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RADIOSENSITIVITY STUDIES IN BASMATI RICE

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Abstract

Three Basmati rice varieties (Basmati 370, Basmati Pak, and Super Basmati) were used to examine varietal differences in radiosensitivity to gamma radiation. Dry seeds of rice varieties were exposed to 150, 200, 250 and 300 Gy of gamma rays. Sensitivity to dose was determined by various measurements on the M_1 generation and on the basis of frequency of various types of chlorophyll mutations obtained in the M_2 generation. With the increase in radiation dose a decrease in germination, seedling height, root length and emergence under field conditions was observed in M_1 generation. In contrast, the gamma rays doses had some stimulatory effects on total spikelets at the maturity stage. These effects were observed in all the gamma radiation treatments in case of Basmati 370 where total spikelets increased above the non-irradiated control. Plant height and seed fertility decreased with increase in gamma radiation dose in an approximately linear fashion. The LD_{50} values for seed fertility were 238, 232 and 223 Gy for Basmati 370, Basmati Pak and Super Basmati, respectively. The effectiveness of the dose in inducing genetical changes was estimated by counting the number of chlorophyll mutations in the M_2 generation. The frequency of chlorophyll mutations increased with the radiation dosage upto 250 Gy which sharply decreased thereafter. Gamma ray dose of 200 and 250 Gy produced the highest mutation frequency for Basmati 370 followed by Basmati Pak and Super Basmati. The albina type of mutation was most frequent in all the three varieties.

Introduction

Ionizing radiations have been successful in inducing genetic variability in rice. Before the start of any sound breeding programme a knowledge of the relative biological effectiveness and efficiency of various mutagens is useful in mutation breeding (Smith, 1972). Various attempts in this direction have been made by different scientists to determine the most effective mutagenic treatment for the induction of desirable traits in rice (Awan & Bari, 1979; Reddy & Rao, 1988; Bansal *et al*., 1990; Katoch *et al.,* 1992; Pillai *et al.,* 1993; Sarawgi & Soni 1993; Kumar, 1998; Sanjeev *et al*., 1998), but mutagenic studies in Basmati rice are meager (Awan & Bari, 1979).

Basmati rice is a premier food grain crop of Pakistan for domestic consumption and export with a production of 1871 thousand tons from an estimated rice area of 1296 thousand hectares and an annual foreign exchange return of US$ 250.6 million (Anon., 1999-2000). In Pakistan only six Basmati rice cultivars viz., Basmati 370, Basmati Pak, Basmati 385, Super Basmati, Shaheen Basmati and Basmati 2000 are approved for general cultivation. So, the present study was undertaken to assess the mutagenic effects of gamma rays and to find out the radiation dose of a given mutagen in order to induce genetic variability in Basmati rice.

Materials and Methods

Three Basmati rice cultivars viz., Basmati 370, Basmati Pak and Super Basmati (*Oryza sativa* L.) were chosen for this study. Paddy seeds of each variety were irradiated with 150, 200, 250, and 300 Gy of gamma rays from the ^{60}Co source at 13% moisture.

After irradiation 10 seeds were sown in four replications per treatment on blotting paper in Petri dishes by using distilled water and germination was recorded after seven days. Seedling height and root length was determined by sandwich blotter technique as described by Myhill & Konzak (1967). Seeds were placed between two wet blotters, which were supported vertically between slots in PVC racks. The racks were placed in plastic trays containing enough water to immerse the lower edge of the filter papers. Twenty seeds were sown per treatment in three repeats along with the controls. The experiment was laid out in randomized method and data were analyzed by two factors completely randomized design.

Five hundred seeds of each irradiated dose along with unirradiated control were sown in the nursery beds. Emergence under field conditions was recorded, and 30 day-old seedlings were transplanted in field as M_1 by keeping plant to row distance 10 cm, in a randomized complete block design with 3 replications. Data for plant height, total spikelets per panicle and fertility percentage were recorded at maturity on 10 randomly guarded plants per treatment per replication. The data were analyzed by analysis of variance in a two factors randomized complete block design. Effectiveness of the mutagenic treatments were calculated by the following formula as used by Konzak *et al*., (1965).

Effectiveness = *Percentage of M_1 mutant panicles (Mp) for chlorophyll mutations / Radiation dose in kR = Mp/kR.*

For chlorophyll mutation studies in M_2 generation, single panicles from each M_1 plant were sown in the field nursery. Chlorophyll mutations were scored 14 days after the germination. Mutation frequency was expressed as number of mutants per 1000 M_2 seedlings. Chlorophyll mutations were grouped into four classes viz., albina (white), Xantha (Yellow), Viridis (light green) and others (with no definite pattern of chlorophyll deformities).

Results and Discussion

M_1 studies

Germination: The germination decreased after gamma irradiation, but the decrease was neither proportional to the increase in dosage nor definite pattern was found in all the three rice varieties (Table 1). Similar results have been reported in rice by Ando (1970), Miah *et al.,* (1970), Pathak & Patel (1988), and in *Plantago ovata* by Sareen & Koul (1999).

Seedling height: Seedling height is widely used as an index in determining the biological effects of various physical and chemical mutagens in M_1 (Konzak *et al*., 1972). The results of the present study showed that the seedling height decreased in decreasing order with the increase of irradiation dose, but the decrease was not proportional to the increase in dosage. The dose of 300 Gy causing 50% seedling height reduction was same in all the three rice varieties (Table 1) indicating that these varieties did not differ in radiosensitivity with respect to seedling height. A linear dependency of seedling height on the dosage of physical and chemical mutagens have been reported by Mikaelsen *et al*., (1968), Siddiq & Swaminathan (1968), Ando (1970), Katoch *et al.,* (1992) and Wang *et al.,* (1995).

Table1. Mean values of germination, seedling height, root length and emergence following gamma radiation.

Variety	Treatments (Gy)	Germination (%)		Seedling height (cm)		Root length (cm)		Emergence (No.)		Correlation (r) dose x emergence
		Actual	% of control	Actual	% of control	Actual	% of control	Actual	% of control	
	Control	100	100	9.37	100	13.05	100	86.6	100	
	150	96.2	96.2	8.29	88.4	10.29	78.8	82.6	95.3	
Basmati-370	200	100	100	6.85	73.1	9.17	70.2	78.2	90.3	-0.825^{NS}
	250	97.5	97.5	6.33	67.5	8.44	64.6	73.3	84.6	
	300	96.2	96.2	4.81	51.5	7.59	58.1	52.4	60.5	
	Control	100	100	10.48	100	12.80	100	86.2	100	
	150	97.5	97.5	10.68	101.9	9.02	70.4	85.1	98.7	
Basmati-Pak	200	98.7	98.7	7.59	72.4	7.76	60.6	75.5	87.5	-0.862*
	250	97.5	97.5	7.05	67.2	6.37	49.7	58.2	67.5	
	300	96.0	90.6	5.32	50.7	5.94	46.4	46.8	54.2	
	Control	98.7	100	9.97	100	15.86	100	90.4	100	
	150	96.2	97.4	8.68	87.0	11.14	70.2	84.0	92.9	
Super-Basmati	200	97.5	98.7	8.67	86.9	10.42	65.6	82.8	91.5	-0.852^{NS}
	250	100	101.3	6.87	68.9	8.81	55.5	58.2	64.3	
	300	96.2	97.4	5.11	51.2	6.32	39.8	44.0	48.6	
LSD values 0.05		3.66		3.72		3.94				

* significant at P=0.05 level
^{NS} Non-significant

Root length and emergence under field conditions

In the present study, reduction in root length and emergence of seedlings under field conditions occurred with each corresponding increase in gamma ray dose (Table 1). The inhibition on roots was more at 150 Gy as compared to higher doses. In case of seedling emergence, 150 and 200 Gy doses did not have drastic effect but emergence decreased significantly at higher doses. The dose causing 50% reduction in root length and emergence was higher in Basmati 370 than Basmati Pak and Super Basmati. From these results it can be inferred that Basmati 370 is more radioresistant than Basmati Pak and Super Basmati.

The coefficient of correlation between emergence under field conditions and radiation dose was found to be significant and negative in Basmati Pak. The reduction in root length and emergence/survival under field conditions with increasing gamma ray doses is in agreement with the results of Siddiq & Swaminathan (1968) who also reported decrease in the percentage of survival and root length of M_1 plants with the increase in radiation dose.

Plant height and spikelet fertility: Data for plant height and spikelet fertility at maturity are presented in Table 2. Maximum reduction in plant height was observed at 150 Gy. There was no significant difference in plant height reduction at different radiation doses within each variety. All the varieties responded similarly with increasing doses of irradiation. Similar results have been reported in rice by Miah *et al*., (1970), Katoch *et al*., (1992) and Sarawgi & Soni (1993). Apparently, reduction in M_1 plant height could be due to the inhibition of DNA synthesis or other physiological damage after mutagenic treatment.

As regard total spikelets per panicle all the radiation doses showed a stimulatory effect in case of Basmati 370, whereas in the other two varieties some doses showed enhancing effects and other have suppressing effects.

Seed fertility decreased with increase in gamma radiation dose (Table 2) in an approximately linear fashion in all the three varieties. Fifty percent fertility reduction occurred at 238 Gy, 232 Gy and 223 Gy in Basmati 370, Basmati Pak and Super Basmati, respectively (Fig.1 a, b, c). So, it is apparent that Basmati Pak and Super Basmati are slightly higher radiosensitive than Basmati 370. Miyahara (1997) reported 250 Gy of gamma rays dose at which seed fertility was halved in rice. Panicle fertility may be influenced by many environmental factors as shown by the control. However, mutagens generally reduce the reproductive ability of the plant and increase the number of sterile florets much more than the environmental effects. The decrease in fertility of rice after irradiation is considered to be due to chromosomal aberrations (Matsuo & Onozawa, 1961). There is an evidenc that the radiation-induced M_1 sterility is partly transferred into later generations (Anon., 1977). A large part of M_1 sterility is caused by physiological damage and consequently not transferred to M_2. In this study, induced floret sterility increased with increasing doses of gamma radiation. These results are in agreement with those of Siddiq & Swaminathan (1968), in which it was reported that gamma rays treatments induced high levels of sterility in M_1 rice plants. The induction of M_1 sterility in rice by gamma radiation has been reported by various workers (Mikaelsen *et al*., 1968; Ando, 1970; Miah *et al.,* 1970; Awan & Bari, 1979; Katoch *et al*., 1992; Sarawgi & Soni, 1993; Sanjeev *et al.,* 1998). The induction of > 71% sterility with the 300 Gy gamma treatment compared to the maximum of 17.5% sterility in the untreated control indicated the high mutagenic potency of gamma irradiation in Basmati rice.

Table 2. Mean values of plant height, total spikelets per panicle and seed fertility at maturity following gamma irradiation.

Variety	Treatment	Plant height (cm)		Spikelets per panicle		Seed fertility	
	(Gy)	Actual	% of control	Actual	% of control	Actual	% of control
	Control	127.3	100.0	95.7	100.0	92.0	100.0
	150	117.6	92.3	99.5	103.9	56.9	61.8
Basmati-370	200	115.5	90.7	107.6	112.4	56.7	61.6
	250	114.2	89.7	98.5	102.9	42.8	46.5
	300	113.6	89.2	103.5	108.1	28.2	30.6
	Control	117.1	100.0	63.2	100.0	95.1	100.0
	150	108.2	92.3	59.7	94.4	62.6	65.8
Basmati-Pak	200	106.2	90.6	59.4	93.9	58.6	61.6
	250	105.3	89.9	59.2	93.6	41.4	43.5
	300	105.6	90.1	61.2	96.8	26.1	47.4
	Control	94.6	100.0	97.4	100.0	82.5	100.0
	150	84.6	89.4	93.4	95.8	56.2	68.1
Super-Basmati	200	88.8	93.8	100.6	103.2	51.9	62.9
	250	84.3	89.1	92.8	95.2	30.1	36.4
	300	84.5	89.3	92.9	95.3	24.6	29.8
LSD 0.05		5.23		10.52		9.59	

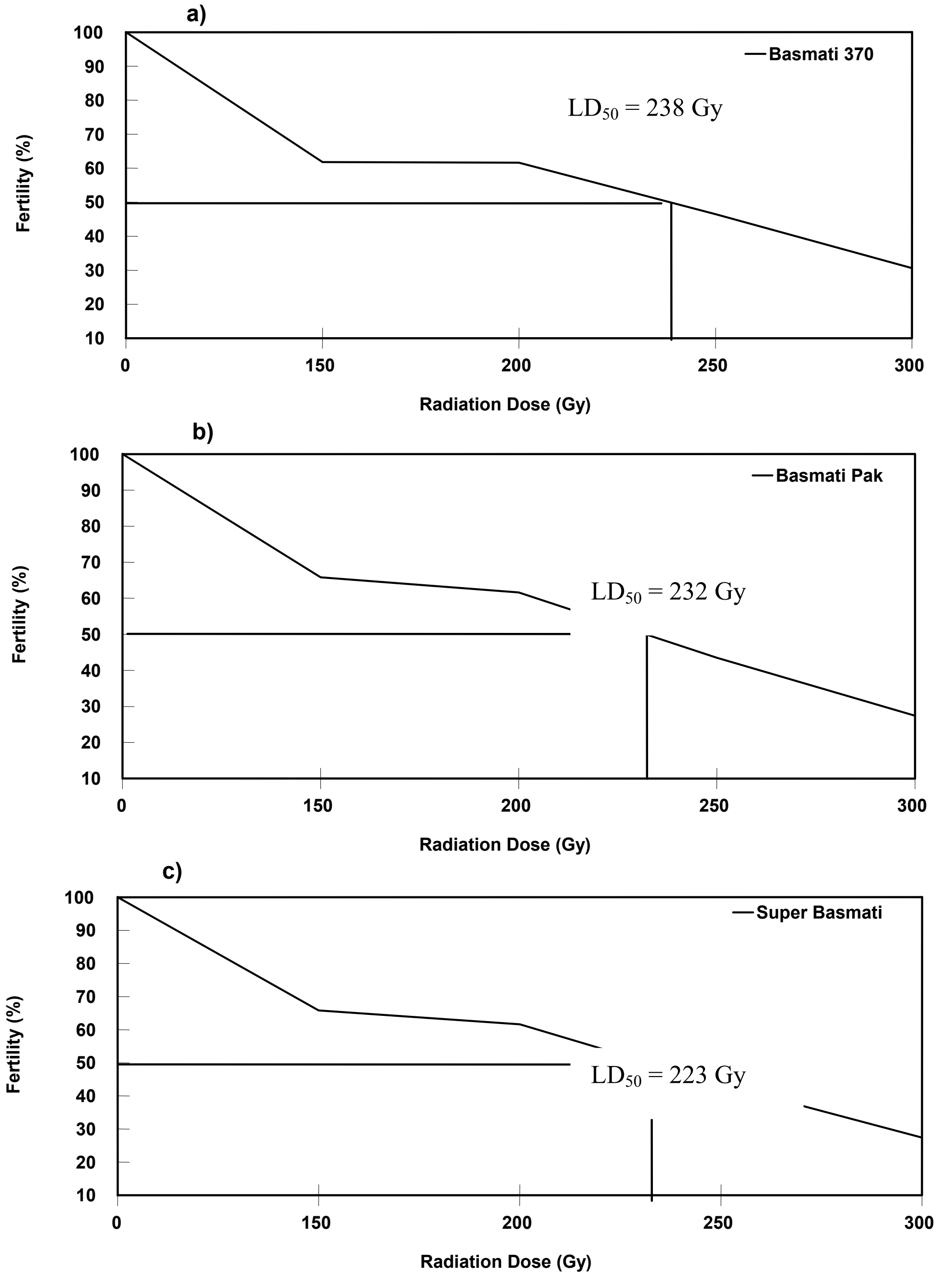

Fig. 1. Effect of gamma radiation on M_1 plant fertility (%) in rice.

M_2 studies

Chlorophyll mutations: The maximum chlorophyll mutations frequency following gamma rays was observed at 200 Gy after which it decreased at higher doses.

In the present investigation, the effectiveness is defined as the frequency of chlorophyll mutations in relation to irradiation dose. The induction of chlorophyll-deficient mutations is directly related to the mutagenic efficiency of a mutagen (Miah *et al.*, 1970), most effective gamma treatment (200 Gy) resulted in producing 75% chlorophyll mutations on M_1 panicle progenies basis (Table 3) and the maximum mutations frequency (8.25%) on M_2 seedling basis was observed in Basmati 370. A similar trend was found in Basmati Pak and Super Basmati. Awan & Bari (1970) have reported that gamma treatment induced a maximum frequency of 40% chlorophyll mutations on M_1 spike basis and 2.23% on an M_2 seedling basis in rice. Sanjeev *et al.*, (1998) observed highest frequency of chlorophyll mutations (2.04%) at 20kR dose of gamma radiation in rice variety Lanjhi.

The increase in mutation frequency has been shown to be accompanied by injuries in M_1 plants (Kawai & Sato, 1966). In general, the chlorophyll mutation frequency increased with increasing dosage upto certain limit, beyond which it exhibited a decline. This shows that a saturation point was reached at a certain dosage. Swaminathan (1961) attributed this decline at higher dose level to the rigor of diplontic and haplontic selections in the irradiated materials.

Individual chlorophyll mutations with respect to various gamma rays doses is given in Table 4a. The mutation spectra obtained in different treatments with gamma rays were pooled together and reported in Table 4b. The data show that in all three rice varieties, the appearance of albina mutations were maximum, followed by Xantha in Basmati 370 and Super Basmati and viridis in Basmati Pak.

Of the total 405 mutant seedlings observed , there were 55% albina, 19% viridis and 18% xantha. The chlorophyll mutation spectrum after treatment with chemical mutagens differs from the spectrum obtained with radiation treatment in barley. Radiation treatments generally produce a higher proportion of albina mutations in comparison to viridis and xantha types (Swaminathan *et al.*, 1962; Awan & Bari, 1979). This agrees well with our results presented in the Table 4b. A spectrum of radiation induced chlorophyll mutations characterized by a higher number of albina mutations than xantha or viridis type was also reported in rice by Mikaelsen *et al.*, (1968), Ando (1970) and Awan & Bari (1979).

For breeding purpose mutagenic treatments with low physiological effects and strong genetic effects are desirable. The ultimate aim of a mutagenic treatment is to induce mutations leading to genetic improvement of a specific trait. In practice, for radiation treatments often a fertility reduction of 50% (LD_{50}) for M_1 plants in comparison with the control plants is taken as a criterion for a promising treatment. In the present studies, LD_{50} for fertility ranged between 200 to 250 Gy in all the three Basmati rice varieties. These doses also produced maximum frequency of chlorophyll mutations on the basis of M_1 panicles and M_2 seedlings. Hence it is concluded that in Basmati 370, Basmati Pak and Super Basmati, 200 and 250 Gy of gamma treatments can be used safely for practical breeding purposes having low effects (physiological effects) with high frequency of vital mutations (genetic effects).

Table 3. Mutagenic effectiveness in M_1 and frequency of chlorophyll mutations in M_2 generation in Basmati rice varieties after gamma irradiations.

Variety	Treatment (dose in Gy)	No. of panicles analyzed	No. of panicles mutated	M_1 panicles segregating for chlorophyll mutations (%)	Effectiveness	No. of M_2 seedling analyzed	Chlorophyll mutants	Frequency of chlorophyll mutations (%)	Chlorophyll mutants per 1000 M_2 seedlings
Basmati-370	Control	20	---	----	----	1410	----	----	----
	150	20	6	30	2.00	1013	22	2.17	21.71
	200	20	15	75	3.75	1115	92	8.25	82.51
	250	20	15	75	3.00	877	70	7.98	79.81
	300	20	12	60	2.00	800	25	3.12	31.25
Basmati-Pak	Control	20	----	----	----	880	----	-----	----
	150	20	4	20	1.33	690	17	2.46	24.63
	200	20	13	65	3.25	584	41	7.02	70.20
	250	20	10	50	2.00	409	24	5.86	58.67
	300	20	6	30	1.00	362	10	2.76	27.62
Super-Basmati	Control	20	----	----	----	1056	----	----	----
	150	20	2	10	0.66	853	16	1.87	18.75
	200	20	6	30	1.50	825	43	5.21	52.12
	250	20	6	30	1.20	566	28	4.94	49.46
	300	20	4	20	0.66	528	17	3.21	32.19

Table 4(a). Spectrum of M_2 chlorophyll mutations.

Variety	Treatment dose in Gy	Albina	Xantha	Viridis	Others	Total no. of mutant seedlings
Basmati-370	150	6	2	12	2	22
	200	37	43	10	2	92
	250	40	----	20	10	70
	300	13	3	2	7	25
Basmati Pak	150	13	1	----	3	17
	200	28	----	13	----	41
	250	12	4	8	----	24
	300	7	----	2	1	10
Super Basmati	150	16	----	----	----	16
	200	19	18	5	1	43
	250	21	2	5	----	28
	300	11	----	----	6	17

Table 4(b). Spectra of chlorophyll mutations in M_2 generation (in %)

Spectrum	Basmati-370		Basmati-Pak		Super-Basmati		Total No. of mutations	
	Actual	%	Actual	%	Actual	%	Actual	%
Albina	96	45.93	60	65.21	67	64.42	223	55.06
Xantha	48	22.96	5	5.43	20	19.23	73	18.02
Viridis	44	21.05	23	25.00	10	9.61	77	19.01
Others	21	10.04	4	4.34	7	6.73	32	7.90
Total No. of mutants	209	100.0	92	100.0	104	100.0	405	100.0

References

Anonymous. 1977. *Manual on Mutation Breeding. Technical Report Series No.119*, IAEA, Vienna, pp.97.

Anonymous. 1999-2000. *Agricultural Statistics of Pakistan*, Govt. of Pakistan, Islamabad.

Ando, A. 1970. Mutation induction in rice by radiation combined with chemical protectants and mutagens. Pages 1-5, in: *Rice Breeding with Induced Mutations II*. IAEA. Vienna.

Awan, M.A and G. Bari. 1979. Mutagenic effects of fast neutrons and gamma rays in rice. *The Nucleus,* 16: 33-38.

Bansal, V., P.C. Katoch and P. Plaha. 1990. Mutagenic effectiveness of gamma rays, ethyl methane sulphonate and their combined treatments in rice. *Crop Impr.*, 17: 73-75.

Katoch, P.C., J.E. Massar, and P. Plaha. 1992. Effect of gamma irradiation on variation in segregating generations of F_2 seeds of rice. *Ind. J. Genet.*, 52: 213-218.

Kawai, T. and H.Sato. 1966. Some factors modifying the effects of radiation in seed treatment in rice. Pp. 151-171. In: *Mutation in Plant Breeding*, IAEA, Vienna.

Konzak, C F., R.A. Nilan, J. Wagner and R.J. Foster. 1965. Efficient chemical mutagenesis. Pp. 49-70, in *The use of Induced Mutations in Plant Breeding.* Report of FAO/IAEA Technical Meeting, Rome, Italy. Pergamon Press, NewYork.

Konzak, C.F., I.M. Wickham and M.J. Dekock. 1972. Advances in methods of mutagen treatment. Pp. 95-119. In: *Induced Mutations and Plant Improvement*, IAEA, Vienna.

Kumar, H.D.M. 1998. Frequency and spectrum of chlorophyll mutations induced by gamma rays in two rice varieties. *Karnataka J. Agric. Sci*., 11:637-640.

Matsuo, T. and Y. Onozawa. 1961. Mutations induced in rice by ionizing radiations and chemicals. Pp. 495-501, in: *Effects of Ionizing Radiations on Seeds,* IAEA, Vienna.

Miah, A.J., I.M. Bhatti, A. Awan, and G. Bari. 1970. Improvement of rice varieties by induced mutations to increase yield per acre and resistance to diseases and to improve seed quality. Pp. 69-76, in: *Rice Breeding with Induced Mutations II*, IAEA, Vienna.

Mikaelsen, K., I. Kiss and K.Osone. 1968. Some effects of fast neutrons and gamma radiations on rice. Pp. 49-54, In: *Neutron Irradiation of Seeds II,* IAEA, Vienna.

Miyahara, K. 1997. Mutation induction in rice by soft X-ray irradiation. *Tech. News Inst. Rad. Breed.*, 58: 2.

Myhill, R.R. and C.F. Konzak. 1967. A new technique for culturing and measuring barley seedlings. *Crop Sci*., 7: 275-277.

Pathak, H.C. and M.S. Patel. 1988. Sensitivity of upland rice genotypes to gamma radiation. *IRRN*, 13: 6.

Pillai, M.A., M. Subramanian and S. Murugan. 1993. Effectiveness and efficiency of gamma rays and EMS for chlorophyll mutants in upland rice. *Annals Agric. Res*., 14: 302-305.

Reddy, T.V.V.S. and D.R.M. Rao. 1988. Relative effectiveness and efficiency of single and combination treatments using gamma rays and sodium azide in inducing chlorophyll mutations in rice. *Cytologia,* 53: 491-498.

Sanjeev, S., A.K. Richharia and A.K. Joshi. 1998. An assessment of gamma ray induced mutations in rice (*Oryza sativa* L.). *Indian J. Genet*., 58:455-463.

Sarawgi, A.K. and D.K. Soni. 1993. Induced genetic variability in M_1 and M_2 population of rice (*Oryza sativa* L.). *Advances in Plant Science*, 6: 24-33.

Sareen, S. and A.K. Koul. 1999. Mutation breeding in improvement of *Plantago ovata* Forsk. *Indian J. Genet*., 59: 337-344.

Siddiq, E.A. and M.S. Swaminathan. 1968. Induced mutations in relation to the breeding and phytogenetic differentiation of *Oryza sativa*. Pp. 25-51. In: *Rice Breeding with Induced Mutations,* IAEA, Vienna.

Smith, H.H. 1972. Comparative genetic effects of different physical mutagens in higher plants. Pp. 75-93. In: *Induced Mutations and Plant Breeding Improvement*, IAEA, Vienna.

Swaminathan, M. S. 1961. Effect of diplontic selection on the frequency and spectrum of mutations induced in polyploids following seed irradiation. Pp. 279-288. In: E*ffects of Ionizing Radiations on seeds,* IAEA, Vienna.

Swaminathan, M.S., V.L. Chopra and S. Bhaskaran. 1962. Chromosome aberrations and the frequency and spectrum of mutations induced by ethyl methane sulphonate in barley and wheat. *Ind. J. Genet*., 22: 192-207.

Wang, C.L., M. Shen, Q.F. Chen and G. Xu. 1995. Preliminary study of mutagenic effects of nitrogen ion implantation in rice. *Acta Agriculturae Nucleatae Sinica*, 9: 13-19.

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