Theor. Appl. Genet. 61, 135–139 (1982)

Response of Excised Embryos of Rice (*Oryza sativa* L.) to X-rays

A. Shome and P. N. Bhaduri

Department of Botany, Burdwan University, Burdwan, W. Bengal (India)

Summary. The response of rice (*Oryza sativa* L.) embryos to X-rays (M_1 to M_3) was studied. By means of irradiating excised embryos, both chlorophyll and macromutation were successfully induced in three genotypes of rice. However, differential responses in terms of mutation frequency, mutation spectrum and optimal levels of X-rays required for induction of mutation (chlorophyll as well as morphological) were found to exist between cultivars. In 'Satika' and 'Ashkhata', LD_{50} values and maximum induced seed sterility are concomitant to optimum level of radiation required for triggering chlorophyll mutation. However, optimum dose for induction of macromutation in 'Satika' and 'Kerangserang' is independent of either LD_{50} and/or induced seed sterility.

Chances of obtaining both dominant and locus specific recessive mutations in the immediate X-ray treated generation (M_1) are large. This indicates the very high degree of effectiveness of the excised embryo irradiation technique with rice.

Key words: *Oryza sativa* – X-irradiation of excised embryos – X-ray effect on M_1 – Mutation frequency

Introduction

Hitherto whole seeds (dry/wet) have mostly been used for induction of mutation and comparatively less attention has been paid to embryo irradiation. Mericle and Mericle (1962) showed that when dry seeds are irradiated, the screening procedures for mutation are usually delayed until the third generation. They found that when barley pro-embryos were irradiated with low doses of irradiation, a higher mutation rate as well as larger 'isomutant carrying sectors' could be obtained even in the M_2 generation. They obtained a comparable mutation frequency of 11% when barley pro-embryos were irradiated with 800 R of ^{60}CO. Their findings compare favourably to those of Gaul (1961a) who treated dry seeds with 20 kR of X-rays. In rice the effect of radiation has been studied from developing embryos in the spikelets of growing plants (Kawai 1962a; Kawai and Inoshita 1965). Kawai (1962a) indicated that if rice panicles were irradiated with semi-acute doses of gamma rays at postmeiotic stage, then a large number of mutations could be obtained.

There is no detailed report concerning the induction of mutation by irradiating excised rice embryos. However, preliminary studies by the authors (Bhaduri and Shome 1969) showed that the response of the individual genotype to radiation could be assessed critically if excised embryos are directly exposed to X-rays and subsequently cultured in nutrient medium, thus eliminating the influence of endosperm or maternal tissues. The study reported here had two principal aims: (1) to determine the effect of X-rays on M_1 – generation performance, and (2) to induce mutation through excised X-irradiated rice embryos following embryo culture technique. Detailed radiosensitivity studies of excised mature and immature embryos of rice in vitro have already been reported (Shome and Bhaduri 1980).

Materials and Methods

Pure line seeds of two 'day-neutral' cultivars, 'Satika' (early; embryo-size small) and 'Ashkhata' (late; embryo-size medium), and a 'short-day' cultivar 'Kerangserang' (late; Javanese rice with big embryo) of rice (*Oryza sativa* L.), obtained from the Rice Research Station, Chinsurah, West Bengal, were utilised in this investigation. Decoated rice grains of these varieties were superficially sterilized with 95% alcohol for 1 min followed by 1 min in a 0.1% mercuric chloride solution. Grains were then rinsed thoroughly in sterile distilled water. Afterwards the sterilized grains were soaked in sterile distilled water for 4 hr and the embryos were excised by a technique previously described (Shome and Bhaduri 1980). The whitish excised embryos were treated in plastic Petri dishes with

0040-5752/82/0061/0135/$ 1.00

Table 1. Survival rate, plant height, number of reproductive tillers, days required to flower, and length of panicle as percentage of controls following X-ray treatments of excised embryos of rice varieties (V_1 = 'Satika', V_2 = 'Ashkhata', V_3 = 'Kerangserang').

Treatment	Percentage of control[a]														
	Plant survival on 21st day			Plant height			Tiller number			Days to flower			Panicle length		
	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3
10 kR	79.1 (75.0)[b]	100.0 (95.8)[b]	100.0 (88.0)[b]	101.1	95.0	90.9	133.3	88.8	100.0	116.0	100.0	101.3	76.4	111.7	97.0
15 kR	45.8 (45.8)	100.0 (95.8)	76.0 (64.0)	100.0	81.6	89.6	122.2	88.8	114.2	117.2	100.0	102.0	88.2	105.8	94.1
20 kR	4.1 (0.0)	67.3 (62.4)	68.0 (56.0)	–	81.6	88.4	–	77.7	100.0	–	100.0	102.0	–	105.8	85.2
25 kR	[c]	40.8 (37.4)	60.0 (28.0)	–	82.4	83.6	–	77.7	85.7	–	102.1	112.4	–	105.8	82.3
30 kR	[c]	14.2 (14.4)	12.0 (0.0)	–	87.5	–	–	122.2	57.1	–	127.1	–	–	11.7	–
35 kR	[c]	[c]	[c]	–	–	–	–	–	–	–	–	–	–	–	–

[a] Value obtained after dividing mean value of treatments by mean value of respective controls × 100

[b] Figures in parentheses indicate survival of plants at harvest (per cent of control)

[c] No survival beyond one week (in vitro)

10 kR to 35 kR of X-rays (125 kV, 25 mA machine) at an interval of 5 kR and at a dose rate of 2,000 R/min. In this experiment a total of 2,100 embryos were excised and in all varieties 100 embryos were treated with each dose or treatment and reared in culture vials (20 cm × 2 cm) containing White's medium (autoclaved at 1.41 kg/cm² for 15 min) at a controlled temperature (20 °C ± 1 °C) with 12 hr daily illumination (approximately 8000 lx) from fluorescent tubes. After 14 days, the culture tubes were placed out of doors. On the 35th day, the seedlings were transplanted to pots in a net house and were raised to adult plants following a standardised method (Shome 1966). Observations on various M_1 characters, beginning with plant height, were recorded. The radiation damage or effect expressed was based on adult plants (M_1) at appropriate stages as per cent of their respective controls. The selfed seeds of each M_1 plant were carefully collected to raise M_2s (to avoid contamination, all the panicles of individual plants were bagged at anthesis). The M_2 was raised as M_1 progeny rows. The seeds were harvested from individual plants of the M_2 lines yielding mutation for further confirmation in M_3. Mutation frequency was calculated on a M_1 and M_2 family basis.

Results and Discussion

Effects of X-rays on M_1 Plants

In general, X-irradiated embryo culture plants showed a minus (–) trend (radiation damage) in M_1 generation growth paramters (Tables 1, 2). However, the most interesting observation is that although X-irradiation reduced tillering in 'Ashkhata' and 'Kerangserang' at certain specific levels of radiation, an increased number of reproductive tillers was noted irrespective of genotype. This indicates the dose specificity nature of the genotype in terms of tillering. However, a maximum number of reproductive tillers coupled with highest seed sterility appeared in 'Ashkhata' at 30 kR, which in turn implies that the side effect of induced sterility can not be ruled out (Tables 1, 2).

Mutation Frequency

Chlorophyll Mutation

Chlorophyll mutation did not appear in the M_1. However, in vitro a few seedlings of 'Satika' and

Table 2. M_1 seed sterility

Treatment	Percentage of seed sterility (± S.E)		
	'Satika'	'Ashkhata'	'Kerang-serang'
0 kR	17.7 ± 1.60	6.5 ± 0.84	15.2 ± 1.38
10 kR	58.4 ± 1.92	37.3 ± 2.66	26.3 ± 2.21
15 kR	70.3 ± 3.34	74.2 ± 4.92	39.7 ± 3.68
20 kR	–	80.5 ± 5.29	46.4 ± 4.69
25 kR	–	81.8 ± 3.63	All plants sterile
30 kR	–	83.4 ± 8.19	–

Table 3. Chlorophyll mutation in M_2[a]

Cultivar	Treatment	Total no. of M_1 families sown	Total no. of M_1 families segregating	Total no. of plants in M_2	Types of chlorophyll mutation			Total no. of plants segregating for mutation	Mutation rate/M_1 family	Mutation frequency/100 M_2 plants
					Albino	Xantha	Striata			
'Satika'	10 kR	64	12	1 788	–	8	4	12	18.7	0.6
	15 kR	56	12	1 620	–	12	–	12	21.4	0.7
'Ashkhata'	15 kR	92	4	2 560	24	6	–	30	4.3	1.1
	20 kR	60	8	1 782	70	6	–	76	13.3	4.2
	25 kR	36	12	734	120	4	–	124	33.3	17.1
Summary of chlorophyll mutations (Combined over both cultivars)				Total	214	36	4	254		
				%	84.2	14.2	1.6			

[a] Only those cultivars and doses are shown in the table where chlorophyll mutation were scored

'Ashkhata' which received 10 kR and 20 kR irradiation respectively showed chlorophyll deficiency and were lethal. In general, M_2 progenies of 'Satika' and Ashkhata' yielded chlorophyll mutation. 'Kerangserang' did not produce any chlorophyll mutation irrespective of X-ray doses. Table 3 shows that the most frequent type of chlorophyll mutation is albino (84.2%) followed by lethal yellow (14.2%) and then striata (1.6%). Albinos appeared immediately after germination and was a lethal mutation. It is interesting to note that the albino seedlings when raised in embryo culture would survive in vitro up to 21–24 days compared to 8–11 days when raised from seeds in pots. The albino segregated in M_2 in a monogenic ratio of 3 : 1 (green : albino). The lethal yellow mutant could only be distinguished 7 days after germination and they survived until the 14th to 24th day. The lethal yellow gave a ratio of 15 green : 1 lethal yellow and appeared to be controlled by duplicate genes. Striata was not distinguishable until 25 days after transplantation in the field and showed normal growth producing fertile spikelets. This mutation appeared to be a somatic one, because in the M_3 progenies, all plants became normal.

It is evident from Table 3 that the total number of M_2 families segregating for chlorophyll mutation in 'Satika' at 10 kR and 15 kR was the same. Although the frequency of mutation was higher at 15 kR (optimal dose), the spectrum of mutation was broader at 10 kR. In the case of 'Ashkhata', the effective dose range for chlorophyll mutation was 15 kR to 25 kR, the lowest (10 kR) as well as highest survival dose did not produce any mutation. Further, in both varieties, chlorophyll mutation frequency increased with increasing X-ray dose and 'Ashkhata' yielded the highest mutant sector, especially at 25 kR (optimal dose). It is of interest to note that LD_{50} values for survival of seedlings ('Satika' and 'Ashkhata') was observed to occur at approximately these doses (optimal doses, Table 1). The present study further revealed that (except 30 kR in the case of 'Ashkhata') an increase in induced seed sterility is concomitant to an increase in the chlorophyll mutation rate (Tables 2–4).

Table 4. LD_{50} (value based on 21 days culture in vitro), maximum seed sterility and their relationship with optimal level of X-rays for mutation

Cultivar	LD_{50} value	Maximum seed sterility occurred at	Optimum dose for induction of mutation	
			Chlorophyll mutation	Macro-mutation
'Satika'	around 15 kR	15 kR	15 kR	10 kR
'Ashkhata'	20 to 25 kR	20 to 30 kR	25 kR	25 kR
'Kerangserang'	slightly above 25 kR	25 kR	no mutation	10 kR

Table 5. Macromutations in M_2[a]

Cultivar	Treatment	Total no. of M_1 families	Total no. of M_1 families segregating	Total no. of plants	Types of macromutation														Total no. of plant mutated	Mutation ra	
					Filiform leaf	Dwarf	Tall	Tall – green	Tall purple	Terminally awned	Awnless	Lax panicle	Red kernel	White kernel & short-day	Bolder grain	Fine grain	Small grain & non-lodging		M_1 family basis	M_2 family basis
'Satika'	10 kR	64	24	1,788	–	12	4	12	8	–	–	–	–	4	–	–	–	40	37.5	2.2
	15 kR	56	8	1,620	–	–	–	–	–	12	–	16	–	–	–	–	–	28	14.2	1.7
'Ashkhata'	20 kR	60	2	1,782	–	–	–	–	–	–	–	1	–	–	–	1	–	2	3.3	0.1
	25 kR	36	6	734	–	–	–	–	–	–	–	13	–	–	1[b]	–	12	26	16.6	3.5
	30 kR	14	2	304	1[b]	–	–	–	–	–	–	1	–	–	–	–	–	2	14.2	0.6
'Kerang-serang'	10 kR	88	4	2,200	–	–	–	–	–	–	1[b]	2	1	–	–	–	–	4	4.5	0.3

[a] Only those doses are given in the table where mutations were scored
[b] Isolated in M_1 generation

Macromutation

A number of morphological mutations (bred true to mutant characters in M_3) were scored in the M_2 (Table 5). However, the most striking observation in the present experiment is the scoring of 3 mutants in the M_1 generation itself and hence these mutants were considered as dominant and/or locus specific recessive mutations. Inductions of locus specific mutation by chemical mutagens (hydrazine and hydroxylamine) have been reported in higher plants, including rice (Jain et al. 1968; Chandrashekar and Reddy 1971; Reddy and Reddy 1972). However, there appears to be no reports of physical mutagens inducing viable dominant and/or locus specific mutation in rice in the immediate treated generation. Hence, the present observation is a new and unique one because 2 out of 3 cultivars produced dominant or otherwise specific types of mutation in the M_1 itself. This indicates the very high degree of effectiveness of the present technique.

Out of 3 M_1 mutants, 2 were viable ('bolder grain' mutant of 'Ashkhata' and 'awnless' mutant of 'Kerangserang') and one 'filiform leaf' (100% pollen sterility; crossing with normal 'Ashkhata' produced only nonviable crumpled seeds, though PMC's was found to be normal) was nonviable. Crossing experiments with 'bolder grain' mutant of 'Ashkhata' × medium grain parent showed the dominance of bolder grain over the medium grain, because F_1 was bolder grained. The F_2 population segregated into 3 bolder grain to 1 medium grain types showing the monogenic dominance of bolder grain over medium grain which was further confirmed by the backcross data. The crosses between the 'awnless' mutant of 'Kerangserang' and the awned parent indicated that awnless was monogenic recessive to awned: F_1 was awned and in F_2, a segregation of 3 awned : 1 awnless was observed confirming that one pair of genes is involved in its inheritance. As the 'awnless' mutant was scored in M_1 and bred truely till M_5, it may be concluded that this mutant has arisen from simultaneous mutations of both alleles of a locus. Therefore, the true breeding 'awnless' mutant of Kerangserang' may be considered in terms of locus specific recessive mutation.

The present study revealed that different cultivars differed in terms of mutation frequency, spectrum of mutation and optimum dose of X-rays required for induction of mutation. Thus, 'Satika' yielded a higher mutation rate as well as a wider spectrum of mutation as compared to 'Ashkhata' or 'Kerangserang' (Table 5). However, the mutation frequency in 'Satika' decreased with increasing X-ray dose and its optimal level required for induction of mutation was indicated to be at 10 kR (a 5 kR less than chlorophyll mutation). In contrast to 'Satika', the rate of mutation was increased in 'Ashkhata' with increasing X-ray dose (up to 25 kR) and optimum dose was 25 kR. It is interesting to note that this same level of radiation (25 kR) also triggered a maximum chlorophyll mutant sector in 'Ashkhata'. In 'Kerangserang', only 10 kR dose produced macromutations. Hence this response to irradiation, at least in the case of rice should be viewed in terms of respective genotype. A similar opinion was also put forward by Shome (1981) while studying the X-ray effect on 11

cultivars of *Hibiscus* spp. (*H. cannabinus* L. and *H. sabdariffa* L.) through excised embryo irradiation technique.

Results also revealed that in two 'day-neutral' cultivars, 'Satika' and 'Ashkhata', LD_{50} and maximum induced seed sterility were concomitant to an optimal level of radiation required for obtaining a greater chlorophyll mutant sector. However, optimum dose for inducing greater macromutation sectors in 'Satika' and 'Kerangserang' was different from both the LD_{50} dose and that inducing highest seed sterility.

In summary, it appears that the probability of obtaining benificial mutations will be greater in the case of the excised embryo because a comparatively lower dose than that required for whole seeds (Bose 1968; the author utilized Satika as one of the varieties in his experiments) can be effectively employed. Moreover, screening for sectors carrying macromutations could begin in the M_1: the chances of their appearing as dominant and/or locus specific recessive mutations are high in the immediate treated generation when grown in vitro because the damage caused to endosperm or maternal tissues by irradiation is eliminated in this method. This indicates a very high degree of effectiveness of excised embryo irradiation, especially with rice.

Acknowledgement

The authors thank the Indian Council of Agricultural Research (I.C.A.R.), New Delhi for financial help.

Literature

Bhaduri, P.N.; Shome, A. (1969): X-ray irradiation to dissected embryos of rice and their mutation spectrum. Curr. Sci. **38,** 297–298

Bose, M.M. (1968): X-ray irradiation studies in a few recommended varieties of rice (*Oryza sativa* L.) of West Bengal. Ph. D. Thesis, The University of Calcutta, India

Chandrashekhar, V.S.G.; Reddy, G.M. (1971): Specific locus mutations in maize by chemical mutagens. Curr. Sci. **40,** 136–137

Gaul, H. (1961a): Studies on diplontic selection after X-irradiation of barley seeds. Symposium on the Effects of Ionizing Radiation on Seeds and their Significance for Crop Improvement, pp 117–138. Vienna: I.A.E.A.

Jain, H.K.; Raut, R.N.; Khamanker, Y.G. (1968): Base specific chemicals and mutation analysis in *Lycopersicon.* Heredity **23,** 247–257

Kawai, T. (1962a): The relation between radio-sensitivity and the developmental stage in rice. Gamma Field Symp. **1,** 37–42

Kawai, T.; Inoshita, T. (1965): Effects of gamma ray irradiation on growing rice plants 1. Irradiation at four main developmental stages. Radiat. Bot. **5,** 233–255

Mericle, L.W.; Mericle, R.P. (1962): Mutation induction by proembryo irradiation. Radiat. Bot. **1,** 195–202

Reddy, G.M.; Reddy, T.P. (1972): Induction of mutations by hydrazine in rice. Indian J. Genet. Plant Breed. **32,** 388–391

Shome, A. (1966): Studies on embryo and tissue culture of crop plants. Ph. D. Thesis, The University of Calcutta, India

Shome, A. (1981): X-ray irradiation to excised embryos of mesta (*Hibiscus cannabinus* L. and *H. sabdariffa* L.). Theor. Appl. Genet. **59,** 11–15

Shome, A.; Bhaduri, P.N. (1980): Radiosensitivity of the excised rice embryo. Cytologia **45,** 705–713

Received January 6, 1981
Accepted May 8, 1981
Communicated by K. Tsunewaki

Dr. A. Shome
Plant Breeding and Genetics Division.
Jute Agricultural Research Institute
Barrackpore (743101)
24 Parganas, W. B. (India)

Dr. P. N. Bhaduri
Emeritus Scientist (I.C.A.R.)
B.C.K.Viswa Vidyalaya
Mohanpur, Nadia, W.B. (India)