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INCREASING MUTATION FREQUENCY AFTER SEED IRRADIATION IN SORGHUM, *SORGHUM BICOLOR* (LINN.) MOENCH

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ABSTRACT

The frequencies of somatic and germinal mutations were first obtained in fast-growing, slow-growing, early-flowering, and late-flowering M_1 plants. Later, the mutation rate was determined from M_1 plants grown in soil known to enhance or retard vegetative growth. In both cases, the mutation rates were markedly higher in slow-growing than in fast-growing plants. The data suggest additional means of increasing the frequency of induced mutations. The employment of uniform techniques for conducting radio-sensitivity studies is proposed for the purpose of obtaining comparable M_1 data for universal usage.

Introduction

Mutations have often been commonly referred to as the "touchstones" of genetic science for having provided means of unravelling new mechanisms that have advanced biological thinking in our time. Moreover, the induction of useful mutations during the past 40 years has led to the official release of hundreds of improved crop varieties in different regions of the world. The demand for more new genotypes to meet the needs of an ever-increasing human population justifies a continuing study of how the induced mutation rate could be increased.

Suitable laboratory techniques have been developed in the 1960's for reducing radiation damage in seed mutagenesis (Nilan and Konzak, 1965; Konzak *et al.*, 1965) thereby improving the efficiency of mutation induction and increasing the rates of mutation in barley. Other methods such as reirradiation (Emery, 1972; Soriano, 1964; Frydenberg and Sandfaer, 1964) and combined radiation and chemical treatments (Lenhouts *et al.*, 1983; Soriano, 1968) have been shown to increase the frequency of mutations. However, the inavailability of even these simple laboratory procedures to most mutation workers, as evidenced by published literature, indicates the need for investigating other means of maximizing the occurrence of mutations in irradiated material.

Most mutation studies have been largely superficial (Nilan *et al.*, 1979) in dealing with the induction and selection of mutants as these gave very little interest

and attention, if at all, to such academic but more substantive problems as increasing the mutation rate, mutagenic specificity (Conger, 1979) and mechanism of action or nature of the mutational event (Nilan *et al.*, 1979).

This study was conducted while the author was engaged in the induction and selection of mutants for resistance to the mosaic disease of sorghum, a disease caused by viruses in many grassoid species, both wild and cultivated. The need for inducing many types of mutations is due to the presence of these plant vectors, some of which are believed to harbor different viral biotypes, viz., plant mutants resistant to one biotype are thought to be susceptible to other biotypes from related vectors growing nearby. Wallace (1965) obtained a marked increase in the frequency of mutations for resistance to the *Helminthosporium* disease in oats when seed moisture content was altered.

The objectives of this work are: (1) To determine the somatic and germinal mutation rates in fast-growing and slow-growing M_1 plants as well as in early-flowering and late-flowering plants; and (2) To devise means of increasing the mutation rate based on the results of the first study.

Materials and Methods

Dormant seeds of sorghum, variety UP Sg-5, were stored in nitrogen atmosphere in a moist dessicator for seven days to bring about a uniform moisture content of 14%. The seeds were placed in sealed plastic bags in nitrogen atmosphere and treated with gamma radiation at the Philippine Atomic Research Center at doses ranging from 10-40 Kr. Unirradiated seeds from the same source were used as controls. Soon after treatment, the seeds were rehydrated in water for two hours at a constant temperature of 30°C, rinsed briefly in water and sown on moist tissue paper in a petri dish to sprout. After 14 days of germination, seedlings that were at least eight cms. tall were transplanted in field rows at the rate of three seedlings per hill at distances of 30 cms. between rows and 20 cms. in the row. In the experiments on increasing mutation frequency, the plots were either weeded or not weeded and fertilizer was either applied or not depending on the requirements of a particular test. When needed, commercial urea fertilizer was applied at the rate of approximately 150 kgms. per hectare two weeks after transplanting.

M_1 plant height was measured 30 days after transplanting and at the time of flowering. Based on the height measurements, the plants were marked either as fast-growers or slow-growers. Fast-growing plants were 30 cms. or more tall 30 days after transplanting and 100 cms. or more tall at the onset of flowering. These height measurement represented growth increments of about 22 cms. or more during the first 30 days of field growth and about 70 cms. or more during the second 30-day growth period.

The slow-growing plants, on the other hand, were only 15 cms. or less tall after the first 30 days of field growth and about 48 cms. or less during the second

30-day growth period. These height measurements represented growth increments of only about seven cms. or less during the first 30 days after transplanting and only about 23 cms. or less during the second 30-day growth period in the field.

The flowering date of the control, based on 50% heading and found to be 60 days after transplanting, was the basis for classifying the early-flowering and late-flowering M_1 plants. The early-flowering plants flowered on or before 60 days after transplanting while the late-flowering plants 88 days or more after transplanting. In both the plant height and flowering date tests, the plants that were intermediate in height and heading date were not used in this particular study.

The plants were scored for the occurrence or absence of somatic mutation or chimera 30 days after transplanting and at heading time. The young panicles were bagged individually to insure self-fertilization, harvested upon maturity, dried for one month under room conditions and stored for about two months for seed dormancy. For the frequency of germinal mutations, i.e. chlorophyll-deficiency seedlings, the panicles with their seeds intact were soaked in water overnight and germinated in small plastic bags in a well-lighted laboratory room. This method is preferred over the seedbed method as it avoids the possible influence of mineral deficiency in the soil on chlorophyll formation.

Results and Discussion

Frequency of somatic mutations

The frequency of M_1 plants bearing somatic mutations is shown in Table 1. The data indicates that the frequency of M_1 plants bearing somatic mutation was higher in slow-growing than in fast-growing plants. While only 1.29-3.13 somatically-mutated fast-growing plants per 100 individuals were found at doses of 20-40 Kr, a frequency of from 6.70-11.02 per 100 slow-growers showed chimeral sectors. No somatic mutation was found in the control and at the dose of 10 Kr. in both fast and slow growers.

Table 1. Frequency of M_1 somatic mutations in fast-growing and slow-growing plants

Dose (Kr)	*Fast-growing*			*Slow-growing plants*		
	Total M_1 plants	*Total mutated plants*	*Mutations per 100 plants*	*Total M_1 plants*	*Total mutated plants*	*Mutations per 100 plants*
0	987	0	–	0	–	–
10	851	0	–	25	0	–
20	155	2	1.29	627	42	6.70
30	38	1	2.63	782	53	11.02
40	32	1	3.13	608	57	9.37

A cursory examination of fresh sections of some mutated sectors showed normal epidermal and spongy layer cells similar in shape, size and arrangement as those in the control. However, the former, more or less, differs from the control in the number and position of chloroplasts as well as in the presence of non-green plastids, i.e., pale-green, yellow and no pigmentation. The occurrence of somatically-mutated sectors in M_1 plants is believed due to diplontic or intra-somatic selection which, according to Gaul (1961) and D'Amato (1965) is the competition for survival between mutagenically-injured cells and normal cells in the apical meristem of the growing plant. When mutated cells comprise a bigger sector of the growing point than normal cells, the chance of their being perpetuated and expressed in the resulting tissues is higher than when such cells are few or absent in the actively dividing region of the apical meristem. Buiatti *et al.*, (1970), earlier reported that the number and size of the mutated sector of the meristem is increased when plant growth is slow and reduced. When the growth of the plant is fast, the growing point contains slightly-injured or normal cells. D'Amato (1965) believed that growing conditions of mutagen-treated plants could affect chimera formation.

Vaughn *et al.*, (1980), observed that several leaf chimeras contained mixtures of cells with both mutant and green chloroplastids bearing abnormal thylakoid aggregations wherein the plastid vacuole allows the bleaching of pigments resulting in white leaf sectors. In general, chimeral sectors are believed to arise from structural alterations in chromosomes (Lesley *et al.*, 1979; Mericle and Mericle, 1971) or to changes in the gene material (Zacharias and Terenberg, 1962) due to radiation treatment.

Interest in somatic mutations lie mainly in their being used as radio-sensitive detectors (Mericle and Mericle, 1971) and as indicators of the occurrence of germinal mutations after mutagenic treatment (Blixt and Gelin, 1965). Mutation breeders, on the other hand, have used somatic mutations or sports in the production of new and improved clones or varieties of asexually or vegetatively propagated plants such as sweet potato, plums, pears, cherries and oranges as well as ornamental plants like roses, carnation, chrysanthemum and tulips (Nybom and Koch, 1965).

The occurrence of plant height variations after irradiation appears to be a universal phenomenon but its importance in the expression of somatic mutations has been largely over-looked. Heretofore, mutation rates after mutagenic treatment have been obtained on the per dose basis without taking into account the marked genetic variations between fast-growing and slow-growing plants.

As shown in Table 2, the frequency of somatic mutations was higher in late-flowering than in early-flowering plants. The mutation frequency at doses of 20-40 Kr in early-flowering plants ranged only from 1.09-2.78 plants per 100 individuals as compared to 7.07-9.45 somatically-mutated plants in late-flowering individuals at the same dose levels. Observations showed that early-flowering is more or less related to fast plant growth. The M_1 plants that flower early like the control invariably bear very slight, if at all, radiation damage affecting these two phases of the life-cycle.

Table 2. Frequency of M_1 somatic mutations in early-flowering and late-flowering plants

Dose (Kr)	*Early-flowering plants*			*Late-flowering plants*		
	Total M_1 plants	*Total mutated plants*	*Mutations per 100 plants*	*Total M_1 plants*	*Total mutated plants*	*Mutations per 100 plants*
0	1,792	0	–	0	0	–
10	1,437	0	–	142	2	1.41
20	1,104	12	1.09	368	26	7.07
30	853	19	2.22	759	62	8.16
40	216	6	2.78	984	93	9.45

Early-flowering in irradiated material has previously been reported (Bhalla *et al.*, 1979) while late-flowering is commonly observed in populations exposed to high radiation doses, i.e., higher than LD-50. Thus the latter is invariably due to radiation injury which is commonly known to affect not only vegetative but reproductive growth as well. In sweet peas, early flowering in some plants has been found through genetic analysis to be due to an induced recessive mutant gene (Wellensiek, 1965).

Frequency of germinal mutations

The frequency of germinal mutations in the form of chlorophyll-deficiency seedlings in the selfed progenies of fast-growing and slow-growing M_1 plants is shown in Table 3. Fast-growing M_1 plants gave a lower frequency of chlorophyll-deficiency seedling mutations of only 3.83-7.97 per 1000 seedlings at doses of 20-

Table 3. Frequency of M_2 seedling mutations in selfed progenies of fast-growing and slow-growing plants

Dose (Kr)	*Fast-growing plants*			*Slow-growing plants*		
	Total seedlings	*Total mutant seedlings*	*Mutations per 1000 seedlings*	*Total seedlings*	*Total mutant seedlings*	*Mutations per 1000 seedlings*
0	24,508	0	–	0	–	–
10	106,675	0	–	3,012	18	5.98
20	19,327	74	3.83	78,563	1,124	14.31
30	4,769	38	7.97	94,141	1,805	19.17
40	4,016	17	4.23	76,304	1,593	20.88

40 Kr as compared to 14.31-20.88 mutants per 1000 seedlings at the same doses from slow-growing plants. The chlorophyll-deficiency seedling mutations consisted of albina, xantha, chlorina, and striat. Moreover, at the dose of 10 Kr, the slow-growing plants also gave 5.98 mutants per 1000 M_2 seedlings while the fast-growing plants did not yield any mutation at that dose. No mutant seedling was found in the selfed progeny of the unirradiated control.

The higher frequency of seedling mutations in the selfed progeny of slow-growing than of fast-growing plants is believed due to diplontic selection (Gaul, 1961) favoring the mutated cells of the meristem. The slow-growing M_1 plants, or those that incurred growth increments of only about seven cms. or less during the first 30-day period of field growth and 23 cms. or less during the second 30 days of growth after transplanting, showed lower growth rates than those of the control. On the other hand, the fast-growing plants had about the same growth rates as the unirradiated control plants of 22 cms. or more during the first 30-day period and about 70 cms. or more during the second 30 days of field growth.

In a study of mutation rates in several varieties of rice, Sharma (1985) obtained an average of 2.70% after a dose of 20 Kr gamma radiation while in soybean; Baradjanegara (1982) reported seedling mutation rates of 0.84% at 20 Kr and 0.51% at 30 Kr and much earlier in barley; and Kivi (1965) obtained a range of 0.10%-1.02% chlorophyll-deficiency seedling mutations at a dose of 20 Kr gamma rays.

Table 4 shows the frequency of germinal mutations in selfed progenies of early-flowering and late-flowering M_1 sorghum plants. As indicated by the data, the early-flowering plants yielded a markedly lower frequency of seedling mutations than the late-flowering plants. The former gave a range of only 1.19-3.94 mutant M_2 seedlings per 1000 seedlings at doses ranging from 20-40 Kr while the latter yielded from 6.21-19.21 mutants per 1000 seedlings at the same dose levels.

Table 4. Frequency of M_2 seedling mutations in sefled progenies of early-flowering and late-flowering plants

Dose (Kr)	*Early-flowering plants*			*Late-flowering plants*		
	Total M_2 seedlings	*Total mutant seedlings*	*Mutations per 1000 seedlings*	*Total M_2 seedlings*	*Total mutant seedlings*	*Mutations per 1000 seedlings*
0	25,165	0	–	0	–	–
10	203,354	0	–	17,821	46	2.58
20	138,552	165	1.19	46,184	287	6.21
30	106,927	254	2.40	95,129	1,834	19.27
40	27,108	107	3.94	123,492	1,585	12.83

Singh and Sinha (1985) similarly found early-flowering plants in four *Gora* rice varieties after gamma seed irradiation. Seedling mutation frequencies ranging from 1.88-11.45% after gamma seed-irradiation were obtained in M_2 soybean (Oh, 1983).

Early-flowering about the same time as the control is more or less an indication of very slight or minimal effects of treatment on the flowering process while late flowering is invariably a symptom of heavy or large-scale radiation damage on the reproductive process. The higher seedling mutation frequency in the progeny of late-flowering plants indicates the occurrence of a larger sector of genetically altered cells in the meristem than in early-flowering plants according to the hypothesis of intrasomatic selection (D' Amato, 1965).

Increasing the mutation frequency

To apply the results of the foregoing study, some experiments were done using cultu-practices commonly known to enhance or retard plant growth like weeding or not weeding the field and application or non-application of nitrogenous fertilizer. Following conventional practice, the mutation frequency was obtained on the per dose basis for easy comparison with previously published mutation data.

Table 5 shows the frequency of somatic mutations in M_1 plants grown in weeded, urea-fertilized plots and in weedy, unfertilized soil. The data indicate that the mutation frequency rates in the weeded, urea-fertilized plots with fast-growing plants ranged only from 0.06-0.18 plants per 100 M_1 individuals at doses ranging from 20-40 Kr as compared to 12.93-26.45 plants per 100 in the unweeded and unfertilized plots with slow-growing plants at the same doses. This indicates a means of increasing mutation frequency as plant growth can more or less be regulated or controlled through modification of the growing conditions.

Table 5. Frquency of M_1 plants bearing somatic mutations when grown in weeded, urea-fertilized or weedy, unfertilized soil

Dose (Kr)	*Weeded, urea-fertilized soil*			*Weedy, unfertilized soil*		
	Total M_1 plants	*No. with somatic mutations*	*Mutations per 100 plants*	*Total M_1 plants*	*No. with somatic mutations*	*Mutations per 100 plants*
0	2,368	0	–	1,673	0	–
10	2,043	0	–	1,249	34	2.72
20	1,872	3	0.16	1,052	136	12.93
30	1,705	3	0.18	837	213	26.45
40	1,592	1	0.06	564	108	19.14

The data in Table 6 shows the frequency of chlorophyll-deficient seedlings in the selfed progenies of M_1 plants grown in weeded, urea-fertilized soil and in weedy, unfertilized plots. The data indicate that plants grown in soil conducive for vegetative growth and were tall and robust gave lower mutation frequencies ranging from only 4.79-8.52 mutants per 1000 seedlings at doses ranging from 20-40 Kr than those grown in weedy, unfertilized plots which were short and thin plants giving mutation rates ranging from 18.49-25.37 mutants per 1000 M_2 seedlings at the same doses. M_1 plants grown in soil not very conducive for vegetative growth are believed to retain their mutated sectors in the meristem through the process of intrasomatic selection (Gaul, 1961). In fast-growing plants, such mutated cells tend to lose out in favor of the fast-dividing slightly injured or normal cells of the meristem.

Table 6. Frequency of chlorophyll-deficiency M_2 seedling mutations in the selfed progenies of plants grown in weeded, urea-fertilized and weedy, unfertilized soil

Dose (Kr)	*Weeded, urea-fertilized soil*			*Weedy, unfertilized soil*		
	Total M_2 seedlings	*No. of mutants*	*Mutations per 1000 seedlings*	*Total M_2 seedlings*	*No. of mutants*	*Mutations per 1000 seedlings*
0	14,726	0	—	9,284	0	—
10	15,087	0	—	9,638	74	7.68
20	13,152	63	4.79	8,901	295	33.14
30	11,968	102	8.52	7,134	247	34.62
40	8,426	57	6.76	6,815	276	40.49

Standardization of radiosensitivity tests

The control of radioactivity at the University of Chicago in the 1940's paved the way, among other things, to radio-sensitivity studies on seeds. After determining the varying degrees of radio-biological damage in seeds, Nilan (1961) and Nilan and Konzak (1961) proposed radiosensitivity tests as means of minimizing such damage and also to increase the efficiency of seed-irradiation and the rate of induced mutations. Hundreds of radio-sensitivity studies on seeds of many plant species have been published since that time but most of them would have been more useful if such data were obtained under uniform and standardized pre- and post-irradiation conditions that minimize extra-radio-biological damage that normally accompanies the seed-irradiation process. Without such conditions, radiosensitivity data even from the same species or varieties are not comparable. There is a need for a universal system of conducting such studies in order to obtain comparable data for universal use. The seed irradiation techniques previously described

by Nilan (1960); Nilan and Konzak (1961); Caldecott (1961); and Soriano (1986) are summarized briefly below:

1. *Use of pure-line seeds.* The seeds to be used in radio-sensitivity studies should come from isogenic lines or plants that have undergone continuous selection and selfing for several generations. Such seeds should be more or less uniform in size and shape and free from disease or fungal marks and other forms of deformities. Pure-line seeds are more or less genetically identical and their radiation response would be similar.

2. *Pre-irradiation techniques.* This consists of storing the seeds in nitrogen atmosphere in dessicators over equal mixtures of potassium chloride and ammonium chloride for seven days to bring about a more or less uniform seed moisture content of 14%. The seeds are prepared for radiation treatment by placing them in glass capsules, vials or plastic bags with nitrogen atmosphere and sealed to prevent the entrance of air about 20% of which is oxygen.

3. *During-irradiation method.* The seed containers are rotated to insure their equal exposure to the desired doses. This aspect of the work is preferably left to the facility technician but in the absence of one, the investigator should have previously calculated the period of exposure and learned the mechanics of operating the irradiation facility be it an X-ray machine or an irradiation facility with Co-60 or Cs-137 source.

4. *Post-irradiation techniques.* Soon after irradiation of the seeds, the seeds are rehydrated in water at a constant temperature of 30°C, rinsed briefly in running tap water and sown directly on moist blotting or tissue paper in a petri dish seeing to it that the seeds have ample spaces between them. Clumping the seeds in the petri dish tends to enhance the oxygen effect.

5. *Root-tip and shoot-tip cytology.* The seeds are sown about 5-10 mms. apart in the petri dish and sprouted in a growth chamber with a constant temperature of about 30°C. Root-tips about 5-10 mms. long are cut with a sharp razor blade and placed in the desired fixative fluid. Shoot-tips are cut when the plumule growth is about 10-15 mms. long and placed in the fixative.

6. *M_1 seedling height.* M_1 seeds are picked at random from the petri dish soon after rehydration and are sown in a blotter "sandwhich" which is placed over a small amount of water in a porcelain or glass pan. The seedlings are sprouted at a temperature of 30°C in a growth room under a florescent illumination of about 120 foot candles. The height of seedlings are measured from the base of the shoot to the tip of the longest leaf. Seedlings that are less than 30% of the control height are discarded as heavily damaged embryos. The LD-50 is obtained from a growth curve of the seedling height means.

7. *M_1 meiotic aberrations.* The types and frequencies of chromosomal configurations at metaphase I and anaphase I are obtained from florets that have been randomly grown and treated similarly as the control in terms of sowing density, planting distance, weeding, cultivation and fertilizer application as these conditions tend to influence diplontic selection. The flower buds are collected at the proper

stage, placed in the fixing fluid and stored preferably at low temperature at least for the first 24 hours after collection.

8. *M_1 plant fertility.* The sources of florets for the study of pollen fertility or stainability and fruits or panicles for seed-set determinations should have been grown under similar conditions as the control, as described in the preceding section. These materials are picked at random in the plot or field row involving at least 20 plants for the fertility data.

9. *M_1 plant height.* The M_1 plant height is obtained from plants grown under the same conditions as the control. The plants are measured from the base to the tip of the longest leaf. Plant height measurements include at least 20 plants per dose and the plants are chosen at random in the plot or row.

Plant height is measured using the metric system to the nearest fraction of a centimeter, like 2.5, 3.8 and 53.6 cms. but not 4, 8, and 51 cms. or 5.18, 39.52 or 131.84 cms.

10. *M_2 chlorophyll-deficiency mutations.* The seed germination method as previously described is recommended. The seedbed method has been critized for so long and the change is called for to render germinal mutation data credible to all sectors of biological research.

Summary and Conclusions

The frequencies of somatic and germinal mutation in the form of chlorophyll deficiency seedlings were higher in slow-growing than in fast-growing plants. Likewise, late-flowering plants gave markedly higher mutation rates than early-flowering plants. M_1 plants grown in weedy and unfertilized soil exhibited retarded growth and gave higher mutation frequencies than the tall and robust plants in weeded and urea-fertilized plots. The adoption of uniform and standard techniques for conducting radio-sensitivity tests on seeds is hereby proposed to obtain comparable M_1 data for universal use.

Literature Cited

Awan, M.A., A.A. Cheena and G.R. Tabin. 1985. Induced mutations for genetic analysis in rice. Rice Genetics (IRRI Sympos., Los Banos). pp. 697-705.

Balkenia, G.H. 1972. Diplontic drift in chimeric plants. *Radiation Botany.* 12: 51-55.

Baradjanegara, A.A. 1972. Mutation breeding in soybeans. Induced Mutations for Improvement of Legume Production (FAO/IAEA Res. Coord. Meeting, Seoul). pp. 155-162.

Bhalla, J.K., S. Bhamdurkar and L. Nizam. 1983. Mutagenic effects of gamma radiation and mutagenic fields on block gram. Proceed. Intl. Congress Rad. Res. (Amsterdam). pp. E-4.

Bixt, A. and O. Gelin. 1965. The relationship between leaf spotting and mutation rate in *Pisum.* Use of Induced Mutations in Plant Breeding (FAO/IAEA Terch. Meeting, Rome). pp. 251-262.

Buiatti, M.S., Baroncelli, R. Tesi and P. Boscariol. 1970. Effect of environment on diplontic sclection in irradiated gladiolus corms. *Radiation Botany.* 10: 531-538.

Caldecott, R.S. 1961. Seedling height, oxygen availability, storage and temperature: Their relation to radiation-induced genetic and seedling injury in barley. Effects of Ionizing Radiations on Seed (FAO/IAEA Meeting, Karlsruhe). pp. 3-24.

Conger, B.V. 1979. Experimental mutagenesis in plants: An introduction. Proceed. 6th Intl. Congress Rad. Res. Tokyo). pp. 562-567.

D' Amato, F. 1965. Chimera formation in mutagen-treated seeds and diplontic selection. *Use of Induced Mutations in Plant Breeding* (FAO/IAEA Meeting, Rome). pp. 303-316.

Emergy, D.A. 1972. Effect of reirradiation on radiosensitivity in peanut (*Arachis hypogaea* L.) *Radiation Botany.* 12: 137-150.

Frydenberg, A. and J. Sandfaer. 1965. The vitality, productivity and radiosensitivity of recurrently irradiated barley populations. *Use of Induced Mutations in Plant Breeding* (FAO/IAEA Tech. Meeting, Rome). pp. 175-183.

Gaul, H. 1961. Studies on diplontic selection after x-irradiation of barley seeds. *Effects of Ionizing Radiations on Seeds.* (FAO/IAEA, Karsruhe). pp. 117-138.

Kivi, E.l. 1965. Some aspects of strelity effect of radiation on the basis of gamma and X-ray treated barley. *Use of Induced Mutations in Plant Breeding* (FAO/IAEA Tech. Meeting, Rome). pp. 151-158.

Konzak, C.F., R.A. Nilan, J.R. Harle and R.E. Heiner. 1961. Control of factors affecting the response of plants to mutagens. *Brookhaven Sympos. in Biology.* 14: 126-127.

Lesley, J.W., M.M. Lesley and R. Soost. 1979. Variegation due to a chromosome loss induced by a gene in the wooly mutant of the tomato. *Jour. Heredity.* 70: 103-108.

Leenhouts, H.P., M.J. Sijsme and K.W. Chagwick. 1983. Synergism between dibromethane and x-rays at low doses of radiation. Proc. 7th Intl. Cong. Rad. Res. (Amsterdam). pp. E:16-17.

Mericle. L.W. and R.A. Mericle. 1971. Somatic mutations in Clone 02 of *Tradescantia, Jour. Heredity.* 62: 323-328.

Nilan, R.A., W.M. Owais, J.L. Rosichan and A. Klienhofs. 1979. Advances in kowledge of mutagenesis at the molecular level in plants. Proceed. 6th Intl. Congress Rad. Res. (Tokyo). pp. 568-574.

_____and C.F. Konzak. 1961. Increasing the efficiency of mutation induction. *Mutation and Plant Breeding.* (NAS-NSRC, Washington, D.C.). pp. 437-460.

_____, C.F. Konzak, R.R. Legault and J.R. Harle. 1961. The oxygen effect in barely seeds. *Effects of Ionizing Radiation on Seeds.* (FAO/IAEA, Karlsruhe). pp. 139-154.

Nybom, N. and A. Koch. 1965. Induced mutations and breeding methods in vegetatively-propagated plants. *Use of Induced Mutations in Plant Breeding.* (FAO/IAEA Tech. Meeting, Rome). pp. 661-675.

Oh, J.H. 1983. Induced mutation for soybean mosaic virus resistance in soybean. *Induced Mutations for Improvement of Legume Production.* (FAO/IAEA Res. Coord. Meet., Seoul). pp. 133-148.

Sharma, H. 1985. Induced mutagenesis in rice. *Rice Genetics (Proceed. IRRI Sympos., Los Banos). pp. 679-695.*

Singh, M.P. and P.K. Sinha. 1985. Induced mutagenesis in native rices. *Rice Genetics.* (Proceed. IRRI Sympos., Los Banos). pp. 719-727.

Soriano, J.D. and A. Micke. 1986. Induction and screening of useful mutations in peanut (*Arachis hypogaea* L.). Proceed. Intl. Meeting Applic. Atomic Energy Agric., Beijing). pp. 56-63. (In Chinese with English summary).

_____. 1968. Modifying the effects of fast neutrons in rice seeds by post-treatment with chemical mutagens. FAO/IAEA Technical *Reports Series* (Vienna). 92: 55-62.

_____. 1964. Increasing the mutation frequency in rice seeds through re-irradiation. *Nat. Appl. Sci. Bull.* 19: 81-90.

Thanh, L.D. and H.G. Rinh. 1985. Effects of diethyl sulfate, ethylene imine and gamma rays

on rice variability in the M_2. *Rice Genetics* (Proceed. IRRI Sympos., Los Banos). pp. 739-750.

Vaughn, K.C., D.L. Kimpel and K.G. Wilson. 1980. Investigation of the plastone of *Chlorophyton.* Jour. Hered. 71: 154-157.

Wallace, A.T. 1965. Increasing the effectiveness of ionizing radiations in inducing mutations at the vital locus controlling resistance to the fungus, *Helminthosporium victoria.* in oats. Use of Induced *Mutations in Plant Breeding.* (FAO/IAEA Tech. Meeting, Rome). pp. 393-398.

Zacharias, M. and L. Ehrennberg. 1962. Induction of leaf spots in leguminous plants by nucleotoxic agents. *Hereditas.* 48: 284-306.