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Gamma ray induced morphological mutations in non-Basmati aromatic rice

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ABSTRACT

Induction of various types of chlorophyll and morphological mutations by three doses (250, 350 and 450 Gy) of gamma rays in two genotypes, developed from Gobindabhog and Tulaipanja was studied. Segregation of chlorophyll and morphological mutants in M_2 generation from normal looking M_1 plants indicated recessive nature of mutations. Frequency of different chlorophyll and morphological mutations was higher in Tulaipanja group. The highest frequency of chlorophyll and morphological mutations was observed in the genotype IET 14143, developed from Tulaipanja, at 450 Gy. Among different chlorophyll mutations albina was the most predominant group. In general, higher mutagenic effectiveness and mutagenic efficiency were found at 450 Gy. Various morphological mutants like grassy leaf, rolled leaf, striped leaf, and broom stick leaf and several other mutants like sterile, early flowering, late flowering, non-flowering, tall, dwarf, semi-dwarf and high yielding mutants were induced with variable frequencies in different genotypes by different doses of mutagen. The frequency of chlorophyll mutations increased with increase in dose of mutagen but no relationship between the doses of mutagen and mutation spectrum could be established in morphological mutation.

Key words: *aromatic rice, gamma ray, induced mutation, morphology,*

Aromatic rice has a special place in world rice market. There is an increasing demand for the production of high grain quality aromatic rice in our country due to rising standard of living and export market. Some non-Basmati traditional aromatic rice cultivars such as Gobindabhog, Tulaipanja, *etc*., are very popular in West Bengal due to their excellent grain quality and aroma. But these cultivars are handicapped by low yield potential. Therefore, there is an urgent need to improve the yield potential of such cultivars. Hybridization method is less preferred due to difficulty in retaining the quality characteristics of aromatic rice. Hence, mutation is one of the important tools for creation of genetic variability in aromatic rice. Therefore, the effectiveness and efficiency of different doses of gamma radiations on four genotypes derived from two aromatic rice cultivars Gobindabhog and Tulaipanja were studied in the present investigation.

MATERIALS AND METHODS

Healthy unhusked seeds of pigmented mutant (designated as G_1) and IET 13541 (designated as G_2) of Gobindabhog and IET 14142 (designated as T_1) and IET 14143 (designated as T_2) of Tulaipanja were irradiated with three different doses of gamma ray *viz*., 250Gy, 350Gy and 450Gy from ^{60}Co source at Central Research Institute for Jute and Allied Fibre (CRIJAF), Barrackpore, West Bengal. Approximately 500 seeds were treated for each dose of gamma ray for each genotype. The unexposed seeds of each genotype were used as control. Seeds from bagged panicles of suspected mutant plants in M_1 generation were sown in nursery. Screening for chlorophyll mutations was done after 7 days of germination in the nursery bed when the first leaf was fully developed. The frequency and spectrum of chlorophyll mutations per 100 M_2 plants were scored at the seedling stage following the classification of Gustafsson (1940). Mutagenic effectiveness and mutagenic efficiency were calculated as per following formulae:

$$\text{Mutagenic Effectiveness} = \frac{\text{Mutation frequency in } M_2}{\text{Dose of Mutagen (Gy)}}$$

$$\text{Mutagenic Efficiency} = \frac{\text{Mutation frequency in } M_2}{\text{Percentage sterility in } M_1}$$

Thirty-day-old seedlings were transplanted in puddled field as progeny-row with one seedling hill^{-1}. The M_2 populations were thoroughly screened at various developmental stages, particularly from flowering to maturity, for different morphological mutations based on visual observations. Mutants were identified on the basis of quantitative changes *viz.,* leaf morphology, spikelet, panicle, flowering time, tiller number, grain number as well as other variation in lodging behaviour and other morphological characters.

RESULTS AND DISCUSSION

Different types of chlorophyll mutations (Table 1) observed in the present study could be grouped into lethal (*albina, xantha and alboxantha*) and non-lethal (*alboviridis, viridis* and *striata*) types. Different types of morphological mutations (Table 3) identified were grassy leaf, rolled leaf, striped leaf, broom-stick leaf, sterile, late flowering, early flowering, non-flowering, dwarf, semi-dwarf, tall and high-yielding mutants. The absence of above chlorophyll and morphological mutants in the M_1 generation and their appearance in M_2 generation indicated the recessive nature of such mutations.

Frequency of chlorophyll mutations increased with increase in the dose of mutagen (Table 1). This is in agreement with the reports of Cheema and Atta (2003). The highest frequency of chlorophyll mutations (4.86%) was observed in IET 14143 at 450 Gy treatment. In all the four genotypes, the frequency of chlorophyll mutations was higher at higher doses. The results indicated that among the different mutant classes induced, *albina* was highest in frequency in all four genotypes. Singh and Singh (2003) reported that the *albina* type of mutants was most frequent among induced rice mutants. Chlorophyll mutations *alboxantha* and *alboviridis* were not observed at different doses in IET 14143 genotypes. In case of IET 14142, *alboxantha* (0.03%) was found only at 450Gy dose. In genotype G_1, *alboxantha* and *alboviridis* were not found in 250Gy dose, and at 350Gy the frequency of *alboxantha* was 0.18% and at 450Gy the frequency of *alboviridis* was 0.03%.

Frequencies of different chlorophyll mutants were higher in Tulaipanja group than that of Gobindabhog group. It was observed that frequency of *albina* was the most frequent chlorophyll mutant followed by *xantha* > *striata* = *viridis* in all the genotypes except T_1 where, *albina* was followed by *striata* > *viridis* > *xantha*. Similar results were reported by Mahabal Ram and Zaman (1972).

The frequency of chlorophyll mutants was found to be independent of mutagenic dose of gamma ray (Bekendam 1961 and Reddi and Rao 1980). Kawai and Sato (1966) found *albina* to be the most frequent type in rice. Swaminathan *et al.* (1962) and Sree Ramla (1970) suggested that differences in the mutation spectrum and rate in different genotypes might be due to differences in the location of genes in relation to the centromere.

The chlorophyll mutants that are of common occurrence have been used as a measure of mutagenic action in the mutation breeding experiments (Kawai 1969). Mohan Rao (1972) and Rao and Rao (1983) suggested that the estimation of mutation frequency on the basis of M_2 plants gave the best estimate of actual mutation frequency. The frequency of mutations expressed in M_2 population basis is more realistic and helpful (Gaul 1964). A wide range of variations in the frequency of chlorophyll mutations in rice following ionizing radiations have been reported by Majeed and Majeed (1997), Shadakshari *et al* (2001).

For any mutation breeding programme, selection of effective and efficient mutagen(s) is very essential to recover high frequency of desirable mutations. The results indicated that the effectiveness of various doses and the response of genotypes varied (Table 2). In all the genotypes studied, mutagenic effectiveness increased with increase in dose except in IET 14142, where 250 Gy had highest mutagenic effectiveness (0.014). This indicated that the mutagenic effectiveness depends upon the genetic background of the material undergoing mutagenic treatment. Mutagenic efficiency is indicative of the proportion of mutations as against associated undesirable biological effects such as gross chromosomal aberration, lethality and sterility, induced by the mutagen in question suggested by Nilan (1967).

The results indicated that 450Gy treatment was

Table 1. Freqency and spectrum of chlorophyll mutations in M_2 generation of pigmented mutant of Gobindbhog Genotype, IET 13541 Genotype, ET 14142 Genotype, IET 14143 genotype

Dose	Total number of M_2-Seedlings	Different chlorophyll mutant classes														Number of mutant-seedlings	Percent of M_2-seedlings
		Albino		Xantha		Viridis		Alboxantha		Alboviridis		Striata		Unidentified			
		x	y	x	y	x	y	x	y	x	y	x	y	x	y		
Pigmented mutant of Gobindbhog Genotype																	
250Gy	11772	154	1.31	25	0.21	2	0.02	7	0.06	11	0.09	5	0.04	2	0.02	206	1.75
350Gy	8789	89	1.01	13	0.15	11	0.13	7	0.08	9	0.1	28	0.32	24	0.27	181	2.06
450Gy	3786	113	2.98	26	0.69	16	0.42	3	0.08	1	0.03	34	0.9	-	-	193	5.1
IET 13541Genotype																	
250Gy	10410	78	0.75	8	0.08	8	0.08	-	-	-	-	14	0.13	-	-	108	1.04
350Gy	9733	103	1.06	18	0.18	-	-	17	0.18	-	-	12	0.12	-	-	150	1.54
450Gy	6237	121	1.94	36	0.58	16	0.26	-	-	2	0.03	27	0.43	-	-	202	3.24
IET 14142 Genotype																	
250Gy	5561	67	1.2	-	-	2	0.04	-	-	4	0.07	39	0.7	-	-	112	2.01
350Gy	3855	77	2	12	0.31	20	0.52	-	-	10	0.26	20	0.52	-	-	139	3.61
450Gy	3958	178	4.49	6	0.15	7	0.18	1	0.03	1	0.03	10	0.25	-	-	203	5.13
IET 14143 Genotype																	
250Gy	4223	118	2.79	11	0.26	4	0.1	-	-	-	-	6	0.14	5	0.12	144	3.41
350Gy	3570	93	2.61	19	0.53	15	0.42	-	-	-	-	12	0.34	-	-	139	3.9
450Gy	2840	138	4.86	20	0.7	2	0.07	-	-	-	-	3	0.11	-	-	163	5.74

x: Number of M_2 -mutant seedling

y: Frequency (Number of individual class 100 seedlings^{-1}

Table 2. Effectiveness and efficiency of gamma-rays as mutagen in four genotypes of rice

Genotype	Treatment	Number of M_2 plants studied	Number of mutant plants in M_2	Mutation rate in M_2(%)	Sterility in M_1 (%)	Mutagenic effectiveness	Mutagenic efficiency
Mutant of	250Gy	11772	206	1.75	53.1	0.007	0.03
Gobindbhog	350Gy	8789	181	2.06	52.63	0.006	0.04
	450Gy	3786	193	5.09	56.09	0.011	0.09
IET 13541	250Gy	10410	108	1.03	52.59	0.004	0.02
	350Gy	9733	150	1.54	53.62	0.004	0.03
	450Gy	6237	202	3.24	51.95	0.007	0.06
IET 14143	250Gy	5561	112	2.01	50.14	0.008	0.04
	350Gy	3855	139	3.61	55.54	0.01	0.06
	450Gy	3958	203	5.13	65.87	0.011	0.08
IET 14143	250Gy	4223	144	3.41	56.2	0.014	0.06
	350Gy	3570	139	3.89	57.83	0.011	0.07
	450Gy	2804	163	5.81	63.87	0.013	0.09

found to be most efficient in all the four genotypes. The highest efficiency (0.09) was observed in pigmented mutant of Gobind bhog and IET 14143 at 450Gy treatment. However, Chand *et al*. (2007) recorded highest efficiency at 25 kR dose of gamma rays in scented rice variety T- 23.

The low values for mutagenic efficiency could be due to the extent of damage in M_1 generation, which determines the mutability of genes, irrespective of the mutagen used as with equal degree of damage, all mutagens and their doses would yield more or less comparable amount of mutations.

The frequency of morphological mutations (Table 3) induced per 1000 M_2 plants was the highest (7.14) in IET 14142 at 450Gy treatment. The results revealed no relationship between the doses of mutagen and mutation spectrum, although some degree of mutation specificity was noticed in certain treatments. Grassy leaf mutant was obtained at 450Gy treatments in all four genotypes and at 250 Gy dose in G_2 and 350 Gy dose in IET 14143. Comparing all four genotypes, high frequency (0.90) of grassy leaf mutant was observed at 450 Gy treatment in IET 14142. All the three doses induced rolled leaf mutant in G_2 and in both the genotypes derived from Tulaipanja group except at 250Gy dose. However, this type of leaf mutant was observed in G_1 only at 250Gy. The frequency of rolled leaf mutants was maximum (0.90) at 350Gy in IET 14142. Striped leaf mutant was found in both the genotypes derived from Gobindabhog at 250Gy. This type of observation was found at 350Gy and 450Gy in IET 14143 and 450 Gy treatment in IET 14142. In all four genotypes, broomstick leaf mutant was observed at 250Gy treatments. Besides, it was also noticed in other treatments such as at 450Gy in G_2, at 350Gy in T_1 and at 350Gy and 450Gy in T_2. The highest frequency (0.93) of broomstick leaf mutant was observed in T_1 genotype at 250Gy. Sterile mutant was found in all four genotypes at all doses except in G_2 at 350Gy. The maximum number of sterile plants was observed in each genotype at 450Gy. Such sterile mutants could be used in heterosis breeding programme. Kowyama *et al*. (1994) and Singh *et al*. (1998) observed sterile mutant in M_2 generation through induced mutation in rice.

Early flowering mutants, which flowered 10-15 days earlier than their respective mother lines, were obtained at very low frequency in all genotypes at different doses. The maximum frequency (0.90) of this mutant was obtained at 450Gy in IET 14142. Such early flowering mutants, although had poor grain numbers, could be used in cross-breeding programme to develop early maturing high yielding lines. Early maturing mutants through gamma irradiations in rice have been reported by Rao and Reddi (1986), Tanisaka *et al*. (1992), Yokoo and Okuno (1993), EI-Shouny *et al*. (1994), Shadakshari *et al*. (2001) and Singh and Singh (2003). The maximum frequency of late flowering plants (days to heading was delayed by one week to one month than their, respective mother lines) was obtained at 450Gy in all genotypes except in G_1 where, maximum late flowering plants were obtained at 350Gy.

Table 3. Spectrum and frequency of morphological mutants in M_2- pigmented mutant of Gobindbhog genotype, IET 13541, ET 14142, IET 14143 genotype generation

	Pigmented mutant of Gobindbhog								IET 13541							
	250Gy (1710)*		350Gy (1440)		450Gy (720)		TOTAL (3870)		250Gy (1710)		350Gy (1680)		450Gy (1350)		TOTAL (4740)	
Mutant characters	x	y	x	y	x	y	x	y	x	y	x	y	x	y	x	y
Late flowering	2	0.52	3	0.78	-	-	5	1.3	-	-	-	-	1	0.21	1	0.21
Early flowering	-	-	-	-	1	0.26	1	0.26	-	-	1	0.21	-	-	1	0.21
Non flowering	5	1.29	6	1.55	2	0.52	13	3.36	1	0.21	2	0.42	6	1.27	9	1.9
Grassy leaf	-	-	-	-	-	0.26	1	0.26	1	0.21	-	-	1	0.21	2	0.42
Rolled leaf	1	0.26	-	-	-	-	1	0.26	1	0.21	1	0.21	1	0.21	3	0.63
Broom stick	2	0.52	-	-	-	-	2	0.52	1	0.21	-	-	1	0.21	2	0.42
Striped leaf	2	0.52	-	-	-	-	2	0.52	1	0.21	1	0.21	-	-	2	0.42
Sterile	1	0.26	1	0.26	2	0.52	4	1.04	1	0.21	-	-	2	0.42	3	0.63
Dwarf	1	0.26	-	-	2	0.52	3	0.78	1	0.21	-	-	1	0.21	2	0.42
Semi dwarf	-	-	2	0.52	3	0.78	5	1.3	1	0.21	1	0.21	2	0.42	4	0.84
Tall	-	-	1	0.26	-	-	1	0.26	-	-	-	-	2	0.42	4	0.84
High yield	-	-	1	0.26	2	0.52	3	0.78	-	-	-	-	2	0.42	2	0.42
Total/Frequency	14	3.62	14	3.62	13	3.36	41	10.59	8	1.69	6	1.27	19	4.01	35	7.38

x: Number of M_2 -mutant plants; y: Mutant frequency per 1000 M_2 plants
* values in the parantheses indicate the total number of M_2 plants screened

	IET 14142								IET 14143							
	250Gy (1500)		350Gy (1350)		450Gy (1440)		TOTAL (4290)		250Gy (1500)		350Gy (1230)		450Gy (630)		TOTAL (3360)	
Mutant characters	x	y	x	y	x	y	x	y	x	y	x	y	x	y	x	y
Late flowering	2	0.47	4	0.93	6	1.4	12	2.8	1	0.3	2	0.6	3	0.9	6	1.8
Early flowering	1	0.23	-	-	2	0.47	3	0.7	-	-	1	0.3	3	0.9	4	1.2
Non flowering	3	0.7	-	-	-	-	3	0.7	-	-	2	0.6	4	1.19	6	1.79
Grassy leaf	-	-	1	0.23	2	0.47	3	0.7	-	-	-	-	3	0.9	3	0.9
Rolled leaf	-	-	2	0.47	2	0.47	4	0.94	-	-	3	0.9	1	0.3	4	1.2
Broom stick	4	0.93	1	0.23	-	-	5	1.16	3	0.9	1	0.3	1	0.3	5	1.5
Striped leaf	-	-	1	0.23	1	0.23	2	0.46	-	-	-	-	1	0.3	1	0.3
Sterile	2	0.47	3	0.7	3	0.7	8	1.87	2	0.6	3	0.9	3	0.9	8	2.4
Dwarf	2	0.47	1	0.23	3	0.7	6	1.4	3	0.9	1	0.3	2	0.6	6	1.79
Semi dwarf	3	0.7	1	0.23	-	-	4	0.94	2	0.6	2	0.6	1	0.3	5	1.5
Tall	-	-	1	0.23	1	0.23	2	0.47	-	-	-	-	1	0.3	1	0.3
High yield	1	0.23	-	-	-	-	1	0.23	1	0.3	-	-	1	0.3	1	0.3
Total/Frequency	18	4.2	15	3.5	20	4.66	53	12.35	12	3.57	15	4.46	24	7.14	51	15.18

x: Number of M_2 - mutant plants; y: Mutant frequency per 1000 M_2 plants
* values in the parantheses indicate the total number of M_2 plants screened

Rao and Reddi (1986) reported late flowering mutants in rice by induced mutation. Non-flowering mutants were obtained in both the genotypes of Gobindabhog group at all treatments. Maximum frequency (1.55) of non-flowering plants was found at 350Gy in G_1. In Tulaipanja, non-flowering plants were obtained at 250Gy in IET 14142 and 350Gy and 450Gy in IET 14142.

The plant height in dwarf mutant was below 90 cm (Chang *et al*. 1985). They possessed smaller panicle coupled with reduction in the length of the first (panicle bearing) inter-node. The maximum frequency of dwarf mutant was found in IET 14143 at 250Gy. Dwarf mutant was observed in both the genotypes of Tulaipanja at all treatments. In Gobindabhog derivatives, there was no dwarf mutant at 350Gy. Dwarf mutants were developed through utilization of the induced mutation method in rice breeding by Alionte and Alionte (1995), Singh *et al*. (1998), Shadakshari *et al*. (2001) and Singh and Singh (2003). Semi-dwarfs are medium statured plants with height of about 90-110 cm when grown under favourable conditions of the tropics (Chang *et al*. 1985). Such mutants were isolated from all genotypes at different treatments. The maximum frequency of semi-dwarf mutants was induced in pigmented mutant of Gobindbhog genotype at 450Gy. Induced semi-dwarf mutants in rice have been reported by Rao and Reddi (1986), Alionte and Alionte (1995), Singh *et al*. (1998) and Singh and Singh (2003). Maximum frequency of tall mutants (at least 10 cm taller than their respective mother genotypes) was recorded in IET 13541 genotype at 450Gy. Rao and Reddi (1986), Singh *et al*. (1998) and Singh and Singh (2003) reported tall mutant in rice through induced mutation.

The high yielding mutants with at least, 10 per cent more yield than their respective mother genotypes were maximally present in pigmented mutant of Gobindbhog at 450 Gy. The high yield of the mutants was not due to one trait only. Cumulative effects of a number of yield attributing traits resulted in high yield. Singh *et al*. (1998), Shadakshari *et al*. (2001) and Singh and Singh (2003) reported high yielding mutants in rice through induced mutation.

The induction of mutations *viz.*, early flowering, sterile, dwarf and semi-dwarf mutants could be useful indirectly in recombination breeding programme for developing high yielding lines, while high yielding mutants could be of immediate use directly as varieties. The non-flowering mutants could be useful in understanding the physiological process of flowering. The possible cause of these morphological mutations or macro-mutations may be chromosomal aberrations, small deficiencies or duplications and most probably gene mutations.

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