the tailoring of useful plants and animals to meet our needs. It may even be necesssary to speed up the controlled evolution of organisms vital to our existence in view of the rapid alterations that humans are causing in the fauna, flora and available habitats over a large part of the earth's surface. Consider, for example, the increasing menace from pathogenic organisms attacking crop plants when relatively uniform homozygous genotypes, as of wheat, are grown over large areas. This situation accounts in part for the strong interest in the induction or selection of disease-resistant mutants in the small grains in this country.

Once the fact is established that it is possible to produce beneficial or progressive mutations—i.e., that not all artificially induced mutations are detrimental—then the question of using the method in agriculture becomes largely one of economics (56). Is it economical to launch a program in plant improvement using radiation to induce mutations? To what particular circumstances is it applicable? Can the desired mutants be produced with sufficient frequency? Can techniques for recognizing and screening the improved forms be refined for practical use?

HISTORICAL RÉSUMÉ

The effects of ionizing radiations on plants have been studied since the early 1900's, but through the 1920's publications on this subject emphasized only morphological and physiological responses. It remained for Muller and Stadler to provide in 1927-28 the first definite evidence that the appearance of sudden heritable changes in plants and animals is greatly increased in frequency by ionizing radiations. That this method might be used to increase variability in crop plants was recognized early by a few geneticists and plant breeders in Sweden, Germany and Russia.

In 1929 Nilsson-Ehle and Gustafsson (37) began experiments using irradiation to induce mutations in cultivated plants. In 1934-35 the first promising mutants in barley—dense-eared, stiff-strawed types called *erectoides*—were produced. In 1940 investigations were started at Svalöf according to a comprehensive program which included induction of X-ray mutations in a number of agricultural plants and also a study of the mechanism of induction.

In Russia, during the 1930's, studies were made on the potentialities of radiation as a tool in plant breeding. Delauney (15) and Sapehin (72) both produced mutations in wheat and published results indicating that the method has definite possibilities. Their studies might well have flowered into genuinely profitable contributions had they not been eclipsed by the chicanery of Lysenkoism.

In Germany the early work of Stubbe on mutations induced in plants by radiation was followed by investigations of a more applied nature (83). In 1942 Freisleben and Lein (23) reported positive results on the induction of resistance to mildew in barley, and by 1944 they had isolated 92 induced mutations in one variety of barley (24). These studies are being continued in Germany, useful mutations having been reported as induced in barley (4, 41), wheat (68), flax (42), soybeans (90) and black currant (5). In Austria, Hänsel and Zakovsky (39) found evidence that by radiation of barley, resistance to mildew may be produced as frequently as lethal albino chlorophyll variants.

The statement is often made that work on the induction of beneficial mutations was not undertaken in this country until after the potentialities had been demonstrated in Europe. However, attention should be drawn to a paper by Horlacher and Killough (44) in 1933 entitled "Progressive Mutations Induced in *Gos-*

sypium hirsutum by Radiations." They say—"Mutations which are progressive have been produced in cotton by x-ray treatment of dry seeds. These mutations consist of:

(1) A mutation from forked leaf shape to normal leaf shape.

(2) A mutation from virescent yellow leaf and plant color to normal green leaf and plant color.

The mutation rate in each case was less than one percent".

Evidence was also presented for reversible mutations, that is, from normal leaf to forked, and from green to virescent yellow leaf. It is of interest to note that radiation of dry seeds was used, the method which today has been most productive of beneficial mutations.

It is true that there was little research or confidence in the potentialities of radiation-induced mutations for agriculture on the part of American plant breeders until the 1950's. In Canada in 1950 Shebeski began a program in induced mutation and, in 1954, he and Lawrence (74) reported the induction of beneficial mutations that conferred stem rust resistance and stiffer straw in barley. Concurrently, Konzak (50) at the Brookhaven National Laboratory and Frey (25) at Iowa State College published positive results on inducing stem rust resistance in oats.

In the past few years a number of workers in this country (76), in Europe (60) and in Asia (12, 45, 46, 49) have reported the induction of beneficial mutations in an increasing variety of materials. The recent interest has developed concomitantly with the expansion of atomic energy programs and the general availability of more sources of radiation.

TYPES OF RADIATION AND THEIR EFFECTS

Six kinds of radiation have been used most extensively to induce mutations. These are: X-rays, λ-ray, β-rays, fast neutrons, slow neutrons, and ultraviolet rays.

The first two, X-rays and λ-rays, are electromagnetic radiations of very short wavelength. Their energy may be absorbed by atoms in the tissue through which they pass, causing an ejection of planetary electrons and thus resulting in ionizations and consequent changes in chemical reactivity. Kinetic energy in the ejected electron produces further ionizations. The biological effects result from paths of ionizations, of relatively low or sparse ion density,

produced along the track of the ejected electron. Genetic alterations may be effected through direct change in the gene molecules or indirectly through other chemical changes in the cell.

Since the biological effects of X-rays and λ-rays are produced by the ejected high-speed electron, it follows that similar results can be obtained by direct bombardment with electrons, i.e., β-particles of comparable energies, but these do not penetrate tissue for more than a few mm. However, certain radioisotopes, such as P^{32}, emit β-particles during atomic disintegration, and can be administered so as to reach the region of actively dividing cells. In addition, since P^{32} is transmutated into sulfur (S^{32}) as it disintegrates, this may have additional disruptive effects, leading to mutation if the radioactive isotope has become incorporated in the genetic material.

Neutrons are electrically neutral particles and in a somewhat different category. Fast neutrons cause ionization indirectly by collision with nuclei of atoms, mostly hydrogen, in the tissue. Their biological effects are almost wholly due to the densely ionizing protons from hydrogen, whereas the effects of β-particles and X-rays are due primarily to sparsely ionizing electrons. Slow neutrons produce their effects as a result of radiations emitted following transmutation reactions that occur when they are captured by nuclei of elements in the tissues penetrated, or by later decay of radioisotopes produced. An important biological reaction is the transmutation of nitrogen in which a proton is emitted and dense ionization is caused in the neighborhood of the capture event.

The third distinctly different kind of radiation that has been used to induce mutations, namely, ultraviolet rays, primarily causes excitation and photochemical reactions through selective absorption by cellular constituents, mostly nucleic acids, in producing genetic effects. UV rays do not penetrate tissues appreciably, so that mutation studies with higher plants using this agent have been confined to analyzing the results of pollen treatments.

Differential biological effects of irradiation have been interpreted mainly in terms of different ion densities and of the relative importance of ionization vs. excitation in the reactions involved. Both gene mutations and chromosome breakage result from the dissipation of the initial radiant energy into ultimate chemical reactions.

INDUCED MUTATIONS VS. CHROMOSOMAL BREAKAGE AND SPONTANEOUS MUTATIONS

The question of whether ionizing radiations, or any other mutagens, are capable of inducing true "point" mutations or "intragenic" mutations, unaccompanied by breaks in and rearrangements of chromosomes, has been a subject of much debate and experimentation. Its eventual resolution depends on further understanding of the relation between the longitudinal cohesive forces holding the chromosome intact and the functioning of the ultimate units of heredity. A detailed discussion of investigations in this field is not within the scope of this paper.

In a recent discussion of the problem in relation to spontaneous mutation, Muller (61) has stated: "The best answer we have to this question lies in the mass of data obtained in X- and gamma ray experimentation on *Drosophila* which shows that, despite the production of clear-cut deficiencies and other structural changes, a very large proportion of the seeming point mutations, and especially of those induced in stages with extended chromosomes are in no known way distinguishable from the mutations that have arisen spontaneously. In fact, experience shows that every spontaneous mutant of *Drosophila* can, if thoroughly searched for, also be found after X-ray treatment." Giles (29) has shown for certain loci in *Neurospora* that changes occur in both directions: induced so-called forward mutations, from wild-type to mutant phenotype, can be induced to back-mutate to an essentially wild type.

It is a concept of considerable practical importance that the gene mutations caused by ionizing radiations can be found, if an intensive enough search is made, to be a counterpart of spontaneous mutations, i.e., those that account for the natural variability of the species. Actually, in application it matters little whether the mutation is a minute rearrangement on the intergenic level or a true intragenic change. Although the spectrum and frequency of mutants may not be exactly the same, artificial induction of mutations from a practical standpoint may be considered as essentially a rapid "passing in review" of the spontaneous mutations that have not been previously incorporated into the genotype at hand. In this sense the expectation is not so much that genuinely novel variants will be produced by radiations; rather it is that any

population of cultivated plant is conceived as only a selected sample of the potential inherent variability of the species and that this potential can be called forth by irradiation or exposure to some other agent of the mutagenic arsenal. A similar spectrum of variations might be picked up in nature if the species were widespread and particularly if it were cross-breeding. The possible inaccessibility of the desired variants and the expense of collecting and maintaining natural variations points up the fact that the use of radiation to produce mutations artificially is, to an important degree, one of economics.

FACTORS WHICH MODIFY GENETIC EFFECTS OF RADIATION

A prime objective in the genetic use of radiation in crop improvement is to be able to control the treatment so that the frequency of desired mutants can be increased and the frequency of undesirable effects reduced. Considering the rather basically different mode of action of the main sources of radiation, it is of interest that the kinds of genetic changes produced show no major differences. However, evidence has been reported that the frequencies and spectra of mutations may differ with the different types of radiations used. Compared to X-irradiation, thermal neutron irradiation is found to produce less response to environmental factors, more uniform growth of treated seedlings, a higher frequency of genetic change relative to the numbers of plants surviving treatment (10), and (with limited data) a significantly different distribution of *erectoides* relative to other viable mutations in barley (66). These differences have been interpreted in terms of treatment with sparsely, as contrasted to densely, ionizing radiations. Thompson, Mac Key, Gustafsson and Ehrenberg (86) found that fast neutrons produced 8.0% seedling mutants per spike progeny in barley, compared to 4.5% with P^{32}, and 2.9% with X-rays.

Results of treating maize pollen with ultraviolet and with X-rays have shown that the former may produce more frequently, though certainly not exclusively, chromatid breaks and so-called intragenic mutations, in so far as they were not associated with pollen defects. X-rays, on the other hand, induce in this material, in many cases if not always, extragenic alterations incidental to chromosome breakage (20).

Modifications of mutagenicity of practical value would include ways of inducing beneficial mutations with a minimum of such effects as chromosomal aberrations, physiological injury and sterility. Accumulating evidence indicates that it will be particularly profitable to ascertain ways by which factors in the organism and in the environment can be manipulated to modify radiation effects.

Four factors in an organism that are known to influence radiosensitivity are its genotype, age of tissue, stage of the chromosomes, and chromosome number. Differences in response to irradiation have been found among species, varieties and genetic strains within a variety. Sparrow and Gunckel (79) have reported widely different tolerances among 79 species of plants to chronic gamma radiation. A daily dose of 30 r per day produced severe effects in the trumpet lily, whereas it required a dosage of 6,000 r per day to cause comparable radiation damage to gladiolus. Gustafsson (35) observed that mong seeds of various cultivated plants treated with X-rays, the "critical dosage" ranged from 5,000 r for seeds of sunflower to 90,000 r for seeds of rutabaga and white mustard. Little is known about the basic reason for these differences, but since varieties and even single gene differences (55, 77) will show different degrees of response, it is clear that the genotype itself can influence radiation effects.

In aged compared to fresh seeds, experimental evidence has been reported that the frequency of induced mutations is greater and the spectrum of types different (34). Young plants are apparently more radiosensitive than mature plants, and meiotic cells have been found to be more sensitive to radiation than mitotic cells (80).

The time of maximum sensitivity during meiosis has been studied by a number of investigators, and, although there is not general agreement, the best evidence with plant material (78) shows that the stages from late prophase to metaphase are most susceptible to breakage of chromosomes by radiation. Lewis (57) reported evidence for recovering different frequencies of change in self-incompatibility following irradiation at different stages of meiosis. Singleton (75) reported that "mutation rate," as shown by loss of endosperm characters in maize, was greater at late stages of pollen development, probably corresponding to pollen

grain mitosis, than at immediately preceding or more advanced stages. There is also a suggestion (51) that chromosome aberrations are relatively more frequent when pollen is irradiated than when seeds are irradiated.

Polyploids are in general more resistant to radiation than related diploids. In wheat and oats it has been shown that the frequency of induced chlorophyll seedling mutations is lower in the species with higher chromosome numbers (81). However, with neutron irradiation in hexaploid wheat, a decidedly higher frequency of mature plant character mutants has been obtained (59) than in diploid barley.

Environmental factors have been found to influence the genetic effects of radiation, and these are of potential importance in practical application, since they afford an opportunity for additional control over the mutation process. The interplay between the effects of moisture, oxygen, storage and temperature are complex. Control of these environmental factors has been shown to have a profound influence on responses in plants from seeds treated with radiations of sparse ion density, but to have little or no modifying effect on densely ionizing treatments.

Moisture content in the embryo of irradiated seeds affects the radiation response in resulting seedlings (9, 17). Early studies with barley showed that soaked seeds were more sensitive to X-radiation than dormant seeds stored under ordinary laboratory conditions. Furthermore, it was reported that in the former the frequency of mutations per *r* dosage was higher (19) and possibly the types of mutants produced were different (39). However, the highest total number of mutations is obtained from high radiation doses to seeds in their more radioresistant dormant condition (17, 36, 58, 85).

The injurious effects of X-radiation on dormant barley seeds increase with the length of time that they are stored after irradiation and before hydration. Hydration in the presence of oxygen with barley seeds of four to eight per cent water content in the embryo results in greater radiation-initiated damage than does hydration in the presence of nitrogen (1, 13). This differential effect occurs in seeds stored up to six hours after irradiation and before hydrating but not thereafter. Therefore there are two phases to the storage effects, an early phase which is modifiable by oxygen and

a later one which is not (13). The injurious post-irradiation effect of hydrating seeds in oxygen is established with 30 minutes of hydration, but it is not observed in seeds with higher water content, as 15 to 16 per cent (13). It is correlated with a greater frequency of chromosomal aberrations, and the results at present favor the interpretation that oxygen, probably by indirect means through active radicals, reduces the frequency of restitution of broken chromosomes. The relation between chromosome breakage by radiation and the oxygen effect has been discussed in a number of recent publications (3, 28, 52, 64, 84, 89).

The temperature of cells during irradiation can influence radiosensitivity. Stadler (82), Kaplan (47) and Nilan (63) as well as Swedish workers (67) have found that when seeds are irradiated at dry ice or liquid air temperatures, the frequency of induced chromosomal aberrations is reduced, whereas the visible seedling mutation frequency may be unchanged or increased. Heat applied to barley seeds of low water content after irradiation enhances the oxygen effect (13), while heat applied prior to X-radiation reduces the injurious effects (11).

Chemical treatments, not in themselves mutagenic, when combined with irradiation affect mutation in specific ways. With regard to differential effects, D'Amato and Gustafsson (14) reported that the proportion of radiation-induced chlorophyll mutations in barley could be altered by pretreatment of the seeds with colchicine. The frequency of two rare mutant types was increased, while the relative number of two common chlorophyll mutant types was decreased. This list of modifying factors is by no means complete (for detailed review, see Nilan, 65). In summary, there is increasing evidence that by altering the chemical and physical conditions before, after or during irradiation, modifying effects may be produced with respect to the frequency of mutation, the differential production of intragenic changes vs. chromosome breaks, and the spectrum of mutations.

Another possible means of attaining differential control over the mutation process is the use of chemical mutagens alone or in combinations with irradiation. Mac Key (60) and others have shown differences in the relative distribution of two chlorophyll mutants, *albina* and *viridis,* in the second generation of barley treated with different mutagens. Radiations (X-rays, fast neutrons,

P^{32}) yield a lower proportion of *viridis* mutants; with mustard, 52 to 60 per cent of the mutants are of *viridis* type; and with nebularine (purine-9-d-riboside) as the mutagenic agent, the relative frequency of the *viridis* type is raised to 85 per cent (18). Differential effects of mutagens on specific loci in *Neurospora* have been reported by Smith and Srb (76a) and Kölmark (49a). Thus there is evidence that a shift in the mutation spectrum can be effected with differences in the fundamental type of mutagenic agent.

The most extensive evidence published to date on the nonrandomness of the mutation process has been afforded by the recent work of Fahmy and Fahmy (21) on *Drosophila*. This evidence is based on three major differences in the mutants recovered after treatment by certain alkylating compounds and X-radiation as regards: (a) the types of visible mutants, (b) the ratio of recessive visibles to lethals, and (c) the distribution of affected loci along the X-chromosome. The chemical mutagens were reported to act on gene loci which are apparently stable to X-radiation, so that with them nearly 200 sex-linked recessive mutations were recovered which are different in phenotype and genetic position from those induced by X-rays. A particular amino acid mustard was most effective in the induction of visible mutations, mutating two to three times as many of this class of mutant relative to lethals as X-rays.

Investigations on more active and specific chemical mutagens separate from or combined with radiation may provide in the future a means to exercise more direct control over the mutation process.

RESULTS IN INDUCING BENEFICIAL MUTATIONS

The direction of natural evolution is toward better adaptation, i.e., there is continual selection for combinations of genes that give maximum adaptation to the species or population. For cultivated plants this adaptation is governed largely by the needs and whims of man, so that there have been accumulated through the past few thousand years the genotypes which to him are most satisfactory. In so far as man's requirements remain fixed, a static optimum might conceivably be eventually reached, and thereafter no further gene changes would be beneficial. But man is continually shifting his requirements for cultivated plants by calling for new uses, providing altered cultural conditions, combatting chang-

ing spectra of pathogenic organisms, and extending the range of cultivation. Agriculture in this country is, for example, based largely on introduced crops. It is evident that there will be continuing demands for new genotypes.

Previously man has ferreted out, recombined and selected from natural variability to meet his needs. The conventional view is that these methods alone will continue to suffice and remain the most economical. Clearly their full potentialities have not yet been completely exploited, e.g., use of the composite cross population methods is essentially untried (40). It is contended by many that by diligent exploration, collection and maintenance of world germplasm of cultivated and allied species—for the purpose of recombining and increasing the frequency of desired genes when needed —the problem of changing requirements and the objective of continuous improvement can be met.

Artificial induction of beneficial mutations in cultivated plants, at least as an auxiliary tool, is a challenge to the conventional contention. The validity and importance of this challenge are yet to be evaluated; but it is clear that the methods are complementary, not in opposition.

The Swedish experiments on induction of beneficial mutations are most advanced, and a summary of a few of the more interesting X-ray-induced mutants produced by that group, which now constitutes a team of over 20 members, has been compiled by Mac Key (60). Mutants of potential agronomic value have been produced in barley, wheat, oats, peas, soybeans, flax, white mustard and rape. Two of the mutant strains have been introduced into the market: Svalöf Primex white mustard in 1950 and Svalöf Regina II summer oil rape in 1953. Since both of these types cross-fertilize naturally, and may therefore be highly heterozygous, these examples of induced beneficial mutations are far from conclusive. For Primex, however, accompanying characteristics in the strain are considered as proof of the existence of radiation effects. For the first time, with the Weibull Strål pea which was marketed in 1957, an X-ray mutant induced in a self-fertilized crop by Swedish scientists was brought into practical use. Ready for commercial use in this country is an improved Navy or pea bean (*Phaseolus vulgaris*) in which earliness and the bush habit were found in irradiated material (16).

The experiences of Gustafsson (36) with barley and other crop plants showed that one in ten offspring of his irradiated seeds carried some definitely recognizable recessive mutation, and that of these mutations something of the order of one in 800 were potentially useful in some way. In the literature on induced beneficial mutations there is a serious paucity of precise information comparing spontaneous and induced mutation rates for these characters. Furthermore, the low rates of mutations obtained require that extreme care be practised to rule out the possibility of contamination. This possible source of error is magnified where radiation-induced chromosomal aberrations cause considerable male sterility.

One of Gustafsson's interesting results with induced mutations was to demonstrate differences in ecological response (38). His *erectoides* mutants in barley were more adapted to a high nitrogen nutritional level, owing to their stiffer straw, than the original variety, whereas the reverse held true for the bright-green mutations.

A summarization of results obtained so far in this country with mutation breeding in cultivated plants will serve to illustrate some of the different potentialities of the method. There are two ways in which a mutation breeding program may be justified for improving morphological and physiological characters, including yield. One of these is to produce new mutations with singular traits of economic value. A second is to create variation of a kind and magnitude upon which selection can be practised to shift the mean in a desired direction more efficiently than would otherwise be possible.

Examples of the first type are the stiff-strawed *erectoides* mutants produced by the Swedish workers. In this country the short-strawed mutants of wheat, oats and barley, some of which have been produced at Brookhaven National Laboratory, as well as the short-strawed rice mutant produced by Beachell (6) in Texas, are radiation-induced variants of potential value in crop improvement programs, particularly to prevent lodging and to facilitate mechanical harvesting.

The investigations of Gregory (31-33) on yield in peanuts afford the most extensive demonstration that variation which is quantitative can be increased by radiation sufficiently beyond the

natural variability to increase the efficacy of selection. He first demonstrated a fourfold increase in genetic variance among progenies of normal-appearing X_2 plants compared to the controls. He next made five best-mutant selections (all of which were normal or wild type in appearance) and conducted yield tests with the X_5 generation in 1953 and 1955. In these tests the mutant lines maintained a measurable superiority.

The stability of the mutants was tested in the X_5 generation by family progeny tests. Fifteen plant progenies were grown from each of ten original selections of vegetatively vigorous normals. The progeny to progeny variance in yield within each family was not significant in eight of the ten progenies, thus demonstrating that they were stabilized for high performance in an advanced inbred generation.

Gregory's results show, therefore, that phenotypically constructive mutations affecting a quantitative character can be induced by radiation and can be effectively accumulated by selection. This is of particular significance, since most of the agronomically important characteristics by which species and varieties are differentiated are controlled in their inheritance by polygenic systems. Two early maturing lines of tomato, produced by X-radiation of seed, were reported by Mertens and Burdick (60a).

Programs for inducing disease resistance in cereals have perhaps attracted the greatest amount of recent attention—a consequence of the agricultural importance of the problem and the successes reported. One probable reason for success relates to the delicate balance between biochemical processes of host and pathogen so that mutations which alter, in the host, the synthesis of nutritive or metabolic requirements of the pathogen may be expected to confer resistance (51). A second and important reason rests on the highly efficient screening methods which are being developed and used so that large populations can be subjected to selection for resistance. An example is the work of Wheeler and Luke (88) who, using toxin produced by the pathogen *Helminthosporium victoriae* as a screening agent, tested two varieties of oats that are susceptible to *Helminthosporium* blight, for resistant variants. In 100 bushels of oats (approximately 45 million grains) so screened, 973 blight-resistant seedlings were obtained; in addition, some of these were resistant to crown rust. This method, which is equally

applicable to investigations on induced and spontaneous mutations, is so efficient and relatively inexpensive that it brings sharply into focus the problem of weighing the economics of planning programs based on screening of spontaneous mutations vs. transfer of mutations through backcrossing vs. artificial induction of mutants.

Disease-resistant types in small grains, the mutation frequency of which has been reported by workers in this country to be increased by radiation, are: in oats, resistance to Victoria blight, stem rust and crown rust; in wheat, resistance to stem rust and stripe rust. The investigators reporting these results are Konzak, Borlaug, Acosta and Gibler (54), Frey (26), Frey and Browning (27), Myers, Ausemus, Koo and Hsu (62), and Wallace (87). The induced disease resistance may be dominant, as in stem rust resistance in wheat and oats; or recessive, as in Victoria blight-resistance in oats and stripe rust-resistance in wheat.

Two other cases of radiation-induced resistance in crop plants reported by U. S. workers are rust resistance in flax by Flor (22) in North Dakota and resistance for leaf spot and stem rot in peanuts by Gregory (33) in North Carolina. There is an interesting contrast in the two diseases of peanut in that the leaf spot pathogen is highly species-specific, attacking only legumes of the subtribe to which peanuts belong; whereas the stem rot pathogen is highly species-general, attacking species of a number of families of flowering plants.

Most of the uses of radiation to induce mutations have stressed the undesirability of the accompanying chromosome breaks and gross rearrangements; furthermore, emphasis has usually been on the advantages of single-gene induced mutations within an accepted commercial variety to overcome the laboriousness of the repeated backcrosses required to transfer a desired character to the accepted residual genotype following hybridization. However, radiation can be used to facilitate the transfer of a desired character where its only source is a species or genus so distantly related that the chromosomes fail to pair with those of the commercial variety and, in addition, carry associated unfavorable characteristics. Under these circumstances the transference by backcrossing is impractical. Practical success can be achieved by transposing to the accepted genotype through chromosome breaking and rejoin-

ing only that part of the foreign chromosome which contains the beneficial character separate from undesirable ones.

A demonstration of this technique, using radiation to break chromosomes, has been afforded by Sears' (73) results in transferring leaf rust resistance from *Aegilops umbellulata,* a wild grass of the Mediterranean region, to wheat. A single non-pairing *Aegilops* chromosome added to wheat provides a high degree of resistance to leaf rust. Wheat plants with this added chromosome were X-rayed prior to meiosis, and translocations between the *Aegilops* and wheat chromosomes were obtained. Data from one of the translocations indicated that the resistance gene was near the centromere of the *Aegilops* chromosome, so that transfer of the entire arm included associated deleterious traits. The one intercalary translocation obtained showed essentially normal pollen transmission, and homozygous plants were distinguished from normal wheat only by their rust resistance and slightly later maturity.

That radiation-induced translocations may have general applications in locating and transferring valuable traits from exotic sources to cultivated plants of refined genotypes, such as improved maize selections, has been advanced by E. G. Anderson (2).

Production of radiation-induced somatic mutations, particularly those affecting fruit or flower, in vegetatively propagated materials is at present being investigated on a rather wide scale in this country, in Canada, in Sweden and in Germany. Either chronic or acute radiation is applied to growing points. The methods are as yet inefficient. The problems of technique relate primarily to the detection and propagation from a multicellular meristem of single-event mutations that must subsequently compete with non-mutated cells to be recognized (48). Research is needed on ways to modify cell selection within an individual. On the other hand, an obvious advantage of the method is that a periclinal chimera which appears in a single growing point can be multiplied clonally. Granhall (30) has reported inducing red fruits in apples and russet-skin in pears. Bishop (7) has produced dark red sports of the Cortland variety of apple. In preliminary experiments with the black currant, Kaplan (48) and co-workers have isolated a number of interesting mutant shoots from X-rayed scions that showed changes in leaves, in internode length, growth habit and fruit characteristic. With

commercial strains of carnation, induced changes have been reported (70) from white to red flowers, red to variegated, and standard double to single-flowered types; in addition there were mutations from the dominant red color to the recessive colors brick red, salmon and white (71). The somatic mutations cited are of potential practical value in horticulture.

Examples of beneficial mutations induced by irradiation are not confined to the higher plants. A group of investigators (43, 69) has developed by successive radiations of the mold *Penicillium* and a stepwise incorporation of best-yielding mutants, a strain which yields approximately nine times more penicillin than the original variety. This result is of considerable economic and medical value.

Although the lethality incident to chromosomal aberrations is generally a detrimental response in radiation usage, it has been employed as a new tool in economic entomology. Males of the screw-worm fly, a pest of goats and cattle in the tropics and subtropics, after being treated with gamma rays can be released in large numbers to compete with normal males. They mate with the females, but, owing to structurally changed chromosomes in the sperms, the zygotes die, thus reducing the population. By repetition of this process the screw-worm was eradicated from the Caribbean island of Curaçao in 1954 (8).

CONCLUSIONS

In conclusion, the use of radiation to produce genetic variants is a new tool of potential value in agriculture, capable of being employed as an adjunct to conventional methods. With greater understanding of the fundamental processes of gene mutation and chromosome breakage, as well as refinements in techniques for differentially modifying radiation effects and detecting desirable variants, this tool may find increasing application. This field of investigation is too new to be fully evaluated now. It is not a panacea that can be expected to revolutionize agriculture, but it shows promise and is worthy of more extensive investigation. Particularly needed are more precise data showing rates of mutation for beneficial mutants in untreated compared to irradiated populations. Considering the unique effects of radiation, emphasis in future research should be placed on appropriate and special cir-

cumstances where it can be used to greater advantage than conventional methods.

We have reached a crossroads and from here on the objective is no longer primarily to test whether beneficial mutations can be produced by irradiation—this has been done. It has been definitely demonstrated that with radiation, alterations can be effected in the mechanisms of heredity (mutation in the broadest sense) that are useful in plant improvement. It has been reasonably well demonstrated that useful mutations in the narrower sense, i.e., changes at the gene (or, at least, minute rearrangement) level, can be increased in frequency by irradiation. In fact, if it is agreed that radiation can cause gene mutations, then to consider that useful mutations are not producible would imply the unlikely alternatives, either that the process of radiation-induced mutation is discriminatory against hereditary changes that man finds useful, or that all desirable mutants have already been accumulated in cultivated plant varieties. Induction by irradiation of entirely new mutations, different from any occurring in nature, has not yet been demonstrated; and, indeed, it may never be possible to do so in a final sense.

The crux of the problem now is—and this is stressed throughout—is it economically feasible to use radiation in plant improvement programs? There is no one answer. Each particular problem requires careful appraisal, and the answers will be different, depending on the material, objectives and circumstances.

BIBLIOGRAPHY

1. ADAMS, J. D., NILAN, R. A., and GUNTHARDT, H. M. After effects of ionizing radiation in barley. I. Modification by storage of X-rayed seeds in oxygen and nitrogen. A preliminary report. Northwest Sci. **29**: 101-108. 1955.
2. ANDERSON, E. G. The application of chromosomal techniques to maize improvement. Brookhaven Symp. Biol. **9**: 23-35. 1956.
3. BAKER, W. K., and VON HALLE, E. S. Evidence on the mechanism of the oxygen effect by use of a ring chromosome. Jour. Cell. & Comp. Physiol., Suppl., 2. **45**: 299-307. 1955.
4. BANDLOW, G. Mutationsversuche an Kulturpflanzen. II. Züchterisch wertvolle Mutanten bei Sommer- und Wintergersten. Züchter **21**: 357-363. 1951.
5. BAUER, R. The induction of vegetative mutations in *Ribes nigrum*. Hereditas.
6. BEACHELL, H. M. [U. S. Dept. Agric., A. R. S., unpublished results.]

7. BISHOP, C. J. Mutations in apples induced by X-radiation. Jour. Hered. **45**: 99-104. 1954.
8. BUSHLAND, R. C., KNIPLING, E. F., and LINDQUIST, A. W. Eradication of the screw-worm fly by releasing gamma-ray-sterlized males among the natural population. Proc. Int. Conf. Peaceful Uses Atomic Energy, Vol. 12: 216-220. 1956.
9. CALDECOTT, R. S. Effects of hydration on X-ray sensitivity in *Hordeum*. Rad. Res. **3**: 316-330. 1955.
10. ————, BEARD, B. H., and GARDNER, C. O. Cytogenetic effects of X-ray and thermal neutron irradiation on seeds of barley. Genetics **39**: 240-259. 1954.
11. ————, and SMITH, L. The influence of heat treatment on the injury and cytogenetic effects of X-rays on barley. Genetics **37**: 136-157. 1952.
12. CHAUDHURI, K. L., and DAS, A. High-yielding X-ray mutations of *Sesamum orientale* L. Science & Culture [India] **19**: 620-622. 1954.
13. [Unpublished results of experiments carried out at Brookhaven National Laboratory by H. J. Curtis, N. Delihas and C. F. Konzak; and independently by R. S. Caldecott at the University of Minnesota].
14. D'AMATO, F., and GUSTAFSSON, Å. Studies on the experimental control of the mutation process. Hereditas **34**: 181-192. 1948.
15. DELAUNAY, L. N. Experimentell erzeugte Mutationen bei Weizen. Verlag Ukr. Akad. landwirt. Wiss. pp. 1-56. 1934.
16. DOWN, E. E., and ANDERSEN, A. L. Agronomic use of an X-ray-induced mutant. Science **124**: 223-224. 1956.
17. EHRENBERG, L. Factors influencing radiation-induced lethality, sterility, and mutations in barley. Hereditas **41**: 123-146. 1955.
18. ————, GUSTAFSSON, Å., and LUNDQVIST, U. Chemical induced mutation and sterility in barley. Acta Chem. Scand. **10**: 492-494. 1956.
19. ————, GUSTAFSSON, Å., LUNDQVIST, U., and NYBOM, N. Irradiation effects, seed soaking and oxygen pressure in barley. Hereditas **39**: 493-504. 1953.
20. FABERGÉ, A. C. The analysis of induced chromosome aberrations by maize endosperm phenotypes. Zeit. Ind. & Abst. Vererb. **87**: 392-420. 1956.
21. FAHMY, O. G., and FAHMY, M. J. Cytogenetic analysis of pre-action of carcinogens and tumor inhibitors in *Drosophila melanogaster*. V. Differential genetic response to the alkylating mutagens and X-radiation. Jour. Genet. **54**: 146-164. 1956.
22. FLOR, H. H. Host-parasite interaction in flax rust—its genetics and other implications. Phytopath. **45**: 680-685. 1955.
23. FREISLEBEN, R., and LEIN, A. Über die Auffindung einer mehltauresistenten Mutante nach Röntgenbestrahlung einer anfälligen reinen Linie von Sommergerste. Naturwiss. **30**: 608. 1942.
24. ————, and ————. Röntgenindurzierte Mutationen bei Gerste. Züchter **16**: 49-64. 1944.
25. FREY, K. J. Artificially induced mutations in oats. Agron. Jour. **46**: 49. 1954.

26. ————. Agronomic mutations in oats induced by X-ray treatment. Agron. Jour. **47**: 207-210. 1955.
27. ————, and BROWNING, J. A. Mutations for stem rust resistance induced in oats by X-ray treatment. Phytopath. **45**: 490-492. 1955.
28. GILES, N. H. The oxygen effect on radiation-induced chromosome aberrations: breakage-versus-recombination hypotheses. Jour. Cell. & Comp. Physiol., Suppl. 2 **45**: 271-284. 1955.
29. ————. Forward and back mutation at specific loci in *Neurospora*. Brookhaven Symp. Biol. **8**: 103-123. 1955.
30. GRANHALL, I. Spontaneous and induced bud mutations in fruit trees. Acta Agric. Scand. **4**: 594-600. 1954.
31. GREGORY, W. C. X-ray breeding of peanuts (*Arachis hypogaea* L.). Agron. Jour. **47**: 396-399. 1955.
32. ————. The comparative effects of radiation and hybridization in plant breeding. Proc. Int. Conf. Peaceful Uses Atomic Energy **12**: 48-51. 1956.
33. ————. Induction of useful mutations in the peanut. Bookhaven Symp. Biol. **9**: 177-190. 1956.
34. GUSTAFSSON, Å. Studies on the genetic basis of chlorophyll formation and the mechanism of induced mutation. Hereditas **24**: 33-93. 1938.
35. ————. The X-ray resistance of dormant seeds in some agricultural plants. Hereditas **30**: 165-178. 1944.
36. ————. Mutations in agricultural plants. Hereditas **33**: 1-100. 1947.
37. ————. Swedish mutation work in plants: background and present organization. Acta Agric. Scand. **4**: 361-364. 1954.
38. ————. Mutations, viability, and population structure. Acta Agric. Scand. **4**: 601-632. 1954.
39. HÄNSEL, VON H., and ZAKOVSKY, J. Röntgeninduzierte Mutanten der Vollkorngerste (*Hordeum distichum nutans*). I. Bestrahlung und Auslese auf Mehltauresistenz. Die Bodenkultur [Austria] **9**: 50-64. 1956.
40. HARLAN, J. R. Distribution and utilization of natural variability in cultivated plants. Brookhaven Symp. Biol. **9**: 191-206. 1956.
41. HOFFMANN, W. Ergebnisse der Mutationszüchtung. *In*: Vorträge über Pflanzenzüchtung. 1951. [pp. 36-53].
42. ————, and ZOSCHKE, U. Röntgenmutationen beim Flachs (*Linum usitatissimum* L.) Züchter **25**: 199-206. 1955.
43. HOLLAENDER, A. The mechanism of radiation effects and the use of radiation for the production of mutations with improved fermentation. Ann. Missouri Bot. Garden **32**: 165-178. 1945.
44. HORLACHER, W. R., and KILLOUGH, D. T. Progressive mutations induced in *Gossypium hirsutum* by radiations. Amer. Nat. **67**: 532-538. 1933.
45. JACOB, K. T. Synopsis of the applied research work of the Botany Department for 1954-1955. Bose Institute [India]. 1955. [pp. 44-91.]

46. JAJIMA, Y., HARADA, C., and OTA, N. On the sex discriminating method by coloring genes of silk-worm eggs. I. Induction of translocation between the W and the tenth chromosome. Jap. Jour. Breed. **1**: 47-50. 1951.
47. KAPLAN, R. W. Einfluss tiefer Temperatur auf die Röntgenstrahlen-Mutationsraten von Gerstenkörnen. Naturwiss. **34**: 316-317. 1948.
48. ————. Über Möglichkeiten der Mutationsauslösung in der Pflanzenzüchtung. Zeits. Pflanzenzucht. **32**: 121-131. 1953.
49. KOBAYASHI, T. [Action of radioactive substances on the plant cells. II. Mutants in sesame induced by double irradiations of beta and X-rays.] Jap. Jour. Genet. **30**: 174. 1955. [In Japanese.]
49a. KÖLMARK, G. Differential response to mutagens as studied by the *Neurospora* reverse mutation test. Hereditas **39**: 270-276. 1953.
50. KONZAK, C. F. Stem rust resistance in oats induced by nuclear radiation. Agron. Jour. **46**: 538-540. 1954.
51. ————. Induction of mutations for disease resistance in cereals. Brookhaven Symp. Biol. **9**: 157-171. 1956.
52. ————. The influence of oxygen on the mutagenic effects of X-rays on maize endosperm loci. Rad. Res. **6**: 1-10. 1957.
53. ————. Genetic effects of radiation on higher plants. Quart. Rev. Biol. **32**: 27-45. 1957.
54. ————, BORLAUG, N. E., ACOSTA, A., and GIBLER, J. Stripe rust resistant mutants obtained from irradiation of Gabo wheat. Phytopath. **46**: 525-526. 1956.
55. LAMPRECHT, H. Röntgen-empfindlichkeit und genotypische Konstitution bei *Pisum*. Agr. Hort. Genet. **14**: 161-176. 1956.
56. LAWRENCE, T. The production of mutations by the irradiation of Montcalm barley. Canad. Jour. Bot. **33**: 515-530. 1955.
57. LEWIS, D. Structure of the incompatibility gene. II. Induced mutation rate. Heredity **3**: 339-355. 1949.
58. LUTKOV, A. N. Chlorophyll mutations and other types of hereditary variations induced in *Hordeum* by X-rays. Bull. Appl. Bot. II. **7**: 223-225. 1937.
59. MAC KEY, J. Neutron and X-ray experiments in wheat and a revision of the speltoid problem. Hereditas **40**: 65-180. 1954.
60. ————. Mutation breeding in Europe. Brookhaven Symp. Biol. **9**: 141-152. 1956.
60a. MERTENS, T. R. and BURDICK, A. B. On the X-ray production of "desirable" mutations in quantitative traits. Amer. Jour. Bot. **44**: 391-394. 1957.
61. MULLER, H. J. On the relation between chromosome changes and gene mutations. Brookhaven Symp. Biol. **8**: 126-147. 1955.
62. MYERS, W. M., AUSEMUS, E. R., KOO, F. K. S., and HSU, K. J. Resistance to rust induced by ionizing radiations in wheat and oats. Proc. Int. Conf. Peaceful Uses Atomic Energy **12**: 60-62. 1956.
63. NILAN, R. A. Relation of carbon dioxide, oxygen and low temperature to the injury and cytogenetic effects of X-rays in barley. Genetics **39**: 943-953. 1954.
64. ————. Post-radiation storage effect on chromosomes in barley seeds X-rayed at normally ineffective dosages. Genetics **40**: 588. 1955.

65. ————. Factors governing plant radiosensitivity. Conf. Radioactive Isotopes in Agric., U.S.A.E.C., TID 7512: 151-162. 1956.
66. NYBOM, N. On the differential action of mutagenic agents. Hereditas 42: 211-217. 1956.
67. ————, LUNDQVIST, U., GUSTAFSSON, Å., and EHRENBERG, L. Biological effects of X-irradiation at low temperatures. Hereditas 39: 445-457. 1953.
68. OLTMANN, W. Züchterische Auswertung röntgeninduzierter Mutationen an physiologischen Merkmalen bei Winterweizen. Zeits. Pflanzenz. 29: 76-89. 1950.
69. RAPER, K. B. Penicillin. U. S. Dept. Agr., Yearbook 1943-1947: 699-710. 1947.
70. RICHTER, A., and SINGLETON, W. R. The effect of chronic gamma radiation on the production of somatic mutations in carnations. Proc. Nat. Acad. Sci. 41: 295-300. 1955.
71. SAGAWA, Y., and MEHLQUIST, G. A. L. The mechanism responsible for some X-ray induced changes in flower color of the carnation, *Dianthus caryophyllus*. Amer. Jour. Bot. 44: 397-403. 1957.
72. SAPEHIN, A. A. X-ray mutants in soft wheat. Bull Appl. Bot., Genet. & Plant Breed. II. 9: 3-37. 1936.
73. SEARS, E. R. The transfer of leaf-rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Symp. Biol. 9: 1-21. 1956.
74. SHEBESKI, L. H., and LAWRENCE, T. The production of beneficial mutations in barley by irradiation. Canad. Jour. Agri. Sci. 34: 1-9. 1954.
75. SINGLETON, W. R. The effect of chronic gamma radiation on endosperm mutations in maize. Genetics 39: 587-603. 1954.
76. ————, KONZAK, C. F., SHAPIRO, S., and SPARROW, A. H. The contribution of radiation genetics to crop improvement. Proc. Int. Conf. Peaceful Uses Atomic Energy 12: 25-30. 1956.
76a. SMITH, H. H., and SRB, A. M. Induction of mutations with beta-propiolactone. Science 114: 490-492. 1951.
77. SMITH, L. Hereditary susceptibility to X-ray injury in *Triticum monococcum*. Amer. Jour. Bot. 29: 189-191. 1942.
78. SPARROW, A. H. Radiation sensitivity of cells during mitotic and meiotic cycles with emphasis on possible cytochemical changes. Ann. N. Y. Acad. Sci. 51: 1508-1540. 1951.
79. ————, and GUNCKEL, J. E. The effects on plants of chronic exposure to gamma radiation from radiocobalt. Proc. Int. Conf. Peaceful Uses Atomic Energy 12: 52-59. 1956.
80. ————, and SINGLETON, W. R. The use of radiocobalt as a source of gamma rays and some effects of chronic irradiation on growing plants. Amer. Nat. 87: 29-48. 1953.
81. STADLER, L. J. Chromosome number and the mutation rate in *Avena* and *Triticum*. Proc. Nat. Acad. Sci. 15: 876-881. 1929.
82. ————. The experimental modification of heredity in crop plants. II. Induced mutation. Sci. Agr. 11: 645-661. 1931.
83. STUBBE, H. Über einige theoretische und praktische Fragen der Mutations-forschung. Abh. Sächs. Akad. Wiss., Math.-naturwiss. Klasse 47: 3-23 + xi. 1952.

84. SWANSON, C. P. The oxygen effect and chromosome breakage. Jour. Cell. & Comp. Physiol., Suppl. 2. **45**: 285-298. 1955.
85. TASCHER, W. R. Experiments with X-ray treatments on the seeds of certain crop plants. Univ. Missouri (Thesis-Ph.D.). 37 pp. 1929.
86. THOMPSON, K. F., MAC KEY, J., GUSTAFSSON, Å., and EHRENBERG, L. The mutagenic effect of radiophosphorus in barley. Hereditas **36**: 220-224. 1950.
87. WALLACE, A. T. [Unpublished results.]
88. WHEELER, H. E., and LUKE, H. H. Mass screening for disease-resistant mutants in oats. Science **122**: 1229. 1955.
89. WOLFF, S., and LUIPPOLD, H. E. Metabolism and chromosome-break rejoining. Science **122**: 231-232. 1955.
90. ZACHARIAS, M. Mutationversuche an Kulturpflanzen. VI. Röntgenbestrahlungen der Sojabohne (*Glycine soja* (L.) Sieb. et Zucc.). Züchter 11: 321-338. 1956.

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Induced mutations – A new paradigm in plant breeding

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Summary

The use of ionizing radiation, such as X-rays, gamma rays and neutrons and chemical mutagens for inducing variation, is well established. Induced mutations have been used to improve major crops such as wheat, rice, barley, cotton, peanuts, and beans, which are seed propagated. Since the establishment of the Joint FAO/IAEA Division of the Nuclear Techniques in Agriculture, more than 1800 cultivars obtained either as direct mutants or derived from their crosses have been released worldwide in 50 countries. In vegetatively propagated plants, many of mutants were derived from irradiating rooted stem cuttings, detached leaves, and dormant plants. According to the FAO/IAEA database, of the 465 mutants released among the vegetatively propagated plants, most are in the floricultural plants and a few in fruit trees. These include chrysanthemum, *Alstroemeria*, dahlia, bougainvillea, rose, *Achimenes*, begonia, carnation, *Streptocarpus*, and azalea. The irradiation of *in vitro* cultured date palm, apple, potato, sweet potato and pineapple now provides a means to treat large populations, which would not have been possible before. Irradiation of micropropagated plants, axillary and adventitious buds, apical meristems, regenerative callus cultures, anthers and microspores, and somatic embryos provides a miniaturized version of trees and seeds in the Petri dish instead of the field. During the last decade, the use of radio-actively labeled probes in recombinant DNA research for cloning and mapping plant genes and transgenesis, particularly for RFLP, microsatellite based DNA fingerprinting, has become a routine procedure. Many homeotic mutants that change floral development have been isolated in *Arabidopsis, Petunia, Antirrhinum* and *Lycopersicon*. Mutants of *Arabidopsis* are being used to analyze genes, which determine response to auxins, cytokinins, gibberellin, abscisic acid and ethylene in plant growth, floral development and senescence, fruit formation and ripening. These mutants are facilitating the isolation, identification and cloning of the genes, which would ultimately help in designing crops with improved yield, increased stress tolerance, longer shelf-life and reduced agronomic inputs. The identification and analysis of mutants by using molecular techniques of DNA fingerprinting and mapping with PCR based markers, such as RAPDs, AFLP and STMS, and mutant tagging shall bring a new dimension in gene technology. Already, mutations can be linked to changes in DNA sequences for some plant traits and to establish molecular maps in structural and functional genomics of crop plants. These in turn would lead to a rapid enhancement of crop yields and quality.

Introduction

The role of plant breeding in increasing food production and provide sustainable nutrition is well recognized. Increased crop yields, based on the use of fertilizers, agro-chemicals to control insect pests, pathogens and weeds, crop rotation and use of agricultural machinery, would not be possible without varieties designed to meet the specific agro-climatic conditions. During the past fifty years, plant varieties coupled with improved management and agronomic inputs have made a very significant increase in the yield of major crops (cf. Swaminathan, 1998). With increasing population and reducing land resources, it

becomes even more important to breed plant varieties which can sustain production under the varied agro-climatic conditions of different regions.

The methods of plant breeding have become increasingly sophisticated since the days of simple selection among natural populations, which consisted of mixtures of genotypes. The populations arose from natural variation and sexual recombination. Modern day plant breeding is based on creating variation, selection, evaluation and multiplication of desired genotypes. To increase efficiency and make short cuts in each step, the plant breeders combine several techniques together. In so doing, plant breeders have the options to use *in vitro* culture for rapid multiplication, molecular methods to select specific genotypes, mutagenesis to enhance variation, controlled environmental conditions to manipulate growth and flowering, computers to assist data processing, and international collaboration to exchange germplasm.

The use of nuclear techniques in plant breeding has been mostly directed for inducing mutations. Since the discovery of X-rays about one hundred years ago, the use of ionizing radiation, such as X-rays, gamma rays and neutrons for inducing variation, has become an established technology. Induced mutations have been used in the improvement of major crops such as wheat, rice, barley, cotton, peanuts, beans, which are seed propagated. Since the establishment of the Joint FAO/IAEA Division of the Nuclear Techniques in Agriculture, more than 1800 cultivars obtained either as direct mutants or derived from their crosses have been released worldwide in 50 countries (Maluszynski et al., 1995). During the last decade, the use of radioactively labeled probes in recombinant DNA research for cloning and mapping plant genes and transgenesis, particularly for RFLP, microsatellite based DNA fingerprinting, has become a routine procedure.

Past achievements

The prime strategy in mutation-based plant breeding has been to upgrade the well-adapted varieties by altering one or two major traits. These include characters such as plant height, maturity, seed shattering, and disease resistance, which contribute to increased yield and quality traits, e.g. oil profile and content, malting quality, and size and quality of starch granules. For example, short height genotypes in rice, wheat, barley and maize have contributed significantly to increasing grain yield because of their resistance to lodging and high planting density. This has allowed the use of relatively high doses of nitrogen application. Many such varieties were derived from induced mutations with radiation and chemicals. For example, the semidwarf rice mutant 'Calrose 76' released in California had short and stiff straw, and made a major contribution to rice production in USA as did the short height mutant of rice 'Basmati 370' in Pakistan. Several rice mutants induced with gamma radiation were released in India as high yielding varieties under the series 'PNR'; some of these were also early in maturity and had short height (Chakrabarti, 1995). An outstanding rice mutant 'Zhefu 802' was grown on more than 10.6 million ha in China during ten years. In Thailand, an aromatic *indica* variety of rice 'RD6', derived from gamma irradiation, was released in 1977. Even after 17 years of its release, this variety is still grown extensively in Thailand. It was planted on approximately 2.4 million ha (15.2 rai) during the 1994–95 wet season, covering 26.3% of the area under rice during the season, and topped the list of rice cultivars grown by the farmers. Another mutant 'RD15', released in 1978, was planted over 0.2 million ha, equivalent to 3.2% of the area under rice (Anonymous, 1995). The induction of thermosensitive genic male-sterile mutant in Japonica rice, which is controlled by a single recessive gene (Murayama et al., 1991), has contributed significantly to develop strategies for the production of hybrid rice varieties. Similar mutants have been induced by gamma rays in *indica* rice '26 Zhaizao' in China (Chen et al., 1993). Such mutants allow production of hybrid seed based on only two lines.

Many induced mutants have been released as cultivars; several others have been used as parents in the pedigree of some of the leading cultivars. The release of high yielding and short height barley mutants as varieties 'Diamant' and 'Golden Promise' have had a major impact on the brewing industry in Europe. These mutants have been used as the parents of many leading barely cultivars released in Europe. For example, more than 90 leading barley cultivars in over 12 countries in Europe were derived from crosses involving 'Diamant'. 'NIAB-78', a high yielding cotton mutant released in 1987, had a major influence on sustaining the growth of textile industry in Pakistan. This cultivar is heat tolerant, has determinate growth habit and escapes bollworm attack due to its early maturity, which made it an ideal cultivar for wheat-cotton-wheat rotation. The release of 'Sharbati Sonora' as a mutant of cv. 'Sonora' gave a variety with better acceptance for grain colour by the consumer in the early years

of 'Green Revolution' in India. Several high yielding, early maturing peanut varieties named 'Yueyou' series were released in China, which were derived from crosses with the radiation induced mutants. A recently released peanut mutant variety 'TG-26', developed at Bhabha Atomic Research Center, Bombay, India yielded 9.4 tons/h nuts at the farm level. The release of flax cultivars with cooking quality oil 'linola', based on induced mutations (Green, 1986; Dribnenki, 1996), is the latest contribution for changing oil quality as had been done before in 'Canola' from rape seed, and high oleic acid in sunflower.

In vegetatively propagated plants, before the development of *in vitro* techniques, many mutants in ornamentals, e.g. *Achimenes*, chrysanthemum, carnation, roses and *Streptocarpus*, were obtained by irradiating rooted stem cuttings, detached leaves, and dormant plants (Broertjes, 1977). The altered flower colour and shape, growth-habit (dwarf or trailing) and other novel phenotype of commercial value were selected. According to the FAO/IAEA database, of the 465 mutants released among the vegetatively propagated plants, most were in the floricultural plants and a few in fruit trees. These included chrysanthemum (187), *Alstroemeria* (35), dahlia (34), bougainvillea (9), rose (27), *Achimenes* (8), begonia (25), carnation (18), *Streptocarpus* (30), and azalea (15) (Maluszynski et al., 1992). Since the effect of mutation in ornamentals is very visible, selection for changed flower colour, shape, and size is easy, and almost anything which is novel has value. Hence, mutation techniques have become a major tool for breeding ornamental plants (Maluszynski et al., 1995). On the other hand, very few mutant varieties have been released in fruit trees. Among these are mutants of apple with changed skin-colour in Austria (Brunner & Keppl, 1991) and disease resistance in Japanese pear in Japan (Sanada et al., 1993), seedless mutants 'Rio Red' and 'Star Ruby' of grapefruit with deep red colour flesh and juice in USA (Hensz, 1991). A spineless mutant of pineapple was reported in Philippines (Lapade et al., 1995). In banana, 'Novaria' an early ripening mutant with enhanced flavour was released in Malaysia (Mak et al., 1996) and two mutants with disease resistance in Cuba and Costa Rica. Recently, mutants have been reported for reduced glycoalkaloid content in potato tubers (Love et al., 1996). However, the technology has yet to be exploited for the improvement of clonally propagated crops such as sweet potato, yams, plantain, strawberry and date palm.

In vitro *culture and mutagenesis*

It is possible to upgrade well-established clones by changing specific traits by incuding mutations. The availability of large populations for mutagenesis is one of the basic pre-requisites to obtain sufficient variation. Mutation techniques in combination with tissue culture and molecular methods provide a powerful technology to improve clonally propagated plants such as banana, plantains, apple, pineapple, date palm, potato, sweet potato, cassava, carnation, chrysanthemums, roses and tulips. Nearly all of these plants can be regenerated and multiplied *in vitro*, allowing the production of large populations in a small space and short time. The irradiation of *in vitro* cultured date palm, apple, potato, sweet potato and pineapple now provides a means to treat large populations, which would not have been possible before. Irradiation of micropropagated plants, axillary and adventitious buds, apical meristems, regenerative callus cultures, anthers and microspores, and somatic embryos provides a miniaturized version of trees and seeds in the Petri dish instead of the field.

In mutagenesis experiments, whether with chemicals or physical mutagens, it is necessary to advance the treated material through few seed generations or vegetative propagations. In seed propagated plants, the recessive mutants are usually selected in the second (M_2) or third (M_3) generation after the treatment. In vegetatively propagated plants, following mutagenesis, several cycles of propagation are needed to obtain homo-histonts or to 'dissolve' chimeras and to obtain 'solid' mutants. It has been suggested that many of the mutants thus generated are sectorial chimeras. The *in vitro* subculture of irradiated material through V_2 to V_4 can be achieved rapidly and without loss of any genotype under disease free conditions. In many plants, such as banana and potato, this procedure can reduce the duration of 5 years in field to less than nine months in the laboratory. In addition, when plants are regenerated from cell suspension cultures capable of producing somatic embryos, the chances are that many of the regenerants would be solid mutants, since only a few and in many cases only single cells give rise to such embryos.

Irradiation of in vitro *cultures*

In many mutagenic studies, gamma ray and X-rays have been used to induce mutations. The key factor in the irradiation of plant material is the dose, which

is the amount of radiation energy absorbed by the material. The unit of measurement of radiation dose is Gray (Gy). One Gy is equal to the absorption of 1 J of energy per kilogram of product irradiated. Radiation doses are divided into three broad categories: high (> 10 kGy), medium (1 to 10 kGy), and low (<1 kGy). The high doses are used for the sterilization of food products, and low doses to induce mutations in seed material, where doses range from 60 to 700 Gy for many seed propagated crops, such as rice, wheat, maize, beans and rape seed. In case of *in vitro* cultured plant material, since only milligrams of tissues and micrograms of cell suspensions are irradiated, the dose levels are much lower. The limited number of available reports suggest that callus cultures are much more sensitive to radiation treatment, and require much lower doses (2 to 5 Gy) than stem cuttings or seeds; with relatively higher doses (15 to 20 Gy) they turn necrotic or lose their regenerative capacity. For example, regenerative callus cultures of date palm above 25 Gy have very poor survival. In potato micropropagated plants, 20 Gy dose gives optimal survival. In sweet potato, 10 Gy is lethal for callus cultures. In garlic, callus proliferation and regeneration is inhibited with doses of 8 to 10 Gy. In sugarcane, 20 Gy dose to callus cultures reduced regeneration by more than 50%, and 60 Gy reduces regeneration to 2.5%, the optimal dose being between 5 to 10 Gy (IAEA, 1997).

Use of induced mutations in basic research

Developmental mutants

Many mutants in *Arabidopsis* are being used to analyze genes, which determine response to auxins, cytokinins, gibberellin, abscisic acid and ethylene in plant growth, floral development and senescence, fruit formation and ripening. These mutants are facilitating the isolation, identification and cloning of the genes, which would help in designing crops with improved yield, increased stress tolerance, longer shelf-life and reduced agronomic inputs.

Induced mutations in *Arabidopsis thaliana*, maize, barely, pea and tobacco have been used to isolate and identify genes, which regulate plant development, particularly the onset of flowering, formation of floral parts, seed and fruit formation, fruit ripening. These mutants involve growth regulators (phytohormones), such as auxins, cytokinins, gibberellins, abscisic acid, ethylene and brassinosteroids. In plants, the study of the biosynthetic pathways and regulation of the phenotype at the molecular level had been a slow process. The fine dissection of loci has been made possible because of the ease with which such mutants can be isolated and identified among mutagenized populations of *Arabidopsis*. In many cases, these genes have been cloned, sequenced and used in transgenic studies to obtain plants with changed traits at the biochemical or morphological level. In addition, T-DNA insertional mutagenesis in *Arabidopsis* has rapidly advanced our knowledge of the physiology, biochemistry and development of the plants. These advances would not have been possible without understanding the process of mutation induction, production of mutants and the basis of mutagenesis.

The mutant analysis of super root (*sur1*) which results in an over-production of free auxins has revealed the genetics of the regulation of auxin synthesis. The *fass* mutants, which have reduced cell elongation in the apical and basal axis, have suggested implications of the locus in auxin conjugation and auxin homeostasis. There are many mutants which affect auxin transport, auxin inhibition, auxin uptake (*aux1, pid, mp, lop1*) and auxin signal transduction (*axr1, axr4*) and many more which have been used to investigate auxin metabolism (Leyser, 1997). Similarly, several mutants with altered response to cytokinins are now available which will contribute to our understanding of the nature of cytokinin action. These mutants include those with elevated cytokinin levels (*amp1*), photomorphogenesis mutants (*det1, cop*), cytokinin resistant mutants, and cell division mutants (Miklashevichs & Waldon, 1997). Plant gene expression can be markedly changed in response to cytokinins with corresponding increase or decrease in the specific transcripts. Such genes are often regulated by additional stimuli such as light and auxins. A large number of cytokinin mutants and related to its metabolism, such as *ckr1, ein2, cry1, stp1, zea3*, have been isolated in *Arabidopsis thaliana*. They are resistant to cytokinins, and have shown that cytokinin-regulated genes may be involved in diverse biological processes, ranging from cell division, photosynthesis, chloroplast development, disease resistance, and nutrient metabolism (Schmülling et al., 1997). An understanding of the key processes is extremely important for breeding crop plants with increased growth rate, improved nutrient uptake, yield and disease resistance.

The elongated rice seedlings infected with *Gibberella fujikuroi* in Japan led to the isolation of crystalline

gibberellic acids by Yabuta & Sumiki (1938). In contrast to other auxins and cytokinins, mutants with altered shoot elongation in pea and maize were used to investigate gibberellin as early as 1955 and 1956. The application of GA_3 to these mutants restored the wild-type phenotypes in the dwarf *le* mutant of pea and dwarf mutants of maize. Since then many mutants have been isolated in *Arabidopsis*, maize, pea, wheat, and rice, which are involved in GA synthesis and enzymes catalyzing GA biosynthetic pathways (*gal-3* in *Arabidopsis; an1* and *d3* in maize, *ls-1* and *lh-2* in pea, *dx* in rice). Some show reduced amylase activity as in GA deficient dwarf mutant in barley. Others are GA responsive mutants as *gai* and *spy* in *Arabidopsis*. Some short height mutants, e.g. *Rht3* in wheat and *D8* in maize are GA-deficient mutants, and do not respond to applied GA3 (Ross et al., 1997). In the improvement of many cereals, such as wheat, rice, sorghum and barley, dwarf mutants (natural or induced) have played a crucial role in producing lodging-resistant and high fertilizer responsive varieties.

The genetic analysis of abscisic acid signal transduction has been based on many ABA-deficient mutants, such as *aba1* in *Arabidopsis* and *aba2* in *Nicotiana plumbaginifolia* which are orthologous as shown by transposon-tagging isolation and mapping. Other mutants with changed sensitivity response to ABA application, such as *abi1, abi2, abi3* and *abi4*, show a marked reduction in seed germination (Merlot & Giraudat, 1997). Such genes are highly valuable for breeding cereal varieties, which sprout *in situ* on the ear during seed maturation.

The *Arabidopsis* mutant *etr1* which confers ethylene synthesis and perception has a major value in increasing the shelf-life of fruits and extended flower-life and delayed senescence as shown by its transfer to tomato and petunia (Wilkinson et al., 1997). Many such mutants, such as *ein, ain* in *Arabidopsis* and *Nr* (never ripe) in tomato, have severely limited or impaired response to ethylene. Such mutants shall have a major role in the international trade of fruits, such as papaya, pineapple, mango, and banana, and cut flowers, which rapidly spoil after ripening.

Many homeotic mutants, which develop defective flowers, have been isolated in *Arabidopsis, Petunia, Antirrhinum* and *Lycopersicon*. Alone or in combination, three groups of genes, A, B, and C regulate the formation of unique organs in the four whorls of dicot flowers. Among these are the floral homeotic gene mutations *DEFICIENS A, GLOBOSA, APETLA3, AGAMOUS* and *PLENA* in *Antirrhinum, GREEN PETALS* in petunia, *PISTILLATA, SQUAMOSA, FLORICULA* and *AGAMOUS* in tomato (designated TAG1 which change floral structures, such as petals, sepals, anthers (Pnueli et al., 1994). Homeotic mutants for leafy cotyledons *lec* obtained through insertional mutagenesis in *Arabidopsis*, are defective in maturation of embryos which remain green (Meinke, 1992). The *fis* mutants, which determine seed development independently of fertilization, have a critical role in understanding apomixis (Chaudhury et al., 1997).

The isolation of the mutants, which determine development of seed, flowers and fruit, has contributed significantly to our understanding of the basic patterns of development in plants. The developmental patterns in crop plants ultimately determine the yield and quality of the crops. The possibility to modify them will open up a new dimension in plant breeding. The recent investigations on the *INDETERMINATE(ID1)* mutant in maize confirm the translocation of signal from the immature leaves to the shoot apical meristem, where it induces flowering (cf. Aukerman & Amasino, 1998). This may be the first molecular clue to the elusive florigen, implicated in the photoperiodic response of flowering in plants.

Mutants for changing starch quality

Starch is the main carbohydrate material stored in the amyloplasts of seeds and tubers, and is the main source of energy in human diet. Most of the starch is derived from a few crops: rice, wheat, maize, sorghum, oats, barley, cassava, potato, sweet potato, banana and plantain. Starch can be divided into two types of macromolecules, amylose and amylopectin. Amylose is an essentially linear molecule of molecular weight between 5×10^5 and 10^6 composed of anhydroglucose units connected through α linkages. Amylopectin has a molecular weight of several millions, and is much-branched polymer formed by anhydroglucose units but additionally with 2 to 4% α-linked branches. Starches from most plants are composed of about 30% amylose and 70% amylopectin.

Mutations which affect starch biosynthesis can dramatically alter the amount of both components, which in turn can change the physico-chemical properties of the starch granules as has been shown in maize, wheat, rice and pea. A large number of starch mutants have been recognized in maize (Creech, 1965; Nelson & Pan, 1995). Among these are the *sugary* (*susu*) in maize (Hannah et al., 1993), which is a debranch-

ing type and the waxy character mutants (*wx* loci). Many mutants have been induced in pea by chemical mutagenesis (Blixt, 1972). At least six loci have been identified in pea, which modify starch composition and use. In five of them, the *regosus* loci (*r*), the dry seeds are wrinkled, like the one described by Gregor Mendel (Mendel, 1865). The mutation analyzed by Mendel is caused by a transposon-like insertion in the gene (Bhattacharyya et al., 1990). The alleles produced by chemical mutagenesis all have single base pair changes (MacLeod, 1994).

Grain texture of wheat 'soft' and 'hard' is controlled by the expression of a single major gene, *Hardness* (*ha*), located on the short arm of chromosome D. Alleles of hardness gene are present on the 5A and 5B chromosomes of hexaploid wheat but are not expressed. Friabilin, which is a 15-kDa marker protein for grain softness (*ha*), consists of two proteins, puroindoline a and b (pinA and pinB). This protein is present on the surface of water-washed starch from soft wheat in high amounts and on hard wheat starch in small amounts. It is absent in durum wheat starch. Recently, the hardness and softness of grain in wheat (*Triticum aestivum* L. em Thell) has been shown to be linked to mutation of glycine to serine in pinB or null mutation in pinA, which leads to the absence of protein pinA. These mutations have been linked to grain hardness. The complete linkage between this mutation in pinB and hard grain texture among 5D chromosome substitution lines suggests that pinB is involved in the control of grain texture. It seems that mutations in either component of friabilin, pinA, or pinB, can change grain hardness (Giroux & Morris, 1998).

Future prospects

Two new sets of technologies, *in vitro* culture and molecular methods have created a new paradigm in the use of mutations in crop improvement. The determination of radio-sensitivity tests, irradiation with optimal doses and multiplication of irradiated material through tissue culture techniques has assumed a new dimension. With *in vitro* culture, milligram quantities of tissues and calli can now be subjected to mutagenesis, which in future may reduce to micrograms when routine methods of regeneration from cell suspension cultures have been established. Presently, there are only a few vegetative propagated plants such as banana, sugarcane and *Alstroemeria* that can be regenerated from cell suspension cultures and that also not on a routine basis. On the other hand, many cultivars of seed propagated crops can now be regenerated from cell suspension cultures; e.g. maize, rice, wheat, barley, and soybean. Cells in suspension cultures often become small clumps rather than single cells. It is anticipated that the radiation dose required to induce mutations in cell suspension cultures would be even lower than that for callus cultures. We indeed look forward to the development for routine cell suspension culture techniques and use of bioreactors to induce mutations both in the seed and vegetatively propagated plants. Equally important would be the development of *in vitro* cell selection systems for resistance to diseases, where toxins can be used in the culture media as has been shown for the selection of herbicide tolerance. The regeneration capacity of such cell suspension cultures and the stable transmission of the selected trait to the derived plant would be the critical test of such systems. Particularly, if such cell suspension systems can be developed from haploid plants derived from microspore cultures, the probability to obtain recessive mutants in homozygous condition would be enhanced many more times. A combination of the existing techniques of anther and microspore culture, cell suspension culture, irradiation of haploid cells, chromosome doubling and regeneration of doubled haploid plants (Szarejko et al., 1995) could be used to obtain the desired genotypes in a short duration.

The identification, and analysis of mutants is based on the use of molecular techniques of DNA fingerprinting and mapping on PCR based markers, such as RAPD (Random Amplified Polymorphic DNA, AFLP (Amplified Fragment Length Polymorphism) and STMS (Sequence-Tagged Microsatellite Sites). Site-specific insertion of a single base into a targeted gene by using chimeric RNA/DNA oligonucleotides as demonstrated in tobacco (Beetham et al., 1999) and maize (Zhu et al., 1999), and mutant tagging shall bring a new dimension in gene technology. Already, mutations can be linked to changes in DNA sequences for some plant traits, and to establish molecular maps in structural and functional genomics of crop plants. These in turn would lead to a rapid enhancement of crop yields and quality. Induced mutations have thus assumed a new dimension, not only in crop improvement but also in exploring biology. Thus, 'after 50 years of dazzling progress, we find ourselves still dependent on the use of mutants for probing the intricacies of biological processes, and on an understanding of the regulation and physiology of mutation for

probing the subtle biological mechanisms responsible for balancing genomic stability with plasticity' (Fox, 1998).

References

Anonymous, 1995. Bureau of Economic and Agricultural Statistics, Bangkok, 1995.

Aukerman, M.J. & R.M. Amasino, 1998. Floral induction and florigen. Cell 93: 491–494.

Beetham, P.R., P.B. Kipp, X.L. Sawycky, C.J. Arntzen & G.D. May, 1999. A tool for functional plant genomics: chimeric RNA/DNA oligonucleotides cause *in vivo* gene-specific mutations. Proc Natl Acad Sci USA 96: 9774–8778.

Bhattacharyya, M.K., A.M. Smith, T.H.N. Ellis, C. Hedley & C. Martin, 1990. The wrinkled-seed character of peas described Mendel is caused by a transposon-like insertion in a gene encoding starch branching enzyme. Cell 60: 115–122.

Blixt, 1972. Mutation genetics in *Pisum*. Agri Hortique Genetica 30: 1–293.

Broertjes, C., 1977. Mutagen treatment and handling of treated material. In: Manual on Mutation Breeding. Second Edition, International Atomic Energy Agency, Tech Rep Ser No 119. Vienna, pp. 160–168.

Brunner, H. & H. Keppl, 1991. Radiation induced apple mutants of improved commercial value. In: Proc Plant Mutation Breed for Crop Improvement. Vol. 1. Internl Symp, IAEA and Food Agric Org of the UN, IAEA SM-311, Vienna, pp. 547–552.

Chakrabarti, S.N., 1995. Mutation breeding in India with particular reference to PNR rice varieties. J Nuclear Agric Biol 24: 73–82.

Chaudhury, A.M., L. Ming, C. Miller, S. Craig, E.S. Dennis & W.J. Peacock, 1997. Fertilization-independent seed development in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 94: 4223–4228.

Creech, R., 1965. Genetic control of carbohydrate synthesis in maize. Genetics 52: 1175–1186.

Dribnenki, J.C.P., A.G. Green & G.N. Atlin, 1996. Linola™ 989 low linolenic flax. Can J Plant Sci 76: 329–331.

Fox, M.S., 1998. Some recollections and reflections on mutation rates. Genetics 148: 1415–1418.

Giroux, M.J. & C.F. Morris, 1998. Wheat grain hardness results from highly conserved mutations in the friabilin components puroindoline a and b. Proc Natl Acad Sci USA 95: 6262–6266.

Green, A.G., 1986. A mutant genotype of flax (*Linum usitatissimum*) containing very low levels of linolenic acid in its seed oil. Can J Plant Sci 66: 499–503.

Hannah, C., M. Giroux & C. Boyer, 1993. Biotechnological modification of carbohydrates for sweet corn and maize improvement. Scientia Horticulturae 55: 177–197.

Hensz, R.A., 1991. Mutation breeding of grapefruit (*Citrus paradisi* Macf.). In: Proc Plant Mutation Breed for Crop Improvement. Vol. 1. Internl Symp IAEA and Food Agric Org of the UN, IAEA SM-311, Vienna, pp. 533–536.

IAEA, 1997. Report 2nd FAO/IAEA Research Co-ordination Meeting on *in vitro* techniques for selection of radiation induced mutants adapted to adverse environmental conditions. International Atomic Energy Agency, Vienna.

Lapade, A.G., A.M.S. Veluz & I.S. Santos, 1995. Genetic improvement of the Queen variety of pineapple through induced mutation and *in vitro* culture techniques. In: Proc Induced Mutations and Molec Techn for Crop Improvement. Internl Symp IAEA and Food Agric Org of the UN, IAEA, Vienna, pp. 684–687.

Love, S.L., T.P. Baker, A. Thompson-Johns & B.K. Werner, 1996. Induced mutations for reduced tuber glycoalkaloid content in potatoes. Plant Breed 115: 119–122.

Leyser, O., 1997. Auxins: Lessons from a mutant weed. Physiol Plant 100: 407–414.

MacLeod, M.R., 1994. Analysis of an allelic series of mutants at the *r* locus of pea. PhD Thesis, Univ East Anglia, UK.

Mak, C., Y.W. Ho, Y.P. Tan & R. Ibrahim, 1996. Novaria – A new banana mutant induced by gamma irradiation. Informusa 5: 35–36.

Maluszynski, M., B. Sigurbjörnsson, E. Amano, L. Sitch & O. Kamra, 1992. Mutant varieties-data bank, FAO, IAEA database. Part II. Mutation Breed Newsl 39: 14–17.

Maluszynski, M., B.S. Ahloowalia & B. Sigurbjörnsson, 1995. Application of *in vivo* and *in vitro* mutation techniques for crop improvement. Euphytica 85: 303–315.

Meinke, D.W., 1992. A homeotic mutant of *Arabidopsis thaliana* with leafy cotyledons. Science 258: 1647–1649.

Miklashevichs, E. & R. Waldon, 1997. Plant mutants with altered response to cytokinins. Physiol Plant 100: 528–533.

Mendel, G., 1865. Versuche über Pflanzen-hybriden. Verhandlungen des Naturforsehenden Vereins in Brünn 4: 3–47.

Merlot, S. & J. Giraudat, 1997. Genetic analysis of abscisic acid signal transduction. Plant Physiol 114: 751–757.

Maruyama, K., H. Araki & H. Kato, 1991. Thermosensitive genic male sterility induced by irradiation. In: Rice Genetics II. IRRI, Manila, Philippines, pp. 227–235.

Nelson, O. & D. Pan, 1995. Starch synthesis in maize endosperms. Ann Rev Plant Physiol Plant Molec Biol 46: 475–496.

Pnueli, L., D. Harven, S.D. Rounsley, M.F. Yanofsky & E. Lifschitz, 1994. Isolation of the tomato Agamous gene TAG1 and analysis of its homeotic role in transgenic plants. Plant Cell 6: 163–173.

Ross, J.J., I.C. Murfet & J.B. Reid, 1997. Gibberellin mutants. Physiol Plant 100: 550–560.

Sanada, T., K. Kotobuki, T. Nishida, H. Fujita & F. Ikeda, 1993. A new Japanese cultivar 'Golden Nijisseike', resistant mutant to black spot disease of Japanese pear. Japan J Breed 43: 455–461.

Schmülling, T., S. Schäfer & G. Romanov, 1997. Cytokinins as regulators of gene expression. Physiol Plant 100: 505–519.

Shen, Y.W., Q.H. Kai & M.W. Gao, 1993. A new thermosensitive radiation induced male-sterile rice line. Rice Genet Newsl 10: 97–98.

Swaminathan, M.S., 1998. Crop production and sustainable food security. In: V.L. Chopra, R.B. Singh & A. Varma (Eds.), Crop Productivity and Sustainability – Shaping the Future. Proc 2nd Internl Crop Science Cong Oxford and IBH Publishing Co Pvt Ltd New Delhi, pp. 3–18.

Szarejko, I., J. Guzy, J. Jimenez Davalos, A. Roland Chaves & M. Maluszynski, 1995. Production of mutants using barley DH systems. In: Proc Induced Mutations and Molec Techn for Crop Improvement. Internl Symp IAEA and Food Agric Org of the UN, IAEA, Vienna, pp. 517–530.

Yabuta, T. & Y. Sumiki, 1938. On the crystal of gibberellin, a substance to promote plant growth. J Agr Chem Soc Jpn 14: 1526.

Wilkinson, J.Q., M.B. Lanahan, D.G. Clarck, A. Bleecker, C. Chang, E.M. Meyerowitz & H.J. Klee, 1997. A dominant mutant receptor from *Arabidopsis* confers ethylene insensitivity in heterologous plants. Nature Biotech 15: 444–447.

Zhu, T.D., D.J. Peterson, L. Tagliani, G. St. Clair, C.L. Baszczynski & B. Bowen, 1999. Targeted manipulation of maize genes *in vivo* using chimeric RNA/DNA oligonucleotides. Proc Natl Acad Sci USA 96: 8768–8773.

Vol 18:

COMPARISON OF X-RAY AND FAST NEUTRON-INDUCED MUTANT SPECTRA. EXPERIMENTS IN ARABIDOPSIS THALIANA (L.) HEYNH.

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INTRODUCTION

Research into the specific effects of mutagens in higher plants indicates that particular mutants are more frequently or even exclusively obtained with a specific mutagen (Ehrenberg et al., 1959; Gustafsson, 1963; McKelvie, 1963; Nilan et al., 1959) and mutagen specificity of X-rays or *gamma*rays and fast neutrons for some individual loci was revealed by genetic analysis of erectoides and eceriferum mutants in barley (Lundquist, 1975; Lundquist et al., 1962; Lundquist et al., 1968; Persson et al., 1969).

Although Conger and Constantin (1974), Gaul (1964) and Nilan (1964) showed that both experimental conditions and mutagen dose affected the mutant spectrum, these factors have not usually been considered in work on mutagen specificity.

The present study about the effect of X-rays or fast neutrons on the mutant spectrum in Arabidopsis was carried out to extend the data on X-ray and fast neutron specificity and to study more closely the effect of dose on mutant spectrum. Moreover, irradiation was applied with or without a dithiothreitol (DTT) pretreatment.

Fast neutrons induce relatively more chromosome breaks and less base damage than X-rays (Ahnstrom, 1977, 1979; Hawkins, 1979). This could possibly explain the difference in induced mutant spectrum. Since the data recorded in the literature (Malvarez et al., 1965; Schans et al., 1979) indicate that irradiation in the presence of -SH compounds reduces the ratio standard breaks/base damage, the effect of DTT on X-ray and fast neutron-induced mutant spectra could elucidate whether the spcificity of radiation type is influenced by its relative frequency of induced strand breaks.

MATERIAL AND METHODS

Plant material. Arabidopsis thaliana (L.) Heynh. is a small, fast growing, self-fertilizing crucifer. Seed stocks used in the experiment were of the mutant erecta of the ecotype "Landsberg" (Redei, 1962).

Pretreatment. To break dormancy, seeds were kept on moist filter paper at 2*degrees*C for 5 days and redried (24*degrees*C, 24 hr). The redried seeds were submerged in tapwater (=0% DTT) or DTT solution (1.2% DTT), at 22*degrees*C, 3 hr before irradiation.

Irradiation. X-ray exposures were 140, 233, 327 and 420 Gy (10 Gy = 1 krad) for seeds submerged in 22*degrees*C tapwater; and 280, 467, 653, and 840 Gy were given to seeds submerged in 22*degrees*C DTT solution. The irradiation was carried out with an MG 301 X-ray machine with an MCN 420 tube, operating at 320 kVp and 10 mA, with an additional filter of 0.25 Cu and 1.0 Al and with a dose rate of 4 Gy/min. Fast neutrons (20, 33, 47 and 60 Gy to seeds submerged in 22*degrees*C 1.2% DTT) were given in the irradiation room of the BARN (Biological Agricultural Reactor Nederlands, Wageningen) with a dose rate 1 Gy/min. The *gamma*contamination was low, only approximately 3 percent on Gy basis. The maximum duration of irradiation was 3.5 hr. In order to have uniform environmental conditions, the seeds were submerged during irradiation as well as before (3 hr pretreatment) and after. From start to finish, the seeds were submerged for 6.5 hr at 22*degrees*C, and subsequently rinsed with tapwater (5min.) and sown.

The treatments described above (18 including the controls, Table 1) were done twice, with an interval of approximately one year. The X-ray and fast neutron dose ranges applied, in the presence or absence of DTT, induced approximately comparable levels of genetic damage, i.e., M 1-ovule sterility (Table 1), M 2-embryonic lethality, frequency of chlorophylls and viable mutants (Dellaert, 1980).

Culture medium and culture conditions. The seeds were sown (equally spaced) in portions of 30 on a standard mineral medium in a petri dish and put to germinate at 24*degrees*C under continuous illumination using fluorescent light tubes, 8000 lux/cm 2. After 8 days the seedlings were transplanted into soil in an air conditioned greenhouse. The culture medium and culture conditions used were as described by Feenstra (1964) and Oostindier-Braaksma and Feenstra (1973).

M 1-generation. In the M 1-generation Moller's embryo test (Moller, 1961, 1963) was applied to silique number 5 or 6 of the main inflorescence for scoring M 1-ovule sterility, M 2-embryonic lethals and chlorophylls (details of the results will be published elsewhere). Since chimerism is progressively lost upwards along the stem (Balkema, 1971) and within flower chimerism does not occur frequently in Arabidopsis (Dallaert, 1980; Ivanov, 1973), chimerism was in the majority of cases avoided by harvesting a single silique from the top of the main inflorescence per M 1-plant for progeny testing. Only well filled siliques were harvested, in order to 1) increase the germination frequency of the M 2-seeds, 2) increase the fertility in the M 2-generation, and 3) decrease the number of deviant M 2-plants caused by chromosomal aberrations, without reducing the mutant frequency (Mesken et al., 1968; Moller, 1966). The percentage M 1-plants harvested are given in Table 1.

Click here for table

Table 1: Treatments applied and plant material used for the determination of X-ray and fast neutron induced mutant spectra in Arabidopsis.

Click here for table

Table 2: Classification of radiation-induced mutants in Arabidopsis according to phenotype (the main groups of the classification system of Burger (1971) and Kranz (1978), mutagen (X-rays versus fast neutrons) and the conditions of the treatment (0% DTT versus 1.2% DTT).

M 2-generation. Viable mutants were scored in the M 2-generation by testing the progenies of the harvested M 1-plants. Five to twelve seeds were sown per M 1-progeny. Cold treatment (5 days at 2*degrees*C; to break dormancy) was given immediately after sowing. The other culture conditions and the culture medium used, were as described above.

For establishing the fertility in the M 2-generation Moller's embryo test was applied to silique number 5 or 6 of the main inflorescence of one randomly selected plant per M 2-line.

Mutant spectrum. Viable mutants in the M 2-generation were defined as flowering plants showing deviations from the wild type in plant morphology and/or leaf colour. A random sample from M 2-lines, which segregated for viable mutants was harvested and progeny tested to verify the mutant type. Based on the phenotype characteristics, the viable mutants were classified into different groups per treatment (Table 2). The mutant groups distinguished conform to the classification system for morphological and seed colour mutants described by Burger (1971) and Kranz (1978). In addition to this classification system the following mutant phenotypes (Table 2) were ditinguished:

- Vital yellow-green mutants (phenotype group 1), in this group all flowering chlorophyll deficient mutants were classified.

- Mutants with more rosette leaves and late flowering, ones as described by Hussein and Van der Veen (1965, 1968) (Phenotype group 6).

- Mutants with hanging pods and short pod-stalks (phenotype group 12), one mutant with this phenotype was described by Burger (1971), but she did not classify this mutant in a separate group.

- Mutants with a different flower morphology but not belonging to the Flosculimut groups described by Burger (1971) and Kranz (1978), such as the sterile "double-flowering" agamous, similar to the one describd by Conrad (1971) (phenotype group 15).

- Gibberellin-sensitive mutants (phenotype group 17), such as described by Koornneef et al. (1977)

To test the effect of the various treatments (radiation type and DTT pretreatment) independently arisen mutants were classified according to three factors, i.e., mutagen, M (X-rays versus fast neutrons), the condition of the treatment, D (0% DTT versus 1.2% DTT), and the mutant phenotype, P (Table 21). Mutants were considered to be independent when they occurred in different M 1 progenies.

The mutant frequency, m, per mutant group was expressed as the mutation frequency per cell (Frydenberg, 1963; Gaul, 1957).

Click here for figure

RESULTS AND DISCUSSION

The effect of pre selection for "well filled" siliques in M 1 on the relation M 1-versus M 2-percentage ovule sterility.

Without selection for fertility in the M 1-generation a large part of the radiation-induced M 1-sterility is transmitted to the M 2-generation (Gaul, 1963; Mesken et al., 1968; Moller, 1966). From the data from Mesken and Van der Veen (1968) it was deduced that without selection for fertility approximately 45 percent of X-ray induced M 1-ovule sterility in Arabidopsis was transmitted to the M 2-generation. In the present experiment, where only "well-filled" siliques were harvested in the M 1-gneration for progeny testing, it was calculated from the M 1- and M 2-ovule sterility data in Table 1, that the relation between the sterility percentages in the two generations can be algebraically described by:

Click here for figure

This means that approximately 12 percent of the M 1-ovule sterility has been "transmitted" to the M 2-generation. This low percentage can be ascribed to the effect of selection for "well-filled" siliques in the M1. However, it should be noted that the effect of selection could not be accurately measured, since no data were available from a direct comparison (at the same time) of the induced M 1-sterility, M 2-sterility without, and M 2-sterility with pre selection for M 1-fertility.

The effect of radiation type on induced mutant spectrum.

Table 2 gives the number of independently arisen mutants, classified to phenotype (P), mutagen (m) and conditions of the treatment (D). The data from a three-way contingency table. To test whether or not the observed mutant spectrum, i.e., the frequencies of the different mutants, was influenced by the type of radiation (X-rays or fast neutrons) and by the pretreatment with dithiothreitol (DTT) a G-test for independence of the three variables (M, D, P) was used (SOKAL et al., 1969). From Table 3 it can be seen that there was a significant ($p<0.005$) association between mutagen and mutant phenotype (M x P). This indicates that the mutant spectrum was significantly affected by the radiation type, i.e., X-rays or fast neutrons, by which the mutants were induced.

Click here for table

Table 3: Analysis of independence P x M x D*

*Analysis of the data from Table 2; P = mutant phenotype group (17 categories, M = mutagen (X-rays or fat neutrons), D = condition of treatment (0% DDT or 1.2% DTT)

After close examination of the data (Table 2), it was possible to group the 17 mutant phenotypes into three, namely: -P I, the phenotype groups in which the number of fast neutron induced mutants was approximately twice (or more) the number of X-ray-induced mutants, i.e., the long hypocotyl mutants, loosely packed rosette leaf mutants, roundish or broad leaf mutants, eceriferum mutants, mutants with hanging pods and short pod stalks, mutants with a different flower morphology, mutants with yellow seeds, and gibberellin-sensitive mutants.

- P II, the phenotype groups in which conversely the number of X-ray induced mutants was approximately twice (or more) the number of fast neutron induced mutants. These were only mutants with closely packed broad leaves and short petioles, and

-P III, the remaining mutant phenotypes.

In none of the phenotype classes was a significant effect of radiation type on mutant spectrum left. This independence was expected because of the criteria used for grouping the mutant phenotypes. It can be concluded that the significant G-value for the P x M association can be attributed to the differential distribution of X-ray-compared to fast neutron-induced mutants over the phenotype classes P I, P II, and P III, respectively (Table 4).

Click here for table

Table 4: Analysis of independence P I, II, III x M x D*

*Analysis of the data from Table 6; P I, II, III = mutant phenotype class (3 categories), M = mutagen (X-rays or fast neutrons), D = condition of treatment (0% or 1.2% DDT)

Click here for table

Table 5: Rank Test (White, 1952) for unpaired measurements after X-ray and fast neutron irradiation. A. the frequency of mutants with closely packed broad leaves with short petioles/100 cells B. the frequency of mutants with loosely packed leaves with long petioles/100 cells C. the frequency of mutants with roundish or broad leaves/100 cells D. the frequency of eceriferum mutants/100 cells E. the frequency of (vital) yellow-green mutants/100 cells

It is statistically unreliable to isolate individual lines from a contingency table and ascribe significance to them. Therefore, to test whether in the dose range studied, specific mutant phenotypes were more frequently induced with either X-rays or fast neutrons, rank-tests (White, 1952) were applied in which the mutant frequencies of specific mutants at various X-ray doses were compared with the ones obtained at various fast neutron doses. From these tests (Table 5) it can be concluded that mutants with closely packed broad leaves and short petioles were more frequently induced with X-rays ($p<0,05$), while mutants with loosely packed leaves and long petioles, and eceriferum mutants were significantly more frequently induced with fast neutrons ($p<0.05$ and $p<0.01$, respectively). For the remaining mutant types of the phenotype class P I, i.e. mutants which were relatively more frequently induced with fast neutrons, no significant differences between the frequencies were found (see for instance the frequencies of mutants with roundish or broad leaves in Table 5). Between the frequencies of X-ray- and fast neutron-induced mutants grouped into phenotype class P III (i.e. mutants which were observed approximately as frequently after X-rays as after fast neutrons such as the yellow-green mutants (Table 5), no significant differences were found.

The X-ray-induced mutant spectrum observed in the present study is in agreement with the spectrum reported by McKelvie (1963) in Arabidopsis, at least as far as similar mutant groups are concerned. McKelvie found a significant higher frequency of mutants with roundish or

broad leaves after X-rays than after EMS treatment.

The effect of dithiothreitol pre-treatment on induced mutant spectrum.

From Table 3 it can be seen that there was no significant association between mutant phenotype (P) and the DTT treatment (D). This suggests that the observed mutant spectrum was independent of the DTT pre-treatment. However, when the effect of DTT on the distribution of the mutants over the phenotype classes P I, P II, and P III was tested, a weakly significant association between the phenotype classes and DTT was found (0.05 Since DTT protects against single as well as double strand-breaks (Chapman et al., 1975) and does not affect the induced base damage (Schans et al., 1979), the observed DTT effect indicates that the ratio strand-breaks/base-damage is higher for P I mutants than for P II and P III mutants.

Click here for table

Table 6: Classification of radiation-induced mutants in Arabidopsis according to phenotype class P I, P II and P III; mutagen (X-rays or fast neutrons) and dithiothreitol treatment (0% or 1.2% DTT)

The mutant frequency per mutant group.

The study of the mutation rate of specific loci might elucidate to what extent mutability is an intrinsic property of the gene or of the organization of the whole genome. Apart from the effect of the experimental conditions, it is found that the mutability of individual loci within an organism may vary by several orders of magnitude (Lundquist, 1975; Persson et al., 1969; Stadler, 1942; Westergaard, 1959;). This indicates that the intrinsic property of the gene and/or the "location" of the gene on the chromosome affects its mutability. It was noticed by LI and Redei (1969) that mutations for thiamine auxotrophy occurred with the same frequency, i.e., 0.75 x 10 E-4 per locus per genome, in Penicillium and in Arabidopsis, which shows that homologous loci can have similar mutability. Unfortunately, very few data are available for the comparison of mutation rates of specific loci across phylogenetic boundaries (Li et al., 1969; Yonezawa, et al., 1975).

To relate the mutant frequencies observed in the present study to those found on other experiments (with Arabidopsis or with other plant species) the mutant frequency per mutant group per cell is given in Table 7. From these values and from the number of loci per mutant group, a rough estimate for the average mutation frequency per locus per cell, i.e. 1 x 10E-3, in the present experiment was determined. Thus, the mutation rate of the "loci" studied was higher than that of the thiamine loci. This may be due to either different experimental conditions (mutagen, dose) or to a different mutability per se. However, it should be noticed that the estimated frequency of the mutation rate per locus per genome by Li and Redei (1969) might be underestimated due to the error annexed with their calculation method. Apart from errors in estimating the genetic effective cell number per M 1-plant progeny, their estimate is dependent on M 1-progeny size. This is in contrast to Gaul's method (Frydenberg, 1963; Gaul, 1963; Yonezawa et al., 1975) which was used in this study.

Click here for table

Table 7: The mutant frequency per mutant group in Arabidopsis after X-ray or fast neutron irradiation, with or without 1.2% dithiothreitol (DTT) pre-treatment.

Apart from the effect of radiation type, the data in Table 7 do not indicate a difference in the mutability of the loci per mutant group per cell.

It is calculated that in the dose range studied, 1 Gy fast neutrons was generally equivalent to 7 Gy X-rays for the induction of mutants. However, for the induction of mutants with loosely packed leaves and long petioles and for eceriferium mutants, 1 Gy fast neutrons was equivalent to 22 and 18 Gy X-rays, respectively, while for the induction of mutants with closely packed broad leaves and short petioles 1 Gy fast neutrons was equivalent to 1.6 Gy

X-rays. In barley it was found that 1 Gy fast neutron was equivalent to 46.1 Gy X-rays for the induction of eceriferum mutants (Lundquist, 1975). Thus, fast neutrons are more effective in barley than in Arabidopsis for the induction of eceriferum mutants. This indictes that, at least for the eceriferum loci in Arabidopsis and barley, 'homologous' loci have a different mutability.

In barley, significant mutagen specificity in the mutability of individual eceriferum and erectoides loci of X-rays or Gamma-rays and fast neutrons was observed (Lundquist, 1975, Lundquist et al., 1962, 1968; Persson et al., 1969). The number of mutants per locus per radiation type in Arabidopsis are still too low to pronounce upon mutagen specificity for individual loci.

Conclusion

The most significant effect observed in the present experiment was the difference between X-ray- and fast neutron-induced mutant spectra. The data are in agreement with the results found in barley and show that the difference in spectra is consistent over dose. It is found that fast neutrons are more efficient for the induction of specific mutants, i.e., the ones belonging to class P I. The fact that the relative frequency of P I mutants was less with a DTT pre-treatment than without, irrespective of radiation type by which the mutants were induced, suggests that P I mutants more often originate from strand-break damage than the P II and P III mutants. Therefore, the data indicate that the difference between X-ray and fast neutron-induced mutant spectra is caused by the relatively higher frequency of breaks after fast neutrons than after X-rays. In this respect it is worth mentioning that Schubert and Rieger (1976) found differences between segmental response of chromosones I and V in Vicia faba for the induction of aberrations by X-rays and fast neutrons. This indicates that certain parts of the chromosomes have a better ability to 'repair' induced strand-breaks than others. This specific 'repair' ability might be the basis for differences between X-ray and fast neutron-induced mutant spectra.

SUMMARY

Arabidopsis Seeds were irradiated with X-rays or fast neutrons, in the presence or absence of dithiothreitol. Well-filled siliques were selected in the M 1-generation, resulting in a good M 2-fertility. In the M 2-generation, where specific mutants were used as parameter, a significant differentc ($p<0.005$) was found between X-ray and fast neutron-induced mutant spectra. X-rays, for instance, induced more mutants with closely packed broad leaves with short petioles, while fast neutrons induced more mtuants with loosely packed leaes iwth long petioles as well as more eceriferum mutants. This difference between the mutability of certain characters by X-rays and fast neutrons was consistent ovr several doses.

The -SH radioprotector dithiothreitol did not significantly influence the mutant spectra observed. However, certain mutant types, notably those more frequently induced by fast neutrons, seem to be less frequent after irradiation in the presence of DTT.

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REFERENCES

Ahnstrom, G.: Radiobiology. In: Manual on mutation breeding. 2nd ed. Technical report series no. 119, IAEA, Vienna, pgs 21-28 (1977)

-- : In: Utilization of induced mutations for crop improvement. Proc. Regional Seminar, Ibadan, Nigeria 1978. IAEA, Vienna, in press (1979)

Balkema, G.H.: Chimerism and diplontic selection. Thesis, Landbouwhogeschool, Wageningen.

Balkema, Rotterdam Kapstadt (1971)

Borger, D.: Arabid. Inf. Serv. 8, 36-42 (1971)

Chapman, J.D., C.J. Gillespie, A.P. Reuvers and D.L. Dugle: Radiat. Res. 64, 365-375 (1975)

Conger, B.V. and M.J. Constantin: In: Biological effects of neutron irradiation. Proc. symp. Neuherberg, 1973, IAEA, Vienna, pgs 417-432 (1974)

Conrad, D. von: Biol. Zentralbl. 90, 109-126 (1980)

Dellaert, L.M.W.: Mutat. Res. 71, 109-126 (1980)

-- : Exp. Environm. Bot. 20, 309-323 (1980)

-- : Theor. Appl. Genet. 57, 137-143 (1980)

Ehrenberg, L., A: Gustafsson and U. Lundquist: Hereditas 45, 351-368 (1959)

Feenstra, W.J.: Genetica 35, 259-269 (1964)

Frydenberg, O.: Radiat. Bot. 3, 135-143 (1963)

Gaul, H.: Z. Pflanzenzucht. 38, 63-76 (1957)

-- : In: Induction of mutations and the mutation process. Symp. Czech. Ac. Sci., Praha, Sept. 1963, pgs. 62-72 (1963)

-- : Radiat. Bot. 4, 155-232 (1964)

Gustafsson, A.: Hereditas 50, 211-263 (1963)

Hawkins, R.B.: Int. J. Radiat. Biol. 35, 1-13 (1979)

Hussein, H.A.S. and J.H. Van der Veen: Arabid. Inf. Serv. 2, 6 (1965)

-- : Arabod. Inf. Serv. 5, 30 (1968)

Ivanov, V.I.: Arabid. Inf. Serv. 10, 31-32 (1973)

Koornneef, M., A. Barbaro and J.H. Van Der Veen: Arabid. Inf. Serv. 14, 14-17 (1977)

Koornneef, M. and J.H. Van Der Veen: Arabid. Inf. Serv. 15, 38-43 (1978)

Kranz, A.R.: Arabid. Inf. Serv. 15, 118-127 (1978)

Li, S.L. and G.P. Redei: Radiat. Bot. 9, 125-131 (1969)

Lundquist, U.: In: Barley Genetics III, Proc. 3rd Intern. Barley Gen. Symp., Pullman, Washington, Washington State Univ. Press, Washington, pgs. 162-163 (1975)

-- and D. von Wettstein: Hereditas 48, 342-362 (1962)

--, P. von Wettstein-Knowles and D. von Wettstein: Hereditas 59, 473-504 (1968)

Malvarez, E.M., E.A. Favret and G.I. Ryan: Boletin Genetico 1, 3-16 (1965)

McKelvie, A.D.: Radiat. Bot. 3, 105-123 (1963)

Mesken, M. and J.H. Van der Veen: Euphytica 17, 363-370 (1968)

Muller, A.J.: Naturwissenschaften 48, 579 (1961)

-- : Biol. Zentralblatt 82, 133-163 (1963)

-- : In: Erwin-Baur-Gedachtnisvorlesungen IV, 89-97 (1966)

Induced mutations in plant breeding and biological researches in Japan

H Nakagawa

Abstract

Two hundred and forty two direct-use mutant varieties generated by using irradiation, chemical mutagenesis and somaclonal variations, have been registered in Japan. About 61% of these were induced by Gamma-ray irradiation, largely due to successful collaboration with the Institute of Radiation Breeding. This high percentage of Gamma-ray irradiated mutants indicates that mutation breeding via Gamma-ray irradiation is an effective and highly successful approach for the generation of commercial cultivars. Some mutant cultivars of Japanese pear exhibiting resistance to diseases induced by Gamma-ray irradiation and development of a unique bioassay by using toxins of fungi was discussed. In addition, 228 indirect-use (hybrid) mutant varieties primarily generated in rice and soybean have found value as parental breeding germplasm resources in Japan. In 2005, two direct-use cultivars and 97 indirect-use cultivars of rice contributed approximately 12.4% of the total area of rice cultivation in Japan. The semi-dwarf gene (*sd-1*) generated in rice is perhaps one of the most significant contributions. For soybean, similar Gamma-ray induced mutants comprised nearly 9.4% of the total cultivation area of soybean in Japan. Molecular genetic studies focused on genome sequencing have become an extremely powerful tool for identifying the genes and for selecting mutants exhibiting specific phenotypes. It is anticipated that molecular genetic interaction will complement gains in mutation breeding on a dramatic scale. Chronic irradiation in the Gamma Field is also considered to be a useful tool for generating mutant resources for future molecular studies especially in rice, and expand its use into the other graminaceous crops which have genomic synteny to rice. There are interesting reports concerning mutations in rice, such as low glutelin content, in which the size and location of deletions and the mechanisms and phenotypes of low glutelin content were elucidated. Chronic irradiation in the Gamma Field is useful to generate mutant resources for molecular researches.

Introduction

After the construction of the Gamma Field, now considered the worlds largest radiation field (**Fig. 1**, 100m radius with an 88.8 TBq ^{60}Co source at the center), the Gamma Room and the Gamma Greenhouse in the Institute of Radiation Breeding (IRB) in 1960's, mutation breeding was accelerated by cooperative research with national and prefectural breeding laboratories, private companies and universities in Japan [1].

In *The New York Times* (*In* "Useful Mutants, Bred With Radiation" by William J. Broad, August 27, 2007), Dr. P. J. L. Lagoda of the Joint FAO/IAEA was quoted to say, "Spontaneous mutations are the motor of evolution. We are mimicking nature in this. We're concentrating time and space for the breeder so he can do the job in his lifetime. We concentrate on how often mutants appear - going through 10,000 to one million - to select just the right one."

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Figure 1 Gamma Field of IRB

The concept and objectives of the IRB's Gamma Field has the same goals for the plant breeder. The facility is used to artificially induce mutations at a higher frequency than it occurs in nature. The radiation dose at the nearest point of the field (10m from the center, ca. 2 Gy/day) is estimated to be about 300,000 times that of normal and natural background radiation. At the farthest point (100m from the center, ca. 0.01 Gy/day), the radiation dosage is about 2,000 times that of normal background radiation. This means that growing plants at the nearest point to the Gamma-ray sources are being treated to a 1,000 year's of accumulated normal background rates of radiation per day. Although we do not know all the genes or mechanism of mutations, radiation breeding has produced many useful mutant cultivars and contributed greatly to the farmers and industries of Japan.

In 1991, the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan launched the Rice Genome Research Program (RGP), with the aim of fully decoding the rice genome in three phases over a 21-year period. With the cooperation of 10 participating countries [2], the genome sequencing of 12 rice chromosomes was completed in 2005 [3]. Following this achievement, molecular genetic studies based on the results of the genome sequencing project became the most powerful tool for selecting mutants of certain characteristics in rice. This is anticipated to revolutionize mutation breeding success in rice, and become applicable to a number of other important crop species.

In this report, the mutant cultivars developed mainly by Gamma-rays are discussed. In addition, their economic impacts in Japan, as well as molecular studies performed to elucidate the mutation at the DNA level are described.

Mutation breeding and released cultivars in Japan

In a 2007 search regarding the number of induced mutation varieties in the IAEA database, China is first in the number of described induced

mutation varieties at 638; India is second listing 272 varieties; and Japan is third with 233 varieties. The total number of mutant cultivars, including direct-use mutant cultivars and indirect-use cultivars, exceed these totals. A selection of mutant cultivars developed in Japan, including the economic impact of these cultivars, and their characteristics are reviewed here.

The number of cultivars developed by mutation breeding

Figure 2 shows the number of direct-use and indirect-use (hybrid) mutant cultivars registered in Japan in each five-year period from 1960 to 2005. The number of direct-use cultivars had been rapidly increasing until 1995, when 67 cultivars were registered in five years (about 13 cultivars per year). This number fell from 2001 to 2005, with only 41 cultivars being registered (about 8 cultivars per year). The number of indirect-use cultivars primarily generated in rice has steadily increased over time and 68 cultivars were registered from 2001 to 2005. This number can be increased if agronomically useful, direct-use cultivars, such as "Reimei" with the *sd1* dwarf gene for rice are developed.

Two hundred and forty two direct-use mutant cultivars comprising 61 species generated through irradiation utilizing Gamma-ray, X-ray and ion beams, chemical mutagenesis and in vitro culture (somaclonal variation), have been registered and released in Japan (**Fig. 3**). More than 61% of these were induced by Gamma-ray irradiation and those induced by somaclonal variation and chemical mutagen, not including those with double chromosome numbers through colchicine treatment, are 15.7% and 6.6%, respectively. Recently, the development of mutant flower cultivars, generated by ion beam irradiation, has been a growing area of mutation induction in Japan.

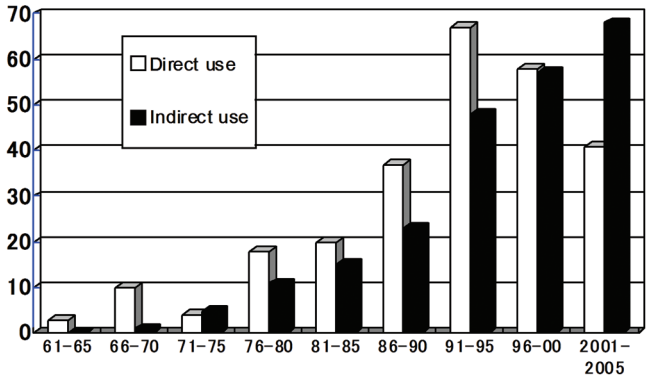


Figure 2 Number of cultivars developed by mutation breeding in each 5-year period from 1961-2005. Total number of direct use cultivars is 212 and that of indirect use cultivars is 230 [4].

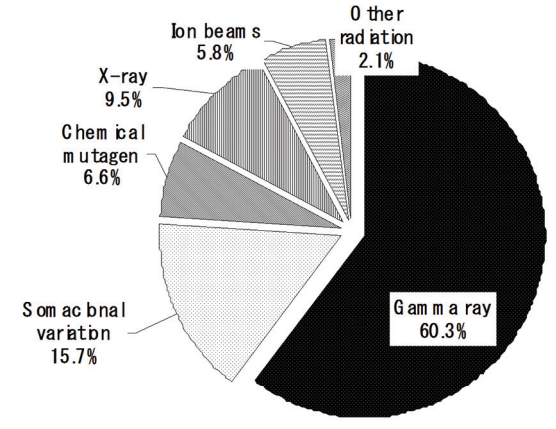


Figure 3 Percentage of total 242 cultivars developed by mutation breeding by using various kinds of methods in Japan (2008). Chemical mutagen does not include colchicine [4].

Table 1 shows the number of registered mutant cultivars of some crops developed by radiation, Gamma-rays, and those irradiated at the IRB, NIAS [4]. There are 50 mutant cultivars of chrysanthemum, 31 of rice, 16 of soybean, 10 of rose, etc. Among them, 100 cultivars have been generated at the IRB and these contributions of the IRB regarding the development and release of superior mutant induced cultivars has been extensive. This high percentage of Gamma-ray irradiated mutants indicates that mutation breeding via Gamma-ray irradiation is an effective and highly successful approach for the generation of commercial cultivars.

The first mutant rice cultivar is "Reimei," which means "dawn" in Japanese, was the first irradiation induced mutant cultivar that illustrated the potential of utilizing Gamma-rays for breeding improvements in Japan. Reduction of plant height, including dwarfism and semi-dwarfism is one of the characteristics that can be induced with high frequency by irradiation and can be easily detected in the field. "Reimei," registered in 1966 [5] was a successful case of an irradiation induced semi-dwarf mutant. This cultivar exhibits a mutation of the *sd-1* locus [6] and shows a culm 15cm shorter than the original cultivar "Fujiminori." The semi-dwarf is associated with the high-yielding ability and recorded the highest yield in Japan in 1967 [5].

Table 1. Number of registered mutant cultivars developed by radiation, Gamma-rays, and those irradiated in the Institute of Radiation Breeding, NIAS [4]

	Mutant cultivars[1]	Radiation	Gamma-rays	IRB[2]
61 Crops	242	188	146	100
Rice	31	14	12	11
Wheat	4	2	2	0
Barley	4	4	3	0
Soybean	16	16	15	9
Chrysanthemum	50	46	32	29
Rose	10	7	7	6
Sea pink (Limonium)	6	6	6	0
Cytisus	8	8	8	8
Apple	2	2	2	2
Japanese Pear	3	3	3	3
Others	108	80	56	32

[1] Total number of mutant cultivars developed by radiation (Gamma-ray, X-ray and ion beams), chemicals (Excluding colchicine treatment), somaclonal variation
[2] Number of mutant cultivars irradiated in the Institute of Radiation Breeding (IRB)

Table 2. Number of indirect use mutant cultivars in Japan (2008)

Rice	Wheat	Barley	Soybean	Tomato	Others	Total
198	3	7	9	3	7	228

In Japan, the total number of indirect-use mutant cultivars is 228, which includes 198 rice, 9 soybean, 7 barley, 3 wheat, 3 tomato, 4 lettuce, 1 eggplant, 1 Japanese lawngrass, 1 mat rush, and 1 mushroom cultivar in 2008 (**Table 2**). Interestingly, among the 198 indirect-use mutant cultivars in 2008, 89 cultivars (44.9%) were derived from the "Reimei" or its offspring. This suggests that agronomically useful mutations can be utilized as parental lines to develop new varieties with this characteristic and transferred efficiently to the farmers' field.

The Economic impact of mutant cultivars in Japan

Figure 4 shows the increase of mutant rice cultivars, which were derived from mutants generated by Gamma-rays, planted in farmers' fields in Japan since 1960. "Reimei" was first cultivated on 61,598 ha in 1968, (http,//ineweb.narcc.affrc.go.jp/). The number of mutant cultivars has

been increasing and 99 mutant cultivars (2 direct-use and 97 indirect-use cultivars) were in cultivation in 2005 [4].

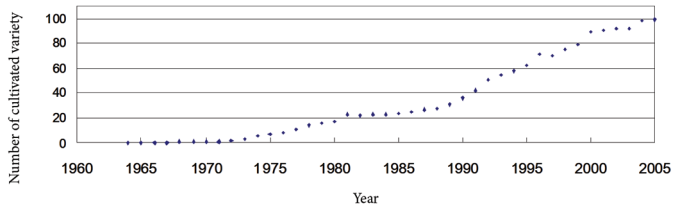


Figure 4 Total number of mutant rice cultivars, which are derived from mutants generated by Gamma-rays, cultivated in farmers' field from 1960 to 2005 in Japan [4].

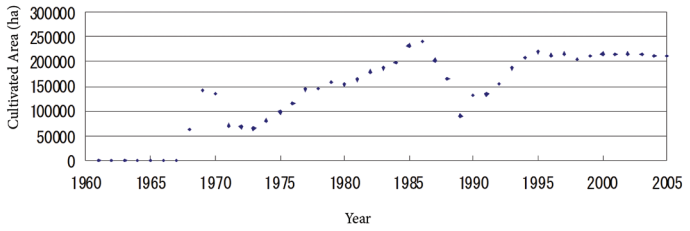


Figure 5 Total areas of mutant rice cultivars, which are derived from mutants generated by Gamma-rays, cultivated in farmers' field from 1960 to 2005 in Japan [4].

Figure 5 shows the total cultivated field of the mutant cultivars, which are derived from mutants generated by Gamma-rays, from 1961 to 2005. This increased after "Reimei" was released for cultivation in 1968. The peak use of mutant induced cultivars reached 250,000 ha in 1986 and was slightly more than 200,000 ha from 1994 to 2005. In 2005, the total cultivated area of mutant cultivars was 210,692 ha, which is 12.4% of total cultivated area of paddy rice (1,702,000 ha) in Japan [4].

The total crude income of farmers selling the brown rice of mutant cultivars also has been increasing as the increase of the cultivated area, although the price of the grain is different in each year. The amount of total income is estimated to be approximately 250 billion Yen (2.34 billion US dollars) in 2005 [4]). The mutant cultivars, which are derived from mutants generated by Gamma-rays and have been cultivated on more than 5,000 ha from 2001 to 2005, are the following 17 cultivars, "Kinuhikari (263,223ha)"; "Haenuki (219,734ha)"; "Tsugaru-roman (106,423ha)"; "Yume-akari (66,491ha)"; "Yume-tsukushi (58,893ha)"; "Aichi-no-kaori (53,697ha)"; "Asahi-no-yume (51,049ha)"; "Mutsuho-mare (46,959 ha)"; "Dontokoi (17,008ha)"; "Yume-shizuku (14,076ha)"; "Mine-asahi (10,698 ha)"; "Yume-hitachi (10,440ha)"; "Yume-minori (9,957ha)"; Aki-geshiki (7,510ha)"; "Aki-roman (7,450ha)"; "Miyama-nishiki (7,242 ha)"; and "Tsukushi-roman (5,533 ha)." The mutant cultivars, which have been cultivated in more than 100000ha of farmers' fields are the following 5 cultivars, "Akihikari (1,410,810ha)"; "Reimei (886,188ha)"; "Kinuhikari (263,223ha); "Haenuki (219,734ha); and "Tsugaru-roman (106,423ha)." Among them, "Reimei" is a direct-use mutation cultivar and the others are indirect-use cultivars [4].

There are 16 direct-use mutant cultivars of soybean registered in Japan since "Raiden" and "Raikou" were developed by Gamma-ray irradiation in 1960. The improved characteristics were early-maturity and late-maturity, yellow hilum, seed-coat color, short-stem, and the number of pods/stem, lipoxygenase-free, low allergen etc. Among them, one cultivar was induced by X-ray and the other 15 were induced by Gamma-rays. The number of indirect-use cultivars is 10. The total cultivated area of mutant cultivars cultivated in the farmers' fields came to 13,283 ha (9.4% of total cultivated area (142,000ha) of soybean in Japan in 2005) and total farmers' crude income was 5.56 billion Yen (ca. 52 million US dollars) [4]. As a result, economic impact of mutant cultivars is huge in Japan.

Some useful mutant varieties by using various screening methods.

Rice

Although rice is not a high protein grain crop, the protein content is ca. 7% when the white rice is cooked. A mutant line with a low content of gulutelin was obtained from the ethyleneimine (EI) treatment to "Nihonmasari." The "LGC-1" was developed from back-crossing this mutant with the original "Nihonmasari" to eliminate undesirable characteristics, such as semi-sterility and semi-dwarfism [7]. The seed protein of the "LGC-1" is composed of mainly of a low amount of digestible glutelin and high amount of indigestible prolamine. This construction of protein is disadvantageous for the digestion of rice grains in humans, though the total amount of protein is mostly similar to the original cultivar. As a result, the "LGC-1" is useful as "low protein rice," and some clinical trials on patients with kidney disease indicate that the variety is a useful and effective daily food for such patients [8]. The defect of the "LGC-1" is its eating quality, and there are the other loci that control the biosynthesis of digestible protein, such as globulin. Therefore, Nishimura, *et al.* [9] induced a mutant named "89WPKG30-433" with a deficiency in globulin from the leading Japanese cultivar "Koshihikari" through Gamma-ray irradiation. They hybridized it with the "LGC-1" and selected "LGC-Katsu" and "LGC-Jun" from the hybrids, whose globulin content was as low as the "LGC-1," where the globulin content is zero. The total digestible protein content tested to about 30% of ordinary rice. As the eating quality is highly improved and digestible protein content is lower than "LGC-1," these two cultivars will greatly help in the dietary management of proteins with chronic renal failure.

Soybean

Takagi [10] identified two major genes, which control radio-sensitivity, in some soybean varieties. When the 50% reduction rate (RD_{50}) of root length was determined with acute irradiation to the seeds or the chronic irradiation to the plants for the entire growth period, radio-sensitivity of a sensitive cultivar, "Shinmejiro," is more than twice that of the resistant variety, "Tachisuzunari." The differences in radio-sensitivity between the varieties to the chronic irradiation in the Gamma Field were controlled by a single recessive gene, *rs1*. Besides, the second recessive gene rs2, which was discovered in "Goishishirobana," whose activity is only expressed following acute seed radiation.

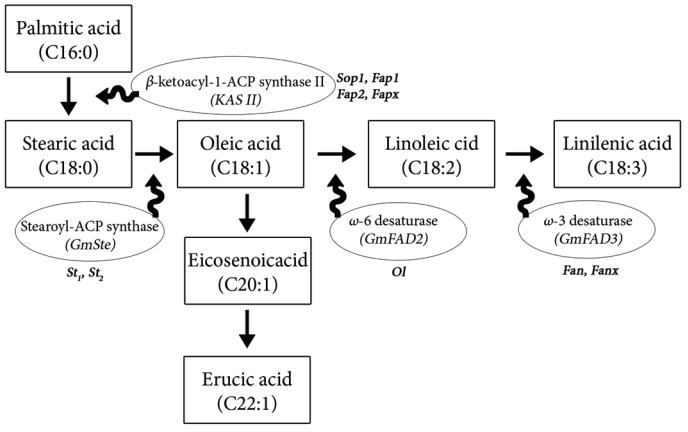


Figure 6 Metabolic pathway and key genes of fatty acid in soybean [13] (courtesy of Prof. Y. Takagi).

Soybean is the most widely used source of edible oil for human con-

sumption. Fatty acids of soybean seeds consist of palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid (**Fig. 6**). Altered unsaturated fatty acid content (elevated oleic acid and reduced linolenic acid) increase the oxidative stability that provides health benefits and improvement of fatty acid contents. This has been one of the most important breeding objectives of soybean. As natural genetic diversity in soybean is limited, mutation induction is one effective approach to induce modification. Through the use of X-rays or chemical mutagens, mutants with different fatty acid compositions, such as reduced and elevated palmitic acid, elevated stearic acid, elevated oleic acid (50%) , and reduced linolenic acid (3%) content were isolated and found to be controlled by major genes (**Fig. 6**; [11-13]).

Soybean seed has three lipoxygenases called *L-1*, *L-2* and *L-3*, respectively [14]. The lipoxygenases are the main factors of the grassy-beany flavor of the products. Soybean lines lacking each of the three genes have been developed. However, no line lacking all three genes has been obtained because of tight linkage between *L-1* and *L-2* [15]. The F_2 seeds derived from a cross between a line without *L-1*, *L-3* and a line without *L-2*, *L-3* were irradiated with Gamma-rays. After surveying 1,813 M3 seeds by using SDS/PAGE, one mutant seed lacking all *L-1*, *L-2*, and *L-3* was selected [16]. A new cultivar "Ichihime" with this unique characteristic was registered and released in 1994 [17].

Italian ryegrass

Mutation breeding has been mainly established in seed propagated, self-pollinated species. Although several methods have been widely used for the screening of mutants in self-pollinated species by the single-seed descent approach [18,19] and by single seed descent (one-plant-one-grain method, Yoshida [20]), these methods have not been applied to cross-pollinated species. Ukai [21] developed a new method for obtaining mutants of cross-pollinated species efficiently in a temperate forage grass, Italian ryegrass (*Lolium multiflorum* L.). The method was called the "Crossing-within-Spike-Progenies Method." This method is composed of 1) taking seeds separately from each spike from a population of plants irradiated with Gamma-rays, 2) sowing the seeds in a hill plot as a spike-progeny, 3) isolating each hill from others at the time of flowering and allowing the open-pollination of plants within hills, and 4) taking seeds from each of the hills and sowing the seeds in hill progenies for the screening of mutants. This procedure is repeated each year. When 300 Gy of Gamma-ray was irradiated to the seed, the frequency of chlorophyll mutations was approximately 70.6% per hill progeny and 1.87% per plant. In contrast, open-pollinated populations exhibited that only 10% per progeny and 0.12% per plant, respectively. This method will be applied to the other wind- or insect-pollinating outcrossing crop species.

Chrysanthemum

In general, it is very difficult to isolate mutants from mutation sectors in vegetatively propagated crops although the maintenance of mutant genotypes is easier than the seed-propagated species. It has been shown that the combined method of chronic Gamma-ray irradiation and tissue culture is very effective in solving this problem. By tissue culturing the floral organs of chrysanthemum (*Chrysanthemum morifolium* Ramat.) plants chronically irradiated in the Gamma Field from the seedling to the flowering stages, many non-chimeric mutants, with various flower colors and shapes, are obtained [22]. From these mutant lines, 10 cultivars were registered. The technology, given the term "radiobiotechnology," is not only effective in obtaining non-chimeric mutants but also effective in producing high mutation frequencies. The method has been utilized to induce mutations in various vegetatively propagated crops and many mutant cultivars have been registered.

Japanese pear and apple resistant to Alternaria disease

A popular cultivar of Japanese pear (*Pyrus serotina* Rehd. var. *culta* Rehd.), "Nijisseiki," which was a leading variety, occupied 28% of the total cultivated area of Japanese pear in 1990 in Japan. The cultivar, however, is highly susceptible to the black spot disease, *Alternaria alternata* (Fr.) Keissier (= *Alternaria kikuchiana* Tanaka), one of the most serious diseases of pear [23]. Growers are required to spray fungicides several times during the growing season to counter the disease. To induce mutations resistant to the disease by Gamma-ray irradiation, small plants of the cv. "Nijisseiki" were planted at every 4 meters from 37 m to 63 m from the ^{60}Co source in 1962 and chronic Gamma-ray irradiation was applied (30 x 10-2 Gy - 4 x 10-2 Gy/day) in the Gamma Field [24]. In 1981, nearly 20 years after the planting, a twig without the symptom of the disease was found in a plant planted at a distance of 53 m from the irradiation source. As it was ascertained that there was no difference in other agronomic characteristics between the mutant and the original variety except for the resistance to the black spot disease, it was registered and released in 1991 with the name "Gold Nijisseiki"[24]. It was registered as the same name in Australia in 2004 (Certificate Number 2533).

Dr. Sanada, one of the breeders of this cultivar, mentioned, "The situation of mutation breeding on fruit trees has been severely criticized because there have been no successful results." Although it took them nearly 20 years to identify a useful mutation and 30 years for the registration, the release of "Gold Nijisseiki" is a monumental achievement for the Gamma Field.

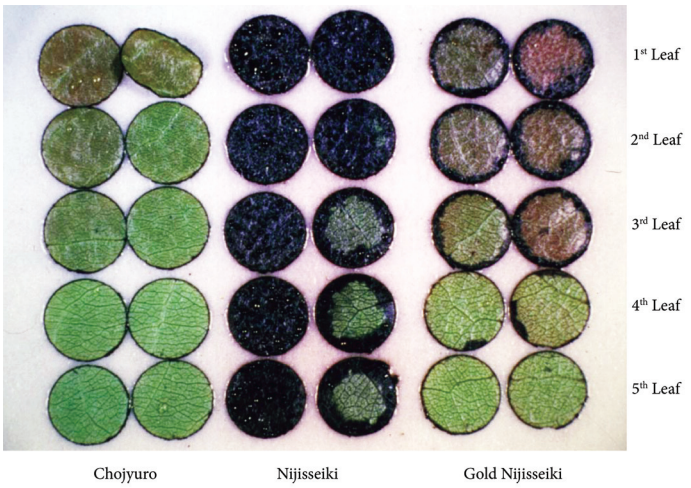


Figure 7 Bioassay of resistant to the black spot disease by using the AK-toxin obtained from the culture of the fungus. Upper to lower leaf disc(1 – 5) means 1 (young) to 5 (older) leaf; cv."Chojyuro", highly resistant; cv. "Nijisseiki", highly susceptible; cv. "Gold Nijisseiki", resistant

At the same time an easy and effective method for the screening of resistance to the fungus has been developed by treating leaf discs (7 mm in diameter) by the AK-toxin produced by the fungus [25]. It was coincidental and lucky for the breeders that Nakashima, *et al.* [26,27] isolated and identified the chemical structure of the toxin named "AK-toxin" produced by the fungus of black spot disease and generating the symptom of black spots on leaves at the same time. As a consequence, the breeding group entered into a cooperative research program with the chemistry group and established this unique method. When the leaf discs are placed on the filter paper soaked with AK-toxin obtained either from the extract of the fungal body or artificial synthesis in a Petri dish, and kept for two or three days, susceptible leaves turned to black and resistant leaves stayed green (**Fig. 7**). After the development of this method, two new mutant varieties, "Osa-Gold [28,29]" and "Kotobuki Shinsui [30]" were developed in a short period of time by using this screening method. The economic effect of this research has been great [4].

These researches suggest that the breeding of fruit trees requires pa-

tience and that development of easy and precise screening methods is a very important addition to the development of methods for mutation induction.

Achievement of biological researches on mutations induced by Gamma-ray irradiation

Deletion size generated by Gamma-ray

Naito, *et al.* [31] studied the deletion sizes of transmissible and non-transmissible mutations induced with Gamma-ray and carbon ion beam irradiation by the sophisticated pollen-irradiation methods in *Arabidopsis*. It has been revealed that most mutants induced with these ionizing irradiations possess extremely large deletions (more than 6 Mbp), most of which are not transmittable to the next generation, as well as small deletions (1 or 4 bp), which are normally transmissible.

In rice, the same tendency was observed in transmissible mutants. Morita (unpublished) researched the frequency of transmission of different mutations possessing different deletion sizes as obtained with Gamma-ray irradiation in rice. Among 11 Gamma-ray induced mutants, one *GluA2* mutant exhibited 1 base pair (bp) substitution, and among 10 mutants with a deletion, the deletion size of 6 mutants, which include *CAO* (*chlorophyllide-a oxygenase*), *GA3os* (*GA3-beta-hydroxylase*), *GluA1* (*glutelin A1*), and *GluA2* (*glutelin A2*) are 1 bp deletion, and those of the other *CAO mutants* and *PLA1* (*Plastochron1*) are 3 and 5 bp deletions, respectively. Those of *GluB4/5* (*Glutelin B4/5*), two α-globulin mutants are more than 10 kbp, 15 kbp, and 90 kbp, respectively. It is very interesting that the Gamma-ray induced mutations transmittable to the next generation are primarily classified into 2 groups, the one with extremely a large deletion and the other with small deletions (1 to 5 bp). We are not sure whether or not it is very difficult to obtain mutants with medium deletion size by Gamma-ray irradiations. However, we are accumulating data to elucidate it.

Different size and location of deletion generates different kinds of phenotypes

In the course of plant evolution, genes are often duplicated in tandem, resulting in a functional redundancy. The analysis of function of these genes by developing double mutants might be difficult because they would be very tightly linked. Mutants of such tandem duplicated genes were investigated for their genotypes and phenotypes. There are reversely repeated two loci, which both codes for mRNA of glutelin production. There are various mutants that exhibit low glutelin contents isolated by SDS-PAGE [7, 32]. The mechanisms of low glutelin contents of mutants that have been studied suggest that the size and the position of deletions generate different characteristics of mutations. Some act as dominant genes or recessive genes, and those relationships between genotypes and phenotypes, etc. are provided as example below.

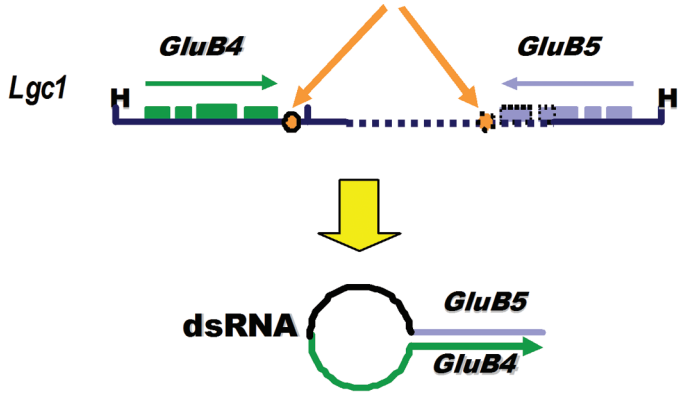


Figure 8 Mechanism of low glutelin in LGC-1 through a deletion at the transcription termination signal and produced double-stranded RNA suppress the glutelin synthesis by RNAi [33] (by courtesy of Prof. M. Kusaba, Hiroshima University).

Mechanism of low glutelin content in the "LGC-1" mutant

The *Low glutelin content* (*Lgc-1*) is a dominant mutation that reduces glutelin content in the rice grain. Glutelin is a major digestible seed storage protein encoded by a multigene family. Kusaba, *et al.* [33] reported that in *Lgc-1* homozygotes, there is a 3.5 kbp deletion between two highly similar glutelin genes that forms a tail-to-tail inverted repeat, that might produce a double stranded RNA molecule, a potent inducer of RNA silencing (**Fig. 8**). As a result, glutelin synthesis is suppressed and the glutelin content is lowered. The *Lgc-1* provides an interesting example of RNA silencing occurring among genes that exhibit various levels of similarity to an RNA-silencing- inducing gene. This was the first report that shows the mechanism of a mutation was RNAi.

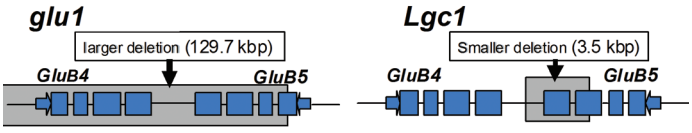
Mechanism of low glutelin content in the "glu1" mutant

The "*glu1*" is a gamma-ray-induced rice mutant, which lacks an acidic subunit of glutelin, a major seed storage protein. Morita, *et al.* [34] elucidated that the *glu1* harbors a 129.7 kbp deletion involving two highly similar and tandem repeated glutelin genes, *GluB5* and *GluB4*. The deletion eliminated the entire *GluB5* and *GluB4* gene except half of the first exon of *GluB5*. As a result, the phenotype of the *glu1* gene is a complete lack of the acidic subunit of glutelin and acts as a recessive gene for low glutelin content in rice grains (**Fig. 9**).

Conclusion

The above examples illustrate that the position and the size of deletions in the same loci have the capacity to dramatically alter the phenotype of mutants through the process of transcription and translation. The *glu1*, which has a large 129.7 kbp deletion, acts as a recessive gene, while the *LGC1*, which has 3.5 kbp deletion including probably a terminal signal of the transcript region acts as a dominant gene.

Furthermore, the *GluB5* and the *GluB4* have the same amino acid sequence in their acidic subunit, suggesting that only the mutation involving both *GluB5* and *GluB4* result in the resultant phenotype. That is the lack of the glutelin acidic subunit deleted in the "glu1" mutant. It probably is very difficult to knock out both loci by chemical treatment or transposon techniques. Sequenced plant genomes exhibited more that 14% of the genes formed tandem array [3, 35]. This finding, however, suggests that Gamma-rays can be an effective mutagen to generate knock-out mutants of both loci and to analyze tandem repeated and functionally redundant genes.



	Phenotype	Dominant or Recessive	Mechanism
glu1	Deletion of 1 acidic subunit	Recessive	Knock-out of 2 genes
Lgc1	Total glutelin contents lower	Dominant	Suppresses glutelin genes via **RNAi.**

Figure 9 Comparison of phenotype, mode of inheritance and mechanism of mutation character between glu1 and Lgc1 mutation with different size and place of deletion in the same region of 2 loci, GluB4 and GluB5 (by courtesy of Dr. R. Morita, IRB, NIAS). glu1,Morita *et al.* [34] ; Lgc1, Kusaba *et al.* [33]

Genetic studies by the useful mutations induced with Gamma-ray chronic irradiation

As the history has shown, spontaneous and induced mutation resources have played an important role not only for mutation breeding but also genetic studies and the elucidation of gene functions.

Phytochrome

Takano, *et al.* [36] have isolated *phytochrome B* (*phyB*) and *phy C* mutants from rice and have produced all combinations of double mutants. Seedlings of *phy B* and *phyB phyC* mutants exhibited a partial loss of sensitivity to continuous red light but still showed significant deetiolation responses. The responses to red light were completely canceled in *phyA phyB* double mutants. These results indicate that *phyA* and *phyB* act in a highly redundant manner to control deetiolation under red light. They also found that mutations in either *phyB* or *phyC* locus causes moderate early flowering under a long-day photoperiod, while monogenic *phyA* mutations had little effect on flowering time. The *phyA* mutation, however, in combination with *phyB* or *phyC* mutation caused dramatic early flowering. Early flowering mutants were generated by chronic Gamma-ray irradiation with dose rates ranging between 3 and 6 Gy/day [36].

Aluminum tolerance

Ma, *et al.* [37] isolated a mutant with highly sensitivity to aluminum concentration from cv. Koshihikari of japonica rice, which has an aluminum resistance [38]. The mutant was induced with chronic Gamma-ray irradiation and exhibited the same phenotype to the wild type with the absence of aluminum. That is, M_1 plants were irradiated in the Gamma Field from seven days before heading to two days after heading under 20 Gy/day for eight days. The root elongation of the mutant, however, was highly inhibited in the presence of 10 μM Al. The mutant also exhibited poorer root growth in acid soil. Genetic analysis showed that the high sensitivity to Al is controlled by a single recessive gene. The gene was mapped to the long arm of chromosome 6.

Conclusion

The Gamma Phytotron was established in Korea in 2005 and the Gamma Greenhouse, approximately doubled the size of the Gamma Greenhouse located at the IRB, Japan, was established in Malaysia in 2008. Both facilities are focused on the induction of mutation by chronic Gamma-ray irradiation to growing plants of important crop species. As described earlier in this report, chronic irradiation is a useful tool for the generation of mutant genome resources that have application toward molecular analysis as well as conventional breeding.

Conclusions

A. M. van Harten [39] describes in "Mutation Breeding -Theory and practical application,"

"An explanation for the decreasing interest in mutation breeding, at least in most "developed" countries, may be that during the past two decades attention has become more and more directed towards studying the possibilities offered to plant breeding by various new molecular technologies…As a result of these developments mutation breeding seems to have lost part of its previous attraction for young researchers."

It is not necessary to mention, however, that mutation breeding is still a very interesting and useful technology for isolating genes and for elucidating gene mechanisms and metabolic pathways in various crops.

The record has also shown that mutation induction is a very useful conventional breeding tool for developing superior cultivars. Today, site-directed mutagenesis *in vivo* or *in vitro* cell can be envisioned and many researchers are conducting programs in this direction.

New fields of science and technologies were developed on the basis of achievements of traditional or classic methods. It is highly desirable that the IRB continues their work while incorporating the new knowledge and technologies. The IRB is well equipped with appropriate facilities and equipment that will contribute to the future mutation breeding developments and be a contributor in solving the problems mentioned in this review.

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BIBLIOGRAPHY

1. Yamaguchi, I. Forty years of mutation breeding in Japan - Research and fruits. *Gamma Field Symposia* **40**, 1-14 (2001).
2. Sasaki, T., Burr, B. International Rice Genome Sequencing Project: the effort to completely sequence the rice genome. *Curr. Opinion Plant Biology* **3**, 138-141 (1998).
3. International Rice Genome Sequence Project. The map-based sequence of the rice genome. *Nature* **436**, 793-800 (2005).
4. Nakagawa, H. Mutation breeding, status quo and future. *Techno Innovation* **68**, 6-12 (2008) (in Japanese).
5. Futsuhara, Y. Breeding of a new rice variety Reimei by gamma-ray irradiation. *Gamma Field Symposia* **7**, 87-109 (1968).
6. Ashikari, M. *et al.* Loss-of-function of a rice gibberellin biosynthetic gene, GA20 oxidase (GA20ox-2), led to the rice 'Green Revolution'. *Breeding Science* **52**, 143-150 (2002).
7. Iida, S. *et al.* A rice (*Oryza sativa* L.) mutant having a low content of glutelin and a high content of prolamine. *Theor. Appl. Genet.* **87**, 374-378 (1993).
8. Mochizuki, T. Hara, S. Usefulness of low protein rice in diet therapy in patients with chronic renal failure. *Jpn. J. Nephrol.* **42**, 24-29 (in Japanese with English summary).
9. Nishimura, M. *et al.* New rice varieties with low levels of easy-to-digest protein, 'LGC-Katsu' and 'LGC-Jun'. *Breeding Science* **55**, 103-105 (2005).
10. Takagi, Y. The second type of gamma-ray sensitive gene RS2 in soybean *Glycine max* (L.) Merrill. *Gamma Field Symposia* **8**, 83-94 (1969).
11. Takagi, Y. *et al.* Construction of novel fatty acid composition in soybean oil by induced mutation. *Gamma Field Symposia* **37**, 17-28 (1998).
12. Anai, T. *et al.* Identification of corresponding genes for three low-a-linolenic acid mutants and elucidation of their contribution to fatty acid biosynthesis in soybean seed. *Plant Science* **168**, 1615-1623 (2005).
13. Takagi, Y., Anai T. Development of novel fatty acid composition in soybean oil by induced mutation. *Oleoscience* **6**(4), 195-203 (in Japanese with English abstract) (2006).
14. Arai, S. *et al.* N-Hexanal and some volatile alcohols. Their distribution in row soybean tissue and formation in crude soy protein concentrate by Lipoxygenase. *Agric. Biol. Chem.* **34**, 1420-1423 (1970).
15. Kitamura, K. *et al.* Inheritance of lipogenase-2 and genetic relationships among genes for lipogenase-1, 2 and -3 isozymes in soybean seeds. *Jpn. J. Breed.* **35**, 413-420 (1985).
16. Hajika, M. *et al.* A line lacking all the seed lipogenase isozymes in soybean (*Glycine max* (L.) Merrill) induced by gamma-ray irradiation. *Jpn. J. Breed.* **41**, 507-509 (1991).
17. Hajika, M. *et al.* A new soybean variety 'Ichinose'. *Bull. Nat. Agric. Res. Cent. Kyushu Okinawa Reg.* **40**, 79-93 (2002) (in Japanese with English summary).
18. Stadler, L.J. Some genetic effects of X-rays in plants, *J. Hered.* **21**, 3-19 (1930).
19. Nybom, N. Mutation type in barley. *Acta Agric. Scand.* **4**, 430-456 (1954).
20. Yoshida, Y. Theoretical studies on the methodological procedures of radiation breeding . I. New methods in autogamous plants following seed irradiation. *Euphytica* **11**, 95-111 (1962).
21. Ukai, Y. Application of a new method for selection of mutants in a cross-fertilizing species to recurrently mutagen-treated populations of Italian ryegrass. *Gamma Field Symposia* **29**, 55-69 (1990).
22. Nagatomi, S. Combined effect of gamma irradiation methods and *in vitro* explant sources on mutation induction of flower color in Chrysanthemum morifolium RAMAT.

Gamma Field Symposia **35**, 51-69 (1996).

23. Nishimura, S. *et al.* Two different phases in pathogenicity of the Alternaria pathogen causing black spot disease of Japanese pear. *J. Fac. Agr. Tottori Univ.* **13**, 1-10 (1978).
24. Sanada, S. *et al.* A new Japanese pear cultivar 'Gold Nijisseiki,' resistant to black spot disease of Japanese pear. *Jpn. J. Breed.* **43**, 455-461 (1993) (in Japanese with English summary).
25. Sanada, S. Selection of resistant mutants to black spot disease of Japanese pear by using host specific toxin. *Jpn. J. Breed.* **38**, 198-204 (1988).
26. Nakashima, T. *et al.* Structure elucidation of AK-toxins, host specific phyto-toxic metabolites produced by Alternaria kikuchiana Tanaka. *Tetrahedron Lett.* **23**, 4469-4472 (1982).
27. Nakashima, T. *et al.* Isolation and structure of AK-toxin I and II. Host-specific phytotoxic metabolites produced by Alternaria alternata Japanese phytotype. *Agri. Biol. Chem.* **49**, 807-815 (1985).
28. Masuda, T. *et al.* Selection of mutants resistant to black spot disease by chronic irradiation of gamma-rays in Japanese pear 'Osanijisseiki. *J. Japan. Hort. Sci.* **66** (1), 85-92 (1997).
29. Masuda, T. *et al.* A new Japanese pear cultivar 'Osa Gold,' resistant mutant to the black spot disease of Japanese pear (*Pyrus pyrifolia* Nakai) induced by chronic irradiation of gamma-rays. *Bull. Natl. Inst. Agrobiological Resources* (Japan) **12**, 1-11 (1999) (in Japanese with English summary).
30. Kitagawa, K *et al.* A new Japanese pear cultivar, 'Kotobuki Shinsui'. *Bull. Tottori Hort. Expt. Stn.* **3**, 1-13 (1999) (in Japanese).
31. Naito, K. Transmissible and non-transmissible mutations induced by irradiating *Arabidopsis thaliana* pollen with γ-rays and carbon ions. *Genetics* **169**, 881-889 (2005).
32. Iida, S. *et al.* Mutants lacking glutelin subunits in rice: mapping and combination of mutated glutelin genes. *Theor Appl Genet* **94**, 177-183 (1997).
33. Kusaba, M. *et al. Low glutelin content1*: A dominant mutation that suppresses the glutelin multigene family via RNA silencing in rice. *Plant Cell* **15**, 1455-1467 (2003).
34. Morita, R. *et al.* Knockout of glutelin genes which form a tandem array with a high level of homology in rice by gamma irradiation. *Genes Genet Syst.* **82**, 321-327 (2007).
35. Arabidopsis Genome Initiative. Analysis of the genome sequencing of the flowering plant *Arabidopsis taliana. Nature* **408**, 796-815 (2000).
36. Takano, M. *et al.* Distinct and cooperative function of phytochromes A, B, and C in the control of deetiolation and flowering in rice. *Plant Cell* **17**, 3311-3325 (2005).
37. Ma, J.F. *et al.* Isolation and characterization of a rice mutant hypersensitive to Al. *Plant Cell Physiol.* **46**, 1054-1061 (2005).
38. Wu, P. *et al.* Genetic control of seedling tolerance to aluminum toxicity in rice. *Euphytica* **97**, 289-293 (1997).
39. Van Harten, A.M. Mutation breeding –theory and practical breeding. Cambridge University Press, Cambridge, UK (1998).

MUTATION BREEDING REVIEW

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OFFICIALLY RELEASED MUTANT VARIETIES - THE FAO/IAEA DATABASE

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ABSTRACT

In the approximately 70 year-old history of induced mutations, there are many examples on the development of new and valuable alteration in plant characters significantly contributing to increased yield potential of specific crops. However, knowledge on the success of induced mutations in crop improvement among geneticists and breeders is usually limited to species of their interest. The present paper contains a comprehensive list of officially released mutant varieties, based on information from plant breeders. The number of mutant varieties officially released and recorded in the FAO/IAEA Mutant Varieties Database before the end of 2000 is 2,252. Almost half of these varieties have been released during the last 15 years. Considering a significant delay in the dissemination of information on newly released varieties and difficulties in the collection of such data, there has been a renaissance in the use of mutation techniques in crop improvement. At the demand of geneticists, plant breeders, and more recently molecular geneticists, for information on released mutant varieties of specific crops, the MVD was transferred to the web site of the FAO/IAEA Joint Division. The MVD will be available on our web pages early in 2001.

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INTRODUCTION

The high efficiency of mutation techniques to generate desired variation in crop plants has been widely proven and documented in many original and review papers. In the approximately 70 year-old history of induced mutations, there are many examples on the development of new and valuable alteration in plant characters significantly contributing to increased yield potential of specific crops. However, knowledge on the successes of induced mutations in crops improvement among geneticists and breeders is usually limited to species of their interest. Often the breeders, using varieties with a desired character as a parent in a crossing programme, are not always aware that the desired gene was obtained by induced mutations, for example genes for semidwarfness in barley, rice and durum wheat, and high oleic fatty acid content in sunflower. The present paper contains a comprehensive list of officially released mutant varieties, based on information from plant breeders, and published in the successive issues of the Mutation Breeding Newsletter. However, the list is far from complete. Many varieties, mainly derived from crosses with parents carrying mutated genes, are published in scientific journals. However, they can only be listed in the FAO/IAEA Mutant Varieties Database (MVD) on the basis of official information obtained from the plant breeder or official authority in the country. At the demand of geneticists, plant breeders, and more recently molecular geneticists, for information on released mutant varieties of specific crops, the MVD was transferred to the web site of the FAO/IAEA Joint Division. The MVD will be available on our web pages early in 2001.

THE MUTANT VARIETIES DATABASE (MVD)

The short history of the FAO/IAEA database on mutant varieties was briefly described in the previous issue of MVD, and published in Mutation Breeding Newsletter (MBNL) 38 (1991) [1]. The idea to collect and transfer information to plant breeders on crop varieties developed with the use of mutation techniques was conceived at almost the same time as the establishment of the Plant Breeding and Genetics Section (PBG), Joint FAO/IAEA Division. B. Sigurbörnsson, the first Head of the PBG Section, began collecting data on mutant varieties in 1963. The first classified list of induced mutant varieties was presented by Sigurbjörnsson at the Pullman Symposium, and published in 1969 [2]. This work was continued over the next 22 years by A. Micke. The original information from the author and plant breeder on new, officially released mutant varieties was transferred to information sheet and kept on files. A comprehensive list of mutant varieties was published by Sigurbjörnsson and Micke in 1974 [3] and this was updated in 1985 [4]. Since the first issue of the MBNL (May, 1972) information on newly released mutant varieties was published at the end of each issue under the title “List of Mutant Varieties”. Filing and retyping the incoming information sheets for the MBNL was done first by Ms. M. Weiner and continued till 1993 by Ms. L. Halgand. In 1980, Sigurbjörnsson and then C. Konzak and B. Donini undertook the establishment of a database on mutant varieties by using mainframe facilities of the IAEA. However, fast development in personal computer technology, together with the large number of suitable software, gave opportunity to organize a database on IBM PC using “DbaseIII Plus” software. The work was initiated by M. Maluszynski in 1987, and has been continued, with the help of Ms. K. Weindl. The MVD was revised by L. van Zanten in 1994 when the Agency introduced MSAccess taking advances in the developer and user interface. On 17 November 2000, the MVD was transferred to a web based system (4D). Programming and system design was undertaken by M. Marsella (Consultant) under the leadership of I. Ferris (FAO/IAEA). Such condensed but full information on mutant varieties should help geneticists, molecular

biologists and plant breeders to asses the value of mutation techniques in germplasm enhancement, and stimulate the use of induced variation.

MUTANT VARIETIES

The number of mutant varieties officially released and recorded in MVD before the end of 2000 is 2,252 (Fig. 1). Almost half of these varieties (1,019) have been released during the last 15 years. Considering a significant delay in the dissemination of information on newly released varieties and difficulties in the collection of such data, there has been a renaissance in the use of mutation techniques in crop improvement.

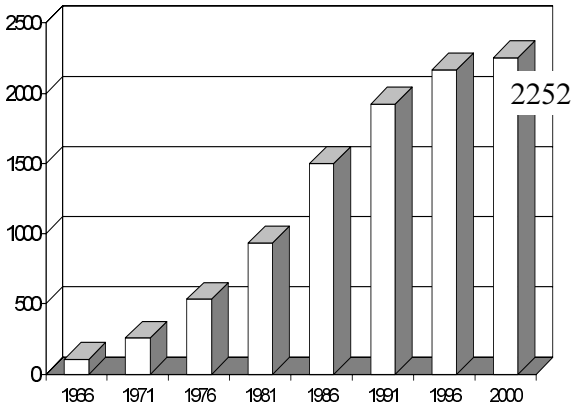

FIG. 1. Cumulative number of officially released mutant varieties, June 2000

In reality, it would be expected that the number of released varieties is much higher than listed, as many mutated genes have been used in cross breeding programmes without indicating the nature of desired genes. This is the case of at least 9 rice varieties in Australia and 2 varieties in Egypt. These varieties were developed through crosses with the gamma-ray induced semidwarf Californian rice variety "Calrose 76". The leading Australian variety "Amaroo", released in 1987, has the sd_1 gene from Carlose 76, as also the variety "Giza 176", released in 1989, and one of the leading varieties in Egypt. Both these varieties were not included in the MVD as the registration forms of these mutant varieties were not available in our files. Modern sunflower varieties or hybrids currently grown in Europe and the USA have a high oleic fatty acid content. The only known and published genetic source for this character has been a mutated gene in variety "Pervenets" developed by Soldatov in 1976 [5]. However, in the MVD only Pervenets (USSR) and “Jingkui 1” (China) were listed under sunflower mutant varieties. It should be expected that in barley, extensively used sources for semidwarfness are mutated genes and mainly *denso* gene induced by x-rays in tall Moravian variety “Valticky” [6]. Barley breeders suggest that more than 150 malting barley varieties in all continents carry the *denso* gene.

Gathering of information on newly released mutant varieties is further complicated by the fact that mutant varieties have been released in approximately sixty countries (Table 1). Additionally in most of these countries induced mutations are used for improvement of various crops, often in different plant breeding stations.

TABLE 1: Number of officially released mutant varieties listed by country

Country	Common name and number of released varieties	Total
Algeria	soybean (1)	1
Argentina	groundnut (2), lemon (1), orange (1), peach (1), wheat (1)	6
Australia	blue lupin (1), lupin (1), oat (2), serradella (1), soybean (1), subterranean clover (1)	7
Austria	apple (1), barley (9), durum (6), faba bean (1)	17
Bangladesh	black gram (1), chickpea (1), jute (1), mungbean (4), oriental mustard (3), rapeseed (2), rice (5), tomato (3), tossa jute (3)	23
Belgium	azalea (8), barley (1), chrysanthemum (7), ficus (2), guzmania (1), potato (1), red clover (1), ryegrass (1)	22
Brazil	chrysanthemum (3), common bean (3), rice (1), wheat (2)	9
Bulgaria	barley (4), durum (4), pepper (3), lentil (1), maize (8), peach (1), pepper (1), soybean (3), sweet pepper (2), tobacco (1), wheat (2)	30
Burkina Faso	rice (2)	2
Canada	apple (2), apricot (1), barley (5), begonia (2), common bean (12), flax/linseed (3), rapeseed (1), rose (2), Russian wildrye (1), sweet cherry (5), tobacco (1)	35
Chile	barley (1), wheat (1)	2
China	alfalfa (1), apple (1), barley (7), bougainvillea (2), canna lilies (4), chinese cabbage (4), chinese garlic (1), chrysanthemum (21), common bean (1), cotton (8), crown vetch (1), cucumber (1), dahlia (2), flax/linseed (3), foxtail millet (1), groundnut (29), jute (1), lotus (3), maize (42), millet (20), mulberry (6), orange/mandarin (5), pea (1), pear (5), radish (1), rapeseed (7), rice (191), rose (35), sesame (1), shadawang (5), sorghum (3), soybean (54), sugar beet (2), sugarcane (2), sunflower (1), sweet potato (4), taro (1), tea (1), watermelon (2), wheat (124), white ramie (1)	605
Costa Rica	common bean (1), cowpea (1), rice (2),	4
Cote d'Ivoire	rice (25)	25
CSFR/Czech Rep.	barley (27), common bean (1), crimson clover (1), maize (3), rose (1), soybean (1), vetch (1), mustard (1)	36
Denmark	barley (21)	21
Egypt	chickpea (1), common bean (1), sesame (2)	4
Estonia	barley (4), potato (1)	5
Finland	barley (4), oat (4), rye (2), wheat (1)	11
France	apple (5), barley (12), black currant (1), carnation (4), dahlia (5), durum (1), forsythia (2), plum (1), rice (5), weigela (3)	39
Germany/ FRG/GDR	alstroemeria (11), azalea (3), barley (44), carnation (4), chrysanthemum (34), common bean (2), faba bean (1), geranium (1), meadow fescue (3), meadow foxtail (2), ribes (1), rose (3), rye (2), snapdragon (1), soybean (1) spinach (1), streptocarpus (22), wheat (2),	138
Ghana	cassava (1)	1
Greece	barley (1), durum (1)	2
Guyana	rice (26)	26
Hungary	chrysanthemum (1), maize (1), rice (3), soybean (1), wheat (1),	7
India	barley (14), bitter gourd (1), black gram (3), bougainvillea (10), castor bean (3), chickpea (4), chinese mustard (1), chrysanthemum (46), citronella (6), common bean (1), cotton (9), cowpea (6), dahlia (11), eggplant (1), egyptian clover (1), gladiolus (2), green pepper (1), groundnut (13), hibiscus (2), hyacinth bean (1), khasianum (1), lentil (1), mulberry (1), mungbean (5), mustard (1), okra (1), opium poppy (1), oriental mustard (3),	259

	papaya (1), pea (1), pearl millet (5), pigeon pea (5), polyanthes (2), portulaca (10), portulaca per. (1), rice (40), ridged gourd (1), rose (15), sesame (3), sorghum (1), sugarcane (5), tobacco (1), tomato (4), tossa jute (3), turmeric (2), wheat (4), white jute (2), wild sage (3)	
Indonesia	mungbean (1), rice (6), soybean (3), tobacco (1)	11
Iraq	barley (7), faba bean (2), rice (3), sesame (3), tobacco (2), wheat (6)	23
Italy	almond (1), common bean (2), durum (13), eggplant (3), green pepper (1), olive (1), pea (6), potato (1), rice (1), sweet cherry (3), vetch (1), wheat (2)	35
Japan	abelia (1), apple (1), azalea (1), azuki bean (1), barley (8), begonia (6), burdock (4), carnation (1), chinese matgrass (1), chrysanthemum (14), creeping bent grass (1), eustoma (3), hibiscus (1), japanese pear (2), job's tears (1), lettuce (2), loquat (1), mat rush (2), mint (1), potato (1), rice (46), rose (3), roselle (4), soybean (6), sugarcane (1), tomato (4), turnip/jpn rape (1), wheat (2)	120
Kenya	cowpea (2)	2
Korea	barley (1), rice (2), sesame (6), soybean (2),	11
Korea, Rep.of	rice (5)	5
Malaysia	banana (1)	1
Mali	sorghum (8)	8
Mongolia	wheat (3)	3
Myanmar	groundnut (1), rice (2), tossa jute (1)	4
Netherlands	achimenes (8), african violet (1), alstroemeria (24), apple (flowers) (1), azalea (3), barley (1), begonia (6), calathea (1), carnation (7), chrysanthemum (80), dahlia (18), euphorbia (1), gladiolus (2), hyacinth (1), kalanchoe (3), lily (2), onion (2), streptocarpus (7), tulip (8)	176
Nigeria	rice (3)	3
Norway	barley (2)	2
Pakistan	chickpea (5), cotton (5), mungbean (9), rapeseed (1), rice (6), wheat (6)	32
Peru	barley (1)	1
Philippines	rice (4)	4
Poland	barley (1), blue lupin (1), chrysanthemum (6), faba bean (5), gerbera (1), pea (14), scarlet runner (1), yellow lupin (1),	30
Portugal	rice (1)	1
Romania	rice (1)	1
Russia	barley (2), millet (1), onion (1), pea (1), tulip (1)	6
Senegal	rice (2)	2
Sri Lanka	groundnut (1), rice (1), sesame (1)	3
Sweden	barley (20), mustard (3), pea (1), rapeseed (2)	26
Switzerland	wheat (1)	1
Thailand	banana (1), carnation (1), chrysanthemum (2), rice (4), soybean (1)	9
Turkey	barley (1), soybean (2)	3
UK	barley (31), streptocarpus (1)	32
Ukraine	barley (1)	1
USA	barley (13), begonia (11), bermuda grass (4), carnation (1), centipedegrass (2), chrysanthemum (1), common bean (26), crapemyrtle (2), crested wheatgrass (1), grapefruit (2), groundnut (1), hop (3), hoya (4), lespedeza (2), lettuce (3), lilac (1), oat (12), peppermint (2), rice (23), rose (2), snapdragon (3), st. Augustine grass (2), tobacco (1), wheat (3)	125
USSR	amarant (1), barley (26), brome grass (1), buckthorn (1), buckwheat (8), castor bean (1), chamomile (1), chrysanthemum (17), common bean (4), cotton (2), cress (1), cucumber (1), durra (1), faba bean (4), fig (1), flax/linseed (3), fodder beet (5), grape (1), iris (5), kale (1), lettuce (1), maize (12), millet (3), oat (3), onion (1), pea (8), pepper (1), plavine (1),	204

	pomegranate (2), poplar (1), rapeseed (2), raspberry (1), rice (6), sainfoin (2), sorghum (1), sour cherry (4), soybean (9), sudan grass (1), sunflower (1), tobacco (4), tomato (2), vetch (1), watermelon (1), wheat (36), white lupin (13), yellow lupin (2)	
Vietnam	groundnut (1), indian jujube (2), maize (2), peppermint (1), rice (18), soybean (5),	29
Yugoslavia	pepper (1)	1

In six countries, the number of released mutant varieties exceeded 100. The top countries on the list are China, India, former USSR and Russia, The Netherlands, USA and Japan (Table 2). However, the list would change if the mutant varieties developed in the former FRG and GDR (in total 138 varieties including one variety recently released in Germany) were combined.

TABLE 2: Number of officially released mutant varieties in the top six countries (total 2,252)

Country	Number of released mutant cultivars	Percent of total
China P.R.	605	26.8
India	259	11.5
USSR + Russia	210	9.3
Netherlands	176	7.8
USA	128	5.7
Japan	120	5.3

The number of mutant varieties released in China and India place Asia at the top of the regional lists. However, it is worth noting that Europe ranks second in the number of mutant varieties, very close to that released in Asia (Fig. 2). This clearly indicates that the enhancement of germplasm through induced mutation techniques is a necessary component of many current breeding programmes.

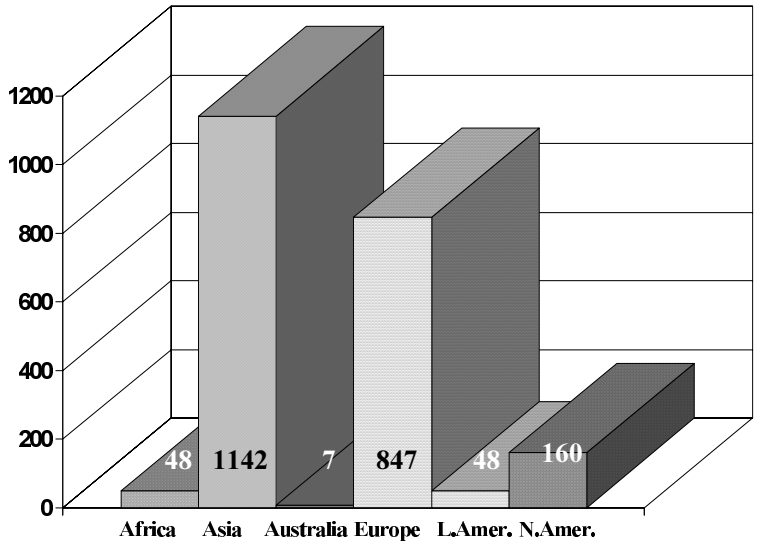

FIG. 2. Cumulative number of officially released mutant varieties in various regions of the world, June 2000.

The list of crop and plant species with induced mutant varieties is a long one and recently reached 175 entities (Table 3) as compared with 154 species in 1995 [7]. This was mainly because of an increase in the application of mutation techniques for the improvement of ornamental and decorative plants (Fig. 3A) in developing countries, where these plants have become important "cash crops". It is remarkable that the number of mutant varieties of vegetatively propagated crops (Fig. 3B) has only slightly increased in spite of the availability of many *in vitro* culture methods, which should have facilitated the development of new varieties. A new FAO/IAEA Coordinated Research Project has been established this year to identify constraints in the production of mutant varieties of fruit trees and to develop methods and protocols for more efficient use of mutation techniques and related biotechnologies. The most significant increase, compared to 1995, [8] was observed in the number of new mutant varieties in crop species (494 new), mainly in seed propagated crops (366 new mutant varieties). The distribution pattern among seed propagated crops did not change very much (Fig. 3C). Mutant varieties of cereals are on the top of the list (1072) followed by legumes (311), industrial (81), vegetables (66), oil crops (59) and other seed propagated crops (111). Significant increase was observed in the number of newly released rice and wheat mutant varieties (Fig 3D). This was mainly based on information from China, where many crop mutant varieties have been recently released. One of the next issues of Mutation Breeding Review will summarize results of the application of mutation techniques in plant breeding in China. In total there are 434 rice and 197 bread wheat accessions in MVD. Progress in the use of induced mutations for oilseed crops improvement was recently reviewed by Bhatia *et al***.** [9].

TABLE 3: Number of officially released mutant varieties in different species

Latin name	Common name	No. of mutant varieties
Abelia sp.	abelia	1
Abelmoschus esculentus (L.) Moench	okra	1
Achimenes sp.	achimenes	8
Agropyron cristatum (L.) Gaertner	crested wheat grass	1
Agrostis sp.	creeping bent grass	1
Allium cepa L.	onion	4
Allium macrostemon Bunge	chinese garlic	1
Alopecurus pratensis L.	meadow foxtail	2
Alstroemeria sp.	alstroemeria	35
Amaranthus sp.	amaranth	1
Antirrhinum sp.	snapdragon	4
Arachis hypogaea L.	groundnut	48
Arctium lappa L.	burdock	4
Astragalus huangheensis	shadawang	5
Avena sativa L.	oat	21
Begonia sp.	begonia	25
Beta vulgaris L.	fodder beet	5
Beta vulgaris L.	sugar beet	2
Boehmeria nivea (L.) Gaudich.	white ramie	1
Bougainvillea sp.	bougainvillea	12
Brassica campestris L.	turnip/jpn rape	1
Brassica juncea L.	oriental mustard	6
Brassica napus L.	rapeseed	15

Brassica oleracea (L.) var. *acephala*	kale	1
Brassica pekinensis Rupr.	chinese cabbage	4
Bromus inermis Leyss.	brome grass	1
Cajanus cajan Millsp.	pigeon pea	5
Calathea crocata	calathea	1
Camelia sinensis Kuntze	tea	1
Canna indica L.	canna lilies	4
Capsicum annuum L.	pepper	10
Carica papaya L.	papaya	1
Chrysanthemum sp.	chrysanthemum	232
Cicer arietinum L.	chickpea	11
Citrullus lanatus Mansf.	watermelon	3
Citrus limon (L.) Burm.	lemon	1
Citrus paradisi Macf.	grapefruit	2
Citrus sinensis (L.) Osbeck	orange	1
Citrus sp.	orange/mandarin	5
Coix lachryma-jobi L.	job's tears	1
Colocasia esculenta Schott.	taro	1
Corchorus capsularis L.	jute	2
Corchorus capsularis L.	white jute	2
Corchorus olitorius L.	tossa jute	7
Coronilla varia L.	crown vetch	1
Cucumis sativus L.	cucumber	2
Curcuma domestica Val.	turmeric	2
Cymbopogon winterianus Jowitt	citronella	6
Cynodon sp.	bermuda grass	4
Cyperus malaccensis Lam.	chinese matgrass	1
Dahlia sp.	dahlia	36
Dianthus caryophyllus L.	carnation	18
Dolichos lablab L.	hyacinth bean	1
Eremochloa ophuiroides Hack	centipedegrass	2
Eriobotrya japonica Lindl	loquat	1
Euphorbia fulgens Karw.	euphorbia	1
Eustoma grandiflorum (Raf.) Shinn.	eustoma	3
Fagopyrum esculentum Gili	buckwheat	8
Festuca pratensis Huds.	meadow fescue	3
Ficus benjamina exotica	ficus	2
Ficus carica L.	fig	1
Forsythia x intermedia	forsythia	2
Gerbera jamesonii Bolus	gerbera	1
Gladiolus sp.	gladiolus	4
Glycine max L.	soybean	90
Gossypium sp.	cotton	24
Guzmania paecockii Ruiz et Pav.	guzmania	1
Helianthus annuus L.	sunflower	2
Hibiscus sp.	roselle	3
Hibiscus sp.	hibiscus	4
Hippophaea rhamnoides L.	buckthorn	1
Hordeum vulgare L.	barley	269
Hoya carnosa R.Br.	hoya	4
Humulus lupulus L.	hop	3
Hyacinthus sp.	hyacinth	1
Ipomoea batatas (L.) Poir.	sweet potato	4

Iris sp.	iris	5
Juncus effusus L.	mat rush	2
Kalanchoe sp.	kalanchoe	3
Lactuca sativa L.	lettuce	6
Lagerstroemia indica L.	crapemyrtle	2
Lantana depressa	wild sage	3
Lathyrus sativus L.	plavine, grass pea	1
Lens culinaris Medik.	lentil	2
Lepidium sativum L.	cress	1
Lespedeza cuneata Dum.	lespedeza	2
Lilium sp.	lily	2
Linum usitatissimum L.	flax/linseed	7
Linum usitatissimum L.	flax	2
Lolium sp.	ryegrass	1
Luffa acutangula Roxb.	ridged gourd	1
Lupinus albus L.	white lupin	13
Lupinus angustifolius L.	blue lupin	2
Lupinus consentini Guss.	lupin	1
Lupinus luteus L.	yellow lupin	3
Lycopersicon esculentum M.	tomato	13
Malus pumila Mill.	apple	9
Malus sp.	apple (flowers)	1
Manihot esculenta (L.) Crantz	cassava	1
Matricaria chamomilla L.	chamomile	1
Medicago sativa L.	alfalfa	1
Mentha arvensis L.	peppermint	1
Mentha arvensis L.	mint	1
Momordica charantia L.	bitter gourd	1
Morus alba L.	mulberry	7
Musa sp.	banana	2
Nelumbo nucifera Gaertner	lotus	3
Nicotiana tabacum L.	tobacco	11
Olea europaea L.	olive	1
Onobrychis viciifolia Scop.	sainfoin	2
Ornithopus compressus L.	serradella	1
Oryza sativa L.	rice	434
Panicum miliaceum L.	millet	4
Papaver somniferum L.	opium poppy	1
Pelargonium grandiflorum hybrid	geranium	1
Pennisetum sp.	pearl millet	5
Phaseolus coccineus L.	scarlet runner bean	1
Phaseolus vulgaris L.	common bean	54
Pisum sativum L.	pea	32
Polyanthes tuberosa L.	polyanthes	2
Populus trichocarpa L.	poplar	1
Portulaca grandiflora L.	portulaca	10
Portulaca grandiflora L.	portulaca per.	1
Prunus armeniaca L.	apricot	1
Prunus avium L.	sweet cherry	8
Prunus cerasus L.	sour cherry	4
Prunus domestica L.	plum	1
Prunus dulcis Webb	almond	1
Prunus persica L.	peach	2

Psathyrostachys juncea (F.) Nevski	Russian wildrye	1
Punica granatum L.	pomegranate	2
Pyrus communis L.	pear	5
Pyrus pyriforia Nakai	japanese pear	2
Raphanus sativus L.	radish	1
Rhododendron simsii Planch.	azalea	2
Rhododendron sp.	azalea	13
Ribes nigrum L.	black currant	1
Ribes sp.	ribes	1
Ricinus communis L.	castor bean	4
Rosa sp.	rose	61
Rubus idaeus L.	raspberry	1
Saccharum officinarum L.	sugarcane	8
Saintpaulia sp.	african violet	1
Secale cereale L.	rye	4
Sesamum indicum L.	sesame	16
Setaria italica (L.) Beauv.	foxtail millet	1
Setaria sp.	millet	24
Sinapis alba L.	white mustard	5
Solanum khasianum Clarke	khasianum	1
Solanum melongena L.	eggplant	4
Solanum tuberosum L.	potato	4
Sorghum bicolor L.	sorghum	13
Sorghum durra Stapf	durra	1
Sorghum sudanense (Piper) Stapf	sudan grass	1
Spinacia oleracea L.	spinach	1
Stenotaphrum secundatum Kuntze	st. Augustine grass	2
Streptocarpus sp.	streptocarpus	30
Syringa vulgaris L.	lilac	1
Trifolium alexandrinum L.	egyptian clover	1
Trifolium incarnatum L.	crimson clover	1
Trifolium pratense L.	red clover	1
Trifolium subterraneum L.	subterranean clover	1
Triticum aestivum L.	wheat	197
Triticum turgidum ssp. *durum* Desf.	durum	25
Tulipa sp.	tulip	9
Vicia faba L.	faba bean	13
Vicia sativa L.	common vetch	3
Vigna angularis Willd.	azuki bean	1
Vigna mungo L.	black gram	4
Vigna radiata (L.) Wil.	mungbean	19
Vigna unguiculata Walp.	cowpea	9
Vitis vinifera L.	grape	1
Weigela sp.	weigela	3
Zea mays L.	maize	68
Ziziphus mauritiana Lam.	indian jujube	2

Of the total 2,252 mutant varieties, 1,585 were developed ‘directly’ after mutagenic treatment and selection in the subsequent generations. However, in many cases mutants or already released mutant varieties have been used as sources of desired characters in cross breeding programmes; in this way, 667 new varieties were developed. Of 1,585 directly

developed mutant varieties, a great majority (1,411) were obtained with the use of radiation as the mutagen (Table 4).

TABLE 4: Number of officially released mutant cultivars developed with different types of radiation

Type of mutagen	Number of released mutant cultivars	Percent of total
Radiation*	1411	100.00
▪ gamma rays*	910	64.49
▪ x-rays*	311	22.04
▪ gamma chronic	61	4.32
▪ fast neutrons**	48	3.40
▪ thermal neutrons	22	1.56
▪ other	24	1.70

**including various treatments*; ***including "neutrons"*

The presented MVD still needs modification and some additions especially for parental varieties used in crosses or improved by mutation characters. Readers are kindly requested to send their comments, questions, suggestions or additional information to the following address:

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REFERENCES

[1] Maluszynski, M., B. Sigurbjörnsson, E. Amano, L. Sitch, and O. Kamra**,** 1991. Mutant varieties - data bank, FAO/IAEA database. MBNL. **38**: 16-21

[2] Sigurbjörnsson, B. and A. Micke , 1969. Progress in mutation breeding. In: Induced Mutations in Plants. IAEA, Vienna. pp.673-698

[3] Sigurbjörnsson, B. and A. Micke , 1974. Philosophy and accomplishments of mutation breeding. In: Polyploidy and Induced Mutations in Plant Breeding. IAEA, Vienna. pp.303-343

[4] Micke, A., M. Maluszynski, and B. Donini**,** 1985. Plant cultivars derived from mutation induction or the use of induced mutants in cross breeding. Mutat.Breed.Rev. **3**: 1-92

[5] Soldatov, K. I., 1976. Chemical mutagenesis in sunflower breeding. In: Proceedings of the VIIth International Sunflower Conference. Vol. 1. Krasnodar. pp.352-357

[6] Bouma, J. and Z. Ohnoutka, 1991. Importance and application of the mutant 'Diamant' in spring barley breeding. In: Plant Mutation Breeding for Crop Improvement. Vol. 1. IAEA, Vienna. pp.127-133

[7] Maluszynski, M., B. S. Ahloowalia, and B. Sigurbjörnsson**,** 1995. Application of *in vivo* and *in vitro* mutation techniques for crop improvement. Euphytica **85**(1-3): 303-315

[8] Maluszynski, M., L. van Zanten, A. Ashri, H. Brunner, B. Ahloowalia, F. J. Zapata, and E. Weck, 1995. Mutation techniques in plant breeding. In: Induced Mutations and Molecular Techniques for Crop Improvement. IAEA, Vienna. pp.489-504

[9] Bhatia, C. R., K. Nichterlein, and M. Maluszynski**,** 1999. Oilseed cultivars developed from induced mutations and mutations altering fatty acid composition. Mut.Breed.Rev. **12**: 1-36

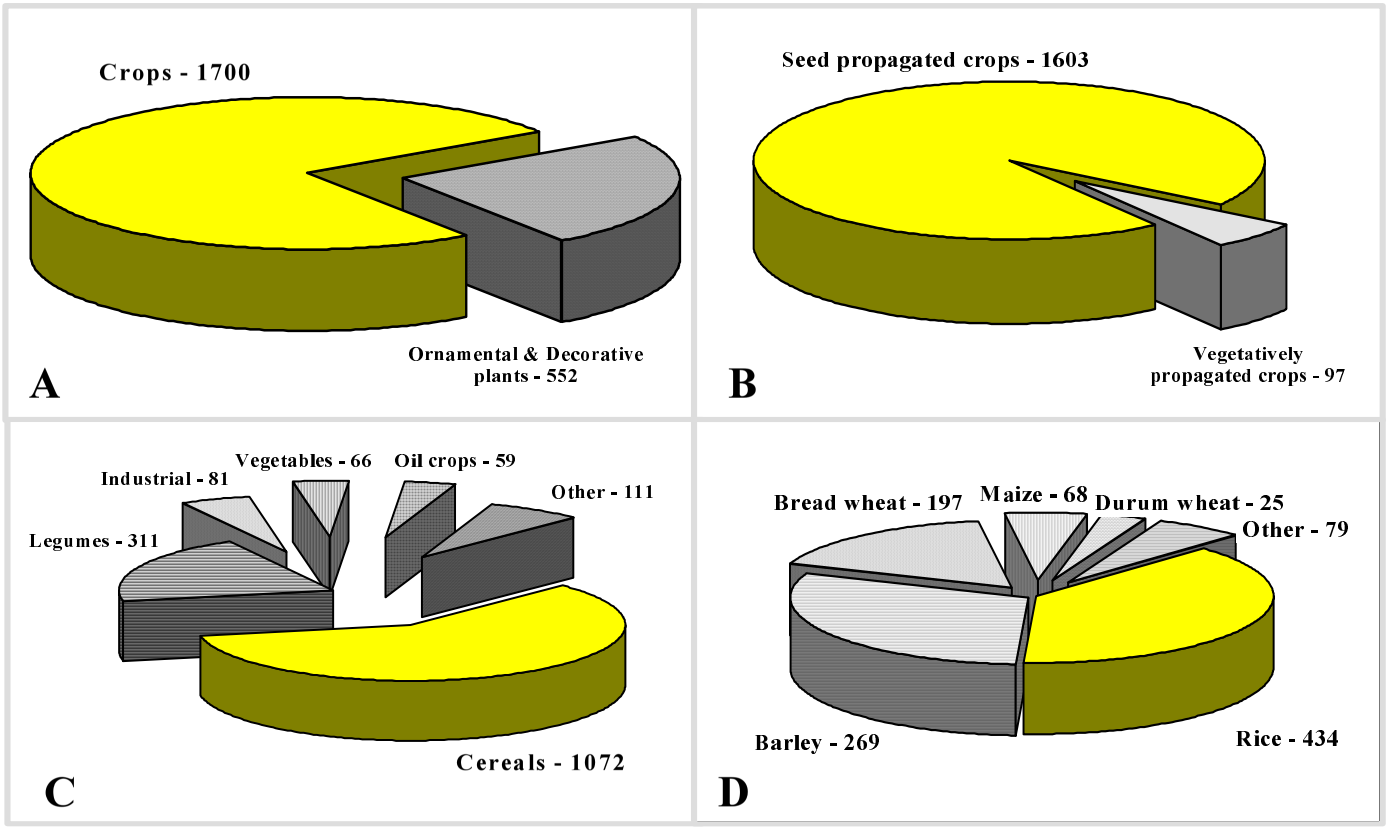

FIG. 3. Number of officially released mutant cultivars in different crop categories: A – ornamental and decorative plants; B – vegetatively propagated; C – major crops; D – major cereals.

FAO/IAEA MUTANT VARIETIES DATABASE

Latin name	Common name	Mutant variety	Country of release	Year of release	Mutagen	Parent variety	Main character induced	MBNL No.
Abelia sp.	abelia	Meifuhanatsukubaneutsu	Japan	1976	gamma rays	Hanazono-Tsukubane	variegated leaves	9
Abelmoschus esculentus	okra	MDU 2	India	1978	DES	Pusa Sawani	yield	33
Achimenes sp.	achimenes	Compact Arnold	Netherlands	1971	x-rays or fN	Paul Arnold	plant architecture	2
		Cupido	Netherlands	1973	x-rays or fN	Paul Arnold	compact growth	17
		Early Arnold	Netherlands	1971	x-rays or fN	Paul Arnold	earliness	2
		Flamingo	Netherlands	1977	x-rays	Tango	plant architecture	17
		Lollipop	Netherlands	1977	fN	Tango	compact growth	17
		Orion	Netherlands	1973	x-rays or fN	Paul Arnold	earliness	17
		Pink Attraction	Netherlands	1977	x-rays	autotetraploid of 'Repelsteeltje'	compact growth	17
		Springtime	Netherlands	1971	x-rays or fN	Paul Arnold	earliness	2
Agropyron cristatum	crested wheat grass	CD-II	USA	1996	cross		vigour	44
Agrostis sp.	creeping bent grass	Springs	Japan	1983	gamma rays	Pencross	heat tolerance	32
Allium cepa	onion	Brunette	Netherlands	1973	x-rays	Grobol	earliness	*
		Compas	Netherlands	1970	x-rays	Grobol	stiffness	1
		KIK-11	USSR	1991	cross		yield	41
		Tabys (KIK-13)	Russia	1993	ENH	Octyabr	yield	41
Allium macrostemon	chinese garlic	Ningsuan 1	China	1990	gamma rays	landrace	yield	n.i.
Alopecurus pratensis	meadow foxtail	Alko	FRG	1983	gamma rays		seed retention	34
		Limosa	FRG	1984	gamma rays		seed retention	34
Alstroemeria sp.	alstroemeria	Appelbloesem	Netherlands	1979	x-rays	King Cardinal	flower colour	31
		Atlas	Netherlands	1984	x-rays	Red Sunset	flower colour	31
		Audino	GDR	1979	gamma rays		earliness	37
		Canaria	Netherlands	1970	x-rays	Orchid Flower	flower colour	*
		Capitol	Netherlands	1977	x-rays	Carmen	flower colour	17
		Chimbotina	GDR	1981	gamma rays		flower colour	37
		Fanfare	Netherlands	1977	x-rays	Carmen	flower colour	17
		Harlequin	Netherlands	1973	x-rays	Paringo's Charm	flower colour	17
		Harmony stabrons	Netherlands	1972	x-rays	Regina	flower colour	17
		Jacqueline	Netherlands	1979	x-rays	Rosario	flower size	31

		Kolibri Blau	GDR	1989	gamma rays		flower colour	37
		Kolibri Gelb	GDR	1989	gamma rays		flower colour	37
		Kolibri Orange	GDR	1989	gamma rays		flower colour	37
		Kolibri Rosa	GDR	1989	gamma rays		flower colour	37
		Kolibri Rot	GDR	1989	gamma rays		flower colour	37
		La Paz	Netherlands	1984	x-rays	Rio	flower colour	31
		La Poza	GDR	1981	gamma rays		flower colour	37
		Lilac Glory	Netherlands	1979	x-rays	Rosario	flower colour	31
		Patricia	Netherlands	1983	x-rays	Pink Triumph	flower colour	31
		Pink Panther	Netherlands	1978	x-rays	Rosario	tallness	31
		Pink Tiger	Netherlands	1983	x-rays	Pink Panther	flower colour	31
		Purple Joy	Netherlands	1979	x-rays	Carmen	flower colour	31
		Quitona	GDR	1981	gamma rays		flower colour	37
		Red Sunset	Netherlands	1979	x-rays		flower colour	14
		Result	Netherlands	1977	x-rays	Carmen	flower colour	17
		Rosali staliro	Netherlands	1975	x-rays	Starosa	flower colour	17
		Rosita stareza	Netherlands	1972	x-rays	Regina	flower colour	17
		Trident	Netherlands	1977	x-rays	Carmen	flower colour	17
		Tucumana	GDR	1981	gamma rays		flower colour	37
		Valiant	Netherlands	1977	x-rays	Carmen	flower colour	17
		Valparaisa	GDR	1981	gamma rays		flower colour	37
		White Wings	Netherlands	1971	x-rays	Orchid Flower	flower colour	2
		Yellow Tiger	Netherlands	1970	x-rays	Orchid Flower	flower colour	*
		Zebra stazeb	Netherlands	1975	x-rays	Orchid Flower	flower colour	17
		Zenith	Netherlands	1977	x-rays	Carmen	flower colour	17
Amaranthus sp.	amaranth	Sterkh	USSR	1992	chemical	[A.panic.x A.nutans]	drought tolerance	41
Antirrhinum sp.	snapdragon	Antirrhinum Juliva	FRG	1961	cross		flower	7
		Bright Butterflies	USA	1966	cross	Antirrhinum divaricata	flower	7
		Little Darling	USA	1966	cross	Antirrhinum divaricata	flower	7
		Madame Butterfly	USA	1966	cross	Antirrhinum divaricata	flower	7
Arachis hypogaea	groundnut	78961	China	1988	cross		earliness	37
		8130	China	1988	cross		seed quality	44
		ANK-G1 (Tissa)	Sri Lanka	1995	gamma rays	Vietnam	yield	43

B 5000	Vietnam	1985	gamma rays	Bacta	seed size	31
BP-1	India	1979	gamma rays	41-C	seed size	31
BP-2	India	1979	gamma rays	41-C	seed size	32
Changhua 4	China	1972	gamma rays	Fuhuasheng	earliness	27
Co 2	India	1984	EMS	Pol-1	yield	26
Colorado Irradiado	Argentina		x-rays	Colorado de Cordoba	yield	7
Fu 21	China	1986	gamma rays	Yueyou 22	yield	29
Fu 22	China	1985	gamma rays		*A. flavus*	37
Ganhua 1	China	1990	gamma rays	Yueyou 551-11	earliness	41
Huayu 16	China	1996	gamma rays		yield	44
Lainong 10	China	1984	laser		earliness	37
Lu 8130	China	1993	cross		pod size	n.i.
Luhua 11	China	1992	laser	hybrid	yield	n.i.
Luhua 13	China	1991	cross		yield	44
Luhua 15	China	1994	cross		seed quality	44
Luhua 6	China	1986	gamma rays	Baisha 1016	earliness	34
Luhua 7	China	1986	gamma rays	Linhua 1	logging resistance	32
MH-2	India	1973	gamma rays		yield	37
N.C.4-X	USA	1959	x-rays	N.C. 4	hull toughness	*
P12	China	1986	cross		yield	37
Shanyou 27	China	1985	cross		uniform	37
Sin Pa detha 1	Myanmar	1982	gamma rays	Magwe-10	earliness	20
SOMNATH	India	1989	cross		earliness	41
TAG-24	India	1991	cross		earliness	41
TG 17	India	1977	x-rays	Spanish Improved	yield	12
TG 3	India	1973	x-rays	Spanish Improved	pod number	12
TG 4	India	1976	x-rays	Spanish Improved	uniform maturity	12
TG-22	India	1994	cross		yield	44
TG-26	India	1996	cross		yield	44
TKG-19A	India	1996	cross		seed size	44
Vikram	India	1973	x-rays	Spanish Improved	seed size	11
Virginia No.3	Argentina	1979	radiation	N.C. 2	pod size	30
Xianghua 1	China	1985	cross		earliness	41

		Xianghuasheng 4	China	1996	gamma rays	Xianghuasheng 2	yield	n.i.
		Yangxuan 1	China	1978	cross			37
		Yeuyou 22	China	1968	cross		dwarfness	25
		Yuexuan 58	China	1978	cross		yield	37
		Yueyou 169	China	1980	cross		plant architecture	37
		Yueyou 187	China	1981	cross		tallness	37
		Yueyou 187-93	China	1982	cross		tallness	37
		Yueyou 33	China	1971	cross		yield	37
		Yueyou 551	China	1972	cross		dwarfness	25
		Yueyou 551-116	China	1975	cross		yield	37
		Yueyou 551-38	China	1975	cross		yield	37
		Yueyou 551-6	China	1975	cross		yield	37
Arctium lappa	burdock	Kobaruto-gokuwase	Japan	1981	gamma rays	Yanagawa-nakate	earliness	21
		Kobaruto-okute	Japan	1981	gamma rays	Yanagawa-nakate	lateness	21
		Kobaruto-wase	Japan	1981	gamma rays	Yanagawa-riso	earliness	21
		Tsuneyutaka	Japan	1986	gamma rays	Yanagawa-risou	thick root	33
Astragalus huangheensis	shadawang	Heifu 2	China	1987	gamma rays	domesticated Shadawang	earliness	n.i.
		Heifu 21	China	1987	gamma rays	domesticated Shadawang	earliness	n.i.
		Heifu 4	China	1987	laser	domesticated Shadawang	earliness	n.i.
		Penyangzaoshudawang	China	1991	gamma rays	Liaoningshadawang	earliness	n.i.
		Zaoshushadawang	China	1983	gamma rays	Shadawang	earliness	31
Avena sativa	oat	Alamo-X	USA	1961	x-rays	Alamo	blight resistance	*
		Bates	USA	1977	cross		shortness	14
		Bay	USA	1995	cross		disease resistance	44
		Belle	USA	1995	cross		disease resistance	44
		Belozernii	USSR	1979	NMH	Orel	shortness	13
		Bob	USA	1977	cross		yield	14
		Centennial	USA	1987	cross		rust resistance	42
		Dolphin	Australia	1984	cross		shortness	28
		Echidna	Australia	1984	cross		shortness	28
		Florad	USA	1959	thN	Floriland	rust resistance	*
		Florida 500	USA	1965	cross		rust resistance	*
		Florida 501	USA	1967	cross		plant type	*

		Gem	USA	1996	cross		disease resistance	44
		Horicon	USA	1990	cross		crown rust	42
		Nasta	Finland	1970	cross		earliness	20
		Ozark	USA	1991	cross		winter hardiness	42
		Puhti	Finland	1978	cross		yield	25
		Ryhti	Finland	1970	cross		yield	*
		Sir-4	USSR	1988	diazoacetylbut	Selma	adaptability	31
		Veli	Finland	1981	cross		yield	32
		Zelonyi	USSR	1976	NEU	Krasnodarskii 73	plant type	13
Begonia sp.	begonia	Aphrodite Joy	USA	1974	gamma rays	Aphrodite Rose	flower colour	17
		Aphrodite Peach	USA	1974	gamma rays	Aphrodite Rose	flower colour	17
		Aphrodite Twinkles	USA	1974	gamma rays	Aphrodite Rose	dwarfness	17
		Big-Cross	Japan	1976	gamma rays	Iron Cross	leaf morphology	9
		Elegance	USA	1975	gamma rays	Aphrodite Rose	double flowers	17
		Enchantress	USA	1974	gamma rays	Aphrodite Rose	flower	17
		Fantasy	USA	1975	gamma rays	Aphrodite Rose	plant architecture	17
		Flambeau	USA	1976	fN	Aphrodite Red	flower	17
		Gin-Sei	Japan	1976	gamma rays	Winter Queen	leaf colour	9
		Heirloom	USA	1975	fN	Schwabenland Pink	flower colour	17
		Hoblanche	Netherlands	1977	x-rays	Vuurgloed	flower colour	17
		Kaede-Iron	Japan	1976	gamma rays	Iron Cross	leaf morphology	9
		Manilla	Netherlands	1983	gamma rays	Grete	flower colour	31
		Manita	Netherlands	1986	gamma rays	Grete	flower colour	31
		Manolito	Netherlands	1986	gamma rays	Grete	flower colour	31
		Mikkel Limelight	USA	1974	fN	Aphrodite Rose	vigour	17
		Mini-Mini-Iron	Japan	1976	gamma rays	Iron cross	plant architecture	9
		Northern Sunset	Canada	1975	x-rays	Renaissance	flower petal	12
		Orange-Iron	Japan	1976	gamma rays	Iron Cross	flower colour	9
		Red Elegance	USA	1975	gamma rays	Aphrodite Rose	flower	17
		Rose Elegance	USA	1975	gamma rays	Aphrodite Rose	flower	17
		Ryoku-Ha	Japan	1976	gamma rays	Winter Queen	leaf morphology	9
		Saanred	Canada	1983	x-rays	Renaissance	flower colour	31
		Tiara	Netherlands	1974	radiation	Clone S01	flower colour	7

		Turo	Netherlands	1973	x-rays	Clone Le1	flower	7
Beta vulgaris	fodder beet	Timiryazevskaya	USSR	1988	chemical	Ekkendorfer	yield	31
		Tymiryazevskaya 87	USSR	1992	gamma rays		yield	41
		Tymiryazevskaya odnos	USSR	1988	EI		yield	41
		Tymiryazevskaya okrug	USSR	1991	EI	hybrid	yield	41
		Umanskii polusakharnyi	USSR	1990	cross		white rhizocarp	41
Beta vulgaris	sugar beet	Tianyan 301	China	1986	cross		quality	n.i.
		Tianyan 302	China	1989	cross		yield	n.i.
Boehmeria nivea	white ramie	Xiangzhu 2	China	1987	gamma rays	Xiangzhu 1	yield	n.i.
Bougainvillea sp.	bougainvillea	Arjuna	India	1976	gamma rays	Partha	variegated leaves	15
		Jaya	India	1977	gamma rays	Jayalakshmi	ornamental type	20
		Jayalaxmi Variegata	India	1977	gamma rays	Jayalakshmi	ornamental type	14
		Lady Hudson of C.V.	India	1979	gamma	Lady Hudson of Ceylon	ornamental type	20
		Los Banos Variegata	India	1990	gamma rays	Los Baños beauty	leaf colour	37
		Mahara variegata	India		gamma rays	Mahara	variegated leaves	43
		Pallavi	India	1986	gamma rays	Roseville's Delight	variegated leaves	31
		Poultoni Variegata	India	1981	gamma rays	Poultoni	variegated leaves	33
		Silver Top	India	1978	gamma	Versicolour	ornamental type	20
		Suicheng 85-2	China	1990	gamma rays	Meiguihong	flower colour	n.i.
		Suvarna	India	1981	gamma	Lady Hudson / Ceylon	flower colour	33
		Yuehong 85-1	China	1990	gamma rays	Meiguihong	flower colour	n.i.
Brassica campestris	turnip/jpn rape	Haya-natane	Japan	1961	colchicine	Michinoku-natane	yield	21
Brassica juncea	chinese mustard	RL 1359	India	1987	cross		earliness	31
	oriental mustard	Agrani	Bangladesh	1991	gamma rays	YS-52	earliness	42
		RLM 514	India	1980	gamma rays	RL-18	yield	17
		Safal	Bangladesh	1991	gamma rays	Line YS 52	yield	42
		Shambal (BAU-M/248)	Bangladesh	1984	EMS	BAU-M/14	shortness	34
		TM-2	India	1978	x-rays	RL-9	pod morphology	43
		TM-4	India	1978	cross		seed colour	43
Brassica napus	rapeseed	Abasin-95	Pakistan	1995	gamma rays	Tower	earliness	44
		Binasharisha-3	Bangladesh	1997	gamma rays		oil content	44
		Binasharisha-4	Bangladesh	1997	gamma rays		oil content	44
		Ganyu 5	China	1977	gamma rays	Shengliyoucai	cold tolerance	32

		Huahuang 1	China	1980	gamma rays		viability	41
		Huyou 4	China	1970	gamma rays	Shengliqinggeng	lodging resistance	27
		Ivanna	USSR	1990	MNH	Jet-Nef	oil content	41
		Regina varraps el. A	Sweden	1953	x-rays	Svalöf's Regina	yield	*
		Regina varraps el. F	Sweden	1962	x-rays	Svalöf's Regina	yield	*
		Stellar	Canada	1987	cross		oil quality	33
		Tismenitskii	USSR	1989	MNH	Gloria	oil content	41
		Xiangyou 11	China	1987	cross		stress tolerance	n.i.
		Xinyou 1	China	1979	gamma rays	Baichenghuangyoucai	seedling growth	27
		Xiuyou 1	China	1978	gamma rays	[Chuannongchangjiao x Qianyou 23]	earliness	32
		Zheyou 7	China	1983	cross		earliness	n.i.
Brassica oleracea var. *acephala*	kale	Vekha	USSR	1990	chemical	Mozgovaya zel.vol.	disease resistance	41
Brassica pekinensis	chinese cabbage	Baicai 9	China	1978	gamma rays	Keer x Feichenghuabai	earliness	25
		Longbai 1	China	1984	gamma rays	F4 line (Jiaoerye x Tongnong)	earliness	30
		Longfuerniuxin	China	1991	gamma rays	Xinnongerniuxin	disease resistance	n.i.
		Longxiebai 1	China	1992	cross		earliness	n.i.
Bromus inermis	brome grass	Fakel 89	USSR	1989	DMS	Morshanskii 760	winter hardiness	41
Cajanus cajan	pigeon pea	Co 3	India	1977	EMS	Co 1	yield	29
		Co 5	India	1984	gamma rays	Co 1	earliness	29
		TAT 10	India	1985	cross		seed size	28
		TAT 5	India	1984	fN	T-21	seed size	28
		Trombay Vishakha-1	India	1982	fN	T-21	seed size	23
Calathea crocata	calathea	Esther	Netherlands	1987	x-rays		flower petal	31
Camelia sinensis	tea	Fufeng	China	1997	gamma rays	Fudingdabeicha	yield	n.i.
Canna indica	canna lilies	Caixiao	China	1986	gamma rays	Dahonghua (root)	flower colour	32
		Caixui	China	1986	gamma rays	Dahonghua (root)	flower colour	32
		Huamei 1	China	1986	gamma rays		flower colour	n.i.
		Xuhong	China	1986	gamma rays	Dahonghua (root)	flower colour	32
Capsicum annuum	green pepper	Albena	Bulgaria	1976	gamma rays	Zlaten medal	fruit morphology	16
		Friari KS80	Italy	1985	EMS		semi-dwarfness	37
		Gornooriahovska kapia	Bulgaria	1997	cross		earliness	44
		Horgoska slatki-X-3	Yugoslavia	1974	gamma rays		fruit quality	33

		Krichimsky ran	Bulgaria	1972	x-rays	Pasardjishka kapia	yield	12
		Ljulin	Bulgaria	1982	cross		hybrid variety	20
		MDU.1	India	1976	gamma rays	K-1	compact growth	10
		Nush-51	USSR	1991	EI	Lastochka	yield	41
		Orangeva Kapia	Bulgaria	1991	x-rays	Pasardjshka kapia	beta carotene	41
		Pirin	Bulgaria	1991	gamma rays	Kurtovska kapia	powdery mildew	41
Carica papaya	papaya	Pusa nanha	India	1986	gamma rays	Ranchi	shortness	30
Chrysanthemum sp.	chrysanthemum	Agnisikha	India	1987	gamma rays		flower colour	37
		Alankar	India	1982	gamma rays	D-5	flower colour	23
		Amber Boston	Netherlands	1978		Pink Boston	flower colour	16
		Anamika	India	1975	gamma rays	E-13	flower colour	15
		Angshoujingshi	China	1989	gamma rays	Fengsehuan	flower colour	n.i.
		Apricot Deholta	Netherlands	1983	x-rays	Delta	flower colour	31
		Apricot Impala	Netherlands	1984	x-rays	Impala	flower colour	31
		Aruna	India	1974	gamma rays	Undaunted	flower colour	15
		Asha	India	1975	gamma rays	Hope	flower colour	15
		Ashankit	India	1974	gamma rays	Undaunted	flower	15
		Babette Gelb	FRG	1988	x-rays	Babette (white)	flower colour	31
		Baiogiku rainb. red	Japan	1985	gamma rays	Seikouno-kurnenai	flower colour	32
		Baiogiku rainb.orang	Japan	1985	gamma rays	Seikouno-kurnenai	flower colour	32
		Baiogiku rainb.peach	Japan	1985	gamma rays	Seikouno-kurnenai	flower colour	32
		Baiogiku rainb.pink	Japan	1985	gamma rays	Seikouno-kurnenai	flower colour	32
		Baiogiku rainb.white	Japan	1985	gamma rays	Seikouno-kurnenai	flower colour	32
		Baiogiku rainb.yello	Japan	1985	gamma rays	Seikouno-kurenai	flower colour	32
		Baiyunyong	China	1991	gamma rays	Changfengwanli	flower type	n.i.
		Basant	India	1975	gamma rays	Paul	flower colour	15
		Basanti	India	1979	gamma rays	E-13	flower colour	23
		Batik	India	1994	gamma rays	Flirt	flower colour	43
		Blue Redemine	Netherlands	1984	x-rays	Redemine	flower colour	31
		Blue Star	Netherlands	1977	x-rays	Pink Star	flower colour	16
		Blue Winner	Netherlands	1975	x-rays	Pink Winner	flower colour	15
		Bright Lameet	Netherlands	1978	x-rays	Lameet	flower colour	14
		Bright Star	Netherlands	1977	x-rays	Pink Star	flower colour	16

Bright Westland	Netherlands	1976	x-rays	Westland	flower colour	15
Bronce Kalinka	FRG	1987	x-rays	Kalinka	flower colour	35
Bronze Byoux	Netherlands	1985	gamma rays	Byoux	flower colour	31
Bronze Charmette	Netherlands	1976	x-rays	Charmette	flower colour	15
Bronze Clinspy	Netherlands	1978	x-rays	Clinspy	flower colour	14
Bronze Miros	Netherlands	1979	x-rays	Miros	flower colour	16
Bronze Redemine	Netherlands	1986	x-rays	Redemine	flower colour	31
Bronze Star	Netherlands	1977	x-rays	Pink Star	flower colour	16
Bronze Westland	Netherlands	1976	x-rays	Westland	flower colour	15
Bronze Winner	Netherlands	1975	x-rays	Pink Winner	flower colour	15
Cherry Deholta	Netherlands	1985	x-rays	Dark Delta	flower colour	31
Chongyangshaoyao	China	1989	gamma rays	Saishaoyao	flower colour	n.i.
Chuntao	China	1991	gamma rays	Zihe	flower colour	n.i.
Colchi Bahar	India	1985	colchicine	Sharad Bahar	flower colour	31
Copper Marconi	Belgium	1985	x-rays	Marconi	flower colour	31
Coral Refla	Netherlands	1986	x-rays	Refla	flower colour	31
Coral Winner	Netherlands	1975	x-rays	Pink Winner	flower colour	15
Cosmonaut	India	1984	gamma rays	Nimrod	flower	26
Cream Clingo	Netherlands	1979	x-rays	Clingo	flower colour	14
Cream Deholta	Netherlands	1985	x-rays	Deholta	flower colour	31
Cream Impala	Netherlands	1984	x-rays	Impala	flower colour	31
Cristiane	Brazil	1995	gamma rays	Repin	flower colour	43
Dalekaya zoezda	USSR	1976	gamma rays	Violet Colour	flower colour	14
Danny Boy	Netherlands	1973	x-rays	Beamsville Pink	flower colour	15
Danny's Cape	Netherlands	1973	x-rays	Beamsville Pink	flower colour	15
Danny's Pearl	Netherlands	1973	x-rays	Beamsville Pink	flower colour	15
Dark Charmette	Netherlands	1976	x-rays	Charmette	flower colour	15
Dark Deep Tuneful	Netherlands	1969	x-rays	Tuneful	flower colour	15
Dark Gaby	FRG	1988	x-rays	Gaby (pink)	flower colour	31
Dark Lymon	Netherlands	1985	x-rays	Lymon	flower colour	31
Dark Mario	FRG	1983	x-rays	Mario (pink)	flower colour	23
Dark Miros	Netherlands	1979	x-rays	Miros	flower colour	16
Dark Oriette	Netherlands	1976	x-rays	Oriette	flower colour	15

Dark Red Marconi	Belgium	1985	x-rays	Marconi	flower colour	31
Dark Torino	Belgium	1985	x-rays	Torino	flower colour	31
Dark Westland	Netherlands	1976	x-rays	Westland	flower colour	15
Dark/Royal Rendez-Vo	Netherlands	1986	gamma rays	Rendez-Vous	flower colour	31
Dr. X	USA	1966	x-rays	Dr. Dave	flower colour	*
Enzett Axillia Gelb	GDR	1988	gamma rays		flower colour	37
Enzett Balina Rot	GDR	1985	gamma rays		flower colour	37
Enzett Balina Weiss	GDR	1985	gamma rays		flower colour	37
Enzett Dilana Gelb	GDR	1977	gamma rays		flower colour	37
Enzett Dilana Rosa	GDR	1979	gamma rays		flower colour	37
Enzett Heli Bronze	GDR	1987	gamma rays		flower colour	37
Enzett Heli Gelb	GDR	1987	gamma rays		flower colour	37
Enzett Mellit Gelb	GDR	1989	gamma rays		flower colour	37
Enzett Minos Bronze	GDR	1985	gamma rays		flower colour	37
Enzett Niva Bronze	GDR	1984	gamma rays		flower colour	37
Enzett Niva Gelb	GDR	1983	gamma rays		flower colour	37
Enzett Niva Lachs	GDR	1984	gamma rays		flower colour	37
Franky Lane	Netherlands	1985	gamma rays		flower colour	13
Fuchengzao	China	1987	gamma rays	Jiangchengluoxia	photoperiod	n.i.
Funny Redemine	Netherlands	1984	x-rays	Redemine	flower colour	31
Funny Rendez-Vous	Netherlands	1986	gamma rays	Rendez-Vous	flower colour	31
Gairik	India	1974	gamma rays	Belur Math	flower colour	15
Gamma	Hungary	1969	gamma rays	Obuda		15
Goldbronze Deholta	Netherlands	1983	x-rays	Deholta	flower colour	31
Golden Byoux	Netherlands	1985	gamma rays	Byoux	flower colour	31
Golden Clingo	Netherlands	1979	x-rays	Clingo	flower colour	14
Golden Cremon	Thailand	1987	gamma rays, *in vitro*	Cremon	flower colour	34
Golden Deholta	Netherlands	1984	x-rays	Deholta	flower colour	31
Golden Geos	FRG	1984	x-rays	Geos	flower colour	35
Golden Luck	FRG	1988	x-rays	Luck	flower colour	31
Hemanti	India	1979	gamma rays	megami	flower colour	16
Himani	India	1974	gamma rays	E-13	flower colour	15
Hoof Lane	Netherlands	1985	gamma rays	Penny Lane	flower colour	31

Huangjuanyun	China	1991	gamma rays	Chuntao	flower colour	n.i.
Indianapolis Yel.Imp	Netherlands	1970	x-rays	Indianapolis Yellow	flower colour	*
Ingrid	Brazil	1995	gamma rays	Repin	flower colour	43
IRB 88-30	Japan	1991	gamma rays	Taihei	flower colour	43
IRB 88-47	Japan	1991	gamma rays	Taihei	flower colour	43
IRB 88-59	Japan	1991	gamma rays	Taihei	flower colour	43
IRB 88-60	Japan	1991	gamma rays	Taihei	flower colour	43
Izetka Filmstar Br.	GDR	1966	x-rays	Filmstar	flower colour	*
Izetka Herbstgold	GDR	1964	x-rays	Izetka Kopenicker Rayonnante	flower colour	*
Izetka Kop.Barb.Gold	GDR	1962	x-rays	Barbarossa	flower colour	*
Izetka Kop.Barb.Rot	GDR	1962	x-rays	Barbarossa	flower colour	*
Izetka Kop.Br.Vogue	GDR	1962	x-rays	Vogue	flower colour	*
Izetka Ma.Cremeweiss	GDR	1966	x-rays	Izetka Marienhain	flower colour	*
Izetka Ma.Dunkelrosa	GDR	1966	x-rays	Izetka Marienhain	flower colour	*
Izetka Ma.Hellgelb	GDR	1966	x-rays	Izetka Marienhain	flower colour	*
Jhalar	India	1975	gamma rays	Undaunted	flower	15
Jingguangsishe	China	1989	gamma rays	Wuguangshise	flower colour	n.i.
Jingsuiqiu	China	1989	gamma rays	011	flower petal	n.i.
Jugnu	India	1991	gamma rays	Lalima	flower colour	43
Kanak	India	1975	gamma rays	Undaunted	flower colour	15
Kansya	India	1974	gamma rays	Rose Day	flower colour	15
Kapish	India	1974	gamma rays	E-13	flower colour	15
Ki-uzushio	Japan	1985	gamma rays	Uzushio	flower colour	32
Kraski oseni	USSR	1976	gamma rays	Violet colour	flower colour	14
KU 1	Thailand	1988	gamma rays, *in vitro*	Hangzhou	flower size	34
Kumkum	India	1982	gamma rays	M-71	flower colour	31
Kunchita	India	1974	gamma rays	Undaunted	flower	15
Lady Amber	Poland	1993	x-rays	Richmond	flower colour	43
Lady Bronze	Poland	1993	x-rays	Richmond	flower colour	43
Lady Pink	Poland	1993	gamma rays	Richmond	flower colour	43
Lady Rosy	Poland	1993	x-rays	Richmond	flower colour	43
Lady Salmon	Poland	1993	gamma rays	Richmond	flower colour	43
Lady Yellow	Poland	1993	gamma rays	Richmond	flower colour	43

Lemon Deholta	Netherlands	1985	x-rays	White Delta	flower colour	31
Liangjihuang	China	1989	gamma rays	Yaohong (leaf callus)	flower colour	n.i.
Lilac Byoux	Netherlands	1985	gamma rays	Byoux	flower colour	31
Lilac Cindy	FRG	1988	x-rays	Cindy	flower colour	35
Lohita	India	1974	gamma rays	E-13	flower colour	15
Main Lane	Netherlands	1985	gamma rays	Penny Lane	flower colour	31
Man Bhawan	India	1982	gamma rays	Flirt	flower colour	23
Mantianxin	China	1990	gamma rays	104 Ju	flower colour	n.i.
Marconi	Belgium	1985	x-rays	Pink cultivar	flower colour	31
Mars	USSR	1976	gamma rays	Privet Zime	flower colour	14
Merkurii	USSR	1976	gamma rays	Privet Zime	flower colour	14
Middelry	Netherlands	1976	x-rays	Horim	flower colour	15
Mikrop	Netherlands	1976	x-rays	Horim	flower colour	15
Milava	Netherlands	1976	x-rays	Horim	flower colour	15
Milonka	Netherlands	1976	x-rays	Horim	flower colour	15
Mirazh	USSR	1976	gamma rays	Lilac-pink	flower	14
Miros	Netherlands	1978	x-rays	Mikrop	flower colour	16
Mlechnyi put	USSR	1976	gamma rays	Privet Zime	flower colour	14
Morning Sun	Netherlands	1978	x-rays	Evening Sun	flower colour	16
Navneet	India	1987	gamma rays	Kalyani Mauve	flower colour	37
Navneet Yellow	India	1993	gamma rays	Navneet	flower colour	43
Nirbhaya	India	1975	gamma rays	Undaunted	flower	15
Nirbhik	India	1975	gamma rays	Undaunted	flower	15
OHB-14	Japan	1991	gamma rays chronic	Taihei	flower colour	43
OHB-8	Japan	1991	gamma rays chronic	Taihei	flower colour	43
Orange Impala	Netherlands	1984	x-rays	Impala	flower colour	31
Orange Lymon	Netherlands	1985	x-rays	Lymon	flower colour	31
Orange Mario	FRG	1983	x-rays	Mario (pink)	flower colour	23
Orange Miros	Netherlands	1979	x-rays	Miros	flower colour	16
Orange Refla	Netherlands	1985	x-rays	Refla	flower colour	31
Orion	USSR	1976	gamma rays	Charodeika	flower colour	14
Pale Remember	Netherlands	1985	gamma rays	Remember	flower colour	31

Peach Deholta	Netherlands	1985	x-rays	Pearl delta	flower colour	31
Pearl Cindy	FRG	1989	x-rays	Lilac Cindy	flower colour	35
Pingal	India	1974	gamma rays	Pink Casket	flower colour	15
Pink Clinspy	Netherlands	1978	x-rays	Clinspy	flower colour	14
Pink Impala	Netherlands	1984	x-rays	Impala	flower colour	31
Pink-Orizuru	Japan	1989	gamma rays	Sei-Orizuru	flower colour	42
Pitaka	India	1978	gamma rays	Kansya	flower colour	14
Pitambar	India	1978	gamma rays	Otome-Zakura	flower colour	14
Plutonii	USSR	1976	gamma rays	Privet zime	flower colour	14
Privet Frantsii	USSR	1976	gamma rays	Excellence	flower colour	14
Purnima	India	1978	gamma rays	Otome-Zakura	flower colour	14
Radii	USSR	1976	gamma rays	Springdawn at Suti dam	flower colour	14
Raktima	India	1998	gamma rays	Shyamal	flower colour	44
Red Lymon	Netherlands	1985	x-rays	Lymon	flower colour	31
Red Marconi	Belgium	1985	x-rays	Pink cultivar	flower colour	31
Repin Rosa	Brazil	1996	gamma rays		flower colour	44
Rohit	India	1979	gamma rays	Kingsford Smith	flower colour	16
Salmon Byoux	Netherlands	1985	gamma rays	Byoux	flower colour	31
Salmon Impala	Netherlands	1984	x-rays	Impala	flower colour	31
Salmon Lymon	Netherlands	1985	x-rays	Lymon	flower colour	31
Saturn	USSR	1976	gamma rays	Charodeika	flower colour	14
Selena	USSR	1976	gamma rays	Springdawn at Suti dam	flower colour	14
Shabnam	India	1987	gamma rays	D-5	flower colour	31
Shafali	India	1975	gamma rays	Undaunted	flower colour	15
Sharad Har	India	1992	gamma rays	Sharad Mala	flower colour	43
Sheela	India	1985	gamma rays	Himani	flower colour	31
Shukla	India	1974	gamma rays	Mrs. H. Gubby	flower colour	15
Shveta	India	1974	gamma rays	Fish tail	flower colour	15
Sijifeng	China	1989	gamma rays	Yaohong	flower colour	
Sijihong	China	1989	gamma rays	Yaohong	flower colour	n.i.
Sijihuang	China	1989	gamma rays	Yaohong	flower colour	n.i.
Sijimohong	China	1986	gamma rays	Yaohong	flower colour	n.i.
Sointse	USSR	1976	gamma rays	Modnitsa	flower colour	14

Sonali	India	1990	gamma rays	Ratna	flower colour	42
Sputnik	USSR	1976	gamma rays	Charodeika	flower colour	14
Subarna	India	1990	gamma rays	Flirt	flower colour	42
Surekha Yellow	India	1992	gamma rays	Surekha	flower colour	42
Svarnim	India	1975	gamma rays	Undaunted	flower colour	15
Tamra	India	1974	gamma rays	Goldie	flower colour	15
Taruni	India	1979	gamma rays	Kingsford Smith	flower colour	17
Torino	Belgium	1985	x-rays	Pink seedling	flower colour	31
Tsezii	USSR	1976	gamma rays	Charodeika	flower colour	14
Tulika	India	1985	gamma rays	M-24	flower colour	31
Uncle Danny	Netherlands	1973	x-rays	Beamsville Pink	flower colour	15
White Cindy	FRG	1989	x-rays	Lilac Cindy	flower colour	35
White Clinspy	Netherlands	1978	x-rays	Clinspy	flower colour	14
White Danusia	Netherlands	1977	x-rays	Danusia	flower colour	13
White Redemine	Netherlands	1984	x-rays	Redemine	flower colour	31
White Refla	Netherlands	1985	x-rays	Refla	flower colour	31
White Remember	Netherlands	1985	gamma rays	Remember	flower colour	31
White Ronny	FRG	1988	x-rays	Ronny (pink)	flower colour	31
White Westland	Netherlands	1978	x-rays	Westland	flower colour	16
White Winner	Netherlands	1975	x-rays	Pink Winner	flower colour	15
Xishihanxiao	China	1991	gamma rays	Chuntao	flower colour	n.i.
Xueyinghong	China	1991	gamma rays	Daguangming	flower type	n.i.
Yalta	USSR	1976	gamma rays	Violet colour	flower colour	14
Yaochuxuean	China	1989	gamma rays	Fenggouhuan	flower colour	n.i.
Yellow Bettina	FRG	1988	x-rays	Bettina (white)	flower colour	31
Yellow Cindy	FRG	1989	x-rays	Lilac Cindy	flower colour	35
Yellow Clingo	Netherlands	1979	x-rays	Clingo	flower colour	14
Yellow Clinspy	Netherlands	1978	x-rays	Clinspy	flower colour	14
Yellow Danusia	Netherlands	1977	x-rays	Danusia	flower colour	14
Yellow Lymon	Netherlands	1985	x-rays	Lymon	flower colour	31
Yellow Redemine	Netherlands	1986	x-rays	Redemine	flower colour	31
Yellow Refla	Netherlands	1986	x-rays	Refla	flower colour	31
Yellow Rendez-Vous	Netherlands	1986	gamma rays	Rendez-Vous	flower colour	31

		Yellow Samba	FRG	1988	x-rays	Samba (white)	flower colour	31
		Yellow Torino	Belgium	1985	x-rays	Pink seedling	flower colour	31
		Yellow Westland	Netherlands	1978	x-rays	Westland	flower colour	16
		Yellow Winner	Netherlands	1975	x-rays	Pink Winner	flower colour	15
		Yingsidai	China	1991	gamma rays	Fenggouhuan	flower colour	n.i.
		Yupiter	USSR	1976	gamma rays	Privet Zime	flower colour	14
		Zitiane	China	1990	gamma rays	104 Ju	flower colour	n.i.
		Zixia	China	1989	gamma rays	Huangjingying	flower colour	n.i.
		Ziyuntuoyue	China	1991	gamma rays	Shuangmantian	flower type	n.i.
Cicer arietinum	chickpea	CM-72	Pakistan	1983	gamma rays	6153	blight resistance	23
		CM-88	Pakistan	1994	gamma rays	C-727	disease resistance	43
		CM-98	Pakistan	1998	gamma rays	K-850		n.i.
		Hyprosola	Bangladesh	1981	gamma rays	Faridpur-1	earliness	19
		Kiran	India	1984	Neutrons	RS-10	erectoid type	26
		Line 3	Egypt	1992	gamma rays, EMS	NECL #055	yield	43
		NIFA-88 (CM-1918)	Pakistan	1990	gamma rays	6153	Ascochyta blight	37
		NIFA-95	Pakistan	1995	gamma rays	line 6151	blight resistance	44
		Pusa 408	India	1985	gamma rays	G-130	yield	29
		Pusa 413	India	1985	gamma rays	G-130	yield	29
		Pusa 417	India	1985	gamma rays	BG 203	yield	29
Citrullus lanatus	watermelon	Gibrid 218	USSR	1984	gamma rays	hybrid Bykovskii 22 x Melitopolskii 143		31
		Huozhou 1	China	1983	cross		quality	n.i.
		Luxigua 1	China	1987	gamma rays	[Taojian 8 x Lemi 1]	earliness	32
Citrus limon	lemon	Eureka 22 INTA	Argentina	1987	x-rays	Frost Eureka	fruit set	44
Citrus paradisi	grapefruit	Rio Red	USA	1984	thN	Ruby Red	fruit colour	37
		Star Ruby	USA	1970	thN	Hudson	seedless	*
Citrus sinensis	orange	Valencia 2 INTA	Argentina	1987	x-rays	Valencia Late	fruit set	44
Citrus sp.	orange/mandarin	Hongju 418	China	1983	gamma rays	Dahongpaohongju (branch)	seedless	27
		Hongju 420	China	1986	gamma rays	Dahongpao (branch)	seed number	34
		Xuegan 9-12-1	China	1983	gamma rays	Xuegan (branch)	seedless	29
		Zhongyu 7	China	1985	gamma rays		seedless	n.i.
		Zhongyu 8	China	1985	gamma rays		seedless	n.i.

Coix lachryma-jobi	job's tears	Hatomusume	Japan	1992	gamma rays	Okayama (local)	earliness	42
Colocasia esculenta	taro	Luyutou 1	China	1993	gamma rays	8501	yield	n.i.
Corchorus capsularis	jute	Binadeshipat-2	Bangladesh	1997	NaN3	CVL-1	fibre yield	44
		Xianghuangma 3	China	1997	gamma rays	Kuanyechangguo	earliness	n.i.
	white jute	Hyb 'C' (Padma)	India	1983	cross		water logging	34
	white jute	JRC-7447	India	1980	x-rays	JRC 212	yield	18
Corchorus olitorius	tossa jute	Atompat-28	Bangladesh	1974	gamma rays	D-154	yield	12
		Atompat-36	Bangladesh	1974	gamma rays	D-154	yield	12
		Atompat-38	Bangladesh	1974	gamma rays	D-154	vigour	12
		IR-1	India	1978	gamma rays	JRO 632	plant vigour	37
		JRO 3690	India	1985	cross		yield	33
		Mahadev TJ-40	India	1983	thN		yield	23
		Shwegontun	Myanmar	1975	gamma rays	C-28	earliness	12
Coronilla varia	crown vetch	Xifuxiaoguanhua	China	1991	gamma rays	Xidexaoguanhua	toxin content	n.i.
Cucumis sativus	cucumber	Altay	USSR	1981	cross		earliness	31
		Ludi 1	China	1981	laser	Jinyan 1	mildew resistance	35
Curcuma domestica	turmeric	BSR 1	India	1986	x-rays	Erode local	rhizome colour	29
		Co 1	India	1983	x-rays	Erode local	rhizome colour	29
Cymbopogon	citronella	Bhanumati (OJC-11)	India	1987	x-rays	Subirrsourav (CKS-CW-S-1)	oil content	35
		Bibhuti (OJC-5)	India	1987	x-rays	Subirrsourav (CKS-CW-S-1)	oil content	35
		Niranjan (OJC-6)	India	1987	x-rays	Subirrsourav (CKS-CW-S-1)	oil content	35
		Phullara (OJC-22)	India	1987	x-rays	Subirrsourav (CKS-CW-S-1)	oil content	35
		Sourav (OJC-3)	India	1987	x-rays	Subirrsourav (CKS-CW-S-1)	oil content	35
		Subir (OJC-31)	India	1987	x-rays	Subirrsourav (CKS-CW-S-1)	oil content	35
Cynodon sp.	bermuda grass	Tifeagle (TW-72)	USA	1995	gamma rays	Tifway II	dwarfness	n.i.
		Tifgreen II	USA	1983	gamma rays		vigour	33
		Tift 94	USA	1995	gamma rays	Midiron	leaf quality	44
		Tifway II	USA	1981	gamma rays	Tifway	nematode	19
Cyperus malaccensis	chinese matgrass	Toyomidori	Japan	1979	gamma rays chronic	Ohi 2	stiffness	21
Dahlia sp.	dahlia	Adagio	France	1970	gamma rays	Aztec	flower colour	17
		Allegro	France	1970	gamma rays	Aztec	flower colour	17
		Altamira	France	1970	gamma rays	Aztec	flower colour	17

Amalfi	France	1970	gamma rays	Aztec	flower colour	17
Annibal	France	1970	gamma rays	Aztec	flower colour	17
Autumn Harmony	Netherlands	1967	x-rays	Arthur Godfrey	flower colour	*
Bichitra	India	1978	gamma rays	Kenya	plant architecture	14
Black Beauty	India	1978		Black Out	plant architecture	14
Dutch Visit	Netherlands	1968	x-rays	Arthur Godfrey	flower colour	*
Explosion	Netherlands	1967	x-rays	Arthur Godfrey	flower colour	*
Governor	Netherlands	1968	x-rays	Authority	flower	*
Gracieuse	Netherlands	1966	x-rays	Salmon Rays	flower colour	*
Happiness	India	1978		Croydon Monarch	plant architecture	14
Holland Jubilee	Netherlands	1967	x-rays	Arthur Godfrey	flower colour	*
Huanghuan	China	1989	gamma rays	Honghua (root + seed)	shortness	n.i.
Jayaprakash	India	1978		Croydon Apricot	plant architecture	14
Jubilee	India	1978	gamma rays	Kenya	plant architecture	14
Jyoti	India	1978	gamma rays	Kenya	plant architecture	14
Maarse's Golden Wond	Netherlands	1972	x-rays	Andries Wonder	flower colour	*
Maarse's Purple Wond	Netherlands	1972	x-rays	Andries Wonder	flower colour	*
Maarse's Red Br.Wond	Netherlands	1972	x-rays	Andries Wonder	flower colour	*
Meiguizi	China	1989	gamma rays	Honghua (root + seed)	flower colour	n.i.
Motive	Netherlands	1971	x-rays	Arthur Godfrey	flower colour	2
Netaji	India	1978		Eagle Stone	plant architecture	14
Ornamental Rays	Netherlands	1966	x-rays	Salmon rays	flower colour	*
Pearl	India	1978		Eagle Stone	plant architecture	14
Pride of Sindri	India	1978	gamma rays	Kenya	plant architecture	14
Progression	Netherlands	1967	x-rays	Arthur Godfrey	flower colour	*
Raymond Smith	Netherlands	1970	x-rays	El Dorado	flower colour	*
Rosy Mist	Netherlands	1967	x-rays	Arthur Godfrey	flower colour	*
Rotonde	Netherlands	1966	x-rays	Salmon Rays	flower colour	*
Selection	Netherlands	1966	x-rays	Salmon Rays	flower	*
Temptation	Netherlands	1968	x-rays	Arthur Godfrey	flower colour	*
Twilight	India	1978	gamma rays	Kenya	plant architecture	14
Vivekananda	India	1978		Croydon Master	plant architecture	14
Wine Herald	Netherlands	1969	x-rays	Holland Herald	flower colour	*

Dianthus caryophyllus	carnation	Accent	Netherlands	1982	x-rays	Benoni	flower colour	31
		Bonitas	GDR	1985	gamma rays		semi-dwarfness	37
		Cerise Kortina	Netherlands	1985	x-rays	Kortina	flower colour	31
		Chaichoompon	Thailand	1983	gamma rays, *in vitro*	White Sim	flower colour	34
		Dione	GDR	1977	EMS	William Sim	flower colour	23
		Enzett Barther Fruhl	GDR	1974	EMS	Arthur Sim	flower colour	23
		Enzett Folklore	GDR	1974	EMS	William Sim	flower colour	23
		Galatee-lonvego	France	1982	gamma rays	Pallas-londorga	Fusarium	33
		Lavendel Kortina	Netherlands	1985	x-rays	Kortina	flower colour	31
		Loncerda	France	1983	gamma rays	Elsy-lodonie	Fusarium	33
		Maiella-lonchabi	France	1982	gamma rays	Pallas-londorga	Fusarium	33
		Pink Kortina	Netherlands	1985	x-rays	Kortina	flower colour	31
		Red Kortina	Netherlands	1985	x-rays	Kortina	flower colour	31
		Royal Red Kortina	Netherlands	1985	x-rays	Kortina	flower colour	31
		Scarlet Bell	Japan	1983	gamma rays	Angel	flower colour	32
		Sim Feu Follet	France	1972	gamma rays	Sim Jaqueline	flower colour	2
		UConn White Sim No.1	USA	1962	gamma rays	White Sim	flower	*
		White Kortina	Netherlands	1985	x-rays	Kortina	flower colour	31
Dolichos lablab	hyacinth bean	Co 10	India	1983	gamma rays	Co 6	yield	29
Eremochloa ophuiroides	centipedegrass	AU Centennial	USA	1983	gamma rays	common centipedegrass	dwarfness	30
		Tifblair	USA	1995	gamma rays		vigour	44
Eriobotrya japonica	loquat	Shiro-mogi	Japan	1981	gamma rays	Mogi	fruit size	21
Euphorbia fulgens	euphorbia	Albora	Netherlands	1976	x-rays		flower colour	15
Eustoma grandiflorum	eustoma	Purple Fantasy	Japan	1996	gamma rays chronic	Pastel Murasaki	flower size	44
		Purple Robin	Japan	1996	gamma rays chronic	Pastel Murasaki	flower colour	44
		Red Robin	Japan	1996	gamma rays chronic	Morgen Rot	flower size	44
Fagopyrum sagittatum	buckwheat	Aelita	USSR	1978	gamma rays	Improved Radekhovskaya	yield	30
		Aromat	USSR	1985	EI	[1557/69 x Madjarska]	stiffness	31
		Chernoplodnaya	USSR	1980	EI	Yubileinaya 2	earliness	40
		Galleya	USSR	1979	gamma rays	Victoria	yield	30

		Kurskaya 87	USSR	1991	cross		cooking quality	40
		Lada	USSR	1979	gamma rays	Improved Radekhovskaya	yield	30
		Podolyanka	USSR	1984	radiation, chemical		compact growth	30
		Skorospelaya 86	USSR	1990	cross		earliness	40
Festuca pratensis	meadow fescue	Fesco	FRG	1982	gamma rays		seed retention	34
		Lifesta	FRG	1981	gamma rays		seed retention	34
		Liforte	FRG	1984	gamma rays		seed retention	34
Ficus benjamina exotica	ficus	Golden King	Belgium	1980	x-rays	Green Ficus	leaf colour	31
		Golden Princess	Belgium	1980	x-rays	Green Ficus	leaf colour	31
Ficus carica	fig	Bol	USSR	1979	gamma rays			18
Forsythia x intermedia	forsythia	Courtadic	France	1984	gamma rays	Vitellina	plant architecture	25
		Courtalyn	France	1984	gamma rays	Linwood	plant architecture	25
Gerbera jamesonii	gerbera	Raisa	Poland	1993	gamma rays	Raisa	flower colour	43
Gladiolus sp.	gladiolus	Red Reflection	Netherlands	1988	x-rays	Peter Pears	flower colour	34
		Shobha	India	1988	gamma rays	Wild Rose	flower colour	34
		Showwinner	Netherlands	1984	x-rays	Applause	flower colour	31
		Tambari	India	1991	gamma rays	Oscar	flower colour	43
Glycine max	soybean	Aida	CSFR	1984	EMS	Smena	earliness	26
		Anji 2	China	1989	laser	hybrid	oil content	n.i.
		Arkadiya Odesskaya	USSR	1986	DMS	VNIIMK 9186	earliness	31
		Bangsa-Kong	Korea	1985	x-rays	CB-27	pod number	26
		Bisser	Bulgaria	1984	gamma rays	Beeson	yield	31
		Boriana	Bulgaria	1981	gamma rays	Beeson	earliness	23
		Cerag No.1	Algeria	1979	gamma rays	B 107/10	earliness	14
		Chudo Gruzii 74	USSR	1974	gamma rays			37
		Dioskuriye	USSR	1980	gamma rays			37
		Doi kham	Thailand	1986	gamma rays	S.J.-4	rust resistance	33
		Dorado	GDR	1988	NMH	Fiskeby V	grain yield	34
		DT-83	Vietnam	1987	EI	Cocchum	seed colour	43
		DT-84	Vietnam	1994	gamma rays	F1 from (DT-80xDH-4)	yield	43
		DT-90	Vietnam	1993	gamma rays	F1 (G7002xCocchum)	yield	43
		Fengdou 1	China	1988	gamma rays	F2 [(Qunxuan 1 x Qunjing) x 5621]	earliness	34

Fengshou 1	China	1970	gamma rays	Ke 56-4253	earliness	27
Fengshou 22	China	1992	gamma rays	Hejiao 77-153	earliness	n.i.
Hefeng 25	China	1992	gamma rays	Hejiao 77	yield	n.i.
Hefeng 33	China	1992	thN	Hejiao 8069	disease resistance	n.i.
Hefeng 36	China	1995	gamma rays	hybrid	earliness	n.i.
Heihe 12	China	1995	fN	hybrid	earliness	n.i.
Heihe 8	China	1989	fN	Heijiao 75-327 strain	adaptability	n.i.
Heihe 9	China	1990	fN	Heijiao 7710 F2	stiffness	n.i.
Heinong 16	China	1970	gamma rays	F3 (Wudingzhu x Jingshanpu)	branching	25
Heinong 28	China	1986	fN	F5 (Heinong 16 x	earliness	30
Heinong 31	China	1987	fN	F4 (Ha 70-5075 x Ha 53)	oil content	32
Heinong 32	China	1987	fN	F4 (Ha 70-5075 x Ha 53)	oil content	32
Heinong 34	China	1988	cross		yield	44
Heinong 35	China	1990	cross		yield	44
Heinong 37	China	1992	thN	hybrid	earliness	n.i.
Heinong 38	China	1992	thN	hybrid	lodging resistance	n.i.
Heinong 4	China	1966	gamma rays	Mancangjing	plant type	25
Heinong 41	China	1997	cross		seed size	44
Heinong 5	China	1967	gamma rays	Dongnong 4	root system	25
Heinong 6	China	1967	x-rays	Mancangjing	tallness	27
Heinong 7	China	1967	x-rays	Mancangjing	branching	25
Heinong 8	China	1967	x-rays	Mancangjing	earliness	25
Heinongxiaolidou 1	China	1989	fN	F2 (7626 x 7634)	grain weight	n.i.
Heinoun 26	China	1975	cross		plant architecture	25
Jidou 8	China	1992	EMS + PMS	Zaoshu 10	earliness	n.i.
Jiyuan 1	China	1986	laser	Gongjiao 6514	drought tolerance	n.i.
Kartuli 7	USSR	1980	gamma rays			37
Kefu 795-832	China	1988	gamma rays, DES	Fengshou 12	tallness	n.i.
KEX-2	Korea	1973	x-rays	Keumkang-Dai-Rip	earliness	4
Kosuzu	Japan	1986	gamma rays	Natto kotubu	earliness	32
Liaodou 10	China	1995	Cross		lodging resistance	n.i.
Liaodou 11	China	1996	Cross		yield	n.i.
Liaodou 3	China	1983	Cross		earliness	27

Liaodou 7	China	1992	gamma rays	hybrid	disease resistance	n.i.
Liaodou 9	China	1993	gamma rays	hybrid	disease resistance	n.i.
Liaoduo 4	China	1992	gamma rays	79 Hong-1	protein content	n.i.
Liaonong 1	China	1988	gamma rays	F2 (Heinong 11 x Tiefeng 9)	earliness	34
Luchezarnaya	USSR	1990	MNH		earliness	40
Ludou 9	China	1993	gamma rays	(7528 x 7405)	plant architecture	n.i.
M-103	Vietnam	1986	gamma rays, EI		yield	44
Mageva (Lastochka-out)	USSR	1991	chemical mutagen		earliness	40
Mufeng 6	China	1987	gamma rays	F2 (Tielingduanyebin x Meiguokelake 63)	earliness	n.i.
Muria	Indonesia	1987	gamma rays	Orba	yield	35
Mushi 6	China	1980	gamma rays	F2 (Fengshu 10 x Jilin 3)	earliness	25
Mutant 2	USSR	1980	gamma rays			37
Nanbushirome	Japan	1977	cross		earliness	21
Ningzheng 3	China	1993	gamma rays	Ningzheng 1	plant architecture	n.i.
Nitrobean-60	Australia	1995	EMS	Bragg	hypernodulation	43
Noventa	Hungary	1989	gamma rays	Altona	earliness	n.i.
Prikarpatskaya 81	USSR	1991	ENH	Kirovogradskaya 2	disease resistance	40
Raiden	Japan	1966	gamma rays	Nemashirazu	earliness	*
Raiko	Japan	1969	gamma rays	Nemashirazu	earliness	*
Ryokusui	Japan	1990	gamma rays	Fukura	lateness	42
S-31	Vietnam	1995	gamma rays, EI	V-74	yield	43
Suilong 12	China	1996	gamma rays	F6 [Suijio 83-432 x (Heihe 4 x Te 7604)]	yield	44
TAEK A3	Turkey	1994	gamma rays	Amsoy 71	oil content	43
TAEK C10	Turkey	1994	gamma rays	Calland	yield	43
Tainung 1(R)	China	1962	thN		vigour	*
Tainung 2(R)	China	1962	x-rays		vigour	*
Tengger	Indonesia	1991	gamma rays	Orba	earliness	42
Tidar	Indonesia	1987	gamma rays	AVRDC No. 29	earliness	35
Tiefeng 18	China	1973	gamma rays	n.i.-15 x 5621	fertilizer response	25

		Tiefeng 19	China	1974	gamma rays	n.i.-15 x 5621	earliness	n.i.
		Tiefeng 24	China	1988	cross		plant architecture	n.i.
		Universal I	USSR	1965	gamma rays	Imeretinskaya	yield	19
		Wase-suzunari	Japan	1983	gamma rays	Okushirome	earliness	32
		Wei 7610-13	China	1983	gamma rays + fN	Fengshouhuang	earliness	32
		Wendou 79012	China	1986	gamma rays		lodging resistance	n.i.
		Yedadou 2	China	1990	gamma rays	(Williams x Sanledaqindou)	disease resistance	n.i.
		Yubian 30	China	1982	x-rays	6825	virus resistance	n.i.
		Yubian 31	China	1982	x-rays	6825	drought tolerance	n.i.
		Yudou 4	China	1987	gamma rays	Heidou	disease resistance	n.i.
		Yudou 9	China	1989	gamma rays	Shangqiu 7068	yield	n.i.
		Zarya	Bulgaria	1984	gamma rays	Zora	earliness	32
		Zhangdou 1	China	1980	gamma rays	Tiefeng 18	drought tolerance	n.i.
Gossypium sp.	cotton	113	China	1985	gamma rays	Liao 6496	earliness	35
		Agdash 3	USSR	1983	gamma rays	Mutant line 9/1	yield	31
		Badnawar-1	India	1961	cross			30
		Chandi 95	Pakistan	1995	gamma rays	NIAB 78	yield	43
		Chuanpei 1	China	1982	gamma rays	Dongtin 1	earliness	34
		DS-1	India	1985	gamma rays	G-27	semi-dwarfness	42
		Emian 15	China	1991	gamma rays	Henan 75	yield	n.i.
		Indore-2	India	1950	x-rays	MU-4 (=Dhar Kambodia)		30
		Jimian 8	China	1984	gamma rays	hybrid	earliness	n.i.
		Khandwa-2	India	1971	cross			30
		Lumian 1	China	1976	gamma rays	F9 (Zhong 2 x 1195)	plant architecture	19
		M.A.9	India	1948	x-rays	Co-2	drought tolerance	30
		MCU 10	India	1982	gamma rays	MCU 4	drought tolerance	29
		MCU 7	India	1971	x-rays	L 1143 EE	earliness	2
		NIAB-26N	Pakistan	1992	cross		yield	n.i.
		NIAB-78	Pakistan	1983	gamma rays	F1 (Deltapine x Ac134)	yield	23
		NIAB-86	Pakistan	1990	cross		yield	n.i.
		NIAB-Karishma	Pakistan	1996	cross		yield	n.i.
		Oktyabr	USSR	1984	cross		compact growth	31
		Pusa Ageti	India	1978	gamma rays	Stoneville 213	ginning capacity	16

		Rasmi	India	1976	gamma rays	MCU 5	photoperiod	16
		Xinhai 2	China	1979	x-rays	66-170	plant architecture	27
		Yanmian 48	China	1985	cross		yield	n.i.
		Yunfu 885	China	1977	gamma rays	Daizimian 15 x Xiaoyemian	earliness	27
Guzmania paecockii	guzmania	Edith	Belgium	1974	gamma rays		leaf colour	17
Helianthus annuus	sunflower	Jingkui 1	China	1987	fN	Mokui	earliness	n.i.
		Pervenets	USSR	1977	DMS	VNIIMK 8931	oil content	13
Hibiscus sp.	hibiscus	Anjali	India	1987	gamma rays	Alipore Beauty	flower colour	31
		Purnima	India	1979	gamma rays chronic	Alipore Beauty	variegated leaves	30
		Shirasagi-no-Yume	Japan	1987	gamma rays		flower colour	33
	roselle	Hiroshima local No.1	Japan	1967	gamma rays	Hiroshima local	tallness	12
		Hiroshima local No.3	Japan	1967	gamma rays	Hiroshima local	tallness	12
		Hiroshima local No.5	Japan	1967	gamma rays	Hiroshima local	tallness	12
		Hiroshima local No.7	Japan	1967	gamma rays	Hiroshima local	tallness	12
Hippophaea rhamnoides	buckthorn	Zyrianka	USSR	1985	gamma rays, MNH	wild form of Altai	yield	28
Hordeum vulgare	barley	7938	China	1984	gamma rays	Zaoshu 3	earliness	n.i.
		AC-Albright	Canada	1993	cross		disease resistance	43
		Acclaim	GDR	1984	cross		yield	37
		AC-Stacey	Canada	1995	cross		earliness	43
		Advance	USA	1979	cross		yield	28
		Akdeniz M-Q-54	Turkey	1998	gamma rays	Quantum	drought tolerance	44
		Akkord	USSR	1987	cross		earliness	31
		Alexis	FRG	1986	cross		powdery mildew	36
		Alf	Denmark	1978	thN	Bomi	shortness	13
		Alis	Denmark	1985	cross		nematode	36
		Allasch	FRG	1963	cross		stiffness	5
		Alpina	Austria	1995	cross		semi-dwarfness	43
		Amagi Nijo 1	Japan	1971	x-rays	Fuji Nijo	earliness	2
		Amalia	Austria	1988	cross		yield	33
		Amazone	FRG	1986	cross			36
		Amei	FRG	1966	cross		stiffness	5
		Amethyst	CSFR	1972	cross		yield	10

Amil	Iraq	1994	gamma rays	Numar	disease resistance	43
Anker	Denmark	1986	cross			37
Anna Abed	Denmark	1979	cross		stiffness	34
Anni	Estonia	1993	cross		drought tolerance	43
Araraty 7	USSR	1983	EI	Caler	lodging resistance	31
Arena	FRG	1983	cross		shortness	36
Ariel	Sweden	1988	cross		stiffness	37
Atlanta	Canada	1977	cross		stiffness	11
Atlas	CSFR	1976	cross		yield	10
Ayr	UK	1986	cross		shortness	34
Bacchus	UK	1981	cross			37
Balder J.	Finland	1960	x-rays	Balder	yield	5
Baraka	Iraq	1994	gamma rays	Baldi	yield	43
Baraka	France	1986	cross		winter type	37
Bastion	USSR	1992	cross		stiffness	41
Beate	FRG	1984	cross		brewing quality	36
Beauly	UK	1983	cross		shortness	34
Berolina	Austria	1982	cross		yield	37
Berta	Austria	1982	cross		yield	20
Betina	France	1970	EMS	Vada	shortness	*
BH-75	India	1983	cross		semi-dwarfness	36
BIOS-1	Russia	1993	cross, DH		lodging resistance	41
Blazer	USA	1974	cross		alpha amylase	10
Blenheim	UK	1987	cross		yield	36
Bonneville 70	USA	1969	thN	Bonneville	threshability	*
Bonus	CSFR	1984	cross		yield	31
Boyer	USA	1974	cross		earliness	10
Camargue	UK	1986	cross		yield	32
Camen	Denmark	1989	cross		yield	37
Camir	Denmark	1985	cross		malting quality	36
Canor	Denmark	1985	cross		malting quality	37
Canut	Denmark	1988	cross		yield	37
Cargine	France	1986	cross			37

Carmen	Austria	1986	cross		yield	29
Carnival	UK	1981	cross			37
Carula	Denmark	1989	cross		malting quality	37
Catrin	Denmark	1985	cross		yield	37
Cheri	FRG	1987	cross		earliness	36
Comtesse	FRG	1987	cross		yield	33
Consista	GDR	1979	cross		yield	32
Corgi	UK	1985	cross			37
Corniche	UK	1985	cross		yield	32
Cromarty	UK	1983	cross		shortness	34
Deawn	USA	1975	cross		shortness	11
Debut	USSR	1982	NEU	Start	yield	20
Defia	GDR	1984	cross		yield	37
Defra	GDR	1984	cross		yield	32
Delita	GDR	1987	cross		yield	32
Denar	CSFR	1969	x-rays	F1 (Celechovicky x Bavaria)		6
Dera	GDR	1982	cross		yield	32
Derkado	GDR	1987	cross		yield	32
Diabas	CSFR	1977	cross			13
Diamant	CSFR	1965	x-rays	Valticky	yield	*
Diana	Bulgaria	1983	gamma rays	Miraj	yield	36
Dinky	Belgium	1987	cross			37
DL-253	India	1981	gamma rays, EMS	Ratna	yield	19
Donan	UK	1983	cross		shortness	34
Dorett	FRG	1985	cross		yield	36
Dorina	GDR	1984	cross		yield	32
Doublet	UK	1983	cross		yield	30
Eight-Twelve	USA	1991	cross		short spikes	41
Elo	Estonia	1989	cross		malting quality	43
Empress	Canada	1983	cross		yield	28
Esk	UK	1985	cross		shortness	34
Eva	Sweden	1972	cross		stiffness	7
Everest	UK	1985	cross			37

Fakel	USSR	1975	EI	Moskovskii 121	shortness	12
Fatran	CSFR	1980	cross		yield	31
Favorit	CSFR	1973	cross		yield	10
Femina	GDR	1984	cross		grain quality	32
Fergie	UK	1990	cross			37
Fleet	UK	1985	cross		yield	37
Formula (=W 7200)	Sweden	1987	cross		shortness	37
Frankengold	FRG	1975	cross			37
Fuji 2-jyo II	Japan	1974	gamma rays, BUdR	Fuji 2-jyo	stiffness	11
Fuxuan 48	China	1985	gamma rays	Zaoshu 3	earliness	n.i.
Galant	Denmark	1984	NaN3	Triumph	proanthocyanine-free content	37
Gamma 4	Japan	1965	gamma rays	Kirin-Choku 1	shortness	*
Gavotte	France	1986	cross			37
Gerlinde	GDR	1979	cross		yield	32
Goldfield	UK	1969	cross			36
Goldmarker	UK	1976	cross		erectoid type	10
Goldspear	UK	1975	cross		erectoid type	10
Gorm	Denmark	1981	cross			37
Grammos	Greece	1969	gamma rays	Rivale	cold tolerance	37
Grisante	UK	1984	cross			37
Grit	GDR	1979	cross		yield	32
Gunilla	Sweden	1970	cross		yield	*
Gunnar	Denmark	1982	cross		earliness	33
Hana	CSFR	1973	cross		yield	10
Hankkija's Aapo	Finland	1975	x-rays	Ta 7990 (a n.i.15 x Staller II)	stiffness	7
Hankkija's Eero	Finland	1975	cross		stiffness	7
Haya-Shinriki	Japan	1962	gamma rays	Aka-Shinriki	earliness	2
Helena	FRG	1983	cross			37
Hellas	Sweden	1967	cross		stiffness	*
Heriot	UK	1983	cross		semi-prostrate	30
Herzo	FRG	1976	cross			37
Hesk	USA	1979	cross		shortness	36

Horal	CSFR	1982	cross		yield	31
Ilka	GDR	1984	cross		yield	32
Inga	Denmark	1982	cross			36
Ingot	UK	1980	cross			36
Jamina	UK	1979	cross			36
Jarek	CSFR	1987	cross		yield	31
Jaspis	CSFR	1986	cross		yield	31
Jenny	Sweden	1980	cross		yield	19
Jianghaidamei	China	1991	gamma rays + microwave	7422	stress tolerance	n.i.
Jupiter	UK	1976	cross		yield	13
Jutta	GDR	1955	x-rays	Peragis mittelfrühe II	yield	*
Jutta	Austria	1983	cross		yield	29
K-2578	India	1980	cross		tallness	36
Karan-15	India	1982	cross		semi-dwarfness	36
Karan-201	India	1984	cross		semi-dwarfness	36
Karan-265	India	1989	cross		semi-dwarfness	36
Karan-3	India	1982	cross		semi-dwarfness	36
Karan-4	India	1983	cross		semi-dwarfness	36
Karat	CSFR	1981	cross		yield	31
Kaskad	USSR	1984	cross		stiffness	31
Kawamizuki	Japan	1979	cross		shortness	21
Kazbek 1	USSR	1983	gamma rays	Dzveltesly	yield	31
Keti	Denmark	1982	cross		yield	20
Kharkovskii 84	USSR	1988	ethyleneoxide	Union	semi-dwarfness	31
Kingspin	UK	1985	cross			36
Koral	CSFR	1978	cross		yield	31
Korinna	GDR	1988	cross		yield	36
Kormovy	Ukraine	1997	EI	Quantum	yield	44
Kosmos	Poland	1977	cross		semi-dwarfness	44
Krassi 2	Bulgaria	1983	cross		shortness	36
Kredit	CSFR	1984	cross		yield	31
Kristina	Sweden	1969	cross		stiffness	*
Krystal	CSFR	1981	cross		yield	31

Kustaa	Finland	1980	cross		earliness	19
Lada	GDR	1979	cross		yield	32
Larissa	GDR	1989	cross		yield	36
Laura	France	1971	cross			37
Leelo	Estonia	1995	cross		yield	43
Leila	France	1984	cross			37
Lenka	GDR	1985	cross		yield	32
Leo-INIA/CCU	Chile	1990	cross		earliness	37
Liisa	Estonia	1981	cross		lodging resistance	43
Lina	Sweden	1982	cross		yield	25
Lupidamei 1	China	1987	gamma rays	Zaoshu 3	photoperiod	n.i.
Lussi (=Vicky)	Sweden		cross		malting quality	37
Luther	USA	1967	dES	Alpine	shortness	*
Madelon	France	1985	cross			37
Maksim	USSR		cross		lodging resistance	37
Mal	USA	1979	cross		lodging resistance	36
Mamluk	USSR	1992	NTMU	line 137/9	earliness	41
Maresi	GDR	1986	cross		yield	32
Mari	Sweden	1962	x-rays	Bonus	earliness	*
Marina	Germany	1994	cross		stiffness	43
Markeli 5	Bulgaria	1976	gamma rays	Beta ketsoras	earliness	14
Mars	CSFR	1983	cross		yield	31
Masakadomugi	Japan	1989	cross		BYMV resistance	35
Matura	FRG	1967	cross			37
Midas	UK	1970	cross		shortness	*
Mikkel	Denmark	1983	cross			37
Milns Golden Promise	UK	1966	gamma rays	Maythorpe	shortness	*
Minak	UK	1976	cross		stiffness	13
Minsk	USSR	1974	gamma rays	Viner	stiffness	6
Mona	Sweden	1970	cross		yield	*
Moskovskii 2	USSR	1984	cross		yield	30
Nadja	GDR	1975	cross		shortness	9
Nairn	UK	1983	cross		shortness	34

Natasha	France	1986	cross		yield	36
Nebi	GDR	1983	cross		yield	32
Nirasaki Nijo 8	Japan	1967	cross		earliness	2
Nomad	FRG	1990	cross			36
Nomini	USA	1992	cross		earliness	42
Noor Al-Qadisyiha 17	Iraq	1995	cross		earliness	43
Noor Al-Qadisyiha 68	Iraq	1995	cross		earliness	43
Novator	USSR		cross		winter hardiness	20
Novum	CSFR	1988	cross		yield	34
Octave	Austria	1986	cross			36
Opal	CSFR	1980	cross		disease resistance	31
Orbit	CSFR	1986	cross		yield	31
Otal	Canada	1981	cross		earliness	43
Othello	UK	1988	cross			37
Pacha	France	1986	cross			37
Pallas	Sweden	1960	x-rays	Bonus	stiffness	*
Pamunkey	USA	1993	cross		semi-dwarfness	43
Patricia	France	1988	cross			37
Peak	UK	1988	cross			37
Pennrad	USA	1963	thN	Hudson	winter hardiness	*
Perelom	USSR	1990	cross		lodging resistance	40
Pernilla	Sweden	1979	cross		earliness	19
Perun	CSFR	1987	cross		yield	31
PL 56	India	1975	EMS	C-164	tillering type	32
Pression	France	1986	cross			37
Prisiv (not released)	USSR		cross		yield	20
Prisma	Netherlands	1985	cross		yield	36
Profit	CSFR	1988	cross		yield	34
Qianlu 1	China	1995	gamma rays	Zaoshu 3	disease resistance	n.i.
Radiation	Korea	1974	thN	Bangju	earliness	5
Radikal	USSR	1988	cross		winter hardiness	31
Rapid	CSFR	1976	cross		yield	9
RD-103	India	1978	cross		shortness	26

RD-137	India	1981	cross		shortness	36
RD-2035	India	1988	cross		shortness	36
RDB-1	India	1972	Neutrons	R.S.-17	shortness	*
Rejkiran	India	1982	cross		shortness	26
Robin	Austria	1986	cross		yield	29
Romi	Denmark	1983	cross			36
Rosie	Denmark	1980	cross			36
Rubin	CSFR	1982	cross		yield	31
Rumba	FRG	1988	cross			36
Rupal	Sweden	1972	cross		shortness	7
Safir	CSFR	1978	cross		shortness	14
Salome	GDR	1981	cross		yield	32
Salve	Sweden	1974	cross		grain size	7
Samir	Iraq	1993	gamma rays	Arivat	yield	43
Secret	Russia	1995	NEU	Monolit	lodging resistance	43
Semal	Denmark	1990	cross		yield	37
Senat	Sweden	1974	cross		stiffness	7
Seru	Sweden	1973	cross			36
Shua	Iraq	1992	fN	Arivat	yield	43
Shyrokolystnii	USSR	1987	NMU+NEU	Obroshynskii-1	tallness	31
Sila	Denmark	1986	cross		stiffness	36
Sissy	FRG	1990	cross		malting quality	37
Skorokhod	USSR	1991	cross		earliness	40
Spartan	CSFR	1977	cross		shortness	14
Spirit	GDR	1986	cross		earliness	32
Stange	Norway	1978	cross		shortness	12
Stella	FRG	1989	cross		brewing quality	36
Taarn	Sweden	1982	cross			36
Taeler	USSR	1991	DMSO	Otra	earliness	35
Tamina	GDR	1982	cross		yield	32
Temp	USSR	1978	ENH	Krasnodarskii 35	earliness	13
Toga	FRG	1986	cross		shortness	36
Tone-nijo	Japan	1990	cross			41

		Troja	Sweden	1981	cross		yield	25
		Trumpf	GDR	1973	cross		shortness	9
		Tuteishy	USSR	1992	cross		lodging resistance	40
		Tuwaitha	Iraq	1992	gamma rays	Arivat	yield	43
		Tyne	UK	1987	cross		shortness	34
		Tyra	Norway	1988	cross		yield	33
		UC 829	USA	1995	cross		semi-dwarfness	43
		UNA-La Molina 95	Peru	1995	gamma rays	Buenavista	earliness	43
		Ursel	FRG	1985	cross			36
		Valerie	France		cross			37
		Vavilon	USSR	1990	cross		lodging resistance	36
		Vega Abed	Denmark	1977	cross		stiffness	34
		Veras	USSR	1992	cross		lodging resistance	40
		Vienna	Austria	1959	x-rays	Probstdorfer Vollkorn VK 41	yield	*
		Visir	Sweden	1970	cross		mildew resistance	*
		VITIM	USSR	1989	cross		lodging resistance	40
		Wandamei 1	China	1991	gamma rays	Zaoshu 3	grain weight	n.i.
		Yanfuaizao 3	China	1977	gamma rays	Zaoshu 3	earliness	25
		Yubilei 100	Bulgaria	1982	cross		yield	36
		Zazerskij 85	USSR		cross			37
		Zenit	CSFR	1985	cross		yield	31
		Zgoda	USSR		cross			37
Hoya carnosa	hoya	Compact Regalis	USA	1980	radiation		leaf colour	31
		Compacta	USA	1980	radiation		leaf colour	31
		Mauna Loa	USA	1980	radiation		leaf colour	31
		Rubra	USA	1980	radiation		leaf colour	31
Humulus lupulus	hop	Crystal	USA		cross		vigour	43
		Santiam	USA	1998	cross	Krasnodar 424	oil quality	44
		Ultra	USA	1995	cross		yield	44
Hyacinthus sp.	hyacinth	Orion	Netherlands	1987	x-rays	Jan Bos	flower colour	34
Ipomoea batatas	sweet potato	Wanshu S-367	China	1998	ion beams	83-367	disease resistance	n.i.
		Yanshu 759	China	1986	fN	(Yanshu 3 x Xushu 18)	starch content	33
		Yanshu 781	China	1986	fN	(Fengshouhuang x Honghong 1)	starch content	33

		Yushu 5	China	1990	gamma rays + NaN3	(Yesheng x Lanyang 203)	disease resistance	n.i.
Iris sp.	iris	Belyi Karlik	USSR	1984	gamma rays		ornamental type	37
		Chistoe Pole	USSR	1984	gamma rays		ornamental type	37
		Marina Raskova	USSR	1984	gamma rays		ornamental type	37
		Marshal Pokryshkin	USSR	1984	gamma rays		ornamental type	37
		Podmoskownaya Osen	USSR	1984	gamma rays		ornamental type	37
Juncus effuses	mat rush	Fukunami	Japan	1984	gamma rays	Asanagi	yield	31
Juncus effusus	mat rush	Seto-nami	Japan	1982	gamma rays	Asanagi	yield	21
Kalanchoe sp.	kalanchoe	Flores	Netherlands	1985	x-rays	Singapur	plant architecture	31
		Lombok	Netherlands	1985	x-rays	Singapur	flower colour	31
		Sumba	Netherlands	1985	x-rays	Singapur	plant architecture	31
Lactuca sativa	lettuce	Blush	USA	1992	EMS	81-1251-C-18-2 (F3)	dwarfness	43
		Evergreen	Japan		32P	Butterhead	heat tolerance	2
		Giantgreen	Japan		32P	Butterhead	heat tolerance	2
		Ice Cube	USA	1992	EMS	81-1251-C-18-2 (F3)	dwarfness	43
		Mini-Green	USA	1992	EMS	81-1251-C-18-2 (F3)	dwarfness	43
		Novogodnii	USSR	1991	EI	Moskovskii parnik	yield	41
Lagerstroemia indica	crapemyrtle	Centennial Spirit	USA		EMS		leaf morphology	28
		Prairie Lace	USA		EMS		sterility	28
Lantana depressa	wild sage	L. dep. bicoloured	India	1986	gamma rays	*Lantana depressa*	leaf colour	37
		L. dep. variagata	India	1986	gamma rays		flower colour	31
		Niharika	India	1986	gamma rays	*Lantana depressa*	leaf colour	37
Lathyrus sativus	plavine, grass pea	Poltavskaya 2	USSR	1980	ENH		drought tolerance	40
Lens culinaris	lentil	Mutant 17 MM	Bulgaria	1999	gamma rays		seed size	44
		S-256	India	1981	radiation	Ranjan	spreading type	20
Lepidium sativum	cress	Vest	USSR	1988	electrons	Uzkolistnyi 3	adaptability	31
Lespedeza cuneata	lespedeza	Interstate	USA	1970	thN		compact growth	*
		Interstate 76	USA	1979	cross		Meloidogyne	16
Lilium sp.	lily	Mies Bouwman	Netherlands	1977	x-rays	Tabasco	flower colour	37
		TX 68-1	Netherlands	1977	x-rays	Tabasco	flower colour	37
Linum usitatissimum	flax	Baltyuchai	USSR	1991	ENH	Vipegantas	disease resistance	41
		M-5	USSR	1991	DMS	Orshanskii 2	disease resistance	41
	flax/linseed	Dufferin	Canada	1979	cross		oil content	18

		Heiya 4	China	1978	cross		earliness	27
		Heiya 6	China	1985	cross		yield	32
		Heiya 7	China	1989	cross		stress tolerance	n.i.
		Linola 989	Canada	1996			oil quality	44
		Redwood 65	Canada	1965	x-rays	Redwood	oil content	5
		Zarja 87	USSR	1988	EI	[LD-147 x Complex]	lateness	31
Lolium sp.	ryegrass	Meritra, R.v.P.	Belgium	1971	colchicine	Lemtal	yield	0
Luffa acutangula	ridged gourd	PKM-1	India	1984	gamma rays	H. 610	yield	32
Lupinus albus	white lupin	Dnepr	USSR	1978	cross		alkaloid content	13
		Drujba	USSR	1984	EMS	Kievskii (rad.mut.)	earliness	31
		Gorizont	USSR	1977	cross			13
		Kievsky Mutant	USSR	1969	radiation	F2 (Hvanchkoly x s.f. Syria)	yield	*
		Martin 2	USSR	1984	cross		Fusarium	31
		Olezhka	USSR	1989	ENH, MNH	Kievskii mutant	alkaloid content	40
		Pyshevoj	USSR	1987	NMU + EI	local line	alkaloid content	31
		Sinii parus	USSR	1991	cross		lodging resistance	40
		Slavutich	USSR	1980	MNH		alkaloid content	40
		Solnechnyi	USSR	1980	chemical		alkaloid content	40
		Start	USSR	1983	gamma rays	White 7	earliness	31
		Ukrainskii	USSR	1981	MNH, EI and DMS		alkaloid content	40
		Vympel	USSR	1982	EI	Rannesp.31 uluchshen	earliness	40
Lupinus angustifolius	blue lupin	Bar	Poland	1991	cross		non-branching	41
		Chittick	Australia	1982	EI	Borre	earliness	20
Lupinus consentini	lupin	Eregulla	Australia	1972	cross		alkaloid content	12
Lupinus luteus	yellow lupin	Aga	Poland	1981	cross		earliness	19
		Kopilovskii	USSR	1985	cross		Fusarium	31
		Narochanskii	USSR	1983	gamma rays	Polish var. R 6025	Fusarium	31
Lycopersicon esculentum	tomato	Bahar	Bangladesh	1992	cross		determinate	42
		Binatomato-2	Bangladesh	1997	gamma rays		yield	n.i.
		Binatomato-3	Bangladesh	1997	gamma rays		yield	n.i.
		Co 3	India	1981	EMS	Co 1	compact growth	29
		Kagyoku	Japan	1985	cross		disease resistance	32

		Kyoryoku-reikou	Japan	1974	gamma rays	(Shugyoku x *L. peruvianum*)	TMV resistance	21
		Kyouryokuogatareikou	Japan	1984	cross		disease resistance	32
		Luch 1	USSR	1965	gamma rays	Pushkinsky	earliness	19
		PKM-1	India	1980	gamma rays	Annanj	yield	32
		Pusa Lal Meeruti	India	1972	gamma rays	Meeruti	fruit ripening	*
		Rannii Nuch	USSR	1983	EI	Jubilejnii 261	earliness	31
		Ryuugyoku	Japan	1985	cross		disease resistance	32
		S.12	India	1969	gamma rays	Sioux	dwarfness	*
Malus pumila	apple	Belrene	France	1970	EMS	Reine des Reinettes	earliness	17
		Blackjoin BA 2 520	France	1970	gamma rays	Jonathan Blackjoin	fruit colour	17
		Courtagold	France	1972	gamma rays	Golden Spur	shortness	30
		Courtavel	France	1972	gamma rays	Starking Delicious	shortness	30
		Golden Haidegg	Austria	1986	gamma rays	Golden Delicious	fruit size	31
		Lysgolden	France	1970	gamma rays	Golden delicious	rust resistance	17
		McIntosh 8F-2-32	Canada	1970	gamma rays	McIntosh	seed colour	1
		Senbatsu-Fuji-2-Kei	Japan	1985	gamma rays	Fuji	fruit colour	37
		Shamrock	Canada	1986	cross		earliness	31
Malus sp.	apple	Donghenghongpinguo	China	1987	gamma rays	Jingguan (seed)	shortness	n.i.
	apple (flowers)	Dovar	Netherlands	1978	x-rays	John Downie	variegated leaves	14
Manihot esculenta	cassava	Tekbankye	Ghana	1997	gamma rays	Isunikakiyan	cooking quality	44
Matricaria chamomilla	chamomile	Podmoskovnaya	USSR	1984	colchicine		lodging resistance	41
Medicago sativa	alfalfa	Xinmu 1	China	1986	gamma rays		cold tolerance	n.i.
Mentha arvensis	mint	Rose mint	Japan	1977	gamma rays	Japanese Mint	yield	15
	peppermint	TN-8	Vietnam	1995	gamma rays	NV-74	oil quality	44
Mentha piperita	peppermint	Murray Mitcham	USA	1976	x-rays	Mitcham	Verticillium	10
		Todd's Mitcham	USA	1971	x-rays	Mitcham	Verticillium	11
Momordica charantia	bitter gourd	MDU 1	India	1984	gamma rays	MC 103	insect resistance	32
Morus alba	mulberry	Fusang 10	China	1980	gamma rays		internode length	27
		Fuzaofeng	China	1992	gamma rays	Yu 151 (branch)	earliness	n.i.
		Ji 7681	China	1988	laser	F1 (Cangxi 49 x Yu 2)	vigour	33
		S54	India	1974	EMS	Berhampore	yield	33
		Sangfu 1	China	1974	gamma rays	Yizhilai	internode length	n.i.
		Shannsang 871	China	1994	gamma rays	hybrid	vigour	n.i.

		Shigu 11-6	China	1995	gamma rays	Husang 32	yield	n.i.
Musa sp.	banana	Klue Hom Thong KU1	Thailand	1985	gamma rays, in vitro	Hom Thong	bunch size	35
		Novaria	Malaysia	1993	gamma rays	Grand Naine	earliness	44
Nelumbo nucifera	lotus	Dandinyuge	China	1997	gamma rays	Xianbeilian 6	flower colour	n.i.
		Dianezhuang	China	1983	gamma rays	Beixianglian	earliness	
		Ruyijiali	China	1997	gamma rays	(Dongguali x Xianbeilian 6)	flower colour	n.i.
Nicotiana tabacum	tobacco	American 307	USSR	1981	cross		leaf colour	41
		American Bahchysarajsk	USSR	1979	NEU	American 181	yield	13
		Baghdad-V77	Iraq	1995	gamma rays	Vargini	yield	43
		Clorina F1	Indonesia	1934	x-rays	Vorstenland	leaf colour	*
		Delhi 76	Canada	1976	gamma rays	Delhi 34	leaf colour	19
		GSH-3	India	1979	cross		leaf quality	30
		Jubilejnyi	USSR	1979	cross		leaf quality	13
		Krupnolystnii B-3	USSR	1979	cross	American 341-62	yield	13
		KY 907	USA	1993	cross		yield	43
		Sumar-V48	Iraq	1995	gamma rays	Vargini	yield	43
		Virginia 0n.i.4	Bulgaria	1986	cross		disease resistance	32
Olea europaea	olive	Briscola	Italy	1981	gamma rays	Ascolana tenera	shortness	19
Onobrychis viciifolia	sainfoin	Kirovogradskij 13	USSR	1986	MNH	Peschanyi	plant architecture	31
		Krasnodarskii 84	USSR	1992	chemical mutagen	Krasnodarskii 2834	yield	41
Ornithopus compressus	serradella	Uniserra	Australia	1971	EMS	Pitman	earliness	*
Oryza sativa	rice	1870	China	1984	gamma rays	Nanjing 33	earliness	n.i.
		202	China	1973	gamma rays	IR 8	leaf size	27
		240	China	1980	gamma rays	Guangbeiguang	earliness	27
		6 B	Vietnam	1986	cross		yield	31
		652	China	1979	gamma rays	129 x Ewan 3	blast resistance	30
		69-280	China	1969	gamma rays	Ainanzhao x Qingxiaojingzao	earliness	27
		7404	China	1977	gamma rays	Xinan 175	shortness	31
		7738	China	1980	gamma rays	Guangbeiguang	earliness	25
		A-20	Vietnam	1990	cross		earliness	42
		A-201	USA	1996	cross		semi-dwarfness	44

Aichinokaori	Japan	1987	cross		yield	42
Aifu 9	China	1966	gamma rays	Aijiaonante	semi-dwarfness	25
Ailiutiaohong	China	1989	gamma rays	Liutiaohong	semi-dwarfness	37
Akichikara	Japan	1986	cross		shortness	32
Akihikari	Japan	1976	cross		semi-dwarfness	11
Amber-Baghdad	Iraq	1994	gamma rays	Amber-33	lodging resistance	43
Amber-Furat	Iraq	1995	gamma rays	Amber-33	earliness	43
Amber-Manathera	Iraq	1995	gamma rays	Amber-33	lodging resistance	43
Arlatan	France	1979	gamma rays	Arlesienne	threshability	18
Atomita 1	Indonesia	1982	gamma rays	Pelita I/1	earliness	21
Atomita 2	Indonesia	1983	gamma rays	Pelita I/1	salt tolerance	23
Atomita 3	Indonesia	1990	gamma rays	No. 627/10-3/PsJ	disease resistance	42
Atomita 4	Indonesia	1991	gamma rays	Cisadane	earliness	42
Au-1	India	1976	gamma rays	IR 8	earliness	29
Aya	Japan	1991	cross		amylose content	42
Baofu 766	China	1988	gamma rays	Baoxuan 3 (PMC)	earliness	n.i.
B-fu 1	China	1982	gamma rays	[(5n.i.0 x Yinnisuitiangu) x BG 90-2]	shortness	29
Binadhan 4	Bangladesh	1998	gamma rays	F2 (BR4 x Iratom 38)	earliness	n.i.
Binadhan 5	Bangladesh	1998	gamma rays	F2 (Dular x Iratom 24)	yield	n.i.
Binadhan 6	Bangladesh	1998	gamma rays	F2 of (Iratom 24 x Dular)	yield	44
Binasail	Bangladesh	1987	gamma rays	Nizersail	tallness	31
Biraj	India	1982	x-rays	OC 1393	lateness	29
BPI Ri 10	Philippines	1983	cross		earliness	42
BPI-121-407	Philippines	1971	gamma rays	BPI-121	earliness	1
Calendal	France	1979	gamma rays	Arlesienne	grain size	18
Calmochi 201	USA	1979	gamma rays	S6	glutinous	15
Calmochi 202	USA	1981	cross		shortness	25
Calmochi-101	USA	1985	cross		photoperiod	28
Calpearl	USA	1981	cross		stiffness	23
Calrose 76	USA	1976	gamma rays	Calrose	shortness	9
Camago-8	Costa Rica	1996	gamma rays	IR-1821	blast resistance	43
Changwanxian	China	1992	gamma rays	hybrid	cold tolerance	n.i.
Changyouzao 1	China	1995	gamma rays	hybrid	earliness	n.i.

Chenzao 5	China	1979	gamma rays	IR 8	earliness	30
Chuukan-bohon Nou-13	Japan	1991	MNU	Kinmaze	amylose content	42
Chuukan-bohon Nou-14	Japan	1991	MNU	Kochihibiki	amylose content	42
Cilosari	Indonesia	1996	cross		yield	44
CNM 20	India	1980	x-rays	IR 8	earliness	18
CNM 25	India	1979	x-rays	IR 8	earliness	18
CNM 31	India	1979	x-rays	IR 8	earliness	17
CNM 6	India	1980	x-rays	IR 8	earliness	18
CRM 49	India	1999	NaN3	IR 50	blast resistance	n.i.
CRM 51	India	1999	NaN3	IR 50	blast resistance	n.i.
CRM 53	India	1999	EMS	IR 50	blast resistance	n.i.
Daisenminori	Japan	1988	cross		lodging resistance	35
Dalris 11	USSR	1988	MNH	Malysh	earliness	31
Danau atas	Indonesia	1988	gamma rays	Seratus malam	blast resistance	35
DB 250	Vietnam	1986	gamma rays	F1 of TB-I x IR-22	adaptability	30
DB-2	Vietnam	1987	ENH	Nep Hoa Vang	earliness	42
DCM-1	Vietnam	1988	MNH	Cuom	semi-dwarfness	42
Dellmont	USA	1992	cross		grain quality	43
Delta	France	1970	gamma rays	Cesariot	grain quality	*
Domannaka	Japan	1992	cross		lodging resistance	42
Dongting 3	China	1976	gamma rays	Aixin 3	semi-dwarfness	21
DT-10	Vietnam	1989	gamma rays, MNH	C4-63	lodging resistance	42
DT-11	Vietnam	1994	gamma rays, NEU	C4-63	disease resistance	43
Ejingnuo 6	China	1986	gamma rays	Guizao 2	blast resistance	31
Enuo 7	China	1994	cross		disease resistance	n.i.
Erfuzao	China	1967	gamma rays	Erjiuai 7	earliness	25
Erjiufeng	China	1985	cross		blight resistance	30
Fu 709	China	1974	gamma rays	Nonghu 6	yield	25
Fu 756	China	1975	gamma rays	Jiangerai	disease resistance	27
Fu 769	China	1976	gamma rays	Jiangerai	disease resistance	27
Fu 8-1	China	1988	gamma rays	8004	blast resistance	37
Fu 8970	China	1995	cross		disease resistance	n.i.

Fubao 201	China	1978	gamma rays	Baoxuan 2	earliness	26
Fuchuerai	China	1978	cross		shortness	37
Fugui 1	China	1980	gamma rays	Guichao 2	earliness	27
Fuheixiangnuo	China	1993	gamma rays	Nongqin 3	earliness	n.i.
Fuhui 06	China	1983	gamma rays	Taiyin 1	earliness	35
Fujihikari	Japan	1977	cross		season-neutral	11
Fulgente	Italy	1973	x-rays	Maratelli	blast resistance	10
Fulianai	China	1966	gamma rays	Liantangzao	semi-dwarfness	25
Fulianzao 3	China	1968	gamma rays	Liantangzao	earliness	27
Fuluzao 1	China	1976	gamma rays	Guangdongai 4 x IR 8	leaf size	27
Funo 402	China	1989	gamma rays	Guichao 2	glutinous	35
Funuo 1	China	1995	cross		earliness	n.i.
Funuo 101	China	1987	gamma rays	Guichao 2	earliness	33
Fushe 31	China	1966	gamma rays	Lucaihao	earliness	25
Fushe 410	China	1974	gamma rays	Chenai 8	blast resistance	27
Fushe 94	China	1971	neutrons	Zhongaizi	earliness	25
Fushenongken 58	China	1973	gamma rays	Nongken 58	disease resistance	29
Fuwan 23	China	1978	gamma rays	Huxuan 19	disease resistance	25
Fuwan 81-548	China	1989	gamma rays	Yuchi 231-8	grain quality	n.i.
Fuxian 6	China	1989	cross		disease resistance	37
Fuxiang 1	China	1978	gamma rays + microwave	Mingshuixiangdao	earliness	27
Fuxuan 1	China	1968	gamma rays	Zhongnong 4	earliness	27
Fuxuan 124	China	1972	gamma rays	Guangxuan 3	blast resistance	25
Fuxuan 3	China	1970	gamma rays	Fuxuan 1	tillering type	25
Fuxuan 8	China	1998	cross		blast resistance	n.i.
Fuyou 130	China	1997	cross		yield	n.i.
Fuyou 63	China	1993	cross		earliness	n.i.
Fuyou 802	China	1998	cross		earliness	n.i.
Fuyou 838	China	1997	cross		earliness	n.i.
Fuyouxiannuo	China	1995	gamma rays	IR 1259	earliness	n.i.
Fuyouxiannuo	China	1995	gamma rays	Nongqin 2	semi-dwarfness	n.i.
Fuyu 1	China	1968	gamma rays	Erjiuai 7	earliness	25
Fuzao 2	China	1969	gamma rays	Erjiuai	earliness	25

Fuzhou 383	China	1989	cross		plant architecture	n.i.
Fuzhu	China	1979	gamma rays	Zhulianai	earliness	25
Gangai A/Fuhui 06 H.	China	1985	cross		fertility rate	35
Ganwannuo	China	1993	gamma rays	SG 8960	grain quality	n.i.
Ganwanxian 23	China	1994	cross		grain quality	n.i.
Ginnosei	Japan	1992	cross		grain size	42
Gongshe 13	China	1969	gamma rays	Laolaiqing	disease resistance	27
Guangdabai	China	1979	laser	Hong 410	earliness	25
Guangfen 1	China	1977	laser	Guangluai 4	earliness	27
Guangfu 1	China	1981	gamma rays + laser	Hong 410	earliness	25
Guifu 3	China	1973	gamma rays	Guiluai 8	earliness	25
Guifunuo	China	1989	gamma rays	Shuangchengnuo	yield	n.i.
Guifuxian 2	China	1992	gamma rays	83-231	grain quality	n.i.
Guiwanfu	China	1988	gamma rays	Baotaiai	cold tolerance	n.i.
Hanahikari	Japan	1975	cross		semi-dwarfness	21
Hangfeng	China	1983	cross		shortness	30
Hangyu 1	China	1998	aerospace	ZR 9	earliness	n.i.
Hari	India	1987	cross		shortness	34
Hatsukogane	Japan	1984	cross		shortness	32
Hayahikari	Japan	1976	cross		stiffness	11
Heiseimochi	Japan	1990	cross		lodging resistance	42
Heugseonchalbyeo	Korea,	1998	gamma rays	Sanghaehyang	grain quality	n.i.
Hirohikari	Japan	1990	cross		stiffness	42
Hongfuzao 7	China	1980	gamma rays	Hong 410	shortness	27
Hongnan	China	1981	gamma rays	F2 (Hongmeizao x Guanxi 1)	earliness	25
Hongtu 31	China	1985	electrons	Hong 410	cold tolerance	31
Houhai	Japan	1976	cross		semi-dwarfness	21
HPU 8020	India	1984	gamma rays	Bala	lateness	29
Hu 2205	China	1987	gamma rays	IET 2938	cooking quality	41
Huangpiai	China	1969	gamma rays	Huangpizhong	semi-dwarfness	25
Huayu 1	China	1990	gamma rays		yield	n.i.
HUR-36	India	1990	gamma rays, EMS	Mahsuri	earliness	42

Hybrid Mutant 95	India	1973	gamma rays	(Jhona 349 x Taichung Native1)	semi-dwarfness	4
Hyokeisake 18	Japan	1972	cross		semi-dwarfness	21
Ibukiwase	Japan	1986	cross		cold tolerance	32
II You 802	China	1996	cross		yield	n.i.
II You 838	China	1995	cross		earliness	n.i.
IIT 48	India	1972	ethyleneoxide	IR 8	earliness	*
IIT 60	India	1972	EMS	IR 8	earliness	*
Ikungbau 4-2	China	1973	x-rays	Ikungbau		37
Indira	India	1980	EMS	Tainan-3	earliness	29
Intan Mutant	India	1988	EI	Intan	photoperiod	35
IRAT 13	Cote d'Ivoire	1978	gamma rays chronic	(63 x 83)	stiffness	11
IRAT 101	Cote d'Ivoire	1976	gamma rays chronic	IRAT 2	adaptability	33
IRAT 104	Cote d'Ivoire	1983	cross		tallness	34
IRAT 109	Cote d'Ivoire	1978	cross		productivity	37
IRAT 110	Cote d'Ivoire	1978	cross		grain quality	37
IRAT 112	Cote d'Ivoire	1983	cross		tillering type	34
IRAT 113	Cote d'Ivoire	1979	gamma rays chronic	Moroberekan	shortness	33
IRAT 114	Cote d'Ivoire	1979	gamma rays chronic	Moroberekan	shortness	33
IRAT 115	Cote d'Ivoire	1979	gamma rays chronic	Moroberekan	shortness	33
IRAT 116	Cote d'Ivoire	1979	gamma rays chronic	Moroberekan	shortness	33
IRAT 117	Cote d'Ivoire	1979	gamma rays chronic	Moroberekan	shortness	33
IRAT 133	Cote d'Ivoire	1978	cross		shortness	35
IRAT 134	Cote d'Ivoire	1978	cross		shortness	35
IRAT 136	Cote d'Ivoire	1978	cross		grain quality	37
IRAT 144	Burkina	1978	cross		yield	34
IRAT 1n.i.	Burkina	1979	cross		shortness	35
IRAT 147	Cote d'Ivoire	1979	cross		grain morphology	37

IRAT 161	Cote d'Ivoire	1980	cross		productivity	37
IRAT 170	Cote d'Ivoire	1984	cross		tillering type	34
IRAT 177 (Cabaçu)	Brazil	1988	spont. from IRAT 79		tallness	34
IRAT 191 (IREM 191)	Guyana	1980	gamma rays chronic	IAC 25	tallness	33
IRAT 192 (IREM 192)	Guyana	1980	gamma rays chronic	IAC 25	tallness	33
IRAT 193 (IREM 193)	Guyana	1980	gamma rays chronic	IAC 25	tallness	33
IRAT 194 (IREM 194)	Guyana	1980	gamma rays chronic	IAC 25	shortness	33
IRAT 195 (IREM 195)	Guyana	1980	gamma rays chronic	IAC 25	tallness	33
IRAT 196 (IREM 196)	Guyana	1980	gamma rays chronic	IAC 25	tallness	33
IRAT 213 = ISA 3	Cote d'Ivoire	1982	cross		grain morphology	37
IRAT 214 = ISA 4	Cote d'Ivoire	1982	cross		yield	37
IRAT 216	Cote d'Ivoire	1985	cross		adaptability	34
IRAT 239 (IREM 779)	Guyana	1980	gamma rays chronic	IAC 25	tallness	33
IRAT 240 (IREM 950)	Guyana	1980	gamma rays chronic	IAC 25	tallness	33
IRAT 241 (IREM 73-2)	Guyana	1983	gamma rays chronic	IAC 5100	tallness	33
IRAT 242 (IREM575-1)	Guyana	1983	gamma rays chronic	IAC 5100	shortness	33
IRAT 243 (IREM 15-2)	Guyana	1983	gamma rays chronic	IAC 5100	tallness	33
IRAT 244 (IREM 12-5)	Guyana	1983	gamma rays chronic	IAC 5100	tallness	33
IRAT 2n.i. (IREM43111)	Guyana	1983	gamma rays chronic	IAC 5100	tallness	33
IRAT 2n.i. (IREM 3n.i.3)	Guyana	1983	gamma rays chronic	IAC 5100	tallness	33

IRAT 247 (IREM 75-1)	Guyana	1983	gamma rays chronic	IAC 5100	tallness	33
IRAT 248 (IREM 2-1)	Guyana	1983	gamma rays chronic	IAC 5100	shortness	33
IRAT 249 (IREM12322)	Guyana	1983	gamma rays chronic	IAC 5100	tallness	33
IRAT 250 (IREM 52-1)	Guyana	1983	gamma rays chronic	Pratao Precoce	tallness	33
IRAT 251 (IREM297-3)	Guyana	1983	gamma rays chronic	Pratao Precoce	tallness	33
IRAT 252 (IREM n.i.-4)	Guyana	1983	gamma rays chronic	Pratao Precoce	tallness	33
IRAT 253 (IREM 50-2)	Guyana	1983	gamma rays chronic	Pratao Precoce	tallness	33
IRAT 254 (IREM 53-2)	Guyana	1983	gamma rays chronic	Pratao Precoce	tallness	33
IRAT 255 (IREM 35-2)	Guyana	1983	gamma rays chronic	Pratao Precoce	shortness	33
IRAT 256 (IREM n.i.-2)	Guyana	1983	gamma rays chronic	Pratao Precoce	shortness	33
IRAT 257 (IREM 4113)	Guyana	1983	gamma rays chronic	Makouta	shortness	33
IRAT 258 (IREM 4114)	Guyana	1983	gamma rays chronic	Makouta	shortness	33
IRAT 268 = IDSA 16	Cote d'Ivoire	1983	cross		grain quality	37
IRAT 269 = IDSA 16	Cote d'Ivoire	1983	cross		grain quality	37
IRAT 320 = IDSA 48	Cote d'Ivoire	1987	cross		grain morphology	37
IRAT 4 (IRAT 51)	Senegal	1968	gamma rays chronic	Sintane Diofor		33
IRAT 5 (IRAT 52)	Senegal	1968	gamma rays chronic	Sintane Diofor		33
IRAT 78 (M18)	Cote d'Ivoire	1976	gamma rays chronic	IRAT 2	leaf morphology	33
IRAT 79 (Mn.i.)	Cote d'Ivoire	1976	gamma rays chronic	IRAT 2	tillering type	33

Iratom 24	Bangladesh	1970	gamma rays	IR 8	earliness	29
Iratom 38	Pakistan	1970	gamma rays	IR 8	earliness	*
IRI #308	Korea,	1970	x-rays	Baekna 18	semi-dwarfness	n.i.
IRI 307	Korea	1970	thN	Palkweng	semi-dwarfness	44
ITA 123	Nigeria	1980	gamma rays		semi-dwarfness	42
ITA 235	Nigeria	1988	cross		RYMV resistance	42
ITA 314	Nigeria	1988	cross		semi-dwarfness	42
Iwate 21	Japan	1988	gamma rays	Sasanishiki	semi-dwarfness	35
Jagannath	India	1969	x-rays	T-141	grain size	0
Jiahezaozhan	China	1997	gamma rays	- (pollen)	grain quality	n.i.
Jiasifu	China	1973	gamma rays	Jiahu 4	earliness	25
Jiguang 2	China	1977	laser	Guangluai 4	shortness	27
Jinfu 1	China	1969	gamma rays	Jinyin 37	earliness	25
Jinfu 48	China	1988	gamma rays	Jinke 5	yield	n.i.
Jinfu 8	China	1969	gamma rays	Xiaozhan 101	earliness	25
Juangyebai	China	1974	neutrons	IR 8	earliness	25
K84	India	1967	gamma rays	T 65	earliness	29
Kagahikari	Japan	1973	cross		earliness	11
Kashmir Basmati	Pakistan	1977	gamma rays	Basmati 370	earliness	10
Katsurawase	Japan	1978	cross		earliness	21
Kefuhong 2	China	1981	cross		earliness	25
Keshari	India	1980	cross		shortness	29
Khao Jao Hawm	Thailand	1998	cross	Shinsu		44
Khushboo	Pakistan	1995	gamma rays	Jaijai 77	grain size	n.i.
Kinuhikari	Japan	1991	cross		lodging resistance	42
Koihime	Japan	1990	cross		lodging resistance	42
KT 20-74	China	1957	x-rays	Ketze	yield	*
Kunihikari	Japan	1987	cross		lodging resistance	33
Lafitte	USA	1995	cross		semi-dwarfness	44
Liaofeng 5	China	1969	gamma rays	Liaogeng 125	earliness	27
Liaoyan 2	China	1992	gamma rays	Toyonishiki	salt tolerance	41
M 112	China	1981	gamma rays	5n.i.0 x Yinnishuitiangu	cold tolerance	27
M 114	China	1981	gamma rays	[(5n.i.0 x Yinnisuitiangu) BG 90-2]	cold tolerance	25

M-101	USA	1979	cross		shortness	15
M-102	USA	1987	cross		lateness	32
M-202	USA	1985	cross		photoperiod	28
M-203 (86-Y-35)	USA	1989	gamma rays	M-40	photoperiod	37
M-204	USA	1992	cross		photoperiod	43
M-301	USA	1980	cross		grain size	18
M-302	USA	1981	cross		shortness	25
M-401	USA	1981	gamma rays	Terso	shortness	19
M7	USA	1977	cross		shortness	13
Madjan	USSR	1987	NMU	line KZROS 356	stiffness	31
Malysh	USSR	1982	EMS	Sirayuki	earliness	40
Marathon	France	1985	gamma rays	Maratelli	blast resistance	30
Megumimochi	Japan	1983	cross		shortness	32
Meisanwu 2	China	1990	gamma rays	Aimeizao 3 x Waixuan 35	disease resistance	n.i.
Mercury	USA	1988	cross		earliness	35
MI-273(m)	Sri Lanka	1971	gamma rays	H4	shortness	29
Milyang 10	Korea	1972	x-rays	Palkweng	semi-dwarfness	*
Mineasahi	Japan	1980	cross		earliness	21
Minnuo 706	China	1991	gamma rays	7056 x IR29	tillering type	35
Minyuan 1	China	1977	gamma rays	Sanyeqi	photonasty	35
Miyama Nishiki	Japan	1978	gamma rays	Takane-Nishiki	grain size	15
Miyanishiki	Japan	1978	cross		earliness	17
Miyukimochi	Japan	1979	gamma rays	Toyonishiki	glutinous	15
Mohan = CSR4	India	1983	gamma rays	IR 8	salt tolerance	37
MT-4	Vietnam	1988	ENH	Moc Tuyen	lodging resistance	42
MT-6	Vietnam	1993	DMS	F1 from IR8 x X6	stiffness	43
Musashikogane	Japan	1981	cross		shortness	21
Mutant 428	USSR	1989	MNH	[Fanu x KUR-127]	lodging resistance	40
Mutashali	Hungary	1980	fN	Dunghan Shali	blast resistance	30
Mutsuhomare	Japan	1986	cross		shortness	32
Mutsuhonami	Japan	1973	cross		grain quality	*
Mutsukaori	Japan	1981	cross		shortness	21
Mutsukomachi	Japan	1981	cross		shortness	21

Nadahikari	Japan	1977	cross		shortness	21
Nangeng 23	China	1967	gamma rays	20025	shortness	27
Nanjing 34	China	1976	gamma rays + microwave	Zhaofeng	shortness	19
Nanzao 1	China	1980	gamma rays	Nanjing 11	earliness	27
NIAB-IRRI-9	Pakistan	1999	fN	IR-6	salt tolerance	n.i.
Niigatawase	Japan	1979	cross		shortness	21
Nijihikari	Japan	1989	cross		lodging resistance	42
NN 22-98	Vietnam	1983	ENH	IR 22	stiffness	30
Nongshi 4	China	1975	fN	IR 20	earliness	27
Norin PL 12	Japan	1991	gamma rays	Reimei	thermosensitive	42
Nucleoryza	Hungary	1972	fN	Cesariot	earliness	2
Nucus 2	USSR	1986	cross		shortness	40
Oltenita	Romania	1992	gamma rays	Krasnodar 424	lodging resistance	44
Oryzella	Hungary	1983	EMS	Chiapelli x Duborszkij 129	earliness	30
Padmini	India	1988	gamma rays	CR 1014	earliness	37
PARC 1	Philippines	1970	gamma rays	IR 8-288-3	grain size	4
PARC 2	Philippines	1970	gamma rays	IR 8-68	earliness	4
PL-56	India	1975	EMS	C-164	tillering type	29
Prabhavati	India	1984	EMS	Ambemohor local	shortness	29
Pusa-NR-162	India	1988	cross		earliness	42
Pusa-NR-166	India	1989	cross		synchronous	42
Pusa-NR-381	India	1989	cross		blast resistance	42
Pusa-NR-519	India	1990	cross		pest resistance	42
Pusa-NR-5n.i.	India	1998	gamma rays	F2 (PNR 125-2 x PNR 130-2)	grain quality	n.i.
Pusa-NR-550-1-2 (JD-8)	India	1997	cross		semi-dwarfness	44
Pusa-NR-551-4-20 (JD-6)	India	1997	cross		semi-dwarfness	44
Pusa-NR-555-28 (JD-10)	India	1997	cross		semi-dwarfness	44
Pusa-NR-555-5	India	1990	cross		earliness	42
Pusa-NR-555-5 (JD-3)	India	1998	cross		yield	44
Pusa-NR-570-17	India	1990	cross		earliness	42
Pusa-NR-571	India	1990	cross		semi-dwarfness	44
Pygmalion	France	1987	chemical	Cigalan	yield	35

Qikesui	China	1986	gamma rays	Hejiang 12	cold tolerance	30
Qinghuaai 6	China	1980	cross		yield	37
Qingwei 1	China	1985	gamma rays		yield	37
Qiufu 1	China	1982	gamma rays	Qiujuai	cold tolerance	31
Quannuo 101	China	1990	gamma rays	hybrid	yield	n.i.
R n.i.2	China	1985	gamma rays	501 Xuan (pollen)	shortness	30
R 817	China	1987	gamma rays	Aishungnuo	glutinous	31
Radiation 85-63	China	1989	cross		tillering type	37
Rasmi	India	1985	gamma rays	Oorpandy	awnless	30
RD 10	Thailand	1981	fN	RD 1	glutinous	18
RD 15	Thailand	1978	gamma rays	Khao Dawk Mali 105	earliness	13
RD 6	Thailand	1977	gamma rays	Khao Dawk Mali 105	glutinous	10
Reimei	Japan	1966	gamma rays	Fujiminori	shortness	0
Rokkonishiki	Japan	1982	cross		grain size	21
S 201	USA	1980	cross		shortness	18
S-102	USA	1996	cross		earliness	44
S2-Calpearl	USA	1987	radiation	Calpearl	shortness	37
S-301	USA	1991	cross		semi-dwarfness	42
Sachiminori	Japan	1978	cross		stiffness	21
Salir	Portugal	1983	gamma rays	Saloio	yield	30
Sattari	India	1983	gamma rays	NSJ 200 x Padma	earliness	29
Savitri	India	1983	cross		daylength	29
SH 30-21	China	1957	x-rays	Shungchiang	yield	*
Shadab	Pakistan	1987	EMS	IR 6	yield	30
Shanyou 371	China	1998	cross		grain quality	n.i.
Shenxiangjing	China	1994	x-rays		blast resistance	n.i.
Shinanosakigake	Japan	1982	gamma rays	Toyonishiki	grain size	21
Shirakabanishiki	Japan	1982	gamma rays	Reimei	grain size	21
Shua 92	Pakistan	1993	gamma rays	Shadab	salt tolerance	42
Shuangchengnuo	China	1980	gamma rays	2004	compact growth	25
Shuangchiang 30-21	China	1957	x-rays	Shuangchiang	yield	30
Shuangfu 1	China	1989	gamma rays	Guichao 2	shortness	n.i.
Shuangke 1	China	1981	cross		earliness	25

Shwethwetun	Myanmar	1981	gamma rays	IR 24	tallness	20
Shwewartun	Myanmar	1975	gamma rays	IR 5	grain quality	12
Sifu 851	China	1985	cross		earliness	30
Suifu 17	China	1979	gamma rays	Suiya 156	shortness	25
Suiwan 2	China	1974	gamma rays	Huxuan 19	tillering type	27
Suzutakara	Japan	1990	EMS	Akenohoshi	earliness	42
Taifu 4	China	1979	gamma rays	Taizhong 3	disease resistance	30
Tangernian	China	1985	gamma rays		yield	37
THDB	Vietnam	1999	gamma rays	Tep Hanh	semi-dwarfness	n.i.
TNDB 100	Vietnam	1997	gamma rays	Tai Nguyen Duc	semi-dwarfness	n.i.
Tsugaruotome	Japan	1990	cross		shortness	42
UNP 9027	Costa Rica	1994	gamma rays	CR 1113	disease resistance	43
Valencia 87	USA	1987	radiation	Calpearl	lodging resistance	37
Vellayani	India	1968	Neutrons	PTB 10		29
VN 10	Vietnam	1975	cross			29
VN 20	Vietnam	1975	cross			29
VN 4	Vietnam	1975	cross		earliness	29
VND 95-19	Vietnam	1998	gamma rays	IR 64	stiffness	n.i.
VND 95-20	Vietnam	1998	gamma rays	IR 64	earliness	n.i.
VND95-26	Vietnam	1995	gamma rays	IR 9729	earliness	44
Vyouwan 3	China	1994	cross		yield	n.i.
Wandao 20	China	1994	ion beams	Eyu 105	grain quality	n.i.
Wandao 25	China	1990	gamma rays	Minggui 1 x Simei 2	earliness	n.i.
Wandao 42	China	1997	ion beams	Taiwanzhongjing	earliness	n.i.
Wandao 44	China	1997	ion beams	hybrid	yield	n.i.
Wandao n.i.	China	1994	ion beams	Zhe 15	earliness	n.i.
Wandao 51	China	1997	gamma rays	hybrid	yield	n.i.
Wanfu 33	China	1980	gamma rays	72-10	earliness	25
Wanfu 8818	China	1997	gamma rays	Yaodao 4	yield	n.i.
Wangeng 257	China	1975	gamma rays	Huxuan 19	fertilizer response	25
Wanhongfu	China	1980	gamma rays	25-1 x Hongmiyouzhan	cold tolerance	27
Wanhua	China	1983	cross		semi-dwarfness	37
Wanjing 3073	China	1990	gamma rays	Sujing 7 x Ewan 5	fertilizer response	n.i.

Weiyouji	China	1983	cross		earliness	31
Wongwangbyeo	Korea,	1998	gamma rays	Seomjinbyeo	disease resistance	n.i.
Wonmibyeo	Korea,	1998	gamma rays	Chuchongbyeo	earliness	n.i.
Wonpyungbyeo	Korea,	1998	gamma rays	Hwaseongbyeo	semi-dwarfness	n.i.
Xangzaonuo 1	China	1984	gamma rays	F2 (IR 29 x Wenqingxuan)	glutinous	30
Xiangfudao	China	1976	gamma rays	Erjiuqing	cold tolerance	25
Xianghu 24	China	1984	cross		blast resistance	35
Xianghu 47	China	1985	cross		panicle size	n.i.
Xianghu 93	China	1984	cross		lateness	n.i.
Xiangjing 832	China	1989	x-rays	Wuxiang 203	shortness	35
Xiangwanxian 7	China	1996	cross		blast resistance	n.i.
Xiangzaoxian 18	China	1995	gamma rays	hybrid	earliness	n.i.
Xiangzaoxian 20	China	1995	gamma rays	hybrid	earliness	n.i.
Xiangzaoxian 21	China	1996	gamma rays + laser	Xiangaizao 7	blight resistance	n.i.
Xiangzaoxian 22	China	1996	cross		grain quality	n.i.
Xiangzaoxian 23	China	1997	cross		earliness	n.i.
Xiangzaoxian 25	China	1997	cross		shortness	n.i.
Xiangzaoxian 28	China	1999	chemical	Zhe 733	earliness	n.i.
Xiangzaoxian 8	China	1988	laser	Xiangaizao 9	earliness	n.i.
Xiangzaoxian 9	China	1989	gamma rays	Hongtu 5	earliness	n.i.
Xiaofuzao	China	1974	gamma rays	Liantangzao	earliness	25
Xieyou 371	China	1999	cross		earliness	n.i.
Xindao 1	China	1986	gamma rays	F2 (Ningxi 62-2 x Panjin 1)	earliness	31
Xiongyue 613	China	1970	gamma rays	Nongken 20	blast resistance	25
Xiushui 04	China	1985	cross		earliness	n.i.
Xiushui 06	China	1984	cross		earliness	n.i.
Xiushui 48	China	1984	cross		blast resistance	35
Xiuxui 117	China	1984	cross		earliness	n.i.
Yangdao 6	China	1997	gamma rays	hybrid	yield	n.i.
Yangfunuo 1	China	1990	gamma rays	IR 29	earliness	n.i.
Yangfuxian 2	China	1991	gamma rays	IR 1529-68-32	yield	n.i.
Yangfuxian 3	China	1993	gamma rays	IR 2415	blast resistance	n.i.

		Yanzhengfu	China	1979	gamma rays	Longzhen 13	yield	37
		Yenhsing-1	China	1963	cross		yield	29
		Yenhsing-2	China	1967	cross		erectoid type	29
		Yifunuo 1	China	1977	gamma rays	IR 8	blast resistance	25
		Youfu 5	China	1980	gamma rays	Siyou 2	earliness	27
		Yuanfengzao	China	1975	gamma rays	IR 8	earliness	19
		Yuanjing 11	China	1990	gamma rays	R 824 x C 80n.i.	earliness	n.i.
		Yuanjing 2	China	1988	gamma rays	Nonghuo 6	yield	n.i.
		Yuanjing 4	China	1993	gamma rays	Suishui 14 x Suishui 27	blast resistance	n.i.
		Yuanjing 7	China	1999	gamma rays	hybrid	grain quality	n.i.
		Yumeminori	Japan	1992	cross		lodging resistance	42
		Zaoyeqing	China	1980	gamma rays	Zaoyeqing 8	panicle size	27
		Zhefu 218	China	1995	cross		earliness	n.i.
		Zhefu 504	China	1999	gamma rays	hybrid	earliness	n.i.
		Zhefu 7	China	1991	gamma rays	Erjiufong	earliness	43
		Zhefu 762	China	1993	cross		disease resistance	n.i.
		Zhefu 802	China	1981	gamma rays	Simei 2	earliness	25
		Zhefu 852	China	1989	gamma rays	Zhefu 802 x Shuiyuan 290	blast resistance	n.i.
		Zhefu 9	China	1990	cross		yield	
		Zhenfu 1	China	1971	gamma rays	Zhenshuai	earliness	25
		Zhengguang 1	China	1979	gamma rays	Taizhongyu 39	YDV resistance	25
		Zhenuo 2	China	1993	gamma rays	R8917	blast resistance	n.i.
		Zhong 156	China	1993	cross		yield	n.i.
		Zhongbao 2	China	1977	fN		earliness	25
		Zhongmounuodao	China	1982	gamma rays	Tianbian 10	glutinous	27
		Zhongtie 31	China	1986	fN	Tieqiu 15	yield	30
		Zhongzhe 1	China	1989	cross		yield	n.i.
		Zhouyou 903	China	1994	cross		grain quality	n.i.
		Zhuchou 40	China	1978	gamma rays	F2 (Zhulianai x Qiuzhen)	cold tolerance	27
		Zijiangnuo	China	1984	cross		yield	n.i.
		Zixiangnuo 861	China	1989	x-rays	Lungjing 2 (germinating seed)	shortness	35
		Zolotistyi	USSR	1989	ENH	[Rossiiskii]	cooking quality	40
Panicum miliaceum	millet	Cheget	Russia	1993	cross		drought tolerance	41

		Kharkovskoe 57	USSR	1987	MNH	Kharkovskoye 37	cooking quality	40
		Lipetskoe 19	USSR	1985	DMS	line No. 947	cooking quality	40
		Lipetskoe 19 *	USSR	1985	DMS, NEH	Line No. 947	earliness	30
Papaver somniferum	opium poppy	BC-28/9/4 (Vivek)	India	1992	gamma rays	Shweta	capsule size	42
Pelargonium	geranium	Dark Mozart	FRG	1988	x-rays	Mozart	flower colour	35
Pennisetum sp.	pearl millet	ICMH n.i.1	India	1986	gamma rays	Tift 23 DB	mildew resistance	30
		New Hybrid Bajra 5	India	1974	gamma rays	Male sterile inbred line Tift 23A	Sclerospora	11
		NHB 3 (hybrid)	India	1975	cross		Sclerospora	37
		NHB 4 (hybrid)	India	1975	cross		Sclerospora	37
		Pusa n.i.	India	1982	radiation	(J104 x K559)	mildew resistance	23
Phaseolus vulgaris	common bean	AC Hensall	Canada	1997	cross		earliness	n.i.
		AC Skipper	Canada	1996	cross		earliness	n.i.
		Albion	USA		cross		earliness	n.i.
		Alfa	CSFR	1972	EMS	Black bean	seed colour	10
		Arapaho	USA	1995	cross		bushy type	n.i.
		Black Magic	USA	1987	cross		seed colour	n.i.
		Blackhawk	USA	1990	cross		seed colour	n.i.
		C-20	USA	1982	cross		seed colour	n.i.
		CAP-1070	Brazil	1986	gamma rays	Carioca	bushy type	34
		Centralia	Canada	1988	cross		earliness	n.i.
		Domino	USA	1987	cross		seed colour	n.i.
		Dresden	Canada		cross		earliness	n.i.
		Eureka	Poland	1991	gamma rays	local ecotype	semi-dwarfness	41
		Fleetwood	Canada	1977	cross		earliness	n.i.
		Frontier	USA	1998	cross		seed colour	n.i.
		FT-Paulistinha	Brazil	1992	cross		yield	42
		Giza 80	Egypt	1980	gamma rays	Fin de Villeneuve	rust resistance	17
		Gratiot	USA	1962	x-rays	Michelite	stiffness	*
		Harkovskaya 8	USSR	1985	gamma rays		seed colour	31
		Harofleet	Canada	1983	cross		earliness	n.i.
		Harokent	Canada	1983	cross		earliness	n.i.
		Huron	USA	1994	cross		seed colour	n.i.
		IAPAR 57	Brazil	1992	cross		GMVD-resistance	40

		JM-126	USA	1986	cross		seed colour	n.i.
		JM-24	USA	1986	cross		seed colour	n.i.
		Kentwood	Canada	1973	cross		earliness	n.i.
		Laker	USA	1983	cross		seed colour	n.i.
		Longyundou 4	China	1994	gamma rays	Heiyundou	yield	n.i.
		Maverick	USA	1997	cross		seed colour	n.i.
		Mayflower	USA	1989	cross		seed colour	n.i.
		Midland	USA	1983	cross		earliness	n.i.
		Mitchell	Canada	1986	cross			34
		Mogano	Italy	1985	EMS	P-224	seed colour	31
		Montalbano	Italy	1985	EMS	P-106	seed colour	31
		Mukhranula	USSR	1982	EI	Mukhranula 4	earliness	40
		NC Alberta Pink	Canada	1998	cross		seed colour	n.i.
		NEP-2	Costa Rica	1975	EMS	San Fernando	seed colour	n.i.
		Neptune	USA	1986	cross		plant architecture	30
		Newport	USA	1995	cross		earliness	n.i.
		Norstar	USA	1993	cross		earliness	n.i.
		Northland	USA	1983	cross		earliness	n.i.
		OAC Seaforth	Canada	1983	cross		earliness	n.i.
		Ouray	USA	1982	cross		bushy type	28
		Pusa Parvati	India	1970	x-rays	Wax podded	earliness	*
		Sanilac	USA	1956	x-rays	Michelite	bushy type	*
		Saparke 75	USSR	1967	gamma rays	Tzanava-31	yield	*
		Seafarer	USA	1967	x-rays	Michelite	earliness	*
		Seaway	USA	1960	x-rays	Michelite	earliness	*
		Stinger	USA	1988	cross		earliness	n.i.
		Suncrest	Canada	1986	cross	Seafarer	earliness	n.i.
		Svetlaya	USSR	1992	MNH	Shchedraya	yield	40
		Swan Valley	USA	1981	cross		seed colour	n.i.
		Unima	FRG	1957	cross		disease resistance	*
		Universal	FRG	1950	x-rays	Granda	earliness	*
		Wesland	USA	1983	cross		earliness	n.i.
Pisum sativum	pea	Agra	Poland	1990	cross		lodging resistance	43

		Bitug	USSR	1990	cross		seed skin quality	40
		Bosman	Poland	1989	cross		afila type	37
		Caoyuan 10	China	1984	gamma rays	Lusecaoyuan	earliness	37
		Diament	Poland	1989	cross			35
		Esedra	Italy	1980	x-rays	Sprinter	lateness	19
		Hamil	Poland	1981	cross		seed tendrilness	18
		Hans	India	1979	EI	P 1163	yield	15
		Heiga	Poland	1986	cross		afila type	30
		Jaran	Poland	1986	cross		afila type	30
		Kwestor	Poland	1991	gamma rays	Paloma	tallness	41
		Mihan	Poland	1983	cross		lodging resistance	26
		Miko	Poland	1989	cross		afila type	35
		Milewska	Poland	1983	cross		lodging resistance	26
		Moskovsky 73	USSR	1974	DES	Nemchonvsky 766	grain size	12
		Navona	Italy	1980	x-rays	Sprinter	lateness	19
		Nemchinovskii 85	USSR	1986	cross		dwarfness	31
		Orphei	USSR	1989	chemical		earliness	40
		Paride	Italy	1988	gamma rays	San Cristoforo	determinate	37
		Piast	Poland	1995	cross		stiffness	43
		Pirro	Italy	1988	gamma rays	Santa Croce	determinate	37
		Priamo	Italy	1988	gamma rays	Alderman	determinate	37
		Ramir	Poland	1985	cross		lodging resistance	26
		Samara	USSR	1992	chemical	Arvika	seed retention	40
		Shikhan	USSR	1984	cross		seed retention	37
		Stral-art	Sweden	1954	x-rays	Kloster	vigour	*
		Streletskii 11	USSR	1985	EI	Zernogradskii mnogop	earliness	31
		Sum	Poland	1979	cross		shortness	15
		Talovets 60	Russia	1993	cross		lodging resistance	41
		Tatarstan 2	USSR	1989	ENH	hybrid seeds	earliness	40
		Trevi	Italy	1985	cross		determinate	35
		Wasata	Poland	1979	gamma rays	Line 5/2	seed tendrilness	15
Polyanthes tuberosa	polyanthes	Rajat Rekha	India	1974	gamma rays	single flowered cv.	leaf colour	14
		Swarna Rekha	India	1974	gamma rays	double flowered cv.	leaf colour	14

Populus trichocarpa	poplar	Donetskii Zolotoi	USSR	1977	gamma rays		variegated leaves	15
Portulaca grandiflora	portulaca	Five Petal	India	1974	gamma rays		flower	20
		Jhumka	India	1974	gamma rays	Karna Pali	flower	14
		Karna Pali	India	1974	gamma rays	Portulaca double	flower	14
		Karna Phul	India	1974	gamma rays	*Portulaca grandiflora*	flower	17
		Lalita	India	1974	gamma rays	Portulaca double	flower	14
		Mukta	India	1974	gamma rays	Portulaca double	flower	14
		Pink colour	India	1974	gamma rays chronic		flower colour	20
		Ratnam	India	1974	gamma rays		flower number	37
		Rosy Green	India	1974	gamma rays chronic		flower morphology	20
		Semi-double	India	1974	gamma rays		flower	20
		Vibhuti	India	1974	gamma rays		flower	14
Prunus armeniaca	apricot	Early Blenheim	Canada	1970	thN	Blenheim	earliness	1
Prunus avium	sweet cherry	Burlat C1	Italy	1983	gamma rays	Bigarreau Burlat	compact growth	31
		Compact Lambert	Canada	1964	x-rays	Lambert	compact growth	*
		Compact Stella 35B11	Canada	1974	x-rays	Stella	compact growth	4
		Ferrovia spur	Italy	1992	x-rays	Ferrovia	shortness	42
		Lapins	Canada	1983	cross		fruit size	25
		Nero II C1	Italy	1983	gamma rays	Durone Nero II	compact growth	31
		Stella	Canada	1968	cross		self-fertile	*
		Sunburst	Canada	1983	cross		fruit size	25
Prunus cerasus	sour cherry	Karlik Samorodka	USSR	1979	gamma rays	Samorodka	dwarfness	18
		Plodorodnaya Michurina	USSR	1977	x-rays	Prunus maackii	fruit set	19
		Polukarlik Orlovskoi	USSR	1979	gamma rays	Orlovskoi Rannei	dwarfness	18
		Polukarlik Turgenevk	USSR	1979	gamma rays	Turgenevka	dwarfness	18
Prunus domestica	plum	Spurdente-Ferco	France	1988	gamma rays	Ente	earliness	35
Prunus dulcis	almond	Supernova	Italy	1987	gamma rays	Fascinello	lateness	32
Prunus persica	peach	Magnif 135	Argentina	1968	gamma rays	Magnif 43	fruit size	*
		Plovdiv 6	Bulgaria	1981	gamma rays	Dupnishka	yield	18
Psathyrostachys juncea	Russian wildrye	Tetracan	Canada	1988	colchicine	(open pollinated)	vigour	43
Punica granatum	pomegranate	Karabakh	USSR	1979	gamma rays			18
		Khyrda	USSR	1979	gamma rays		dwarfness	18

Pyrus communis	pear	Chaofu 1	China	1989	gamma rays	Chaoxianyangli	shortness	n.i.
		Chaofu 10	China	1989	gamma rays	Chaoxianyangli	quality	
		Chaofu 11	China	1989	gamma rays	Chaoxianyangli	lateness	n.i.
		Chaofu 2	China	1989	gamma rays	Chaoxianyangli	quality	
		Fuxiangyanghongdli	China	1983	gamma rays	Xiangyanghong	disease resistance	n.i.
Pyrus pyriforia	japanese pear	Gold Nijisseiki	Japan	1993	gamma rays	Nijisseiki	disease resistance	44
		Kotobuki Shinsui	Japan	1996	gamma rays	Shinsu	disease resistance	44
Raphanus sativus	radish	Qingfu	China	1981	gamma rays	Luoyanglutouqing	yield	n.i.
Rhododendron simsii	azalea	Ingana	Belgium	1984	gamma rays	Inga	flower colour	31
		Osta	FRG	1986	x-rays	Bertina	flower colour	28
Rhododendron	azalea	Adinda	Belgium	1972	x-rays	Karl Glaser	flower colour	6
		Aleida	Netherlands	1978	x-rays	Vuyck's Scarlet	flower colour	14
		Cobalt	Japan	1973	gamma rays	Takasago	dwarfness	*
		Enzet-Rokola	GDR	1969	x-rays	Mme. John Haerens	flower colour	*
		Enzet-Rolko	GDR	1969	x-rays	Ernst Thiers	flower	*
		Eroica	Belgium	1974	gamma and x-rays recurent	Knut Erwen	flower colour	6
		Mevr. R. de Loose	Belgium	1974	gamma and x-rays recurent	de Waele's Favorite	flower colour	6
		Mira	Belgium	1972	gamma and x-rays recurent	Euratom	flower colour	6
		Odilia	Netherlands		x-rays	Silvester	flower colour	34
		Pastorale	Belgium	1973	gamma and x-rays recurent	de Waele's Favorite	flower colour	6
		Saidjah	Belgium	1972	gamma rays	Euratom	flower colour	6
		Sierra Nevada	Belgium	1974	gamma and x-rays recurent	de Waele's Favorite	flower colour	6
		Stefan	Netherlands		x-rays	Silvester	flower colour	34
Ribes nigrum	black currant	Burga	France	1979	gamma rays	Noire de Bourgogne	earliness	29
Ribes sp.	ribes	Westra	FRG	1968	x-rays	Westwick Choice	erectoid type	17
Ricinus communis	castor bean	Aruna	India	1969	thN		earliness	*
		Khersonskaya 10	USSR	1981	chemical		oil content	41
		RC8	India	1978	gamma rays	Rc 1188-54	earliness	11
		Sowbhagya (157-B)	India	1976	cross		earliness	11

Rosa sp.	rose	Abhisarika H.T.	India	1975	gamma rays	Kiss of Fire	flower colour	26
		Angara	India	1975		Montezuma	plant architecture	14
		Beijingzhichun	China	1990	gamma rays	Hongyizhujiao x	flower colour	n.i.
		Beiyumudan	China	1986	gamma rays	Yilishahuanghou (branch)	flower colour	n.i.
		Binghua	China	1986	gamma rays	Beixuaishai x Wuhui (branch &	flower colour	n.i.
		Bridal Sonya	Japan	1985	gamma rays	Sonia	flower colour	32
		Caiyemingxin	China	1986	gamma rays	Mingxin (branch)	leaf morphology	n.i.
		Chuanxiu 1	China	1990	gamma rays	Yangjige (rooted cuttings)	flower colour	n.i.
		Chuanxiu 2	China	1990	gamma rays	Yangjige (young graft)	flower colour	n.i.
		Chuanxiu 3	China	1990	gamma rays	Yangjige (rooted cuttings)	flower colour	n.i.
		Chuanxiu 4	China	1990	gamma rays	Guanghui (rooted cuttings)	flower colour	n.i.
		Chuanxiu 5	China	1990	gamma rays	Guanghui (young graft)	flower colour	n.i.
		Chuanxiu 6	China	1990	gamma rays	Yilishabei (rooted cuttings)	flower colour	n.i.
		Chuanxiu 7	China	1990	gamma rays	Tengheping (graft)	flower colour	n.i.
		Chunyanqifei	China	1989	gamma rays	Ai (branch)	flower colour	n.i.
		Curio	India	1986	gamma rays	Imperator	flower colour	31
		Desi	GDR	1965	x-rays	Gloria Dei	flower colour	*
		Flamingo Queen	Canada	1976	x-rays	Queen Elizabeth	flower colour	17
		Haleihuixin	China	1985	gamma rays	Zhandihuanghua x Haixia (branch & seeds)	flower colour	n.i.
		Hepingzhiguang	China	1986	gamma rays	Heping (branch)	flower colour	n.i.
		Hongdu	China	1984	gamma rays	Lanxia x Lanyue (b & s)	flower colour	n.i.
		Honghuo	China	1986	gamma rays	Ouxiliya x Guonong (b & s)	flower colour	n.i.
		Hongyu	China	1989	gamma rays	Lushimei (branch)	flower colour	n.i.
		Huangjiao	China	1989	gamma rays	Yalishanda (branch)	flower colour	n.i.
		Jiguang	China	1984	gamma rays	Fengheping (branch)	flower colour	31
		Jubian	China	1990	gamma rays	Mubiao	flower colour	n.i.
		Jujing	China	1984	gamma rays	Mohong x Guonong (b & s)	flower scent	n.i.
		Light Pink Prize	India	1989	gamma rays	First Prize	flower colour	37
		Lihui	China	1985	gamma rays	Rongguang (branch)	flower colour	n.i.
		Lubaoshi	China	1984	gamma rays	Beixuaishan x Shiwaitaoyuan (b & s)	sunlight tolerance	n.i.
		Luxin	China	1990	gamma rays	Beixueshan x Luyun (F1 seed)	flower colour	n.i.

		Luye	China	1987	gamma rays	Beixuaishan x Dajiangzhang (b & s)	flower colour	n.i.
		Madhosh	India	1975	EMS	Gulzar	flower colour	14
		Milena	CSFR	1964		Elizabeth Rose	flower colour	14
		Misu-ohmiya	Japan	1990	gamma rays	Queen Elizabeth	flower colour	42
		Nanhailanghua	China	1984	gamma rays	(Langhua x Nanghai)	flower colour	31
		Ohmiyabito	Japan	1990	gamma rays	Queen Elizabeth	flower colour	42
		Paula	USA	1960	gamma rays	Queen Elizabeth	flower colour	31
		Permoser	GDR	1970	radiation	Kordes Perfecta	flower colour	7
		Pink Contempo	India	1986	gamma rays	Contempo	flower colour	31
		Pink Hat	USA	1960	gamma rays	unnamed floribunda	flower colour	31
		Pink-Ilseta	FRG	1985	x-rays	Perl-Ilseta	flower colour	28
		Pusa Christina	India	1975	gamma rays	Christian Dior	flower colour	26
		Qingchunshihuo	China	1989	gamma rays	Yan (branch)	leaf morphology	n.i.
		Saroda	India	1983	gamma rays	Queen Elizabeth	flower colour	23
		September Wedding	Canada	1964	radiation	Montezuma	flower colour	14
		Sharada	India	1983	gamma rays	Queen Elizabeth	flower colour	42
		Shouhong	China	1984	gamma rays	Mohong x Heping (b & s)	flower colour	n.i.
		Striped Christian Di	India	1975	gamma rays	Christian Dior	flower colour	26
		Striped Contempo	India	1983	gamma rays	Contempo	flower colour	37
		Sukumari	India	1983	gamma rays	America's Junior Miss	flower colour	23
		Tangerine Contempo	India	1983	gamma rays	Contempo	flower colour	23
		Twinkle	India	1986	gamma rays	Imperator	flower colour	31
		Xiaguangwandao	China	1984	gamma rays	Lushimei (branch)	flower colour	31
		Xinchao	China	1990	gamma rays	Yidenjing x Yitongji (b & s)	flower colour	n.i.
		Yanhong	China	1984	gamma rays	Mohong x Huancai (b & s)	flower duration	n.i.
		Yellow Contempo	India	1983	gamma rays	Contempo	flower colour	23
		Zhaoyang	China	1984	gamma rays	Yanyangtian x Dajiangzhang (b & s)	flower colour	n.i.
		Zhengzhondajiangzhang	China	1986	gamma rays	Dajiangzhang (branch)	flower colour	n.i.
		Zhengzhouchunse	China	1989	gamma rays	Yalishanda (branch)	flower colour	n.i.
		Zhenjie	China	1984	gamma rays	Xinyong (branch)	flower colour	31
Rubus idaeus	raspberry	Kolokolchik	USSR	1991	ENH	Karnaval (seeds)	disease resistance	41

Saccharum officinarum	sugarcane	Co 6608	India	1966	gamma rays	Co 449	red rot resistance	12
		Co 8153	India	1981	gamma rays	Co 6304 x Co 6806	juice quality	30
		Co 85017	India	1985	gamma rays	Co 740	adaptability	31
		Co 85035	India	1985	gamma rays	Co 740	earliness	32
		Co 997 mutant	India	1967	gamma rays	Co 997	red rot resistance	12
		Guifu 80-29	China	1989	gamma rays	Guitang 72-28	earliness	n.i.
		Nanei	Japan	1981	gamma rays	Ni 1	stalk size	19
		Yuetangfu 83-5	China	1992	gamma rays +	Yuetang 71-210	sugar content	n.i.
Saintpaulia sp.	african violet	Halley	Netherlands	1985	gamma rays	Superba	flower colour	31
Secale cereale	rye	Donar	GDR	1981	PMS	Petkuser Winterroggen Stamm 267/70	shortness	23
		Hankkija's Jussi	Finland	1975	gamma rays	Vjatka	winter hardiness	7
		HJA 6902	Finland	1981	gamma rays	Vjatka	lodging resistance	35
		Pollux	GDR	1981	PMS	Petkuser Winterroggen Stamm 267/70	shortness	23
Sesamum indicum	sesame	Ahnsankkae	Korea	1985	x-rays	Early Russian	disease resistance	29
		ANK-S2	Sri Lanka	1995	gamma rays	MI-1	disease resistance	43
		Babil	Iraq	1992	gamma rays	local variety	earliness	43
		Cairo White 8	Egypt	1992	gamma rays	Giza 24	non-branching	42
		Eshtar	Iraq	1992	gamma rays	local variety	capsule size	43
		Kalika (BM 3-7)	India	1980	EMS	Binayak	semi-dwarfness	17
		Ningya 10	China	1982	gamma rays	Yanza 10	earliness	32
		Pungsankkae	Korea	1996	cross		determinate	43
		Rafiden	Iraq	1992	gamma rays	local variety	earliness	43
		Seodunkkae	Korea	1997	NaN3	Danbaeckkae	disease resistance	44
		Sinai White 48	Egypt	1992	gamma rays	Giza 24	seed colour	42
		Suwon 155	Korea	1998	gamma rays		oil quality	44
		Suwonkkae	Korea	1992	cross		protein content	42
		UMA	India	1990	chemical	Kanak	uniform maturity	43
		USHA	India	1990	chemical	Kanak	yield	43
		Yangbaeckkae	Korea	1995	NaN3	Danbaeckkae	oil quality	42
Setaria italica	foxtail millet	Lugu 7	China	1987	gamma rays	Lugu 2	shortness	33
Setaria sp.	millet	Angu 221	China	1978	gamma rays	Ange 4	earliness	27
		Changwei 74	China	1974	gamma rays	Shuilihun	glutinous	29

		Changwei 75	China	1975	gamma rays	Changwei 69	blast resistance	29
		Chigu 4	China	1987	fN	Shaogu 1	grain quality	n.i.
		Fugu 3	China	1989	gamma rays	Honggu	yield	n.i.
		Fugu 4	China	1992	gamma rays	Honggu	yield	n.i.
		Fugu 6	China	1999	gamma rays	Fugu 3	lodging resistance	n.i.
		Jingu 15	China	1981	gamma rays		earliness	n.i.
		Jingu 21	China	1991	gamma rays		lodging resistance	n.i.
		Longgu 27	China	1988	fN	Lanfan 1	panicle size	n.i.
		Longgu 28	China	1989	fN	Yuan 12n.i.	drought tolerance	n.i.
		Longgu 29	China	1992	fN	Ji 12n.i.	lodging resistance	n.i.
		Lugu 2	China	1991	gamma rays	Jinfeng 69	yield	n.i.
		Nunxuan 11	China	1985	cross		drought tolerance	n.i.
		Nunxuan 12	China	1986	fN	Xiaoyijiu	drought tolerance	n.i.
		Nunxuan 14	China	1992	gamma rays	hybrid	lodging resistance	n.i.
		Yugu 6	China	1995	cross		disease resistance	n.i.
		Zhangnong 10	China	1966	gamma rays	Hongshizhu	grain morphology	27
		Zhangnong 11	China	1966	gamma rays	Hongshizhu	logging resistance	27
		Zhufu 1	China	1974	gamma rays	Moligu	adaptability	27
Sinapis alba	mustard	RLM 198	India	1975	radiation	RL 18	oil content	7
		Seco	Sweden	1961	cross		yield	6
		Svalof's Primex	Sweden	1950	x-rays	Svalöf's White mustard	yield	*
		Trico	Sweden	1967	x-rays		yield	6
		Zlata	Czech Rep.	1996	x-rays	Prerovska Bila	earliness	43
Solanum khasianum	khasianum	RRL-20-2	India	1975	gamma rays	Dehradun local	solasodine	13
Solanum melongena	eggplant	Floralba	Italy	1985	EMS	Florida Market	shortness	32
		Macla	Italy	1983	EMS	Florida Market	shortness	32
		Picentia	Italy	1983	EMS	Lunga Violetta	shortness	32
		PKM 1	India	1985	gamma rays	Puzhuthikathiri	yield	32
Solanum tuberosum	potato	Desital	Italy	1987	gamma rays	Desirée	skin colour	31
		Konkei No.n.i.	Japan	1973	x-rays		skin colour	*
		Mariline 2	Belgium	1968	x-rays	Mariline	yield	*
		Sarme	Estonia	1993	cross		lateness	43
Sorghum bicolor	sorghum	Co 21	India	1977	x-rays	CSV-5	yield	29

		Djeman	Mali	1998	gamma rays	CSM 228	grain colour	44
		Djemanin	Mali	1998	gamma rays	CSM 228	grain colour	44
		Donetskaya 5	USSR	1984	DMS	Krupnosemyannaya 3	shortness	31
		Fambe	Mali	1992	gamma rays	CSM 388	lodging resistance	44
		Gnome	Mali	1998	gamma rays	IPS 0001	lodging resistance	44
		Gnoumanin	Mali	1998	gamma rays	CSM 228	grain colour	44
		Jinfu 1	China	1970	gamma rays	Jingza 5	grain quality	27
		Jinza 1	China	1973	cross		lodging resistance	25
		Longfuliang 1	China	1979	gamma rays	Xinliang 7	earliness	25
		Sadje	Mali	1998	gamma rays	Isunikakiyan	earliness	44
		Sofin	Mali	1998	gamma rays	CSM 388	earliness	44
		Tiedjan	Mali	1998	gamma rays	CSM 228	panicle size	44
Sorghum durra	durra	Volzhskoye 4	USSR	1989	MNH		shortness	40
Sorghum sudanense	sudan grass	Mironovskaya 8	USSR	1990	cross		earliness	41
Spinacia oleracea	spinach	Lavewa	FRG	1987	EMS	Früh-Remona	nitrate content	37
Stenotaphrum	st. Augustine grass	TXSA 8202	USA	1985	gamma rays	Floratam	disease resistance	31
		TXSA 8212	USA	1985	gamma rays	Floratam	disease resistance	31
Streptocarpus sp.	streptocarpus	Albatros	Netherlands	1973	colchicine	mutant 7111 of Maerssen's White	flower morphology	17
		Aurora	FRG	1979	x-rays	Neptun rosa = Carmen	flower colour	37
		Blue Nymph	Netherlands	1969	x-rays	Constant Nymph	flower colour	*
		Blue Windor	FRG	1986	x-rays	Margaret	flower colour	31
		Burgund	FRG	1978	x-rays	Juwel	flower colour	14
		Cobalt Nymph	Netherlands	1969	x-rays,	Constant Nymph	plant architecture	*
		Dark Windor	FRG	1987	x-rays	Margaret	flower colour	31
		Dolly	FRG	1979	x-rays	Neptun blau = Cupido	plant architecture	37
		Freya	FRG	1979	x-rays	Neptun rosa = Carmen	flower colour	37
		Gloria Rot	FRG	1978	x-rays	Gloria rosa	flower colour	14
		Helle Glocke	FRG	1979	x-rays	Nadja	flower colour	37
		Jewel	FRG	1978	x-rays	Laura	flower colour	14
		Kefora	FRG	1977	x-rays	Constant Nymph	plant architecture	10
		Margaret	UK	1974	x-rays	Constant Nymph	earliness	17
		Mini Nymph	Netherlands	1969	x-rays	Constant Nymph	plant architecture	*
		Minidor	FRG	1987	x-rays	Mini Nymph	flower colour	31

		Mutara	FRG	1977	x-rays	Constant Nymph	plant architecture	10
		Nanna	FRG	1979	x-rays	Neptun blau = Cupido	compact growth	37
		Neptun Rosa	FRG	1978	x-rays	Neptun	flower colour	14
		Netta Nymph	Netherlands	1969	x-rays	Constant Nymph	flower colour	*
		Nicky	FRG	1979	x-rays	Neptun	flower colour	16
		Purple Nymph	Netherlands	1969	x-rays,	Constant Nymph	flower	5
		Rosalie	FRG	1979	x-rays	Juwel	flower colour	37
		Rosalinda	FRG	1978	x-rays	Juwel	flower colour	14
		Selene	FRG	1979	x-rays	Hera	flower colour	37
		Snow-white	Netherlands	1973	x-rays	Maerssen's White	dwarfness	*
		Vando	FRG	1987	x-rays	Cynthia	flower colour	31
		Violetta	FRG	1977	x-rays	Constant Nymph	flower colour	10
		Weisse Glocke	FRG	1978	x-rays	Helle Glocke	flower colour	14
		White Windor	FRG	1985	x-rays	Margaret	flower colour	31
Syringa vulgaris	lilac	Prairie Petite	USA	1995	thN		dwarfness	44
Trifolium alexandrinum	egyptian clover	BL-22	India	1984	gamma rays	Mescavi	lateness	26
Trifolium incarnatum	crimson clover	Cardinal	CSFR					6
Trifolium pratense	red clover	Rotra, R.v.P	Belgium	1967	colchicine		yield	*
Trifolium subterraneum	subterranean clover	Uniwager	Australia	1967	EMS	Geraldton	isoflavons content	*
Triticum aestivum	wheat	092	China	1966	gamma rays	Nanda 2419	earliness	25
		1161	China	1966	gamma rays	Nanda 2419	cold tolerance	25
		352	China	1983	laser	470	earliness	27
		503	China	1975	gamma rays	Jiulan	tillering type	27
		62-10	China	1985	fN	Abbodanza	rust resistance	30
		62-8	China	1985	fN	Abbodanza	rust resistance	30
		77 L15	China	1983	laser	F1 (Zhengyin 1 x Shangjian)	stiffness	27
		78 A	China	1986	gamma rays	-	grain quality	n.i.
		Albidum 12	USSR	1984	gamma rays	Triticum-Agropyron hybrid 870	cold tolerance	31
		Altimir 67	Bulgaria	1979	gamma rays	Skorospelka x Mexipak	disease resistance	16
		Bakhtawar-92	Pakistan	1994	gamma rays		disease resistance	44
		Bel'chanka 5	USSR	1992	cross		lodging resistance	40
		Birlik	USSR	1989	cross		lodging resistance	40
		BR4	Brazil	1979	cross		yield	26

Carolina	Chile	1981	gamma rays	Collafen	yield	19
Changwei 19	China	1978	gamma rays	Maoyinafu	disease resistance	25
Changwei 20	China	1978	gamma rays	Maoyinafu	disease resistance	25
Changwei 51503	China	1983	gamma rays	Xiangyang 1 x Heimangmai	tillering type	27
Chuanfu 1	China	1982	beta rays	Chuanyu 5	earliness	27
Chuanfu 2	China	1989	gamma rays	F1 (Chuanfu 1 x 78-2882)	disease resistance	37
Chuanfu 3	China	1989	gamma rays	F1 (Bamai 18 x 79P-600)	disease resistance	37
Chuanfu 4	China	1993	gamma rays	(Chuanfu 1x 78-2882)	yield	n.i.
Claudia (=Mv 8)	Italy	1979	cross			16
Darkhan-35	Mongolia	1992	cross		protein content	44
Darkhan-49	Mongolia	1995	cross		yield	44
Deda	USSR	1983	MNH	Motchynave	earliness	31
Dnestryanka	USSR	1989	cross		shortness	40
Els	FRG	1960	x-rays	Erli x Lichti früh x Triticum	shortness	9
Emai 6	China	1966	gamma rays	Nanda 2419	rust resistance	25
Emai 9	China	1980	gamma rays	selected line from Emai 6	Gibberella	27
Eritrospermum 103	USSR	1982	gamma rays,	Lutestsens 62	earliness	40
Fuer	China	1977	gamma rays	Keshibaipi x 774 Strain	rust resistance	27
Fuou 1	China	1974	gamma rays	Ourou	rust resistance	n.i.
Fusheabo 1	China	1987	fN	Abo	rust resistance	37
Ganchun 20	China	1998	gamma rays	hybrid	grain quality	n.i.
Guifu 12	China	1986	cross		rust resistance	n.i.
Hankkijas Taava	Finland	1978	gamma rays	Ruso	yield	13
Heichun 2	China	1979	cross		earliness	27
Henong 1	China	1985	gamma rays	Yangmai 1	yield	30
Hezu 8	China	1992	gamma rays	Zhefu 908 (immature embryo)	yield	41
Humai 3	China	1978	gamma rays	Yangmai 1	earliness	27
IAS 63	Brazil	1974	cross		yield	19
Inna	USSR	1991	cross		lodging resistance	40
Intesar	Iraq	1992	gamma rays	Saber Beg	yield	43
Iratom	Iraq	1992	gamma rays	Saber Beg	yield	43
Jauhar-78	Pakistan	1979	fN	Nayab	yield	18
Jiaxuan 1	China	1974	gamma rays	Maoyingafu	salt tolerance	27

Jienmai 2	China	1970	gamma rays	Beijing 6	earliness	25
Jihe 02	China	1993	cross		drought tolerance	n.i.
Jimai 28	China	1988	gamma rays	Fanxiu 4	cold tolerance	n.i.
Jingfen 1	China	1976	gamma rays	Shijiazhuang 63	earliness	25
Jingmai 34	China	1990	gamma rays		drought tolerance	n.i.
Jingmai 35	China	1991	gamma rays	K239 x 5084	shortness	n.i.
Jinmai 22	China	1982	cross		earliness	35
Jinmai 23	China	1980	gamma rays	(Fengchan 2 x Bima 4) x Nanda 2419	earliness	n.i.
Kazanskaya 84	USSR	1992	MNH	[Velut.97xAlbid.114]	winter hardiness	40
Kexing 15	China	1972	gamma rays	landrace	rust resistance	27
Khara-86	Mongolia	1986	gamma rays	Orkhon	earliness	44
Kharkovskaya 90	USSR	1991	cross		lodging resistance	40
Khersonskaya 86	USSR	1991	cross		lodging resistance	40
Kiran-95	Pakistan	1996	cross		yield	n.i.
Kiyanka	USSR	1981	dES	Mironovskaja jubilee	yield	25
Kormovaya 30	USSR	1983	NMH	Belotcherkovskaya	silage quality	31
Lewis	USA	1964	thN	Mo. W6185	stiffness	*
Ljubov	USSR	1985	laser	Leningradka	yield	35
Longfumai 1	China	1984	fN	(Xinshuguang 1 x Liaochun 8)	earliness	30
Longfumai 2	China	1986	gamma rays	(Nongxi 35 x Ke 250)	earliness	32
Longfumai 3	China	1987	gamma rays	(Nongfu 77-4096 x S-A-25)	earliness	32
Longfumai 4	China	1988	gamma rays	(Heizia 266 x Ke 79F3-392)	earliness	n.i.
Longfumai 5	China	1992	beta rays	Jiusan B 29-4	earliness	41
Longfumai 6	China	1994	gamma rays	hybrid	disease resistance	n.i.
Longfumai 7	China	1996	gamma rays	K202 (young spike)	grain quality	n.i.
Longfumai 9	China	1999	gamma rays	Kejian 23	grain quality	n.i.
Lumai 11	China	1988	cross		drought tolerance	n.i.
Lumai 16	China	1990	laser	(Gao 8 x Yanda 72-629)	lodging resistance	n.i.
Lumai 20	China	1993	gamma rays	321E (pollen)	earliness	n.i.
Lumai 4	China	1983	laser	70-4-92-1	earliness	32
Lumai 5	China	1984	cross		shortness	32
Lumai 6	China	1984	laser	70-4-92-1	earliness	32
Lumai 8	China	1985	cross		yield	32

Luten 1	China	1968	gamma rays	Huixianhong	semi-dwarfness	25
Lutestsens 7	USSR	1991	cross		seed retention	40
Meshenskaya	USSR	1989	MNH	[Chern.xMiron.Yubil.]	winter hardiness	40
Moskovskaya 70	USSR	1991	cross		lodging resistance	40
Moskovskaya nizkosteb.	USSR	1990	cross		lodging resistance	40
Motsinave 100	USSR	1980	gamma rays			37
Mriya Khersona	USSR	1989	cross		lodging resistance	40
Mv 8	Hungary	1978	cross		shortness	16
Nanjing 3	China	1976	gamma rays	St 1472/506	shortness	19
Nanyang 75-6	China	1979	gamma rays ,	F2 (St 2422/n.i.4 x Neixiang 5)	uniformity	25
Nechinovskaya 86	USSR	1991	cross		lodging resistance	40
Neimai 5	China	1979	gamma rays	[(Ourou x Liaochun 1) x Ruluo]	earliness	27
Nemchinovskaya 52	USSR	1990	cross		lodging resistance	40
NI-5643	India	1975	radiation	[New Thatch x NI-284-S]	earliness	19
Ningmai 3	China	1973	gamma rays	St1472/506	shortness	25
Nishte-95	Pakistan	1995	gamma rays			44
Novosibirskaya 67	USSR	1969	gamma rays	Novosibirskaja 7	stiffness	*
NP 836	India	1961	x-rays	NP 799	awned	*
Odesskaja 75	USSR	1975	cross		shortness	14
Odesskaja Polukarlik	USSR	1975	cross		semi-dwarfness	14
Omskaya ozimaya	USSR	1989	EI		winter hardiness	40
Payne	USA	1981	cross		disease resistance	19
Pitikul	USSR	1982	cross		lodging resistance	40
Polukarlik 3	USSR	1985	cross		lodging resistance	40
Polukarlikovaja-49	USSR	1979	cross		shortness	13
Progress	USSR	1984	cross		lodging resistance	40
Pusa Lerma	India	1971	gamma rays	Lerma rojo 64-A	grain colour	*
Qicheng 115	China	1985	gamma rays	F1 (Qifu 04 x Yaan 74-550)	stiffness	32
Qichun 1	China	1971	cross		drought tolerance	27
Qinchun 415	China	1993	gamma rays	Abuo	stress tolerance	n.i.
Qinghai 570	China	1996	gamma rays	hybrid	grain quality	n.i.
Qinmai 6	China	1983	laser	F1 (Zhengying 1 x Shanqian)	stiffness	n.i.
Qunzhong 42	China	1968	gamma rays	Nannoundaheimang	earliness	25

Rabia	Iraq	1994	gamma rays	F3 (Saber Beg x HD)	yield	43
Sali	Iraq	1994	gamma rays	F3 (Saber Beg x Lachis)	yield	43
Schedraj Polesja	USSR	1987	MNH	Poleskaya 70	yield	31
SGT 17	USSR	1980	gamma rays			37
Shannongfu 63	China	1980	gamma rays	F4 (Youbao x Ourou)	earliness	19
Sharbati Sonora	India	1967	gamma rays,	Sonora 64	grain colour	6
Shirowase komugi	Japan	1977	gamma rays	Shirogane komugi	plant type	21
Sibirskaya niva	USSR	1992	EI	PPG-186	winter hardiness	40
Sinvalocho Gama	Argentina	1962	gamma rays	Sinvalocho	rust resistance	*
Sirius	FRG	1969	cross		stiffness	9
Skifyanka	USSR	1992	chemical	sel. from Spartanka	lodging resistance	40
Soghat 90	Pakistan	1991	NaN3	Pavon	disease resistance	42
Spartanka	USSR	1988	cross		lodging resistance	40
Spinnaker	Italy	1987	fN	Anza	lodging resistance	37
Stadler	USA	1964	thN	Mo. W6243	earliness	*
Taifu 1	China	1966	gamma rays	Nounda 183	earliness	25
Taifu 10	China	1968	gamma rays	F2 (Nongda 183 x Neixiang 5)	drought tolerance	27
Taifu 15	China	1968	gamma rays	Nongda 183	earliness	27
Taifu 22	China	1968	gamma rays	F2 (Nongda 183 x Neixiang 5)	tillering type	27
Taifu 23	China	1968	gamma rays	F2 (Nounda 183 x Neixiang 5)	drought tolerance	25
Tambo	Switzerland	1985	gamma rays	(Probus x Bankuti)xHoeser 52	shortness	30
Tammuz-2	Iraq	1992	fN	F2 (Mexipak x Saber Beg)	yield	43
Tammuz-3	Iraq	1992	fN	F2 [Saber Beg x (Mexipak x Abughjraib 3)]	yield	43
Tatara	Pakistan	1996	gamma rays		drought tolerance	44
Wanmai 32	China	1997	ion beams	Yangmai 158	plant type	n.i.
Wanyuan 28-88	China	1979	gamma rays	F2 (St2422/n.i.4 x Neixiang 5)	shortness	25
Wanyuan 75-6	China	1979	gamma rays,	F2 (St2422/n.i.4 x Neixiang 5)	earliness	27
Wei 9133	China	1993	fN	70-4-92-1	lodging resistance	n.i.
Weifu 6757	China	1986	gamma rays	F1 (Taishan 1 x Shanqianmai)	rust resistance	32
Wuchun 3	China	1973	cross		drought tolerance	27
Xiaoyan 6	China	1979	laser	St 2422/n.i.4 x Xiaoyan 96	rust resistance	27
Xifu 3	China	1977	gamma rays	NP 824	disease resistance	27
Xifu 4	China	1980	cross		drought tolerance	37

Xifu 5	China	1985	cross		yield	37
Xifu 6	China	1989	fN	Xifu 4	earliness	n.i.
Xifu 7	China	1989	gamma rays	Xifu 4	earliness	n.i.
Xifu 8	China	1991	gamma rays	(Afunuoer x Fan 7)	spike size	n.i.
Xinchun 2	China	1984	gamma rays	(Siete Cerros x Qichun 4)	earliness	32
Xinchun 3	China	1986	gamma rays	(Siete Cerros x Qichun 4)	yield	n.i.
Xingchun 6	China	1993	cross		yield	n.i.
Xingchun 7	China	1997	cross		yield	n.i.
Xingdong 19	China	1995	gamma rays	hybrid	disease resistance	n.i.
Xinongmai 2	China	1993	gamma rays	77-2882	earliness	44
Xinshukuang 1	China	1971	gamma rays	F3 (Abo M4 x Ourou)	disease resistance	25
Yanfuzao	China	1984	gamma rays ,	Yekaola	earliness	n.i.
Yangmai 158	China	1993	gamma rays	hybrid	yield	n.i.
Yannoun 685	China	1974	cross		rust resistance	25
Yuanchun 7112	China	1975	cross		yield	18
Yuandon 2	China	1982	gamma rays	[12040 x Afunuoer]	earliness	27
Yuandong 1	China	1979	gamma rays	[Zaoyang x Dongfenhong 3]	earliness	25
Yuandong 3	China	1989	gamma rays	hybrid	rust resistance	30
Yuandong 772	China	1977	gamma rays	[11141 x 12040]	yield	18
Yuandong 7848	China	1978	gamma rays	[12040 x Aurora]	yield	18
Yuandong 94	China	1984	gamma rays	[12040 x Ourou]	earliness	30
Yuanfeng 1	China	1968	gamma rays	Bima 4	cold tolerance	25
Yuanfeng 2	China	1969	gamma rays	Bima 4	cold tolerance	25
Yuanfeng 3	China	1972	gamma rays	Afu	cold tolerance	25
Yuanfeng 4	China	1978	gamma rays	Taishan 1	shortness	25
Yuanfeng 5	China	1985	gamma rays	[(Nuofulin 13 x Youbao 57) x Xiayingsu]	earliness	37
Yuangnong 53	China	1971	gamma rays	F3 (Yuangnong 39 x Ourou)	stiffness	18
Yuangnong 61	China	1971	gamma rays	F3 (Yuangnong 39 x Ourou)	yield	18
Yuanyuan 18-37	China	1987	gamma rays ,	F1 (St2422/n.i.4/Neixiang 5)	yield	n.i.
Yubileinaya 75	USSR	1992	cross		seed size	40
Yumai 12	China	1988	gamma rays	Bounong 7023	earliness	n.i.
Yumai 4	China	1984	gamma rays	Afu strain	earliness	n.i.
Yumai 43	China	1996	gamma rays	hybrid	disease resistance	n.i.

		Yunfu 2	China	1982	cross		earliness	27
		Yunfuzao	China	1980	gamma rays	[(Fengchen 2 x Bima 4) x Nanda 2419]	earliness	25
		Yunnat odesskii	USSR	1989	cross		lodging resistance	40
		Yuyuan 1	China	1979	gamma rays	F2 (St2422/n.i.4 x Neixiang 5)	earliness	25
		Zenkouzikomugi	Japan	1969	gamma rays	Igachikugo-Oregon	earliness	*
		Zhangchun 10	China	1987	cross		lodging resistance	n.i.
		Zhangchun 12	China	1990	gamma rays	Gamma 47-3-1	earliness	n.i.
		Zhangchun 13	China	1991	cross		shortness	n.i.
		Zhangchun 14	China	1991	cross		earliness	n.i.
		Zhangchun 17	China	1998	gamma rays	hybrid	earliness	n.i.
		Zhangchun 18	China	1998	gamma rays	hybrid	drought tolerance	n.i.
		Zhemai 3	China	1983	laser	E-70	earliness	32
		Zhemai 4	China	1989	laser	[1-3-2 x 9-14-3-1]	spike number	n.i.
		Zhemai 5	China	1991	gamma rays	[Zheng 7495 x Anhui 11]	earliness	n.i.
		Zhengliufu	China	1976	gamma rays	Zhengzhou 6	drought tolerance	25
		Zhonga 1	China	1969	gamma rays	Afu	cold tolerance	27
		Zhonghong 1	China	1977	fN	Hongmang	grain quality	n.i.
		Zlatostrui	Bulgaria	1985	gamma rays	F2 (Mexican 225 x Sadovo 1)	yield	32
T. turgidum ssp. *durum*	durum	Arpad	Austria	1987	cross		shortness	30
		Attila	Austria	1980	cross		shortness	16
		Augusto	Italy	1976	cross		yield	10
		Cargidurox	France	1981	EMS	K6800707	shortness	21
		Castel del Monte	Italy	1969	fN	Grifoni	stiffness	*
		Castelfusano	Italy	1968	thN	Capelli	stiffness	*
		Castelnuovo	Italy	1971	x-rays	Carigliano	stiffness	*
		Castelporziano	Italy	1968	thN	Capelli	stiffness	*
		Creso	Italy	1974	cross		stiffness	6
		Febo	Italy	1982	cross		yield	37
		G-0367	Greece	1970	thN	YG-3688	shortness	16
		Gergana	Bulgaria	1984	gamma rays		lodging resistance	37
		Giano	Italy	1982	cross		yield	37
		Grandur	Austria	1980	cross		shortness	16
		Icaro	Italy	1987	fN	Anhinga	shortness	35

		Lozen 76	Bulgaria	1982	cross		yield	20
		Mida	Italy	1974	cross		stiffness	6
		Peleo	Italy	1988	cross		shortness	37
		Probstdorfer Miradur	Austria	1978	cross		yield	13
		Signadur	Austria	1984	cross		shortness	26
		Sredetz	Bulgaria	1988	cross		yield	33
		Tito	Italy	1975	cross		stiffness	6
		Ulisse	Italy	1988	cross		shortness	37
		Unidur	Austria	1984	cross		stiffness	29
		Zeveryana	Bulgaria	1986	cross		shortness	33
Tulipa sp.	tulip	Den' Pobedy	Russia	1993	chemical	London	decorative flower	41
		Dominique	Netherlands	1985	x-rays	Lustige Witwe	flower colour	31
		Estella Rijnveld	Netherlands	1954	x-rays	Red Champion	flower colour	17
		Faraday	Netherlands	1949	x-rays	Fantasy	flower colour	17
		Ivette	Netherlands	1985	x-rays	Lustige Witwe	flower colour	31
		Orange Charles	Netherlands	1985	x-rays	Charles	flower colour	31
		Rimo	Netherlands	1985	x-rays	Lustige Witwe	flower colour	37
		Santina	Netherlands	1985	x-rays	Lustige Witwe	leaf morphology	37
		Yvonne	Netherlands	1985	x-rays	Lustige Witwe	flower colour	31
Vicia faba	faba bean	Babylon	Iraq	1994	gamma rays	Ekwadelgii	disease resistance	43
		Bronto	Poland	1989	gamma rays	Nadwislanski	yield	37
		Chabanskye	USSR	1985	cross		earliness	31
		Dino	Poland	1987	gamma rays	Nadwislanski	shortness	31
		Karna	Austria	1983	gamma rays	Kornberg Kleinkönige	yield	29
		KYU-82	USSR	1987	chemical	line 2193 (Germany)	disease resistance	31
		Martin	Poland	1994	cross		earliness	43
		Prikarpatskie 4	USSR	1986	ENH,MNH,D	[Prikarpatskie 2]	yield	40
		Severinovskie 1	USSR	1992	MNH	[KYU-82 x Fribo]	protein content	40
		Stego	Poland	1987	gamma rays	Nadwislanski	shortness	31
		Tinos	Poland	1992	cross		determinate	41
		Ti-Nova	GDR	1986	cross		terminal	30
		Tuwaitha	Iraq	1994	gamma rays	local variety	disease resistance	43
Vicia sativa	vetch	Nechinovskaya 84	USSR	1989	DES	VIR K-33583	leaf size	40

		Nikian	Italy		EMS	Mirabella	branching	43
		Toplesa	CSFR	1995	cross		vigour	43
Vigna angularis	azuki bean	Beni-nambu	Japan	1978	gamma rays	Mombetsu 26	earliness	21
Vigna mungo	black gram	Binamash-1	Bangladesh	1994	gamma rays	BINA Acc.B-10	disease resistance	43
		Co 4	India	1978	MMS	Co 1	earliness	29
		TAU 1	India	1985	cross		yield	28
		TPU-4	India	1992	cross		grain weight	42
Vigna radiata	mungbean	Binamoog-2	Bangladesh	1994	cross		seed size	43
		Binamoog-3	Bangladesh	1997	gamma rays		yield	n.i.
		Binamoog-4	Bangladesh	1997	gamma rays		yield	n.i.
		Binamoog-5	Bangladesh	1998	gamma rays		yield	n.i.
		Camar	Indonesia	1987	gamma rays	Manyar	Cercospora	42
		Co 4	India	1982	gamma rays	Co-1	yield	29
		ML 26-10-3	India	1983	gamma rays	ML-26	YMV resistance	33
		MUM-2	India	1992	EMS	K-851	yield	43
		NIAB Mung 121-25	Pakistan	1985	gamma rays	RC 71-27	earliness	30
		NIAB Mung 13-1	Pakistan	1986	gamma rays	6601	earliness	29
		NIAB Mung 19-19	Pakistan	1985	gamma rays	Pak 22	earliness	30
		NIAB Mung 20-21	Pakistan	1986	gamma rays	Pak 22	earliness	29
		NIAB Mung 51	Pakistan	1990	gamma rays	[6601x1973A]	earliness	42
		NIAB Mung 54	Pakistan	1990	gamma rays	[6601x1973A]	earliness	42
		NIAB Mung 92	Pakistan		cross		disease resistance	44
		NIAB Mung 98	Pakistan		cross		seed size	44
		NIAB Mung-28	Pakistan	1983	gamma rays	Pak 17	earliness	23
		Pant Moong 2	India	1982	gamma rays	ML-26	virus resistance	23
		TAP-7	India	1982	gamma rays	S-8	earliness	23
Vigna unguiculata	cowpea	Co 5	India	1986	gamma rays	Co-1	nutritional	29
		Cowpea-88	India	1990	radiation		yield	37
		ICV 11	Kenya	1985	gamma rays	ICV 1	semi-erect type	28
		ICV 12	Kenya	1985	gamma rays	ICV 1	yield	28
		Uneca-Gama	Costa Rica	1986	gamma rays	Centa	yield	34
		V16 (Amba)	India	1981	DMS	Pusa Phalguni	yield	25
		V240	India	1984	DMS	Pusa Phalguni	yield	25

		V37 (Shreshtha)	India	1981	DMS	Pusa Phalguni	yield	25
		V38 (Swarna)	India	1984	DMS	Pusa Phalguni	yield	25
Vitis vinifera	grape	Fikreti	USSR	1986	gamma rays	Marandi	earliness	32
Weigela	weigela	Couleur d'Automne Co	France	1979	gamma rays	La Printemps	variegated leaves	25
		Courtadur	France	1980	gamma rays	Bristol Ruby	compact growth	31
		Rubivif Courtavif	France	1980	gamma rays	Bristol Ruby	flower colour	25
Zea mays	maize	CE 200	CSFR	1979	gamma rays	synthetic population	yield	17
		CE 268	CSFR	1979	gamma rays	synthetic population	yield	17
		CE 330	CSFR	1979	gamma rays	synthetic population	yield	17
		Changdan 3	China	1985	cross		earliness	n.i.
		Collectivnii 210 ATV	USSR	1984	cross		earliness	30
		De 2205 SC	Hungary	1987	cross		earliness	37
		DT-6	Vietnam	1990	gamma rays,	Tuxpeño	earliness	43
		DT-8	Vietnam	1990	cross		earliness	43
		Guidan 15	China	1991	cross		earliness	n.i.
		Huafeng 100	China	1976	gamma rays	[Hua 160 x Fengke 1]	ear lower on stem	41
		Hybrid ChK 3 -18 TV	USSR	1991	cross		earliness	41
		Hybrid ChKG 280 MV	USSR	1992	cross		disease resistance	40
		Jidan 1	China	1967	cross		blight resistance	27
		Jidan 101	China	1967	cross		root system	25
		Keduo 6	China	1991	cross		yield	n.i.
		KNEJA-510 (hybrid)	Bulgaria	1982	cross		yield	32
		KNEJA-641 (hybrid)	Bulgaria	1982	cross		yield	32
		KNEJA-666 (hybrid)	Bulgaria	1987	cross		silage quality	32
		KNEJA-674	Bulgaria	1989	cross		yield	41
		KNEJA-HP-556(hybrid)	Bulgaria	1981	cross		protein content	32
		KNEJA-HP-633(hybrid)	Bulgaria	1980	cross		protein content	32
		KNEJA-M-712 (hybrid)	Bulgaria	1987	cross		yield	32
		Knezha MHP 556	Bulgaria	1982	cross			37
		Kollectivnyi 210 (hy	USSR	1982	cross		earliness	40
		Kollektivnyi 100 TV h	USSR	1988	cross		earliness	40
		Kollektivnyi 100SV	USSR	1988	cross		earliness	41
		Kollektivnyi 225 MV h	USSR	1990	cross		earliness	40

Kollektivnyi 244 MV h	USSR	1986	cross		earliness	40
Kollektivnyi 95 M h	USSR	1992	cross		earliness	40
Krasnodarskii 303 VK	USSR	1984	cross		lodging resistance	40
Lauyu 5	China	1985	cross		earliness	31
Liaoyangbei	China	1991	gamma rays	population	disease resistance	n.i.
Liaoyuan 1	China	1988	cross		disease resistance	n.i.
Longbaoyu 1	China	1990	cross		yield	n.i.
Longfuyu 1	China	1984	cross		yield	31
Longfuyu 2	China	1987	cross		grain quality	n.i.
Longfuyu 3	China	1992	cross		disease resistance	n.i.
Ludan 50	China	1998	cross		yield	n.i.
Lude 5	China	1991	gamma rays	hybrid	stress tolerance	n.i.
Luyu 12	China	1993	cross		disease resistance	n.i.
Luyu 3	China	1980	cross		disease resistance	25
Luyu 5	China	1987	cross		earliness	33
Luyuan SC 4	China	1976	gamma rays	Wu SC early	yield	19
Luyuan SC 9	China	1987	cross		earliness	33
Luyuandan 1	China	1976	cross		disease resistance	25
Luyuandan 14	China	1997	cross		lodging resistance	n.i.
Luyuandan 16	China	1995	cross		disease resistance	n.i.
Luyuandan 3	China	1976	cross		disease resistance	27
Luyuandan 4	China	1976	cross		earliness	27
Luyuandan 5	China	1993	cross		earliness	n.i.
Luyuandan 7	China	1981	cross		cob size	25
Luyuanshan 2	China	1981	cross		disease resistance	25
Mudan 7	China	1983	cross		earliness	n.i.
Xiangsan 1	China	1980	cross		disease resistance	27
Xinnongfuyu 1	China	1987	cross		vigour	n.i.
Xinongdanjiao 1	China	1991	cross		disease resistance	n.i.
Xinyu 3	China	1986	cross		grain quality	n.i.
Yuan 74-751	China	1974	gamma rays +	Tangszupintou x Ye 2	plant type	18
Yuan 79-171	China	1979	gamma rays	Kung 70 (pollen)	shortness	18
Yuan 79-418	China	1979	fN	(A96 x Daqiu 36 x B 64)	earliness	18

		Yuanlian 5	China	1980	cross		earliness	25
		Yuanqi 123	China	1978	cross		earliness	33
		Yuanqi 722	China	1978	cross		earliness	33
		Yuanwu 02	China	1975	gamma rays	Wudanzao	earliness	41
		Yubileinyi 60 (hybri	USSR	1982	cross		stiffness	40
		Yubileinyi 60 MV h	USSR	1986	cross		earliness	40
		Zhongyuandan 32	China	1997	cross		quality	n.i.
		Zhongyuandan 4	China	1982	cross		earliness	25
Ziziphus mauritiana	indian jujube	Dao tien	Vietnam	1986	MNH	Tien Phien	earliness	34
		Ma hong	Vietnam	1986	MNH		fruit morphology	34

*/ Sigurbjörnsson and Micke, 1974
n.i./ will be published in next issues of MBNL

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STIMULATIVE EFFECTS OF X-RAYS ON PLANT GROWTH[1]

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(WITH FOUR FIGURES)

Introduction

During the period since the discovery of x-rays by RÖNTGEN in 1895, a vast amount of work has been done in which these radiations have been used for clinical diagnosis and therapy. The practical applications of x-rays in medicine and surgery make it necessary to know the effect which x-rays produce upon the living organism. Many investigators have suggested on the basis of general observations that small doses of x-rays may stimulate cellular activity and growth, but convincing proof of such action has been wanting. In more recent years such claims have been discounted in favor of the belief that x-rays are always more or less destructive in action, and tend to retard growth.

It is not the purpose of this preliminary report to survey the literature dealing with the effects of x-ray treatments upon plants. It has been found that every part of the plant body can be profoundly modified by appropriate treatments. Cytological and histological examination of treated cells and tissues reveals striking changes in the organization of the protoplasm and of organs derived from the treated meristems. Most frequently the results described are of a destructive nature. The protoplasm is partially disorganized; chromosomes are vacuolated or fragmented; the cell division mechanism functions imperfectly, showing unequal distribution of chromosomes, non-disjunctions, translocation of pieces of chromosomes from one to some other non-homologous chromosome, etc. Gene changes may be produced, often injurious in character, with resulting lethal effects and tendency to sterility. The results obtained by MCKAY and GOODSPEED (**5**) on cotton are typical. Many mutations have been induced in maize and barley (**7, 8**), and tobacco (**1**), but it has been questioned whether there are any progressive evolutionary changes induced by x-ray treatments.

All vegetative parts are subject to injury by x-rays. Root tips may become bulbous and swollen, with tumor-like enlargements in which giant cells may occur. Stems become fasciated under strong treatments. Leaves are injured readily; they become asymmetric and crumpled in appearance, develop deep sinuosities, and often show irregular development of chlorophyll. The sunflower shows these injuries in typical fashion, the leaves becoming pocked and marked as though they were suffering from a mosaic

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

disease. Even the flowers of plants rayed in seed or seedling stages may show fasciation or various teratological modifications. Some of these have been described for the sunflower and tomato by JOHNSON (**2, 3**).

On the other hand, one can find a dozen or more claims in the literature that x-rays in small doses are stimulative. In some cases increased yields have been claimed for crops grown from x-rayed material. Such claims have been reinvestigated in some cases, and the stimulative effects denied. JOHNSON (**4**), for instance, has not been able to substantiate such claims made for the potato. However, some increase of yield has been reported for x-rayed potatoes at the New Jersey Agricultural Experiment Station. PATTERSON and MULLER (**6**) have found that induced point mutations in *Drosophila* (presumably caused by chemical changes in the genes) may cause increased vigor in some cases. They argue in favor of the possibility of progressive x-ray mutations with endless eventual potentialities.

As a result of our experiences with the use of x-rays on plants it is believed that stimulative effects may be consistently obtained if appropriate conditions are employed. Possibly these stimulative phenomena have not been regularly detected in the past because the intensity of the radiations have been too great, or possibly because the x-ray beam contained too large a proportion of long wave-length radiation. Deleterious effects are consistently obtained in our work when unfiltered radiations are used, and we believe that these harmful effects mask the stimulation that occurs when the beam is properly filtered. Filtration of the radiation, of course, affects the wave-length constitution of an x-ray beam profoundly. It not only reduces the intensity of each wave length throughout the x-ray spectrum, but also changes the relative proportion of the energy supplied by each wave length throughout the spectrum. The shorter radiations suffer much less absorption than the longer radiations; and for practical purposes the longest x-rays are so strongly absorbed by aluminum or copper filters that filtration through such metal plates practically removes them from the beam.

Since filtration affects both the intensity and relative composition of the beam, and since we have not yet differentiated these effects in our work, we are not in position to discuss the nature of the x-ray action. Until further experiments are done we cannot say whether the stimulating effects that are obtained when the beam is filtered are due to the fact that harmful long wave-length rays are removed, or whether they simply indicate that stimulation follows low intensity irradiation, regardless of wave length, and is masked by injury if the intensity is greater, regardless of wave length.

Believing that the dosages in common use for treatment of plants were much too large, we have used very small doses. The intensity of the radia-

tions used is expressed in Röntgen units measured with a Wulf ionometer,[2] the measurements being taken in air without the effect of back-scattering of the beam by solid material. We are indebted to DR. PAUL C. HODGES, Röntgenologist of the University of Chicago, for the calibration of our instrument, and for many helpful suggestions.

In these preliminary experiments we are using about 100 pk. KV., 5 ma., 1-mm. aluminum screen. Under these conditions the instrument delivers about 38 r-units per minute at a point 30 cm. from the target, the distance used in these experiments. Our experimental material is exposed on cellucotton pads in glass dishes resting on a lead-covered table. It undoubtedly received slightly higher doses than were computed in air because of a slight amount of back-scattering of the radiations. But the computation of the dose in air is a standard method of measuring the dosage. In some instances our best results have been obtained with 1 minute or less, a total of 30–40 r-units. In most cases maximum stimulation has been obtained with not more than 2 or 3 minutes; and with 4 or 5 minutes the effect is already one of retardation of growth.

It is evident at once that investigators who have been using from one to ten erythema doses as light doses, are using extremely heavy doses. The erythema dose is a rather rough unit of measurement, and may be defined as that dose of x-rays that just fails to produce a detectable change in the normal human skin. It is at best a vague designation, but is still much used. It seems much better to adopt the more accurate r-unit. It is generally accepted that the physical equivalent of the erythema dose is approximately 600 r. The Holzknecht is also used in expressing x-ray doses, and this is approximately 120 r.

The optimum dosage for different kinds of plants is probably specific, and must be determined by experiment for each species and varietal strain. A number of common plants seem to respond best to dosages between 30 and 120 r.

Methods

In order to make it possible to repeat our procedure, the details of preparation of the seeds for treatment are given. Seeds of such plants as corn, wheat, oats, and sunflower have been used. They are placed for 24 hours in a moist chamber upon a layer of cellucotton saturated with distilled water, and kept at a temperature of about 22° C. The seeds are used without sterilization, and lie in contact with the wet substrate on one side, and in contact with moist atmosphere on the other side. They are not

[2] Small-chamber instruments of this sort are intended primarily for use with higher voltages and are somewhat inaccurate at lower voltages. Eventually the calibration will be checked with large-chamber instruments that are relatively insensitive to voltage change.

submerged during the period of preliminary imbibition and germination. At the end of 24 hours the seeds of all four species show incipient germination. The radicles protrude through the pericarps and enable one to know that the seeds are alive. At this stage the material for treatment and for controls is selected. Twenty or more seeds as nearly at the same stage of germination as possible (estimated by equal length of protruding radicles) are chosen and divided into two lots. One lot is left untreated, the other is placed upon fresh saturated cellucotton and treated at once for 1–5 minutes. Optimum effects are often obtained with 1, 2, or 3 minutes of treatment, according to species. Sunflower seems best at 3 minutes, corn possibly at 2 minutes, and some varieties of wheat at 2 minutes. In some cases wheat gives good results at 30 to 45 seconds or 1 minute. As soon as the raying is completed, controls and treated seeds are both planted in the same type of soil, or in sand culture, or on fresh saturated cellucotton in a moist chamber, depending upon the nature of the experiment. In the case of respiration experiments, controls and treated seeds are placed on a wet substrate in the respirometer immediately after treatment. During treatment the glass covers of the moist chambers or petri dishes are removed so that the only screen is the metallic aluminum screen. In the case of sunflower seeds the pericarps of the fruits are removed before treatment. They are also removed from the controls before planting. We have tried to avoid any differences except that of the treatment itself. Selection of seeds is practiced only to obtain material of uniform physiological activity for the controls and treatments.

Results

Wheat

The first tests with Marquis spring wheat indicated that it is sensitive to small doses of x-rays. The treated plants were decidedly more vigorous than the controls when the period of exposure was from 45 seconds to 1 or 2 minutes. By the time the plants were several weeks old (in soil culture), the treated individuals were taller and of ranker growth. The greatest difference was in the degree of tillering. The untreated plants showed 50 per cent. with one tiller each, while the treated plants showed 100 per cent. with two tillers each. Figure 1 shows the general appearance of the plants on September 17, after several weeks of growth.

Tests with Minhardi and Trumbull wheat gave us the impression at the time that the hardier variety (Minhardi) was less easily influenced by x-rays. The Minhardi wheat in the first tests seemed to show little stimulation, while Trumbull, a moderately hardy variety, showed plainly that its early development was hastened by treatment, but not so much as the Marquis spring wheat. At the present time we are not certain as to the order of

these varieties with reference to degree of stimulation.[3] It is possible that varieties more stable toward cold treatments may also be more stable toward x-ray action. We believe the dosage is specific for each variety, and that a longer treatment may possibly be required by the hardier varieties to produce a given amount of stimulation.

Corn

The most interesting results were obtained with Madison Yellow Dent corn. It was noted that grains which had been treated emerged from the soil more rapidly. On September 22, seeds which had been imbibing water for 24 hours were treated 1–5 minutes, one series screened by aluminum, another treated without metallic screen. A third series, untreated, served as controls. Five days later the seeds treated through the screen showed 84 per cent. of emergence; the unscreened treated seeds showed 72 per cent.;

Fig. 1. Influence of x-rays on growth of wheat: Pot at left rayed 1 minute; at right, 45 seconds. Controls in middle pot. For other conditions see text.

and of the controls only 60 per cent. had emerged. Treated seeds kept in petri dishes always showed a more rapid elongation of coleoptiles than untreated seeds. We have removed such coleoptiles from the seeds at the end of three days and determined the fresh and dry weight of the coleoptiles. Treated seeds showed from 5 to 26 per cent. greater fresh weight than the controls, and from 3 to 16 per cent. greater dry weight. This suggests the possibility that there is a more rapid utilization of the endosperm reserves in seeds that have been treated.

When the treated corn seeds were grown for a few weeks, some very important differences were noted. Figure 2 shows corn grown from seeds treated 1–5 minutes under an aluminum screen. While the growth differences are visible, and somewhat irregular, the main differences in this set are not visible to the eye in the photograph. The plants treated for

[3] Work on these varieties of wheat is being continued by Miss Bessie Zabelin.

TABLE I

INFLUENCE OF X-RAYS ON GROWTH OF CORN

TREATMENT	STEM DIAM.		FRESH WT. ROOTS		DRY WT. ROOTS		FRESH WT. TOPS		DRY WT. TOPS		CHLOROPHYLL* FRESH WT.		CHLOROPHYLL* DRY WT.		
	mm.	%	*gm.*	%	*gm.*	%	*gm.*	%	*gm.*	%	%	*mg./cm.²*	*mg.*	%	%
Control ...	5.63	100.0	54.7	100.0	5.11	100.0	49.7	100.0	4.655	100.0	0.1006	0.0125	5.03	0.874	100.0
1 min.	5.85	103.9	58.8	107.5	4.76	93.1	65.4	131.6	6.03	129.5	0.1341	0.0163	6.70	1.22	139.6
2 min.	6.73	119.5	54.7	100.0	4.79	93.7	86.1	173.2	7.67	164.7	0.1688	0.0219	8.44	1.43	163.6
3 min.	6.15	109.2	54.2	99.0	3.69	72.2	63.9	128.5	5.85	125.6	0.1517	0.0201	7.58	1.34	153.3
4 min.	6.47	114.9	60.6	110.8	4.80	93.9	86.6	174.2	7.30	156.8	0.1573	0.0205	7.86	1.57	179.6
5 min.	5.45	95.2	62.2	113.7	5.66	110.7	65.1	130.9	6.44	138.3	0.1407	0.0178	7.03	1.21	138.4

* Chlorophyll determinations according to GUTHRIE, made by Mr. G. B. ULVIN.

short periods (1–3 minutes) had thicker stems than the controls, or those treated 5 minutes. The treated plants looked and felt slightly more succulent, and were darker green in color. The fresh green weight of the tops was obviously greater in the treated plants than in the controls. Without detailed discussion we present in table I such differences as were measured. The chlorophyll differences need further investigation, as this darker green color was not noticed in the oats, wheat, and sunflowers.

The irregular growth of the 3-minute plants in figure 2 may have been caused by a defect in the instrument which was not discovered and corrected until after several lots of seeds had been treated. In table I the most important data are those on dry weight increase (column 11) and those on chlorophyll increase (column 16).

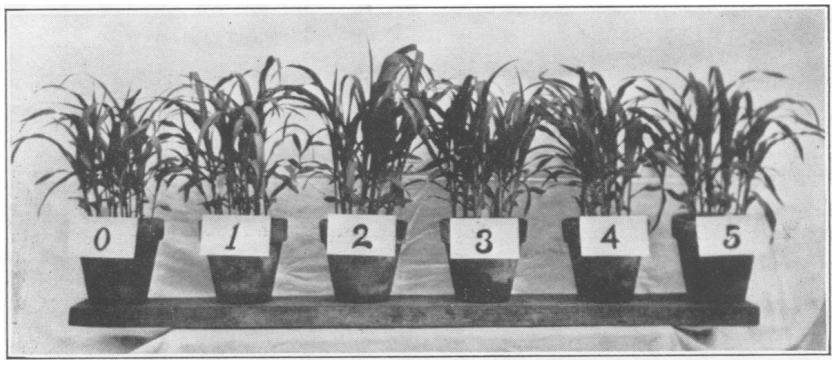

FIG. 2. X-rays and the growth of corn. Control at the left. Time of treatment in minutes indicated on the pots. For other conditions see text.

In table II are presented data on the moisture content of the roots and stems. While the differences are small, they affect roots and tops alike.

TABLE II

WATER CONTENT OF X-RAYED CORN PLANTS

TREATMENT	ROOTS		TOPS	
	DRY WEIGHT	WATER	DRY WEIGHT	WATER
	per cent.	*per cent.*	*per cent.*	*per cent.*
Control	9.34	90.66	8.58	91.42
1 min.	8.09	91.91	8.32	91.57
2 min.	8.75	91.25	8.03	91.97
3 min.	6.78	93.22	8.25	91.75
4 min.	7.92	92.08	7.87	92.13
5 min.	9.14	90.86	9.06	90.94

With light doses, the dry weight percentage decreases and the water content increases. Even these small differences are large enough so that the practiced eye and touch can detect the greater succulence of the plants from seeds treated for 1–3 minutes.

Oats

Only one experiment has been performed with oats. The seeds were from a laboratory sample without name. The increased growth of treated seeds was irregular, as in the case of corn, but plainly visible in all of the treated material. Figure 3 shows the results with plants from seeds

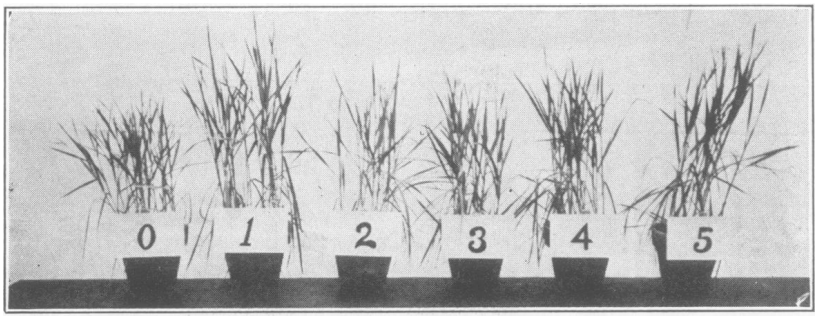

Fig. 3. X-rays and the growth of oats. Control at the left. Time of treatment in minutes indicated on pots. For other conditions see text.

rayed through a 1-mm. aluminum screen at 30 cm. for the periods of time marked on the pots. A defective contact in the machine is believed to have been responsible for the irregular behavior at 2, 3, and 4 minutes, but even these showed increased growth in height and thicker culms than the controls.

Sunflower

The sunflowers were treated after the x-ray machine had been repaired. In figure 4 the controls and treated plants show an excellent curve of height growth. In the photograph the 2-minute and 4-minute plants were omitted. They were perfectly intermediate between 1 and 3 minutes, and 3 and 5 minutes respectively. The 10-minute plants were rayed without the screen. These unscreened plants show the symptoms of burning described by Johnson (**2**). The leaves are asymmetrical, distorted, pocked as if they had mosaic, and the plants are greatly stunted. The screened plants show none of these ill effects; leaves are normal in every way, and growth more rapid. The group of plants rayed 3 minutes blossomed first, indicating a slight shortening of life history by the treatment.

Some attention has been given to the carbohydrate metabolism and respiration of treated seeds. Under the methods we are using, a slightly more rapid liberation of sugar is detectable from the reserves of corn, and a

slightly more rapid respiration of rayed seedlings. The increases are not very striking, and we feel that the data are too meager to be published at present. It seems hardly possible that the increased rate of emergence of seedlings, increased rate of growth, etc., could take place without some increase in respiration rate. This may be controlled in part by the concentration of sugar in the protoplasmic environment. The first tests on diastatic activity, however, showed distinct depression of the enzyme by x-ray treatment. Much more extensive tests must be made on sugar concentration, respiration, and enzyme activity with material more favorable than corn for this purpose.

Conclusion

From the results obtained in these preliminary experiments it is concluded that if the x-rays are properly filtered to decrease the intensity of the beam, or to decrease the proportion of the longer radiations, and if the quantity of energy used is adjusted to the specific requirements of the

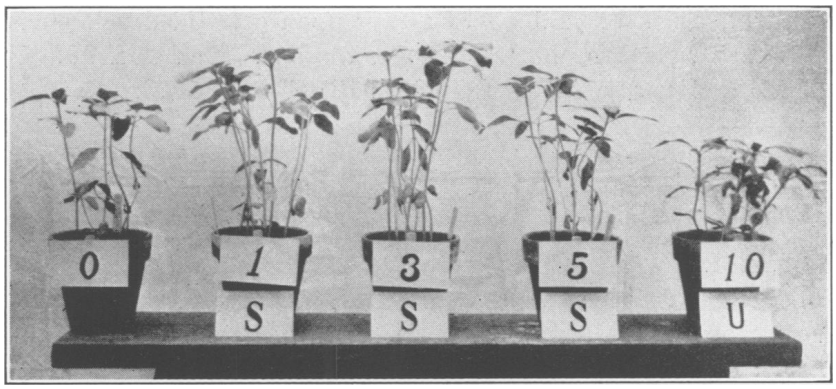

FIG. 4. X-rays and growth of sunflowers. Control at left. Time of treatment in minutes indicated on pots. Plants at right unscreened. For other conditions see text.

plants by control of the duration of radiation, and of the voltage and amperage used, plants can be stimulated to show increased growth rates.

Summary

1. A few preliminary experiments are described which indicate that under appropriate conditions of treatment, x-rays produce stimulative effects upon plant growth. Wheat, corn, oats, and sunflower seedlings have been used.

2. The seeds were treated in an early stage of germination after soaking for 24 hours in a closed moist chamber on a substrate of cellucotton saturated with water. The seeds are not submerged during soaking, but are wet on one side, and in contact with air.

3. The conditions which we believe necessary for such stimulative action are: the use of metallic screens, high voltage and low amperage, and brief exposures. The total dosage for stimulation does not much exceed 100 r-units. Even with the 1-mm. aluminum screen sunflowers given 150–200 r-units were overtreated. Optimum growth occurred with about 115 r-units (3 minutes).

4. There is some evidence of increased sugar content and increased respiration of treated seedlings.

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LITERATURE CITED

1. GOODSPEED, T. H. The effects of x-rays and radium on species of the genus *Nicotiana*. Jour. Hered. **20**: 243–259. 1929. See also Science **67**: 46. 1928, and Natl. Acad. Sci. Proc. **14**: 66–69. 1928.
2. JOHNSON, EDNA L. Effects of x-rays upon growth, development, and oxidizing enzymes of *Helianthus annuus*. Bot. Gaz. **82**: 373–402. 1926. See also Amer. Jour. Bot. **15**: 65–76. 1928.
3. ————. Effect of x-irradiation on growth and reproduction of tomato. Plant Physiol. **6**: 685–694. 1931.
4. ————. Tuberization of potatoes increased by x-rays. Science **68**: 231. 1928.
5. MCKAY, J. W., and GOODSPEED, T. H. The effects of x-radiation on cotton. Science **71**: 644. 1930.
6. PATTERSON, J. T., and MULLER, H. J. Are "progressive" mutations produced by x-rays? Genetics **15**: 495–577. 1930.
7. STADLER, J. L. Genetic effects of x-rays in maize. Natl. Acad. Sci. Proc. **14**: 69–75. 1928.
8. ————. Mutations in barley induced by x-rays and radium. Science **68**: 186–187. 1928.

Protocol for X-ray mutagenesis of plant material: seed

Background

Induced mutagenesis in plants dates back to the beginning of the 20th century. Physical mutagenic treatments have included gamma, X-ray and neutron irradiation. In the 1950s there was a global spread of gamma irradiators for plant mutagenesis, especially to create desired mutants for plant breeding. Protocols for gamma irradiation were optimised and many mutant plant varieties have been released. The plant mutant variety data base (http://mgvs.iaea.org/) However, gamma sources (usually the radioactive isotopes: Cobalt-60 and Cesium-137) have become security risks and strict international regulations are imposed on: 1) the shipment of gamma sources, 2) the production of gamma sources and 3) the refurbishment of old gamma irradiators (Mastrangelo et al., 2010). These restrictions now limit gamma irradiation for plant mutagenesis. The Plant Breeding and Genetics Laboratory (PBGL) of the FAO/IAEA has therefore embarked on a series of investigations aimed at optimizing X-rays for plant mutagenesis. Our initial studies have focused on developing procedures and adapting an existing commercially available X-ray machine, the RS-2400, which has been used extensively in the FAO/IAEA Insect Pest Control Laboratory to produce sterile male insects for SIT (Parker and Mehta, 2007; Mastrangela *et al*., 2010; Figure 1)

In order to obtain even sample irradiation, X-ray machines require rotation of the sample in the X-ray beam. In the RS-2400 X-ray irradiator samples are placed in canisters which orbit the X-ray source, in addition the canisters also rotate longitudinal along their axis (Figure 1).

The RS-2400 X-ray irradiator (produced by RAD Source Technologies Inc., USA) is a self – contained low-energy irradiator, which operates at 150 kV and 45mA to give a dose rate (to water) in a centre of the rice filled canister 14.1±0.7Gy/min (rice is used as a irradiation dummy as its density is close to that of other seeds such as rice, barley, wheat). The Specific dose rate (SDR) at that location is 0.0376 Gy/kJ^{-1} or 2.26Gy min^{-1} kW^{-1}. Samples are placed inside canisters (5 canisters in the RS-2400) which are suspended by cradles that revolve in a vertical plane around the fixed horizontal X-ray tube (Figure 2). A specific dose is achieved by setting up a control panel with the required amount of kWs to produce the required radiation absorbed dose. The RS-2400 is currently used in the sterile insect technique in 2 countries (Brazil and Costa Rica) and under installation in 2 others (Pakistan and Burkina) Faso) and is easily adapted for plant mutation induction. Here we describe a protocol for seed irradiation.

Adaptation of the RS 2400 irradiator - sample canister

Each sample canister of the RS 2400 is 178 mm in diameter by 167 mm in length, which gives a volume about 3.5 litres (Figure 3). To achieve more uniform dose by hardening the photon spectrum, 0.5 mm steel has been placed in all canister (Parker and Mehta, 2007). RS-2400 offers a possibility of 5 irradiator canisters for a total volume of 17.5 litres (Figure 2b).

The volume of the sample canisters of the RS 2400 is too large for seed samples of many crop species, e.g. the small grain cereals (rice, wheat, maize, etc.) in which a 1 litre volume may contain 15 thousand seed. Seed irradiation for mutagenesis typically involves sample sizes ranging from 3,000 – 50,000 seed. Additionally, it is important in X-ray irradiation that samples are packed tightly to minimize air space and to maintain near uniform field of X-rays through the entire sample, therefore a range of sample container sizes is required. Various containers may be used, e.g. 0.4 to 0.7 litre and these can be set inside the standard sample canisters of the RS 2400 using plexglass brackets (Figure 4).

1. Dose optimization:

The radiation dose is the radiation absorbed by the samples after the completion of the treatment. The standard 3.5 litre sample canister of the RS-2400 has been calibrated for dose uniformity using instant cooking rice to fill the canister because the density of pupae and of instant rice is quite similar, 0.46 and 0.44 g cm^{-3} respectively (Mehta and Parker, 2012). Uniformity is achieved when all (5) canisters are filled with instant cooking rice during the treatment. For seed irradiation, the seed samples need to be packed tightly in an appropriate sample container which is placed into a standard RS 2400 canister and the remaining empty space filled with instant rice (Figures 5, 6a and 6b).

2. Determining the Relative Biological Effectiveness of X-rays

A prerequisite in developing an X-ray irradiation protocol for seed treatment is to determine sample radio-sensitivity and thereby optimum dose for mutagenesis. These studies are described in detail in Bado *et al.* (2012); an outline is given here. The effectiveness of X-ray irradiation was assessed through the Relative Biological Effectiveness (RBE) by measuring growth of M1 seedlings. The RBE for a given test irradiation was calculated as the gamma radiation dose required to produce the same biological effect as a standard x-ray radiation treatment. Seedling height or hypocotyl length as a percentage of control seedlings (from untreated seed, M_0) were plotted against the absorbed dose and growth reduction of 30% and 50% (GR30 and GR50, respectively) were estimated base on the linear regression analysis. Tests were carried out on a range of seed samples of barley, lupin, sorghum and wheat (Table 1).

Table 1: X-ray irradiation doses giving growth reductions of GR30 and GR50 in M1 seedlings of barley, lupin, sorghum and wheat. The RBE with respect to equivalent gamma ray treatments is also given.

Crop	Variety	Gamma ray dose in greys		X-ray dose in greys		1/RBE	
		GR30	GR50	GR30	GR50	GR30	GR50
Barley	Rum	249.8	400.3	187.2	347.5	0.75	0.87
	ASCAD 176	121.4	281.4	191.2	338.0	1.57	1.20
Wheat	Hourani	222.3	314.1	35.5	146.8	0.16	0.47
	ASCAD65	244.0	350.6	195.7	281.1	0.80	0.80
	Um Quis	249.1	352.7	88.36	187.82	0.35	0.53
Sorghum	Koden	246.8	406.4	226.3	349.5	0.92	0.86
Lupin	LG-15	401	826	499	991	1.24	1.20
	LG-46	586	1037	473	909	0.81	0.88
	LG-92	622	1129	628	1047	1.01	0.93
	LAE-1	451	897	423	873	0.94	0.97
	AU 11257-19/1	430	786	468	806	1.09	1.03

These studies indicate that lower X-ray doses are required compare to gamma doses to produce the same biological effect. This was also reported in sterile insect work (Mastrangelo et al., 2010).

3. Protocol

Seeds sample preparation

Step 1

Prior to irradiation the seeds are kept at least for 3 days, in a desiccator with 60% glycerol for moisture equilibration to 12-15% (Figure 7).

Step 2

Seed are packed into appropriately size sample containers to minimize air space (Figures 4). Different seed samples may be placed in paper bags before placing into the sample container to avoid sample contamination or mixing (Figure 8). For small samples, small containers or Petri dishes (size depend of the adaptor groove pre-defined) may be used and the samples are immobilized by packing with tissue paper (Figure 9)

Step 3

The packed sample container is fitted with brackets and fixed into position inside a standard RS 2400 canister. The void volume is filled with instant cooking rice. (Figures 10 and 11).

Step 4

Canisters are placed into the irradiator (Figure 12) and the required irradiation dose is given

Post treatment activities

Post treatment activities are the same as for other physical and chemical induced mutagenesis (including gamma ray) (Kodym and Afza 2003 and Mba et al., 2010).

Radiosensitivity checks

Grow up M1 to produce M2 seed

Screen for mutations, evaluation

Entry into breeding programmes

Examples

Country	Crop	Variety	Dose used (Gy)	Purpose (target trait)	Mutants detected	Current generation of population/varietal development
Jordan	Barley	Rum	200	Smooth awns	Plant heading time , maturity, grain yield and any change in softness of awns	M2
		ASCAD 176	150	Increased production under drought condition	Plant heading time, maturity and grain yield	M2
	Wheat	Hourani	200	Increased production	Plant heading time, maturity and grain yield	M2
		ASCAD65	250	Increased production and plant height	Plant heading time, maturity and grain yield	M2
		Um Quis	200	Improved smut disease tolerance	Plant heading time maturity, grain yield and tolerance to smut	M2
Eritrea	Sorghum	Koden	200 and 400	High yielding , striga resistance and drought tolerance	Diverse phenotypes mutants (seeds, seedling, panicle structure, early tillering)	M4
Poland	Lupin	LG-15	250, 500, 750, 1,000 and 1,500	Radiation test and induce genetic variability in the species and line	Changes in plant architecture, type of growth (from indeterminate to determinate)	M2
		LG-46	250, 500, 750, 1,000 and 1,500	Radiation test and induce genetic variability in the species and line	Changes in plant architecture, type of growth (from indeterminate to determinate)	M2
		LG-92	250, 500, 750, 1,000 and 1,500	Radiation test and induce genetic variability in the species and line	Changes in plant architecture, type of growth (from indeterminate to determinate)	M2
		LAE-1	250, 500, 750, 1,000 and 1,500	Radiation test and induce genetic variability in the species and line	Restricted-branching and any change in growth habit, early maturity, better yield potential, potential to regenerate androgenic plants in *in vitro* culture	M2
		AU 11257-19/1	250, 500, 750, 1,000 and 1,500	Radiation test and induce genetic variability in the species and line	Restricted-branching and any change in growth habit, early maturity, better yield potential, potential to regenerate androgenic plants in *in vitro* culture	M2
Senegal	Jatropha	Bacary Sarre	100, 200, 300, 400, 500	Radiation test, high yielding, good quality and oil content	Currently under screnning	M4
Jamaica	Ginger	Jamaica Native	8 and 10	Development of rhizome rot resistance	Chlorotic abnormalities (10 Gy)	M1V4
		China Blue	8	Development of rhizome rot resistance	Plantlets recently subcultured to M1V4	M1V4
		Jamaica Yellow	8	Development of rhizome rot resistance	Plantlets recently subcultured to M1V4	M1V4
Kenya	Artemisia	Artemisia spp	150	Good quality and quantity biochemical (artemisin)for malaria management	Currently under developpment	M2
	Barley	Barley	250	Increased yield and adaptation to harsh environments	Currently under development	M2

Acknowledgements

Protocol prepared by S. Bado, B.P. Forster in collaboration with Nawal Alhajaj (Jordan), Negusse Abraha Russon (Eritra), Kamila Kozak (Poland), Ann-Marie Smith (Jamaica), Ibrahima Diedhiou (Senegal) and Miriam Kinyua (Kenya).

References

Mastrangela T., Parker A.G., Jessup A., Pereira R., Orozco-Davila D., Islam A., Dammalage T. and Walder J.M.M. (2010). A new generation of X-ray irradiators for Insect Sterilisation. J. Econ. Entomol. 103(1): 85-94.

Mehta K. and , Parker A.,(2011) Characterization and dosimetry of a practical X-ray alternative to self-shielded gamma irradiators. Radiation Physics and Chemistry 80: 107–113.

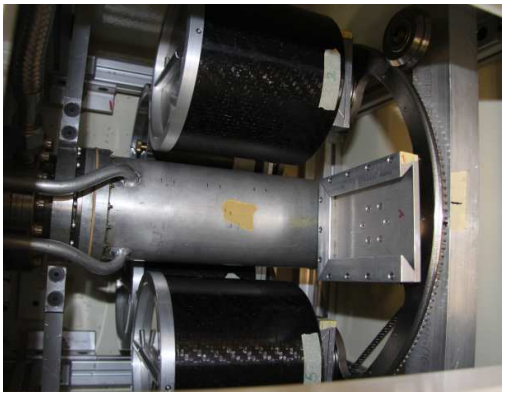
Novak F.J. and Brunner H. (1992). Plant breeding: Induced mutation technology for crop improvement. IAEA Bulletin 4: 25-33.

Parker A. and Mehta K. (2007). Sterile Insect Technique: A model for dose optimization for improved sterile insect quality. Florida Entomologist 90(1):88-95.

Kishor Metho, Ph D. X-ray radiation vs. Gamma radiation. (http://radsource.com/applications/sterile_insect_technique_sit_sir)

Gamma vs X-ray comparison (http://radsource.com/applications/sterile_insect_technique_sit_sir).

Figure 1: X-ray irradiator RS-2400, sample canisters are loaded and unloaded from the top,



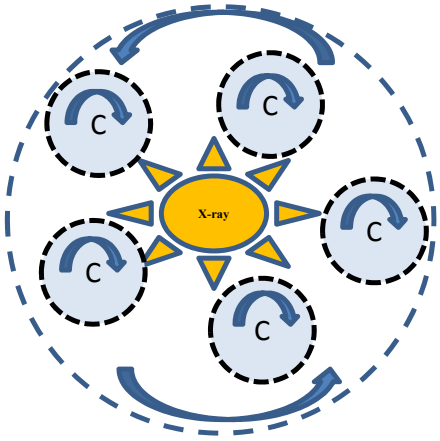


Figure 2a: X-ray tube (centre) with orbiting and rotating sample canisters. Figure 2b: Arrangement of sample canisters (5) around the X-ray tube

Figure 3: Sample canister and the cover, the interior of the canister is lined with a steel film to harden the X-ray beam.

Figure 4: Brackets of varying sizes to fit different sample containers (0.7 litres, 0.4 litres and Petri dishes), brackets are cut from 5 mm PMMA

Figure 5: Seed sample packed inside a contained and fixed in place using tissue. The container is fitted with brackets.

Figure 6a RS 2400 standard sample canister (left, 3.5 litre volume) and a sample container with adaptors (right, 0.4litre volume).

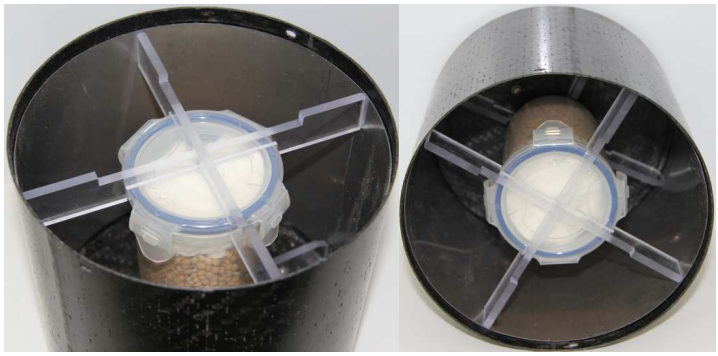


Figure 6b: Seed samples packed inside a sample container with adaptors to set inside the RS 2400 standard canister.

Figure 7: Desiccation treatment of seed to standardise moisture content to 60% glycerol

Photograph 8: Different seed samples in paper bag sealed in small container ready for different dose treatment.

Photograph 9: Small seed samples may be packed into Petri dishes using tissue paper.

Figure 10: The prepared sample container is placed inside a standard RS 2400 canister where it is fixed in position by adaptor brackets. The space between the sample container and the canister is filled with instant cooking rice.

Photograph 11: Canister of samples with spare volume filled with by instant rice and cover canister by side.

Figure 12: Placement of canister in Irradiator and closing the chamber.

SOME GENETIC EFFECTS OF X-RAYS IN PLANTS*

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THIS paper summarizes the results of a series of experiments on the genetic effects of X-rays, conducted at the University of Missouri during the last four years. The experimental material has included various plant species, chiefly barley (*Hordeum vulgare*) and maize (*Zea mays*). Space will not permit the detailed presentation of experimental methods or data in this summary, but fuller accounts of the individual experiments have been, or will be, published separately.

Mutation Induced by X-Rays and Radium

The effects of X-ray and radium treatments on the frequency of mutation in barley were determined by the method illustrated in Figure 1. The treatments were applied to dormant or germinating seeds, which were then planted at sufficient distance to permit the development of several tillers. The tillers develop from the axils of the lower leaves, and each terminates, as does the central culm, in a seed-bearing head. Since the primordia of the tillers are separate in the embryo at the time of treatment, a mutation occurring at this time affects only a single culm. (In much-tillered plants a secondary tiller often develops from an earlier tiller, and a single mutation may affect both).

The occurrence of a mutation is detected by growing a self-fertilized progeny from each head. The mutant character segregates in the progeny of a single head, and its absence in the progenies of other heads of the same plant shows that it has resulted from a genetic change occurring during the development of the plant treated. Ancestral hybridity, pollen contamination, or other complications could account for the segregation of an unexpected character in the progeny of a treated plant, but in any such case the character would segregate similarly in all head progenies of the plant concerned.

In the first series of experiments, in which about 2,800 head progenies from X-rayed and radium-treated plants were examined, 53 mutations were found. In the untreated control, including about 1,500 head progenies, no mutations were found. Of the 53 mutations 48 were recognizable in the seedling stage. The methods of treatment and the mutant seedling characters have been reported elsewhere.[4]

Since about 90 per cent of the mutations found could be recognized in the seedling stage, the use of seedling mutations alone as an index to mutation rate is feasible. This permits the determination of mutation rates on a large scale. Each head progeny may be examined for seedling segregation in a planting occupying about 15 square inches for a period of 10 days, and tests may be made in the greenhouse at any season of the year. The occurrence of seedling mutations is, in fact, determined more accurately under these conditions than in the regular field plantings, since the less viable mutant types sometimes fail to emerge under field conditions. Seed remnants of all head progenies are saved and those from mutating plants are planted in the field for further study.

By this method quantitative studies of mutation rate may be made with

*These experiments were supported in part by a grant from the National Research Council, Committee on Effects of Radiation on Living Organisms.

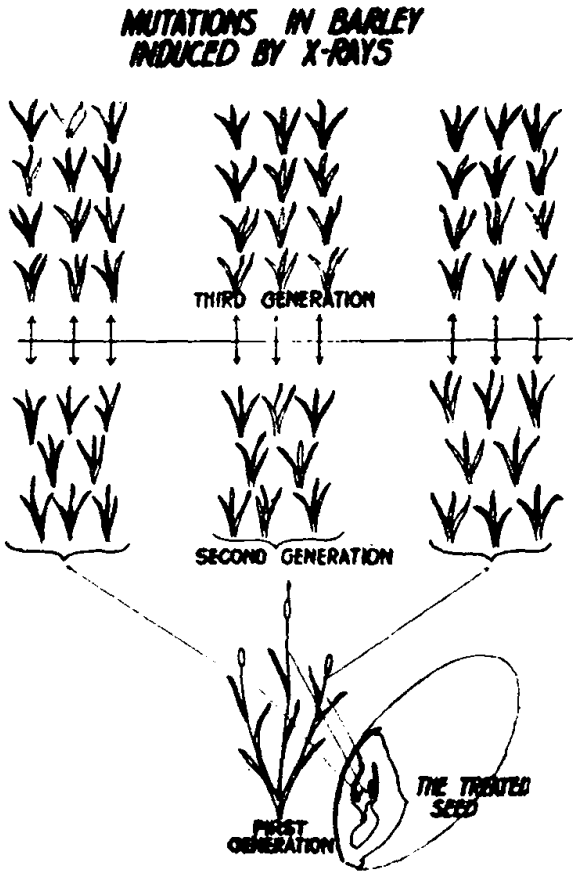
ordinary "visible" mutations. During the fall of 1928 seedling progenies were grown in the greenhouse from about 20,000 head progenies. Only distinct and conspicuous seedling characters were counted as mutants, in order to avoid fluctuation in the standard. About 250 of these were found. The evidence discussed below on the relation of mutation rate to dormancy, temperature, dosage, etc., is derived from these experiments.

The Induced Mutations

The mutant seedling characters recorded were chiefly chlorophyll abnormalities. The predominance of this type of mutant is due in part to the method of study, since special attention was given to characters showing clear-cut segregation in small progenies during the first few days after emergence. This method discriminates against any but the most extreme of morphological variations. A few clear-cut morphological variants, however, are included.

Of the total number of seedling mutants found, some 300 in all, white seedlings made up about 60 per cent. Another 5 per cent were "virescent white"; that is, white seedlings which gradually develop green color. About 15 per cent were yellow seedlings of various shades, of which about one-third were more or less virescent. Intermediate shades of greenish-yellow made up another 10 per cent, and the remaining 10 per cent included miscellaneous types such as "striped", "banded", "fine - striped", "green - striped", "tarnished", "spotted", "tapering", spindling", "shriveled", etc. The mutants grouped as virescent whites, yellows, and greenish-yellows included many phenotypically distinct types. Presumably these as well as the white seedlings included mutations at many different loci.

Most of the mutant seedling characters are lethal and almost all are unfavorable to growth, as would be expected in types conspicuously different from the normal. The striped seedlings are fairly vigorous, and a few of the virescents develop almost normally in later growth.



TECHNIC IN DETERMINING MUTATION RATE IN BARLEY

Figure 1

A recessive mutation induced by irradiation of the seed makes one tiller of the resulting plant heterozygous. In the second generation the selfed progeny of this tiller segregates the mutant character. Some of the normal plants of this progeny are heterozygous, and segregate the same mutant in the next generation. Since the primordia of the three heads are separate in the embryo at the time of treatment, the other head progenies of the treated plant are unaffected by the mutation.

Mutations having no conspicuous effect in the seedling stage but causing distinct variations in later development could have been detected in only about 4,000 head progenies which have been grown to maturity. Seven of these were found, including three for "pale green," two for "non-glaucous," one for "fine-stripe," and one for "dormant," a type resembling winter barley in habit of growth.

DIRECTLY-INDUCED STRIPING

Figure 2

A barley chimera resulting from a direct cytoplasmic effect of X-ray treatment. The two tillers on the right had broad yellow stripes, but the heads were entirely green. The progeny was unaffected. In a similar plant in which a yellow stripe passed through the head, the seed of the striped sector transmitted the defect.

Tests of almost all of the mutations found in the first series of experiments have been carried through one or more generations following that in which the mutant character first appeared. In all of these cases the head progeny segregating the mutant includes normal plants which segregate the same mutant in the next generation. The few mutants which reach maturity breed true. In the few cases tested by crossing on untreated normal plants, the mutant characters are transmitted through the pollen, reappearing as recessive segregates in the F_2 of the hybrid. In other words, the genetic behavior is as would be expected on the assumption that the mutation is a change of a dominant gene to the corresponding recessive in a somatic cell of a homozygous plant.

In the original segregating head progeny the proportion of plants showing the mutant character is usually less than 25 per cent, and the proportion of plants which are found heterozygous is less than 50 per cent. In later generations the ratios are approximately normal. The probable reason is that only a part of the head is derived from the cell in which the mutation occurred.

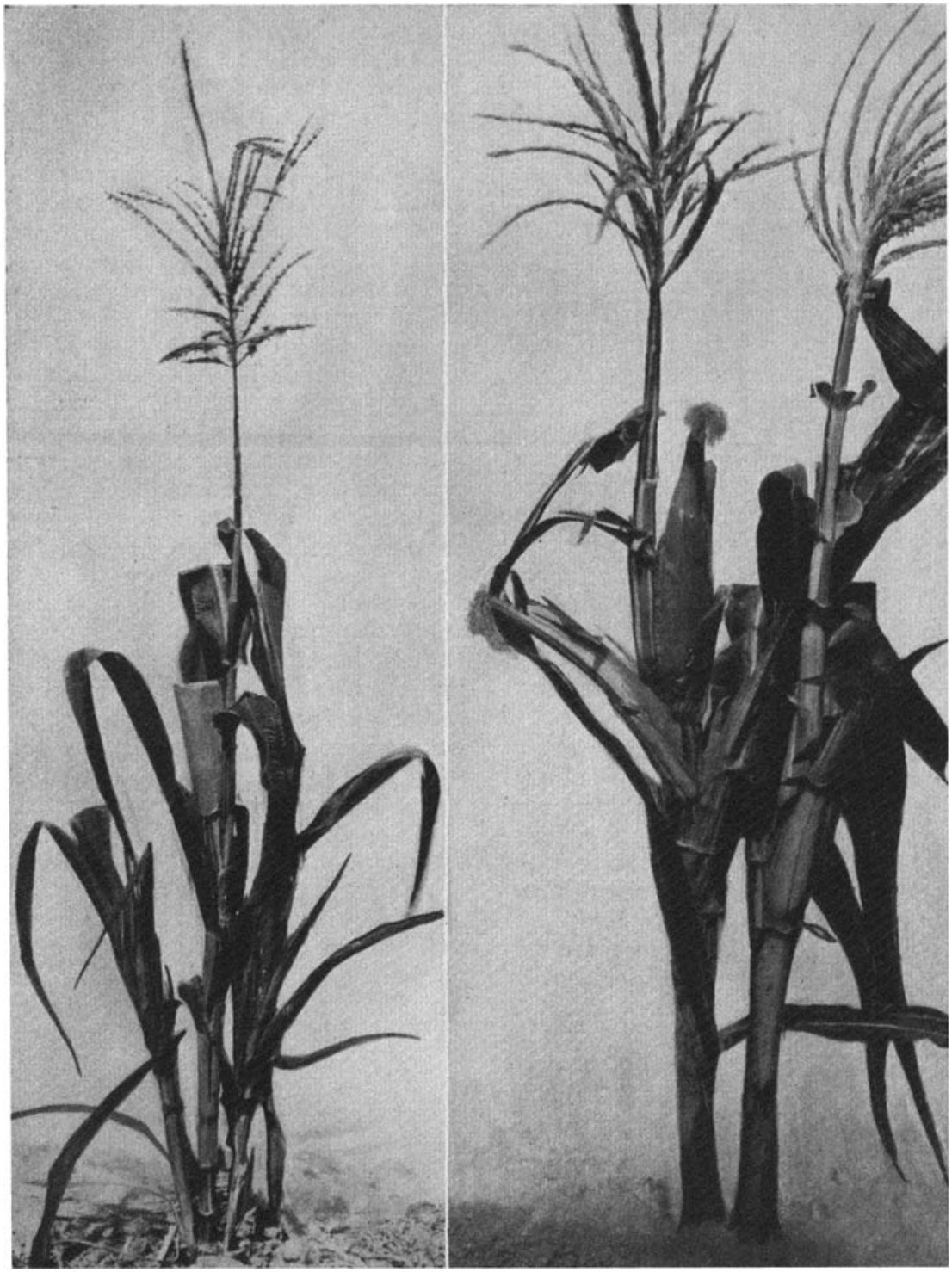
Not infrequently two different mutant characters segregate in the same head progeny. The frequency of such cases is greater than would be expected from chance coincidence.

No clear case of a dominant mutation has yet appeared. All of the mutations tested beyond the first segregating generation are unquestionably recessive. The remainder cannot be classified positively without further test, but none of them has behaved as would be expected of dominants. A dominant mutation should affect not only the progeny of the tiller concerned, but also the phenotype of the tiller itself. All of the treated plants, including many not yet tested for recessive mutation, were carefully examined for somatic variations such as might result from the occurrence of dominant mutations in the primordia of tillers. Among more than

CHIMERAS INDUCED BY X-RAYS

Figures 3 and 4

Two maize chimeras resulting from X-ray treatment of the mature seed. Both are purple plants heterozygous for the gene *B b*, and each has a sector of green tissue derived from a cell in which *B* was lost. The extent of such sectors indicates the amount of tissue which may be derived from a single cell in the embryo of the mature seed.



CHIMERAS PRODUCED BY IRRADIATION OF DEVELOPING EMBRYO

Figures 5 and 6

Two maize chimeras resulting from irradiation 6 days after pollination. Both plants were heterozygous for the gene *G g*, for golden plant color. The lighter colored areas in the figure are "golden," and are derived from a cell in which *G* was lost.

23,000 treated plants only two variations affecting seed-bearing culms were found. One of these was the plant pictured in Figure 2. Two of its tillers (on the same side of the plant) had distinct broad yellow stripes on the leaf blades and sheaths but not on the head. The progeny of these two tillers was entirely normal. The other plant had a similar yellow stripe on the upper sheaths and blades of the main stalk, beginning as a narrow line but broadening on the upper leaves and appearing on several spikelets of the head. The seed of these spikelets gave yellow, green, and sectorially yellow-and-green plants. Probably both cases resulted from some direct cytoplasmic effect of the X-ray treatment. A third case was a plant with broad white stripes on two late tillers, which never headed and which therefore could not be genetically tested. No other indication of dominant mutation has been found. From the tests already completed it is evident that the total number of recessive mutations from these plants will be over 1,000.

The possibility is not excluded that the recessive condition resulting from X-ray treatment is merely the absence of the dominant gene; that is, that the mutation induced is simply the destruction of a gene. But the outward effects and genetic behavior of these mutations, so far as studied, are identical with those of "normal" recessive genes, and this conception may be applied equally well to "normal" recessive mutation.

Tests of the genetic identity and linkage relations of several of the induced mutations have been undertaken by Prof. F. J. Stevenson, of the Unicersity of Minnesota, in connection with his studies of inheritance in barley. The material is technically rather difficult, and routine determination of the linkage relations of large numbers of mutant genes is not practicable.

Induced Mutation In Maize

In experiments in which detailed genetic study of the induced mutations is desirable, maize is now being used, because of its advantages for genetic analysis. Mutations similar to those found in barley are induced in maize by X-ray treatment. Since maize is naturally cross-pollinated, the treated plants must be self-pollinated artificially, and it is therefore not so well suited as is barley to the determination of mutation rates on the extensive scale necessary in quantitative studies.

The portion of the plant which will be affected by a mutation occurring in a single cell of a mature seed is not likely to include both the tassel and the ear, and consequently the mutations induced by treatment of mature seed usually do not segregate in the second generation. A mutation affecting either the tassel or ear, but not both, results in the production of heterozygous plants in the second generation and segregation in the third. By using only tiller ears and pollinating each with pollen from the same tiller, the chance of a second generation segregation is increased. A more satisfactory method is to apply the treatment not to the mature seed, but to the young embryo, at so early a stage that an induced mutation can affect the entire plant.

The portion of the plant affected by mutations resulting from treatment of the embryo at various stages of development is shown in chimeras produced by the treatment of embryos heterozygous for certain plant characters. The plants shown in figures 3 and 4 are from seeds X-rayed just before planting. They are heterozygous for purple plant color, and each shows a sector of green tissue developed from a cell in which a plant-color gene (B) was lost. The genetic nature of these cases is discussed in a later paragraph. The point of interest in the present connection is the extent of the tissue which may be affected by a genetic change resulting from treatment at this stage of development. Several chimeras of this sort have occurred in cultures grown from X-rayed seed. The affected tis-

ABERRANT PLANTS DUE TO IRRADIATION

Figures 7 and 8

A defective plant from a seed X-rayed at fertilization. The plant at the left is a normal plant of the same culture. Plants are similarly affected by irradiation on the day after fertilization.

A japonica-striped maize plant from the irradiation of an embryo heterozygous for *J j* shortly after fertilization. The entire plant is derived from a cell in which *J* was lost.

sue in these plants varies rather widely in extent. Plant 1252-27, pictured in Figure 3, has the largest portion of tissue affected, while plant 1214-55 (Figure 4) has one of the smallest sectors observed. In the former plant, part of both the tassel and the ear are affected, while in the latter apparently no portion of either the tassel or the ear is included in the affected sector.

Even when treatments are applied as early as the sixth day after pollination chimeras are produced. The plants shown in Figures 5 and 6 were produced in this way. In these two cases the gene responsible is *G g*, for golden plant color. Both plants have broad streaks of golden tissue in both the main stalk and the tillers. None of the plants from seed treated at this stage showed a recessive character affecting the entire plant. On the other hand, treatments applied on the first or second day after pollination have produced no chimeras, but have given several plants entirely recessive for a character heterozygous in the seed treated. One of these is shown in Figure 8.

In studies of induced mutation in maize the X-ray treatment is applied to seeds heterozygous for genes marking several or all of the chromosomes. These are treated in the field on the second day after pollination. The plants grown from these seeds are selfed. The induced mutations thus segregate in a progeny segregating also for the chromosome markers, and linkage relations are roughly indicated in the generation in which the mutant characters first appear. So far as possible, genes for endosperm and seedling characters are used as chromosome markers, in order to permit the determination of mutation frequency and linkage relations in seedling progenies.

Rarity of Induced Mutation in Wheat and Oats

Treatment of common wheat and oats by the methods described above for barley gives a very different result. The effect on the treated generation is similar, and the killing dose is not markedly different, but in the following generations there is little or no evidence of induced mutation.

Common oats of the two cultivated species, *Avena sativa* and *Avena byzantina* were treated in several experiments. Selections of the varieties Kherson and Fulghum were used. The conditions of treatment varied somewhat in these experiments, but were such as would have yielded about 50 seedling mutations if applied to barley on the same scale. Only one mutation was found in the oats, a white seedling segregation in Kherson oats treated as dormant seed.

Common wheat (*Triticum vulgare*) was treated on a scale which would have yielded about 40 mutations in barley. Harvest Queen, a winter wheat, and Marquis, a spring wheat, were used. No mutations were found.

It is probable that the cause of the lower mutability of oats and wheat is gene reduplication, connected with their higher chromosome number. Each of the genera Hordeum, Avena, and Triticum includes species with chromosome numbers of 7", 14", and 21", but the cultivated barleys belong to the 7" group in Hordeum, while the cultivated oats and wheats belong to the 21" groups in Avena and Triticum. Whether the 21" condition has arisen by simple chromosome reduplication or by some more complex process involving the combination of unlike but related sets of 7", it is probable that most genes would be present in duplicate or triplicate in the haploid complement of 21 chromosomes. If a dominant gene *A* were present in triplicate *AAA*, recessive mutation of one *A* could have no visible effect, for the duplicate dominants would still be present to maintain the dominant condition. The coincidental mutation of the three homologous genes would probably be so rare as to be negligible. A moderate rate of mutation might result from the presence of some genes in the *aaA* condition, as a result of previous mutations occurring in the course of the past evolution of the species.

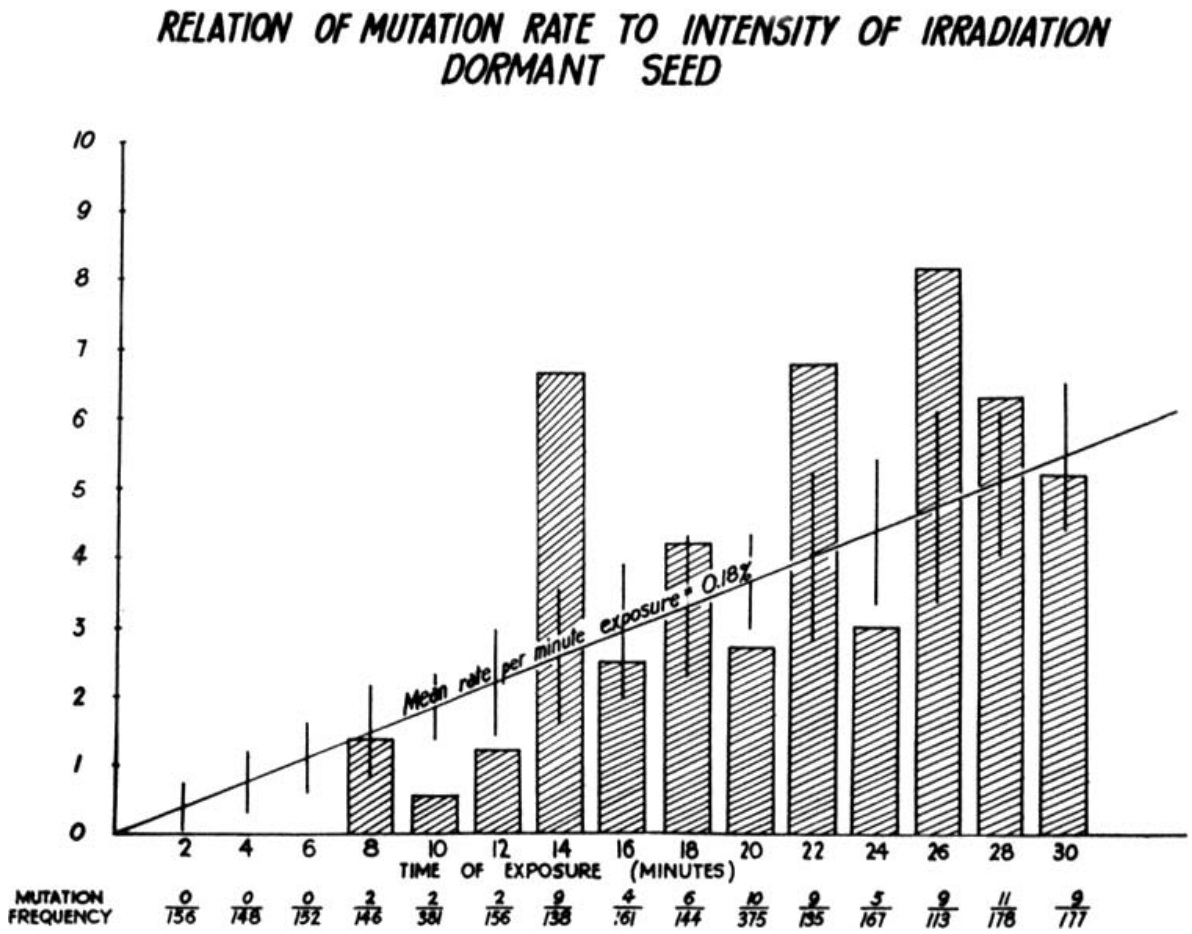


Figure 9

Relation of mutation frequency to dosage. The rate of mutation is proportional to the total intensity of the radiation applied, within the limits of sampling error. Detailed explanation in text.

If this hypothesis is correct the species of Avena and Triticum with 7 pairs of chromosomes should mutate at rates comparable with those of common barley. Tests of these species, as well as some of the 14″ species, are now in progress.*

Mutation Induced in Dormant Tissue

It is well known that the physiological effects of X-rays are in general intensified in actively growing tissue. In attempting to induce mutation by seed treatment with X-rays, experiments were made first with seeds germinating under optimum conditions, and the treatments were applied intermittently in order to increase the number of dividing cells exposed to treatment. But our present fragmentary knowledge of the nature of the gene gives us no reason to assume that the effect of X-rays on mutation would vary with the metabolic activity of the cell. If the gene is a constant entity and its mutation a chemical change energized by radiation, it might reasonably be expected to mutate under irradiation as readily in dormant as in active cells. A consistent difference in the rate of mutation found in cells at different levels of metabolism might serve as a clue to the physical nature of the mutation process.

Dormant and germinating seeds of barley were given identical X-ray

*These tests have been completed and published.

treatments in three experiments.[5] The dose was near the limiting intensity for germinating seed. Dormant seed are much more resistant to injury by irradiation and will withstand 15 to 20 times as heavy a dose.

The average rate of mutation in the plants treated as germinating seeds in these experiments was about four times as high as that in plants treated as dormant seeds. In each case the rate was determined from about 2500 head progenies, and the difference was unquestionably significant from the statistical standpoint. The number of mutations found after irradiation of the dormant seeds was large enough also to indicate positively that mutations were occurring at a rate significantly higher than that in untreated seeds. When the dose applied to dormant seeds was doubled the rate of mutation was approximately doubled also. The double dose applied to germinating seeds was almost completely lethal.

Because of the greater tolerance of the dormant seed, it was possible to apply a dose 10 times the unit dose without appreciable injury to the plants. This resulted in an increase in the mutation rate roughly proportional to the increase in dosage. The mutation rate thus secured was more than double that which followed the irradiation of germinating seeds. In spite of the higher mutability of germinating seeds, it is likely that the maximum sub-lethal dosage of dormant seeds will be much more effective in inducing mutation than that of germinating seeds. Their greater tolerance is more than enough to compensate for their lower rate of mutation.

When seeds which have been irradiated while dormant were stored for two weeks before planting, the percentage of mutations was not appreciably changed. The most heavily irradiated seeds, though apparently uninjured when planted immediately after treatment, decreased greatly in viability during the storage period. In seeds given lighter doses there was no noticeable loss during storage, either in germination or in vigor of growth.

Increased moisture content alone is not responsible for the increased mutability in germinating seeds. The "germinating seeds" referred to above were seeds soaked in water for 6 hours and kept on moist blotters in covered dishes for 18 hours before treatment. When seeds were irradiated immediately after the soaking period the mutation rate was not significantly higher than that in dormant seeds.

By changing the length of the period of soaking, the moisture content of the seed may be widely varied. Identical treatments were applied immediately after soaking to five lots of seed with moisture content of approximately 15, 20, 25, 30, and 40 per cent. The rate of mutation was not appreciably affected.

Temperature of the seeds during irradiation has no pronounced effect on the rate of induced mutation. Germinating seeds irradiated at 10°, 20°, 30°, 40°, and 50° Centigrade showed no significant difference in mutation rate. The limits of sampling error in the experiment admit the possibility of a small effect of temperature, but it is clear that the high temperature coefficient characteristic of many biological reactions does not apply. Similar experiments with dormant seed gave the same result.

Relation of Mutation Frequency to X-Ray Intensity and Wave-Length

The radiation applied in the experiments summarized above varied in both intensity and wave length. In most cases the total intensity applied was well below the tolerance limit of the seed. The wave length range in almost all cases was that of unfiltered radiation at voltages of 54 to 108 K. V. P.

To determine the relation of total intensity to mutation rate, dosage trials were made with both dormant and germinating seeds. Dosage was varied by changing the duration of the

treatment, other factors affecting radiation intensity being constant. (108 K. V.P., 4 m.a. tube current, 18 cm. target distance, no filter).

Fifteen doses were compared in the trial with dormant seed, ranging from 2 to 30 minutes. The heaviest dose had no appreciable effect on the viability of the seed or the vigor of the plants. Later trials showed that a 60-minute exposure under these conditions killed about one-half of the plants.

The frequency of mutation in plants given these treatments is shown in Figure 9. Mutation frequency increased approximately in direct proportion to dosage. Considering all of the data the mean rate of mutation per minute of exposure was 0.18%. This determines the slope of the line in the graph. If mutation rate is proportional to dosage the mutation frequency from any dose should be indicated within the limits of sampling error by the slope of this line. The probable error of the expected mutation percentage for each dose in a population of the size used is indicated by the vertical lines, and the mutation percentages actually found are indicated by the vertical columns. For example, the expected mutation percentage for an exposure of 20 minutes, in a population of 375, is 3.6 ± 0.7; the observed percentage is 2.7. As the graph shows, the actual mutation rates did not deviate from strict proportionality more than might reasonably be expected as a result of sampling fluctuations.

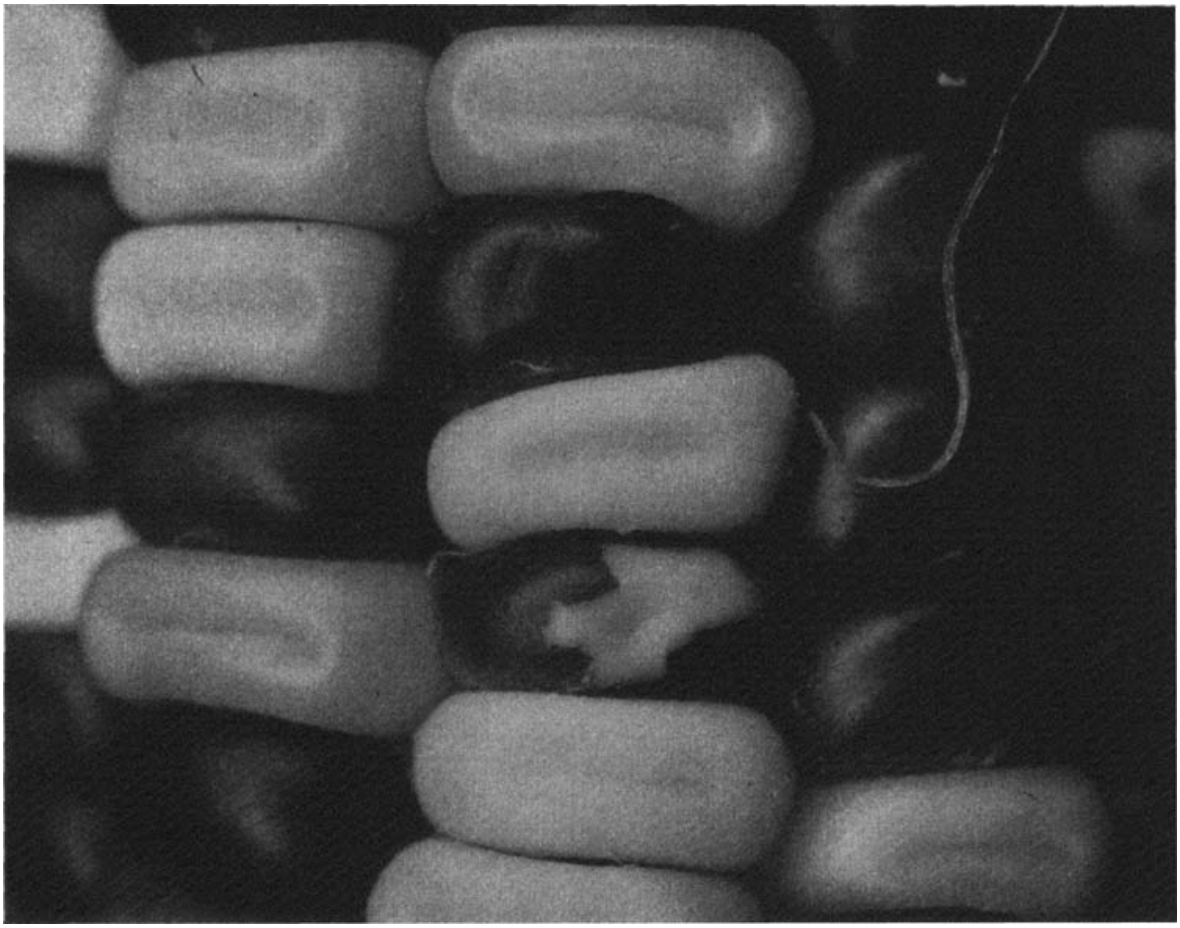
The absence of mutation in the cultures given the three lowest doses might suggest the possibility of a threshold intensity below which mutations do not occur, but in other experiments with low dosage mutations have been found. In the experiments with temperature mentioned above, 1270 progenies from seeds given an exposure of 108 seconds under the same conditions as in the dosage trials yielded 4 mutations, and 1005 progenies from a 216-second treatment yielded 7 mutations. These rates are proportional to those found in the dosage trial.

In the dosage trial with germinating seeds the range in exposure was from 15 to 360 seconds. All exposures above 135 seconds reduced viability, but even at 360 seconds some plants produced seed. The relation of mutation rate to dosage was similar to that found in dormant seed. The mean rate of mutation per minute of exposure was 1.17%, about 6 times that found in dormant seeds.

X-rays through a wide range of wave lengths appear to be about equal in power to cause mutation, when applied in intensities equal in power to ionize air. Equal intensities, as measured by ionization tests, were applied to both dormant and germinating seed of barley at 40, 56, 81, 98, and 116 K.V.P. Mutations were induced by all of the treatments, and the frequency of mutation did not differ significantly. Later, through the kindness of Dr. Gustav Bucky, of New York City, it was possibly to apply accurately measured doses of the ultra-soft X-rays, or "grenz rays," emitted by the Bucky soft X-ray tube. Mutations were induced by this radiation, both at 10 K.V.P. and at 7 K.V.P. At the latter setting no radiation of wave length shorter than 1.76 Å. is included in the emission spectrum.

Effects of X-Rays on Chromosome Distribution

In quantitative studies of the chromosomal effects of X-ray treatments the material chiefly used was mosaic endosperm of maize. A mosaic endosperm is an endosperm chimera in which a portion differs from the remainder in one or more characters, due to a genetic change occurring in early development. Emerson has shown that the phenomenon is due, in most cases at least, to a chromosomal disturbance, since linked genes are usually lost together, while unlinked genes are not. Since the triploid endosperm nucleus contains two identical sets of chromo-



ENDOSPERM CHIMERA DUE TO CHROMOSOME IRREGULARITY

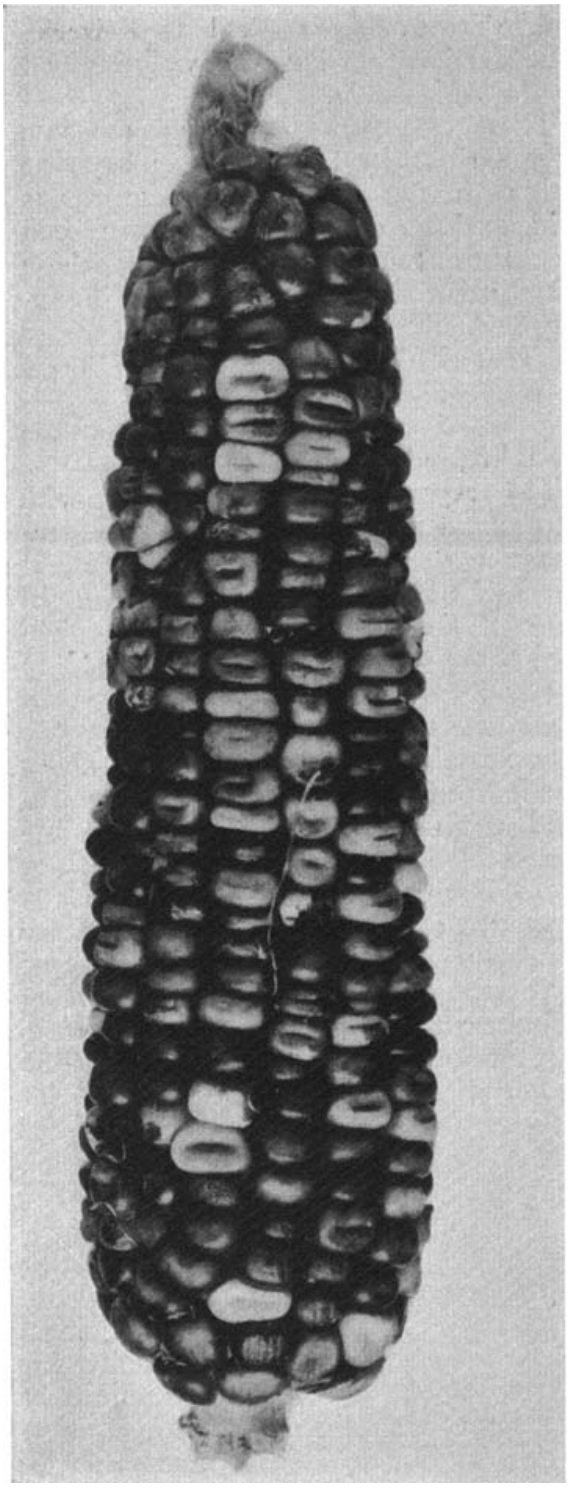
Figure 10

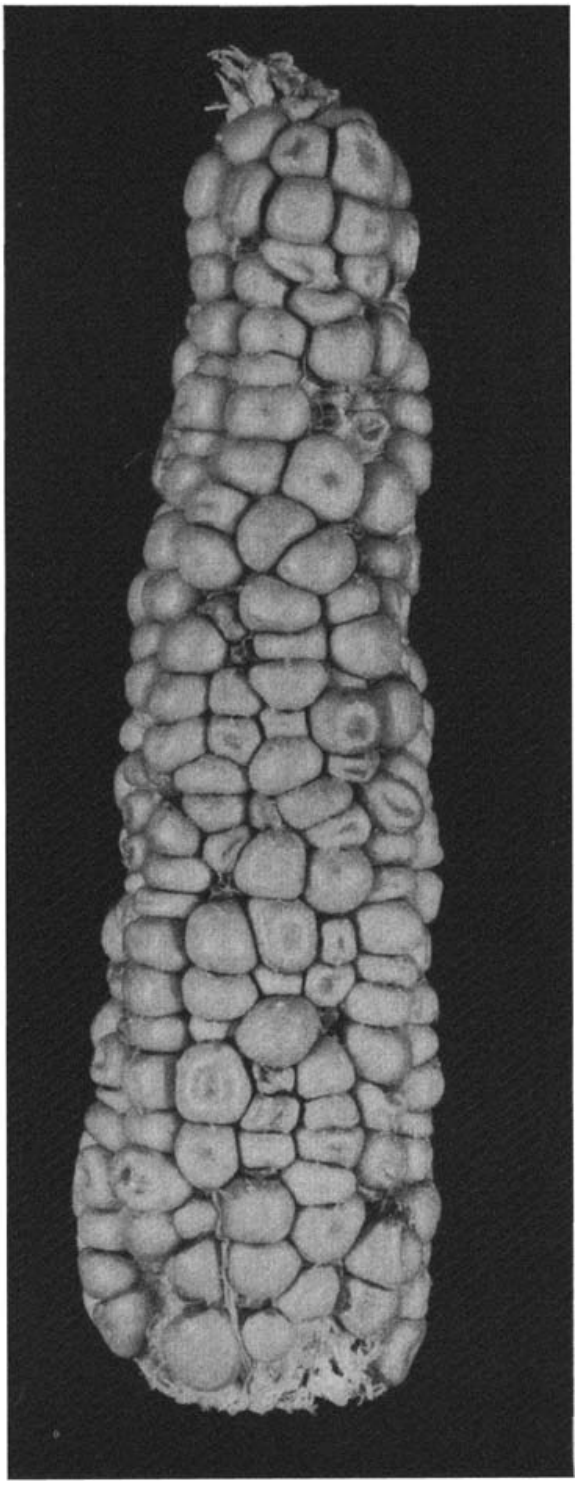
Mosaic endosperm in maize produced by irradiation. In a seed heterozygous for the linked genes *C*, *Sh*, and *Wx*, a sector comprising one-fourth of the endosperm has lost the three dominant genes, and is colorless, shrunken and waxy (*c sh wx*). The cause is a chromosomal irregularly in early endosperm development.

somes from the female parent, the loss of a dominant gene of maternal origin has no visible effect. Mosaics occur only for characters recessive in the female parent and dominant in the male. The cytological nature of the chromosome aberration causing mosaic endosperm is unknown.

Figure 10 illustrates mosaic endosperm in a grain heterozygous for the linked genes *C c*, *Sh sh*, and *Wx wx*. About one-fourth of the grain is colorless, shrunken and waxy (*c sh wx*) while the remainder is colored, smooth, and starchy (*C Sh Wx*). At an early stage in endosperm development a chromosome or portion of a chromosome carrying the three dominant genes was lost or inactivated, and the tissue derived from the cell affected lacks the three characters dependent on the lost genes.

Seven of the 10 maize chromosomes carry genes permitting their identification in endosperm mosaics, but the *C-Sh-Wx* chromosome is the only one with more than one gene suitable for accurate work with mosaic endosperm. Mosaics of each of the 7 chromosomes have been observed in untreated material. Their frequency varies rather widely in different families grown under comparable conditions. There is also wide variation in the frequency of mosaics for different characters in the same ears For example, in 9 unrelated families of *A C r su*, the average frequency of *r* mosaics was 0.59%, and that of *su* mosaics 0.05% If the mosaics are due to losses of an entire





MOSAIC AND DEFECTIVE SEEDS

Figure 11

High frequency of mosaic endosperm in an ear X-rayed at the time of fertilization.

Figure 12

Defective seeds from X-rayed pollen. Many of these seeds are germless. The seed parent was untreated.

chromosome, this difference would indicate that one chromosome is lost much more readily than another. It seems likely, however, that mosaics are caused, in some cases if not in all, by the loss of only part of the chromosome. In material heterozygous for *C c* and *Wx wx*, the majority of colorless mosaics are waxy, but sectors in which *C* has been lost without *Wx* are not uncommon, either in X-rayed or untreated material.

When an ear is X-rayed at about the time of fertilization a great increase in the frequency of mosaics occurs. Data have previously been published[3] showing a twentyfold increase in the mosaic rate, and larger increases occurred in some later experiments. The ear shown in Figure 11 illustrates the high frequency of mosaics in X-rayed ears. This ear was produced by the cross *A A R R c c sh sh wx wx pr pr Su su* × *A A R R C C Sh Sh Wx Wx Pr Pr Su Su*. Mosaics can show only for the paternal *C-Sh-Wx* and *Pr* chromosomes and in half of the grains for the paternal *Su* chromosome. If the 30 chromosomes present are being lost with an average frequency equal to that of these three, the frequency of chromosome loss was 12 times the frequency of the mosaics on this ear.

In X-rayed ears with numerous mosaics, aberrations involving unlinked genes are occasionally found. In the ear shown in Figure 11 two grains appear which are partly colorless-starchy and partly colored-sugary. Each of these resulted from a cell division in which one of the daughter cells lost *C* while the other lost *Su*. The frequency of such cases is probably no higher than would result from chance coincidence, though more extensive data would be desirable on this point.

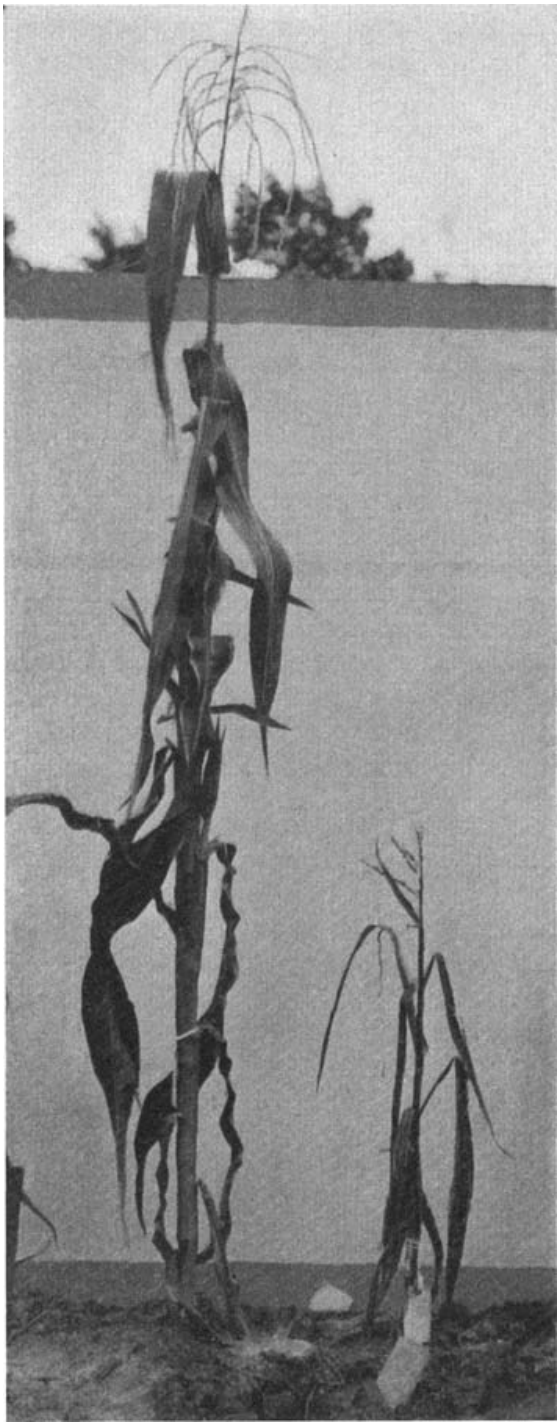
When treatment is delayed until the day after fertilization, the mosaic spots showing recessive characters are smaller and more numerous. When the same treatment is applied on the fifth day after fertilization, they are so small and numerous that the seeds appear stippled and the individual spots cannot be distinguished without magnification. Treatments applied two weeks after fertilization have no visible effect on the endosperm.

These treatments cause chromosome irregularities in the young embryo as well as in the young endosperm, as is evidenced in plants grown from seed of the X-rayed ears. Among these there occur (1) plants normal in appearance but with approximately 50 per cent defective pollen, (2) distinctly defective plants, and (3) plants showing recessive characters for which the seeds treated were heterozygous.

(1) Apparently normal plants with partially defective pollen are very frequent in these cultures. Usually about one-half of the pollen is defective. In most of the plants the defective pollen grains are empty, but in some they are partly filled with starch. In almost all cases the ovules of these plants are also partially defective, producing about one-half of a full set of seed distributed at random over the ear. The affected plant, whether self-fertilized or crossed with a normal plant, yields a progeny of which about 50 per cent are similarly affected. Brink and Burnham[1] have analyzed a similar case occurring in untreated maize. From the genetic evidence they ascribe the phenomenon to chromosomal translocation. It is interesting in this connection that Muller and Painter[2] have found translocation common in X-rayed Drosophila.

(2) The defective plants are of various types, but are all distinctly inferior to the normal plants in vigor of growth. Some never reach the flowering stage. Those which do produce partially defective pollen and few seeds. One of the more vigorous of these plants is shown in Figure 7 in comparison with a normal plant of the same progeny.

(3) In cultures heterozygous for certain characters a few defective plants showing the recessive character have been found. The japonica-striped plant shown in Figure 8 was one of these. When treatment is delayed sectorial chimeras showing the recessive character in a portion of the plant are pro-



DEFECTIVE PLANT FROM X-RAYED POLLEN

Figure 13

A typical defective plant from seed of a normal plant pollinated by X-rayed pollen. The plant on the left is a normal plant of the same culture.

duced, as shown in Figures 3-6. The pollen in affected plants or sectors is partially defective. These plants apparently represent the loss or inactivation of a chromosome or section carrying the dominant allelomorph of the character involved. Some characters are much more frequently lost in this manner than others.

By a study of the chromosome complement of plants of this sort it may be possible to identify the visible chromosomes with specific linkage groups in maize. The induction of chimeras by X-ray treatment at various stages of development will be a useful method for the study of the developmental morphology of the plant.

Dr. L. F. Randolph has undertaken a cytological study of the effects of these treatments, and has found cytological evidence of a chromosomal irregularity associated with semi-sterility and of chromosomal or sectional deficiency in many of the defective plants.

Chromosomal effects of X-rays are shown also in the results of pollen treatments. An ear of an untreated plant pollinated with heavily-treated pollen is shown in Figure 12. A considerable proportion of the seeds are distinctly defective. Many of these are germless. The viable seeds yield both normal and defective plants. A typical defective plant from treated pollen is shown in comparison with a normal plant of the same progeny in Figure 13. Among the defective plants produced by the use of X-rayed pollen Dr. Randolph has found several lacking an entire chromosome and others lacking a portion of a chromosome. Experiments designed to identify genetically the chromosomes eliminated are now in progress.

Induced Mutation in Plant Breeding

The practical value of induced mutation in the improvement of crop plants has been much overrated, at least as regards immediate application. It is true that progress in plant breeding is dependent on the occurrence of germinal variation, and that the rapidity of germinal change may be greatly increased by X-ray treatment. But it

does not follow that increasing the frequency of mutation a hundredfold will expedite the progress of plant improvement in proportion. In spite of the rarity of mutation under natural conditions, a great wealth of germinal variation is now available in every crop plant species. This is the result of the natural mutation of the past, on so vast a scale as to dwarf the most elaborate experiment. The genes which have survived in the varieties of today are those which have stood the rigorous test of natural selection. All of these are available to the breeder. By hybridization according to principles now well understood, they may be brought into almost any desired combination.

The variations resulting from induced mutation are in most cases unfavorable. Probably this is true of mutations occurring naturally as well, and it is reasonable to expect that among artificially induced mutations a small proportion of favorable variations will be found. But the rare favorable mutation is likely to be accompanied by unfavorable mutations induced by the same treatment. The result is a plant heterozygous for several genes, mostly undesirable. From this heterozygote a desirable combination may be extracted, but in most cases a heterozygote of greater promise could be produced by well-directed hybridization.

If the mutations induced by X-rays are qualitatively identical with those occurring naturally, there is little chance of producing experimentally variations which have not already occurred in nature. Characters of value in breeding are more likely to be found in the varietal collection than in the progeny of X-rayed plants, and here they will occur without the many undesirable gene mutations and chromosome aberrations characteristic of the X-ray progenies. If mutations could be induced selectively the outlook for practical application would be much brighter, but the evidence thus far gives no indication of selective action. A more thorough study of this possibility is needed.

There are, however, certain special cases in which induced mutation, even in the present state of knowledge, offers a fair possibility of successful application. These are in general cases in which hybridization is not feasible or in which a character dependent on a single gene-change is particularly important. Two examples will serve for illustration.

Modern corn breeding employs almost exclusively the technic of "selection in self-fertilized lines". By continuous selection through several generations of inbreeding, as much as possible of the best germ-plasm is concentrated in a few inbred strains, which are later to be used in the production of vigorous first-generation hybrids. Of the thousands of inbred strains which have been produced in this way, a few represent extremely valuable gene-complexes. Their further improvement is difficult, for as inbreeding proceeds they approach complete homozygosity and offer little opportunity for selection. If new variation could be introduced by induced mutation, even though most of the variations were unfavorable, some further improvement by selection might be possible. In cooperation with Dr. J. R. Holbert and Dr. J. G. Dickson, of the United States Department of Agriculture, a study is now being made of the effects of induced mutation on variability in two exceptionally good inbred strains of corn.

Another promising application of induced mutation is in the breeding of the tree fruits. The established fruit varieties are complex heterozygotes maintained by vegetative propagation. Most of them originated as chance seedlings of unknown parentage. Under seed propagation the type is lost at once. For this reason and because of the long reproductive cycle, controlled hybridization is under a great disadvantage in the breeding of these plants. The selection of bud variations is a much simpler method of improvement, for a desirable variation may be propagated at once by grafting. A few important varieties have originated in this

way, but somatic mutation is too rare in most fruit species to provide material for the systematic application of this method. Some of the characters determining fruit quality are known to be dependent on single gene-differences, and it seems not unlikely that induced mutations may result in bud variations of breeding value. In cooperation with Dr. A. E. Murneek, of the University of Missouri, a study is being made of the effects of X-ray treatment on bud variations in the apple.

Literature Cited

1. Brink, R. A. and C. R. Burnham. Inheritance of semi-sterility in maize. *Am. Nat.*, 63, pp. 301-316. 1929.
2. Muller, H. J. and T. S. Painter. The cytological expression of changes in gene alignment produced by X-rays in Drosophila. *Am. Nat.*, 63, pp. 193-200. 1929.
3. Stadler, L. J. Genetic effects of X-rays in maize. *Proc. Nat. Acad. Sciences*, 14, pp. 69175. 1928.
4. ————. Mutations in barley induced by X-rays and radium. *Science*, 68, pp. 186-187. 1928.
5. ————. The rate of induced mutation in relation to dormancy, temperature, and dosage. *Anat. Rec.*, 41, pp. 97. 1928.
6. ————. Chromosome number and the mutation rate in Avena and Triticum. *Proc. Nat. Acad. Sci.*, 15:876-881. 1929.

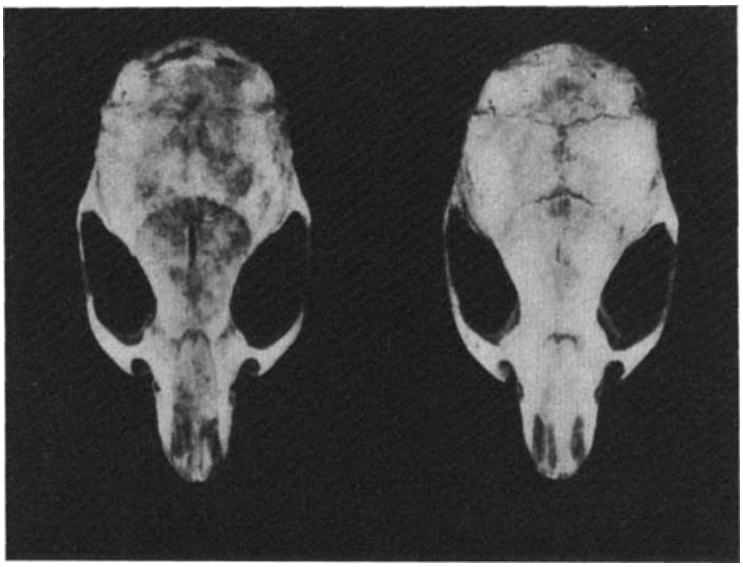
"PARTED PARIETALS" IN MICE

A Dominant Hereditary Character of the House Mouse, *Mus musculus*

Clyde E. Keeler
Bussey Institution, Forest Hills, Mass.

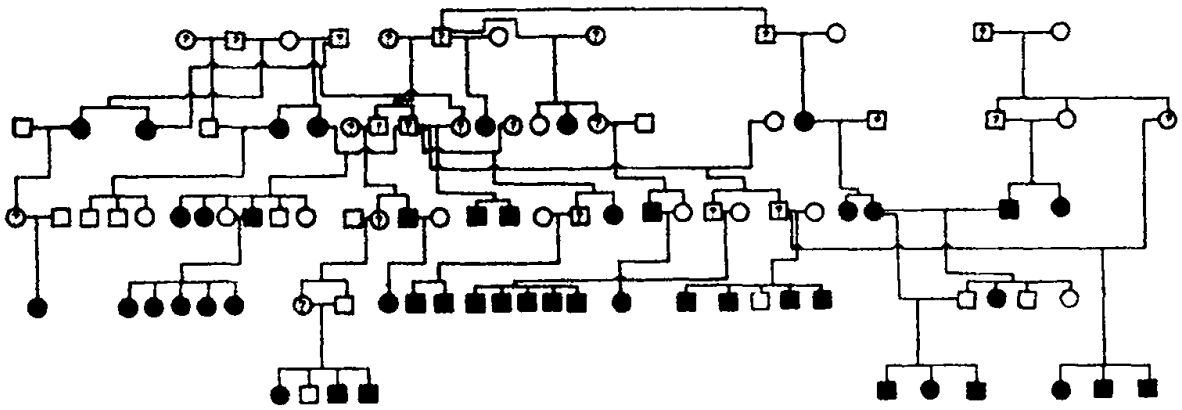
In the year 1924 many skulls of house mice from different laboratory stocks were collected and cleaned for studies of cranial differences existing between distinct strains and also for investigation of the asymmetry caused by early removal of one eye (unpublished results).

It was discovered that in many mice belonging to my redless strain the median suture separating the parietal bones was spread apart in an unusual fashion (Figure 14). This cranial variation seems to be associated with the presence of a blood vessel, pierc-



"PARTED PARIETALS" AND NORMAL
Figure 14

At left is a skull with "parted parietal," an aperture in the median suture, between the eyes, through which a blood vessel passes. In the normal mouse skull (right) the aperture and the blood vessel are lacking.



INHERITANCE OF PARTED PARIETALS

Figure 15

Chart showing relationships of 100 individuals. "Parted parietals" appears to be a simple dominant to normal skull.

ing the skull at an angle, passes to the exterior between these bones. In other individuals both spread suture and vessel are lacking.

Matings had been made both among these rodless mice *inter se* and also with unrelated mice lacking the "parted parietals." The skulls from offspring of several generations following these crosses were cleaned and examined. From the data in hand a pedigree chart has been prepared (Figure 15) showing the genetic relationship and inheritance of "parted parietals" among one hundred individuals.

In Figure 15 squares represent males, circles females. Blackened symbols indicate affected mice. Normals are represented by hollow symbols. Hollow symbols bearing question marks denote individuals the skulls of which have not been examined.

"Parted parietals" is not associated with sex because the trait appears with equal frequency in both sexes. It is apparently inherited as a dominant unit character because (1) offspring of two affected parents are not all affected, (2) offspring of an affected parent and a normal of unrelated strain not bearing "parted parietals" may be all affected, some affected, or none affected. Only the last-named condition would be compatible with behavior as a recessive character.

Population of U. S. Nearly 120 Million

THE population of the continental United States, as of July 1, 1928, according to the estimate of the National Bureau of Economic Research, made public through the release of advance figures taken from a copyrighted statement. issued by the National Bureau, was ,119,306,000, or a growth since the same date ten years ago of 14,299,000.

These figures are embodied in a 500-page report which the National Bureau will publish within a few days, entitled The National Income and Its Purchasing Power, and are revealed in a comprehensive tabulation of the number of persons in this country who are gainfully employed, making those who earn money in the form of salaries or wages, or those whose incomes are derived from the conduct of enterprises which they personally control.

The New York Times

SCIENCE

Useful Mutants, Bred With Radiation

By WILLIAM J. BROAD AUG. 28, 2007

Correction Appended

VIENNA — Pierre Lagoda pulled a small container from his pocket and spilled the contents onto his desk. Four tiny dice rolled to a stop.

"That's what nature does," Dr. Lagoda said. The random results of the dice, he explained, illustrate how spontaneous mutations create the genetic diversity that drives evolution and selective breeding.

He rolled the dice again. This time, he was mimicking what he and his colleagues have been doing quietly around the globe for more than a half-century — using radiation to scramble the genetic material in crops, a process that has produced valuable mutants like red grapefruit, disease-resistant cocoa and premium barley for Scotch whiskey.

"I'm doing the same thing," he said, still toying with the dice. "I'm not doing anything different from what nature does. I'm not using anything that was not in the genetic material itself."

Dr. Lagoda, the head of plant breeding and genetics at the International Atomic Energy Agency, prides himself on being a good salesman. It can be a tough act, however, given wide public fears about the dangers of radiation and the risks of

genetically manipulated food. His work combines both fields but has nonetheless managed to thrive.

The process leaves no residual radiation or other obvious marks of human intervention. It simply creates offspring that exhibit new characteristics.

Though poorly known, radiation breeding has produced thousands of useful mutants and a sizable fraction of the world's crops, Dr. Lagoda said, including varieties of rice, wheat, barley, pears, peas, cotton, peppermint, sunflowers, peanuts, grapefruit, sesame, bananas, cassava and sorghum. The mutant wheat is used for bread and pasta and the mutant barley for beer and fine whiskey.

The mutations can improve yield, quality, taste, size and resistance to disease and can help plants adapt to diverse climates and conditions.

Dr. Lagoda takes pains to distinguish the little-known radiation work from the contentious field of genetically modified crops, sometimes disparaged as "Frankenfood." That practice can splice foreign genetic material into plants, creating exotic varieties grown widely in the United States but often feared and rejected in Europe. By contrast, radiation breeding has made few enemies.

"Spontaneous mutations are the motor of evolution," Dr. Lagoda said. "We are mimicking nature in this. We're concentrating time and space for the breeder so he can do the job in his lifetime. We concentrate how often mutants appear — going through 10,000 to one million — to select just the right one."

Radiation breeding is widely used in the developing world, thanks largely to the atomic agency's efforts. Beneficiaries have included Bangladesh, Brazil, China, Costa Rica, Egypt, Ghana, India, Indonesia, Japan, Kenya, Nigeria, Pakistan, Peru, Sri Lanka, Sudan, Thailand and Vietnam.

Politically, the method is one of many quid pro quos the agency, an arm of the United Nations in Vienna, offers client states. Its own agenda is to inspect ostensibly peaceful atomic installations in an effort to find and deter secret work on nuclear weapons.

Plant scientists say radiation breeding could play an important role in the

future. By promoting crop flexibility, it could help feed billions of added mouths despite shrinking land and water, rising oil and fertilizer costs, increasing soil exhaustion, growing resistance of insects to pesticides and looming climate change. Globally, food prices are already rising fast.

"It's not going to solve the world food crisis," said J. Neil Rutger, former director of the Dale Bumpers National Rice Research Center in Stuttgart, Ark. "But it will help. Modern plant breeders are using every tool they can get."

The method was discovered some 80 years ago when Lewis J. Stadler of the University of Missouri used X-rays to zap barley seeds. The resulting plants were white, yellow, pale yellow and some had white stripes — nothing of any practical value.

But the potential was clear. Soon, by exposing large numbers of seeds and young plants, scientists produced many more mutations and found a few hidden beneficial ones. Peanuts got tougher hulls. Barley, oats and wheat got better yields. Black currants grew.

The process worked because the radiation had randomly mixed up the genetic material of the plants. The scientists could control the intensity of the radiation and thus the extent of the disturbance, but not the outcome. To know the repercussions, they had to plant the radiated material, let it grow and examine the results. Often, the gene scrambling killed the seeds and plants, or left them with odd mutations. But in a few instances, the process made beneficial traits.

In the 1950s and 1960s, the United States government promoted the method as part of its "atoms for peace" program and had notable successes. In 1960, disease heavily damaged the bean crop in Michigan — except for a promising new variety that had been made by radiation breeding. It and its offspring quickly replaced the old bean.

In the early 1970s, Dr. Rutger, then in Davis, Calif., fired gamma rays at rice. He and his colleagues found a semi-dwarf mutant that gave much higher yields, partly because it produced more grain. Its short size also meant it fell over less often, reducing spoilage. Known as Calrose 76, it was released publicly in 1976.

Today, Dr. Rutger said, about half the rice grown in California derives from this dwarf. Now retired in Woodland, Calif., he lives just a few miles from where the descendants grow, he said.

A similar story unfolded in Texas. In 1929, farmers stumbled on the Ruby Red grapefruit, a natural mutant. Its flesh eventually faded to pink, however, and scientists fired radiation to produce mutants of deeper color — Star Ruby, released in 1971, and Rio Red, released in 1985. The mutant offspring now account for about 75 percent of all grapefruit grown in Texas.

Though the innovations began in the United States, the method is now used mostly overseas, with Asia and Europe the leading regions. Experts cited two main reasons: domestic plant researchers over the decades have already made many, perhaps most of the easiest improvements that can be achieved with radiation, and they now focus on highly popular fields like gene splicing.

“Most scientists here would say it’s pretty primitive,” Norman T. Uphoff, a professor of government and international agriculture at Cornell University, said of the method. “It’s like being in a huge room with a flashlight.”

But the flashlight is cheap, which has aided its international spread.

Today, the process usually begins with cobalt-60, a highly radioactive material used in industrial radiography and medical radiotherapy. Its gamma rays, more energetic than X-rays, can travel many yards through the air and penetrate lead.

Understandably, the exposure facilities for radiation breeding have layers of shielding. Scientists run small machines the size of water heaters that zap containers full of seeds, greenhouses that expose young plants and special fields that radiate row upon row of mature plants. In Japan, one circular field is more than 650 feet wide. A shielding dike some 28 feet high rises around its perimeter.

Dr. Lagoda said a rust fungus threatened the Japanese pear, a pear with the crisp texture characteristic of apples. But one irradiated tree had a branch that showed resistance. He said the Japanese cloned it, successfully started a new crop and with the financial rewards “paid for 30 years of research.”

The payoff was even bigger in Europe, where scientists fired gamma rays at barley to produce Golden Promise, a mutant variety with high yields and improved malting. After its debut in 1967, brewers in Ireland and Britain made it into premium beer and whiskey. It still finds wide use.

“The secret,” reads a recent advertisement for a single malt Scotch whiskey costing $49.99 a bottle, is “the continued use of finest Golden Promise barley and the insistence on oak sherry casks from Spain.”

The atomic agency in Vienna has promoted the method since 1964 in outreach programs with the Food and Agriculture Organization of the United Nations, in Rome.

Starting roughly a decade ago, for instance, the atomic agency helped scientists fight a virus that was killing cocoa trees in Ghana, which produces about 15 percent of the world’s chocolate. The virus was killing and crippling millions of trees.

In the city of Accra on the Atlantic coast, at the laboratories of the Ghana Atomic Energy Commission, the scientists exposed cocoa plant buds to gamma rays. The mutants included one that endowed its offspring with better resistance to the killer virus.

The scientists planted the resistant variety on 25 farms across Ghana “with no evidence of a resurgence,” M. R. Appaih, executive director of the Cocoa Research Institute of Ghana, told the agency.

The atomic agency had similar success in the Peruvian Andes, where some three million people live on subsistence farming. The region, nearly two miles high, has extremely harsh weather. But nine new varieties of barley improved harvests to the point that farmers had surplus crops to sell.

In 2006, Prof. Gomes Pando won the Peruvian prize for Good Government Practices for her work on the radiation mutants.

In Vietnam, the agency has worked closely with local scientists to improve production of rice, a crop that accounts for nearly 70 percent of the public’s food energy.

One mutant had yields up to four times higher than its parent and grew well in acidic and saline soils, allowing farmers to use it in coastal regions, including the Mekong Delta.

Last year, a team of 10 Vietnamese scientists wrote in an agency journal, Plant Mutation Reports, that the nation had sown the new varieties across more than one million hectares, or 3,860 square miles. The new varieties, they added, “have already produced remarkable economic and social impacts, contributing to poverty alleviation in some provinces.”

Dr. Lagoda said that radiation breeding, though an old technology, was undergoing rapid growth. New methods that speed up the identification of mutants are making radiation breeding even more popular, he said.

“Now it becomes interesting again,” he said of the method. “It’s not a panacea. It’s not the solution. But it’s a very efficient tool that helps us reduce the breeding time.”

Spreading the secret, Dr. Lagoda added as he played with his tiny dice, “is very gratifying because we really, really help people.”

Correction: September 1, 2007

An article in Science Times on Tuesday about the use of radiation to produce mutations of crops misstated the properties of Japanese pears. Although Japanese pears have the crisp texture characteristic of apples, they are not a cross between apples and pears. They are pears, from the species Pyrus pyrifolia.

A version of this article appears in print on , on page F1 of the New York edition with the headline: Useful Mutants, Bred With Radiation.

RESEARCH ARTICLE
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Gamma Radiation Induced Mutations in Mungbean

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ABSTRACT: Seeds of mungbean varieties KPS 2, VC 6468-11-1B, their F_1 and F_2 were treated with gamma rays (Cs-137 source) at the dose of 500 Gy. The M_1 seeds were sown in the field with the controls (non-irradiated seeds) and bulk-harvested. The M_2 seeds were sown to observe their characters and number of mutants in each population. Among over 430,000 plants observed, irradiated F_1 population gave the highest frequency of mutants at 0.168%, followed by F_2, VC 6468-11-1B, and KPS 2 at 0.165%, 0.152%, and 0.142%, respectively. Mutant characters were grouped as chlorophyll, leaf, flower, and pod mutants. Chlorophyll mutations included albino, coppery leaf, light-green leaf, variegated leaf, waxy leaf, white streak leaf, and xantha leaf. Leaf mutations were lanceolate leaf, narrow-rugose leaf, multiple leaflet, round-cuneate leaf, unifoliate leaf, and wrinkled leaf. The flower mutant was cock's comb raceme while the pod mutant was a lobed one. All mutants were purified for genetic study and possible uses of the traits.

KEYWORDS: *Vigna radiata*, mungbean, gamma rays, mutants.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) (2n=2x=22) is a self-pollinated legume originated in South Asia. It is an economically important crop in India, Pakistan, Thailand, Vietnam, Myanmar, and China with the combined planted area of over 5 million ha. The crop is considered rather wild as it still gives low seed yield (<1 t/ha), with uneven maturity. This opens an ample room for mungbean breeders to improve the crop. Besides natural genetic variation available in mungbean germplasm collections, mutation techniques are proven useful in obtaining novel traits and creating genetic variability. Gamma irradiation as a mutagen can induce useful as well as harmful mutation in plants[1,2]. Singh and Sharma[3] isolated a few pentafoliate and tetafoliate mutants from the gamma rays- and ethyl methanesulphonate (EMS) - treated mungbean. These mutants showed a significant increase in dry matter production, total chlorophyll content and yield, as compared to their parents in M_2 and M_3 generations. Santos[4], and Bahl and Gupta[5] described the mutant characters and their inheritance in mungbean and reported that variegated, multifoliata, xantha, chlorina, albino, unifoliata were each controlled by a recessive gene. Variation in quantitative traits by mutation breeding was also reported by several scientists[6-11]. The major traits were seed yield, seed size, pods per plant, seeds per pod, days to maturity, and plant height. Additionally, Wongpiyasatid *et al*[12] reported an improvement in resistance to powdery mildew, Cercospora leaf spot, and cowpea weevil through gamma radiation induced mutation.

The objective of this study is to induce mutation in four mungbean populations using gamma radiation to determine the mutation frequency, observe the mutant traits and purify them for possible uses.

MATERIALS AND METHODS

Seeds of the parental lines, 'Kamphaeng Saen 2' (KPS 2) designated as P_1, and VC 6468-11-1B designated as P_2 were obtained from the Asian Region Center of the Asian Vegetable Research and Development Center (ARC-AVRDC), Kasetsart University, Kamphaeng Saen, Nakhon Pathom, Thailand. KPS 2 is a popular Thai mungbean cultivar sown over 150,000 ha annually, owing to its high yielding, shiny seed coat with moderately large seed size (~66 g per 1000 seeds), green hypocotyl, and moderately resistant to powdery mildew and Cercospora leaf spot diseases. VC 6468-11-1B is an elite breeding line with a dull seed coat and a large seed size (~70 g per 1000 seeds), purple hypocotyl, and resistant to both diseases.

Crosses were made using KPS 2 as the female parent. The parents and F_1 seeds were sown in the successive

season. All F_1 seedlings had purple cotyledons confirming that they were derived from crossed seeds, since the purple hypocotyl is dominant to the green one. Another set of F_1 seed was also made in parallel to the production of F_2 seeds. Thus, all four mungbean populations (P_1, P_2, F_1, and F_2) were finally obtained in that same season. The initial M_0 seeds were determined for germination percentage in each population and converted to the seed weight of 156, 187, 159, and 212 g for KPS 2, VC6468-11-1B, F_1, and F_2 respectively. Each amount is equivalent to ~2500 seeds that can readily germinate.

The gamma irradiator used in this study is installed at the Gamma Irradiation Service and Nuclear Technology Research Center (GISC), Kasetsart University, Bangkok. It was manufactured by J.L. Shepherd & Associates, under the Model MARK 1-30, Serial No. 1116, loaded with 4500 Curies of Cs-137 having a half-life of 30.12 years. The gamma irradiator was calibrated to irradiate 500 Gy of gamma rays to the seed lots for 82 minutes. The rate of 500 Gy was found to produce much variance while leaving over 60% of the surviving plants[13]. The M_1 seeds were sown in the field surrounded by non-irradiated population as the control. The M_2 seeds were bulk-harvested in each population. There were 7.76, 5.12, 11.02, and 8.72 kg from KPS 2, VC6468-11-1B, F_1, and F_2, respectively. The seeds were drilled in rows, after which the mutants were periodically observed right after germination. In each visit to the field, the mutant plants were marked with bamboo sticks for subsequent observations. Data were recorded on characters and number of the

Table 1. Amount of M_2 mungbean seed sown, number of seedlings germinated, and number of mutants found in the populations of KPS 2, VC6468-11-1B, their F_1 and F_2.

Populations	M_2 seeds sown (kg)	No. of seedlings germinated	Mutant Type					Total	Percent of mutants
			Albino(lethal)	Chlorophyll	Leaf type	Flower	Pod		
KPS	27.76	127,880	113	27	35	0	7	182	0.143
VC6468-11-1B	5.12	81,708	45	26	45	0	8	124	0.152
F_1	11.02	134,607	164	16	35	1	10	226	0.168
F_2	8.72	89,647	105	8	29	0	6	148	0.165
Total	**32.62**	**433,842**	**427**	**77**	**144**	**1**	**31**	**680**	**0.157**

Table 2. Types and number of mutants found in M_2 plants of the four mungbean populations.

Mutant characters	Populations				Total
	KPS 2	VC 6468-11-1B	F_1	F_2	
1. Chlorophyll mutation					
Albino	113	45	164	105	427
Coppery leaf	1	0	0	0	1
Light green leaf	2	2	3	0	7
Variegated leaf	2	3	4	3	12
Waxy leaf	2	6	5	0	13
White streak leaf	1	2	2	3	8
Xantha leaf	19	13	2	2	36
2. Leaflet mutation					
Lanceolate leaflet	2	2	2	0	6
Multiple leaflet	29	37	29	27	122
Narrow-rugose leaflet	2	1	0	0	3
Round-cuneat leaflet	0	0	0	1	1
Unifoliate leaf	2	0	0	1	3
Wrinkled leaf	0	5	4	0	9
3. Flower mutation					
Cock's comb raceme	0	0	1	0	1
4. Pod mutation					
Lobed pod	7	8	10	6	31
Total	**182**	**124**	**226**	**148**	**680**

Table 3. Description of the mutant characters found in M_2 plants of the four mungbean populations.

Mutant characters	Character descriptions
1. Chlorophyll mutation	
Albino	Entirely white leaves. Seedlings survived for less than 2 weeks after germination
Coppery leaf	Copper-like color leaflet beginning from flowering till harvesting
Light-green leaf	Lighter green leaves as compared to normal leaves
Variegated leaf	Persistent variegated yellow-green leaves
Waxy leaf	Normal leaf shape with pale waxy leaflet
White streak leaf	White streak from edge to middle vein
Xantha	Orange yellow to light yellowish white, survived for only 2-3 weeks after germination
2. Leaflet mutation	
Lanceolate leaf	Elongated middle leaflet with broader lateral leaflets
Multiple leaf	Compound leaf with 4 - 9 leaflets per leaf
Narrow-rugose leaf	Narrow and elongated leaflet
Round-cuneat leaf	Short petiole, round leaf, did not set pod
Unifoliate leaf	Single leaf, did not set pod
Wrinkled leaf	Leaf has wrinkled character
3. Flower mutation	
Cock's comb raceme	Raceme look like cock's comb, did not set pod
4. Pod mutation	
Lobed pod	Distinct lobes on pod possibly due to semi-sterility

mutants. At maturity, each mutant plant was individually harvested. The remaining plants were bulk-harvested for M_3 seeds and sown for further observation.

Field cultural practices on this experiment were conducted based on standard management for mungbean grown in Thailand. Briefly, the seeds were drilled in rows of 50 cm apart at the rate of 20 seeds per a meter. Weeds were controlled by pre-emergence spraying of Imazathapyr at 250 g(ai)/ha. Late weeds were eradicated by hand weeding twice at 15 and 30 days after sowing. Insects were controlled by spraying with triazophose (Hostathion 40% EC) at the rate of 40 cc per 20 liters of water when the insect population was building up beyond the threshold level. Irrigation water was applied during the cropping season as needed.

Results and Discussion

Since the gamma rate of 500 Gy was almost at Lethal Dose-50 (LD-50) for mungbean[13], the M_1 seed lost its germination up to 20-30% from the effect of irradiation. Some seedlings showed either albino or xantha leaf, and died prematurely. A number of mutant plants were identified in M_2 generation and the mutation percentages in KPS 2, VC6468-11-1B, F_1, and F_2 population were 0.142, 0.152, 0.168, and 0.165, respectively (Table 1). The percentages were much smaller than that reported by Srichot[13] and Thongpimyn[14] who found the mutant rate of up to 1-4% in both qualitative and quantitative traits. In our experiment, no distinct mutant plants were found regarding yield components, possibly due to such a low mutant rate.

The mutants found were mainly of leaf chlorophyll mutation such as albino, coppery leaf, light-green leaf, variegated leaf, waxy leaf, white streak leaf, and xantha leaf. Leaf mutations were lanceolate leaflet, narrow-rugose leaflet, multiple leaflet, round-cuneat leaflet, unifoliate leaf and wrinkled leaf. Flower mutation gave looks like cock's comb with pollen sterility. Similar mutants were also reported by Lamseejan *et al*[15], Santos[4], and Srichot[13]. A lobed pod mutation with fewer seeds per pod was also found. This trait may associate with partial sterility, causing constriction at the point where there was undeveloped seed. The number of mutants found and their descriptions are shown in Table 2 and 3. These mutants were not found in the control populations. Therefore, they were considered the real mutants and not the results of genetic recombination between the parental lines.

Characteristics of leaflet mutants are shown in Fig 1, while those of the other types are given in Fig 2. The unifoliate leaf mutant was also sterile, in agreement with that reported by Santos[4]. The mutant produced numerous flower buds but failed to open. The round-cuneat leaflet mutant produced flowers but its pollen scattered all over the corolla and thus expressed partial sterility. However, coppery leaf, variegated leaf, waxy leaf, white steak leaf, lanceolate leaflet, narrow-rugose leaflet, multiple leaflet, and wrinkled leaf were fertile

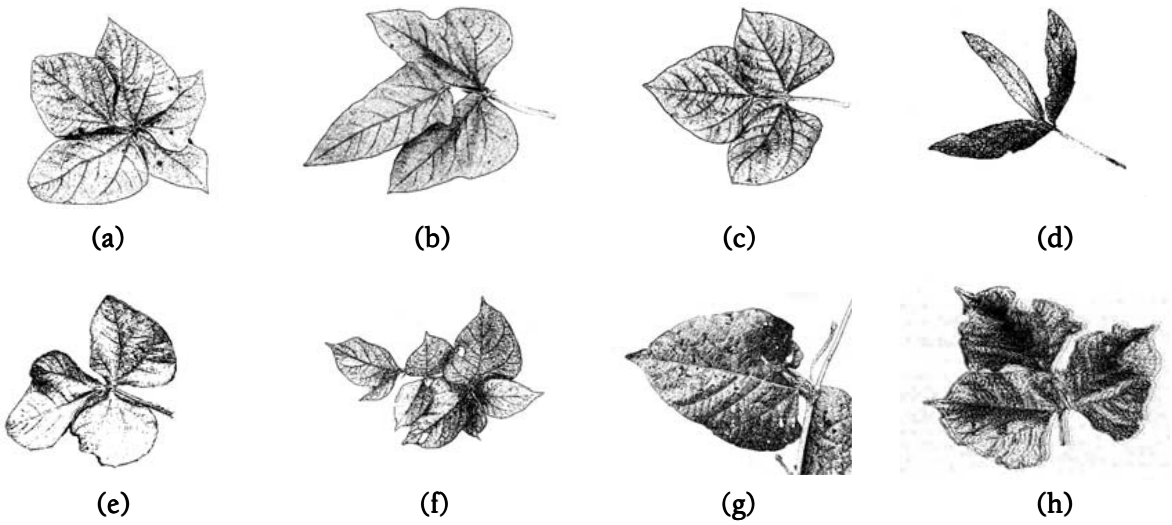

Fig 1. Leaf mutant variation found in the M_2 plants: (a) five multiple leaflet, (b) lanceolate leaf, (c) normal trifoliate leaf, (d) narrow-rugose leaf, (e) round-cuneat leaflet, (f) seven multiple leaf, (g) unifoliate leaf, (h) wrinkled leaf.

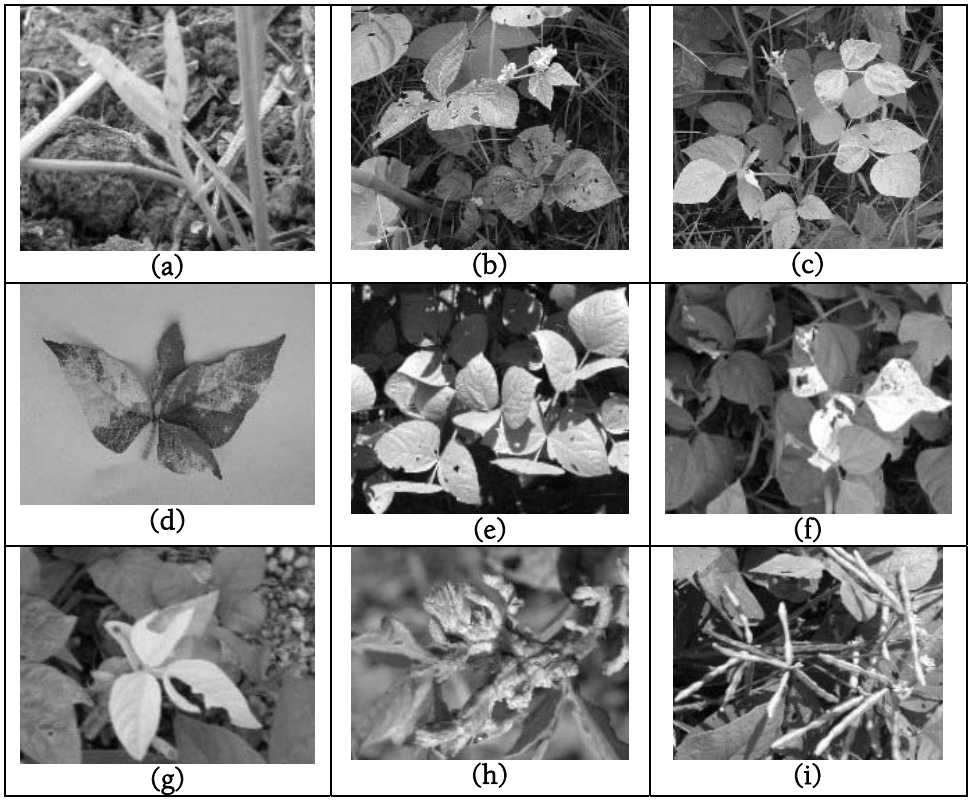

Fig 2. Chlorophyll, flower, and pod mutations found in the M_2 plants: (a) albino, (b) coppery leaf, (c) light-green leaf, (d) variegated leaf, (e) waxy leaf, (f) white streak leaf, (g) xantha leaf, (h) cock' s comb raceme, (i) lobed pod due to sparse seed set.

with low yield. The variegated leaf and narrow-rugose leaf mutants produced only few pods while waxy leaf produced pods with lean seeds. These mutants have been reported by a number of scientists,[1,3-6,13,15] but we have found them all in one experiment, possibly due to the high population used (up to 433,842 seedlings).

Although not statistically significant, the rate of mutation was slightly higher in F_1 and F_2 as compared to the parents, since the progenies are more heterozygous than the parents. The heterozygous genotypes have more possible target alleles to mutate than the pure line parents. However, the mutation rate in this experiments is rather low and thus the result needs to be confirmed in more experiments. The mutant

plants were individually harvested for 2 consecutive generations to establish pure mutant lines for further studies. All mutants were bred-true and can be utilized in breeding and genetic study. Some multiple leaflet lines set profuse pods that might be useful as a marker for mungbean yield improvement in the future.

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References

1. Gupta PK (1996) Mutation breeding in mungbean. *In* Asthana AN and Kim DH (Eds.) Recent Advances in Mungbean Research. Indian Society of Pulses Research, Kanpur, India, pp 124-36.
2. Micke A and Donini B (1993) Induced mutations. *In* Hayward MD, Bosemark NO and Romagosa I (Eds.) Plant Breeding Principles and Prospects. Chapman and Hall, London, pp 52-62.
3. Singh VP and Rashmi Sharma (1993) γ-Rays-and EMS-induced leaf mutants in mungbean (*Vigna radiata* (L) Wilczek). *Curr. Sci.* **65**, 636-8
4. Santos IS (1969) Induce of mutations in mungbean (*Phaseolus aureus* Roxb.) and genetic studies of some of the mutants. Induced mutations in plants. IAEA, Vienna, pp. 169-79.
5. Bahl, JR and Gupta PK (1982) Inheritance of two induced lethal chlorophyll mutations in mungbean. *Curr. Sci.* **53**, 147-8.
6. Ekasomtramage Theera (1982) Induce mutations in mungbean by gamma irradiation. M.S. Thesis, Kasetsart University, Bangkok (in Thai with English abstract).
7. Singh VP and Yadav RDS (1982) Gamma induced yellow testa colour mutant of green gram cv T44. *Curr. Sci*. **51**, 891-2
8. Srinives P, Hual-alai N, Saengchot S and Ngampongsai S (2000) The use of wild relative and gamma radiation in mungbean and blackgram breeding. Proc 7th MAFF International Workshop on Genetic Resources, 13-15 October 1999. AFFRC and NIAR, Japan.
9. Wongpiyasatid A, Chotechuen S, Hormchan P, Ngampongsai S, Lamseejan S and Pichitporn S (1998) Mutant mungbean lines from radiation and chemical induction. *Kasetsart J. (Nat. Sci)* **32**, 203-12.
10. Wongpiyasatid A, Chotechuen S, Hormchan P, Ngampongsai S and Promcham W (2000) Induced mutations in mungbean breeding: regional yield trial of mungbean mutant lines. *Kasetsart J. (Nat. Sci.)* **34**, 443-9.
11. Yaqoob M and Rashid A (2001) Induce mutation studies in some mungbean (*Vigna radiata* (L.) Wilczek) cultivars. Online *J. Biol. Sci*. **1**, 805-8.
12. Wongpiyasatid A, Chotechuen S, Hormchan P and Srihuttagum M (1999) Evaluation of yield and resistance to powdery mildew, *Cercospora* leaf spot and cowpea weevil in mungbean mutant lines. *Kasetsart J. (Nat. Sci.)* **33**, 204-15.
13. Srichot Wanipa (1982) Radiation effects in mungbean. M.S. Thesis, Kasesart University, Bangkok (in Thai with English abstract).
14. Thongpimyn Bandit (2002) Mungbean improvement by gamma radiation. M.S. Thesis, Suranaree University of Technology, Nakhon Ratchasima (in Thai with English abstract).
15. Lamseejan, S, Samutkupt S and Wongpiyasatid A (1983) Gamma ray induce mutants in mungbean. *J. Agri. Sci.* **16**, 446-54 (in Thai with English abstract).

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Mutation breeding by ion implantation

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Ion implantation as a new mutagenic method has been used in the rice breeding program since 1986, and for mutation breeding of other crops later. It has been shown, in principle and in practice, that this method has many outstanding advantages: lower damage rate; higher mutation rate and wider mutational spectrum. Many new lines of rice with higher yield rate; broader disease resistance; shorter growing period but higher quality have been bred from ion beam induced mutants. Some of these lines have been utilized for the intersubspecies hybridization. Several new lines of cotton, wheat and other crops are now in breeding. Some biophysical effects of ion implantation for crop seeds have been studied.

1. Introduction

The induced mutation has been emphasized in crop breeding since 1927 [1], the year Mueller observed the X-ray induced genetic mutation, and has been successfully used for breeding many improved varieties of different kinds of crops. Though various kinds of mutagenic source, such as γ-ray, laser ray, neutron, chemomophasis etc. have been developed, people still insist trying to develop better ones with high mutation rate, wider mutational spectrum and, if possible, user controlled. In 1972, FAO/IAEA began to sponsor an international research program to develop new mutagenic sources for improving the mutational spectrum and increasing the mutation rate. Ten countries have taken part in it. Since then, the neutron induced mutation has become one of the main methods.

In 1986 a program of ion beam induced mutation breeding for rice was started by Institute of Plasma Physics, the fusion research centre in China, collaborating with the Institute of Rice, AAAS, to explore this mutagenic way [2]. In the next section we will report the results obtained from this program. The preliminary conclusion we could get from this four years experience is that the ion beam is a very attractive mutational source.

2. Experiments

The ion beam implantation system we have used has been described elsewhere [3], a high-current dc ion source of the ORNL type (heat cathode, reflective arc, double-plasma ion source) [4]. At 50 keV ion energy, the N^+ current could reach 150 mA. The target plate is 2 m from the ion source, and the uniformity of ion distribution within an area of 15 cm diameter on the plate could be near 90%. The parameters used in our experiments were: 30–50 keV N^+, $(0.5–2)\times10^{16}$ ions/cm^2. The target plate on which the dry seeds were put on with the embryo part facing the ion beam, is temperature controllable by a water cooling system.

In 1986–1987 three rice lines, Luwuhong, CO12 and 02428, which are very popular in China, have been selected to be implanted. The latter two are varieties of Indica rice. In the first generation M1, the irradiation damage and lethality was observed and a relative survival rate as high as 90% was found (table 2). The setting rates of M1 of implanted rice are remarkably lower than that of the control group. In M2, the hereditary variation and mutational effect have been observed and analyzed in detail. A very high mutation rate has been obtained (table 1) even though the implantation parameters were not optimal.

Table 1
Mutation rate of M2 [5]

Varieties	Chlorophyl [%]	Ripe stage [%]	Plant height [%]	Fertility [%]	Other [%]	Total M.R. [%]
Luwuhong	0.21	3.4	3.4	0.0	1.7	8.6
CO12	0.39	0.0	0.0	2.1	0.0	2.4
02428	0.59	5.9	0.4	0.0	7.5	14.4
Average	0.39	3.1	1.3	0.7	3.1	8.5

0168-583X/91/$03.50

Table 2
Survival rate in M1 of implanted seeds

Varieties	M1 [%]	CK [%]	Rel. S. R. [%]
Luwuhong	78.4	89.3	87.8
CO12	79.7	85.7	93.3
02428	77.7	82.4	94.4
Average	78.7	85.8	91.7

Table 3
New lines from Luwuhong

New line	Number of ears	Number of grains	Setting rate	Weight per 1000 grains [g]	Production per plant [g]	Growth rate per plant [%]
J903	12.0	138.7	70.5	22.4	26.2	50.8
J910	10.7	165.5	77.0	24.0	32.6	86.9
J911	9.0	124.5	77.4	23.8	20.7	18.2
J912	11.0	130.0	69.2	21.3	21.1	20.7
CK	9.3	100.0	86.6	21.5	17.4	00.0

It has been found that the lower the setting rate of M1, the higher the mutation rate in M2. The mutants with good agro-characters, such as high yield rate, broader disease resistance, leaf-type, ear-type and plant-type, have been selected from M2. Some of these mutants have been bred to be new lines through several generations of breeding in the south of China. Since 1988, some lines of rice, such as Zhe 15, and other crops began to be implanted and then bred.

3. Results and analysis

Till now, three new lines from irradiated 02428, four from CO12 and four from Luwuhong have been bred. They have been greatly improved with respect to their control groups in high grain yield, 20–40% higher than the control group in small-area trials (table 3), broader disease resistance, and plant-type, leaf-type and ear-type, etc. It should be reported that a new line has been selected from ion-implanted Zhe 15 rice. It not only has the characteristics of high grain yield (150 grains per ear and 6 spikes on the tiller in average) and of resisting White Leaf Disease, but also has high-quality rice. It is hopeful that the contradiction between high grain yield and good quality of rice could be solved.

The chlorophyl damage rate of ion implantation in our case is 1.02%, 0.3% and 0.37% for Luwuhong, CO12 and 02428 from M1, respectively. All the chlorophyl damaged plants, except a yellow one, could not survive due to the low photosynthetic efficiency. The survived yellow plant from Luwuhong was very small and the leaves could begin to change the colour to green in the flowering. These characteristics have been repeated in posterities. This yellow plant was intercrossed with the mutants from 02428, and a new hybridized combination

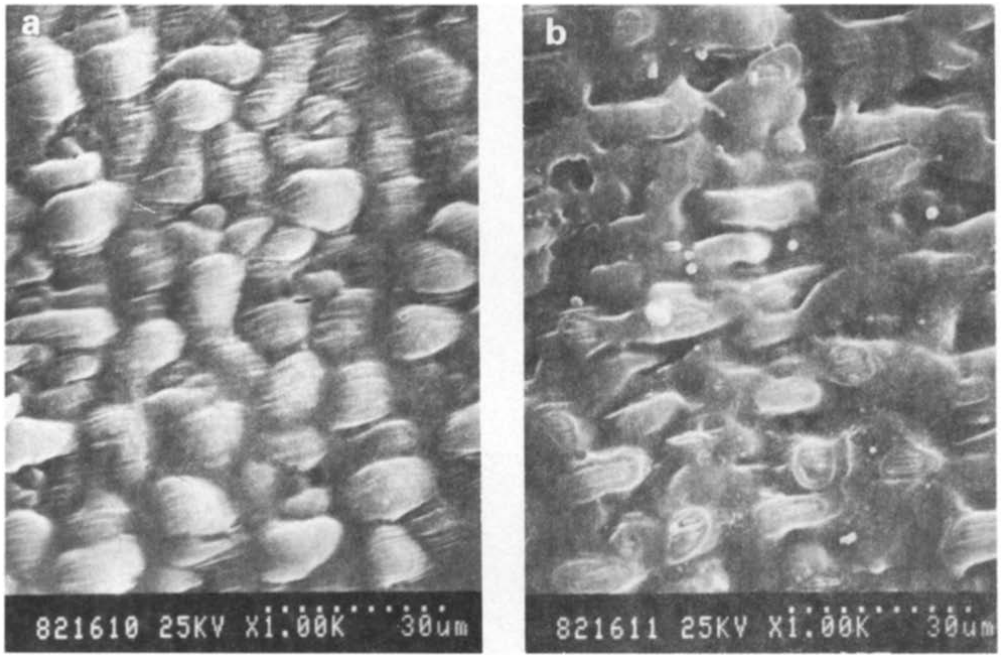

Fig. 1. Photographs of surface cells of soy bean damaged by N^+ implantation; (a) without N^+ implantation, (b) with N^+ implantation.

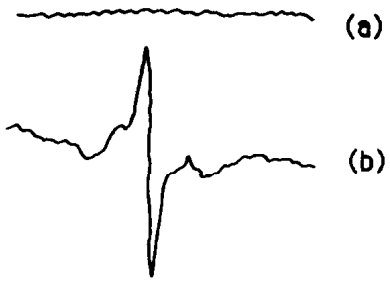

Fig. 2. The free radicals of maize seed induced by N^+ implantation; (a) nonirradiated part of maize, (b) irradiated part of the same seed.

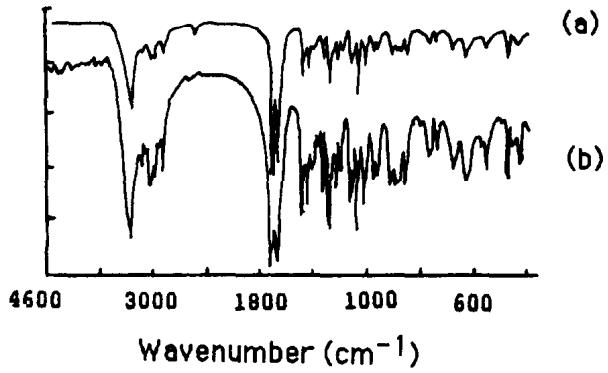

Fig. 4. Infrared spectra of thymine; (a) with N^+ implantation, (b) without N^+ implantation.

has been obtained. It is 80 cm high, 17 spikes on the tiller and 107 grains per ear in average, and 98% fertility. It seems possible that the problem of supercompatibility of plant height with growth period in rice hybridization for the subspecies could be solved. Moreover, it is easy to dig up the bastard from the hybridized combination due to the yellow label.

As is stated above, ion implantation is a very effective tool to improve crops. It is desirable to obtain a higher mutation rate and a wider mutational spectrum with a higher survival rate for the mutation breeding. How can researchers explain the phenomena of the higher mutation and survival rate in improving crops induced by ion implantation? We studied the interaction between incoming ion and seeds from the view point of physics during the breeding. The results show that the induced damage of a seed by the ion beam was only in a partial zone of the surface of the seed, and in the zone the irradiated damage was also serious it could be repaired because of both high LET and slow ion depositing. This is greatly different from other ray mutation in which the mutational zone is usally random. The surface features of the seed with or without ion implanting were observed using scanning electron microscopy (fig. 1). Some of their walls were cut off so that the cytoplasm was exposed [6]. The radicals induced by the ion beam in the embryo part of the seed could be observed by ESR. Fig. 2 shows the ESR spectrum of a maize seed, nonirradiated (a) and irradiated (b). All the radicals produced by the ion beam were in a partial zone irradiated by the ion beam, and could be preserved in dry seed for a long period (18 months after irradiation the concentration of radicals still was the same).

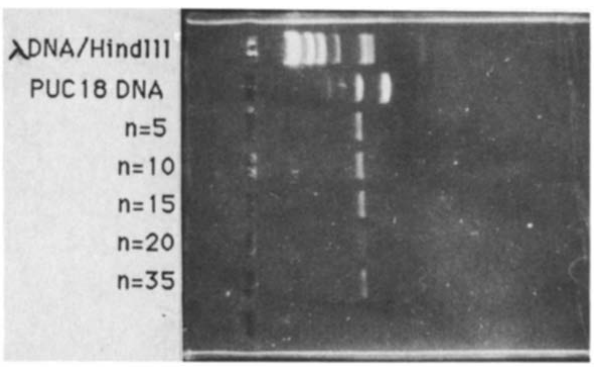

Fig. 3. Electrophoresis spectra of DNA irradiated by N^+ beam. The dose is 10^{15} ions/cm^2 for $n=1$.

The nitrogen ion irradiation has many effects on DNA. The electrophoresis measurements showed that the nitrogen ion could very effectively change the superhelical structure of DNA to an open-circular structure, the latter of which is very stable under the N^+ irradiation (fig. 3). The DNA with linear structure could very easily be broken by a nitrogen ion. Very low dose of irradiation was enough to break the double chain of DNA. ESR also could test the radicals produced by N^+ ions in DNA. The concentration of the radicals in DNA and dry seed was weakly reduced.

The change of the infrared spectrum of thymine due to N^+ irradiation (fig. 4) has been studied carefully. The appearance of a new peak at 2360 cm^{-1}, the changes of the peaks at 3320 and 3160 cm^{-1}, and the splitting of the 1696 cm^{-1} peak, have shown that some NH, CN and CH groups have been partially broken down and some new amino constructions were formed by bonding of irradiated biomolecules with incoming N^+ ions. The spectrum intensity within 1300–400 cm^{-1} was reduced. This could show the breakage of bonds in NH, CN, CH and CO groups.

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References

[1] H.J. Muller, Science 66 (1927).
[2] Yu Zengliang et al, Anhui Agri. Sci. 1 (1989) 17.
[3] Yu Zengliang et al, Nucl. Sci. Eng. 6 (1986) 167, in Chinese.
[4] Yu Zengliang, He Jianjun, Zhou Jin and Deng Jianguo, Vac. Sci. Technol. 9 (1989) 379, in Chinese.
[5] Wu Yuejin et al., Anhui Agri. Sci. 2 (1989) 15.
[6] Yu Zengliang, Deng Jianguo and He Jianjun, Anhui Agri. Sci. 1 (1990) 12.