

## Induction of Chlorophyll and Morphological Mutations through Gamma Ray in Traditional Aromatic Cultivar Tulaipanja

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The induced mutation of the traditional aromatic cultivar may provide useful alternative or complement to natural variation which may be used directly in mutation breeding or as a source of germ plasm in hybridization programme. Induced mutations irradiated through gamma ray in aromatic cultivar Tulaipanja were studied for chlorophyll and other morphological characters in the M<sub>2</sub> generation. The frequency of chlorophyll mutations was high in higher doses. Among the chlorophyll mutants studied, albina was the most frequent, followed by alboxantha, alboviridis, xantha, viridis and striata. The mutation efficiency and the mutagenic effectiveness of the mutagen is more in the lower dose. The semi-dwarf mutants were more prevalent followed by dwarf and semitall-I mutants. The number of height mutants is much more in lower dose than that of higher dose. Among the morphological mutants, a number of mutants with broom stick leaf and few mutants with grassy leaf, rolled leaf, striped leaf were obtained. Besides these, delayed flowering mutants were obtained in low frequency in both the doses while the early flowering mutants were obtained only in the lower dose. The desirable dwarf or semi-dwarf early flowering mutants may be utilized directly or for recombination breeding, whereas the high yielding lines screened may be used directly as aromatic cultivar provided if the performance in the later generation is good.

**Keywords:** Aromatic rice; Chlorophyll mutants; Induced mutation; Morphological mutants.

India is well acquainted for the production and supply of aromatic rice in the national and international market. Tulaipanja is a traditional non-basmati aromatic cultivar which is cultivated in the agro-climatic condition of West Bengal, but it is somehow neglected for its poor yield. The induced mutation of this aromatic cultivar may provide useful alternative or complement to natural variation which may be used directly in mutation breeding or as a source of germ plasm in hybridization programme. Large random variation can be produced through induced mutation in short

time in a chosen genetic background. But one of the most important disadvantage of induced mutation is the undesirable effects produced by the pleiotropic action of the mutant gene or simultaneous mutation of closely linked genes. Mutagenic treatments of seeds with different doses showed a definite increase or decrease in sensitivity to treatments which can be shown as mutation frequency in early generation like M<sub>2</sub>. Mutagenic efficiency is the proportion of mutation in relation to understandable changes like lethality, injury or sterility and mutagenic effectiveness is a measure

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of the frequency of mutation induced by a unit dose of mutation<sup>1</sup>. To understand such desirable and undesirable changes as well as the frequencies of various kinds of mutations in early generation like M<sub>2</sub> of aromatic traditional non-basmati rice after treatment with various doses of mutagen is very much useful to select the effective mutagen along with its proper doses for the study of mutation breeding and its effectiveness in crop improvement programme to evolve high yielding aromatic rice.

### MATERIALS AND METHODS

Dry bold and unhusked seeds of traditional aromatic non-basmati rice cultivar 'Tulaipanja' were irradiated with two different doses of gamma ray viz., 200 Gy and 300 Gy to raise M<sub>1</sub> generation. The number of seeds treated for each treatment was five hundred. Seeds of Tulaipanja which were not exposed to treatment involved in this investigation were used as control. Seeds of each M<sub>1</sub> single plant along with control Tulaipanja harvested separately and individually were used to grow M<sub>2</sub> generation. Seeds from each M<sub>1</sub> plant along with control were soaked in water separately and incubated for germination. The germinated seeds from each M<sub>1</sub> plant along with control were sown in separate individual earthen pots with requisite agricultural practices to generate M<sub>2</sub> plants. After seven days of germination, chlorophyll mutations were screened. At this stage, the first leaf was fully developed. The total number of M<sub>2</sub> seedlings in each earthen pot, the number of chlorophyll mutations and their types were counted and recorded. The chlorophyll mutants were classified following Gustafsson<sup>2</sup>. A single seedling per hill was then transplanted in the field to generate individual progeny row. The M<sub>2</sub> populations were thoroughly screened in various developmental stages and identified for chlorophyll and other morphological mutations based on visual observations and quantitative data. Mutants

were also identified on the basis of changes in morphological characters. The following formulae were used to estimate the mutation efficiency and mutagenic effectiveness:

$$\text{Mutagenic Efficiency} = \frac{\text{Mutation Frequency in } M_2}{\text{Percentage of sterility in } M_1} \text{ and}$$

$$\text{Mutagenic Effectiveness} = \frac{\text{Mutation Frequency in } M_2}{\text{Dose of mutagen}}$$

### RESULTS AND DISCUSSION

The mutation frequencies based on M<sub>2</sub> generation were found to be most effective to consider actual frequency for mutation breeding programme<sup>3</sup>. The plants thoroughly examined for deviation of characters from the parent and the suspected mutants were screened. The M<sub>2</sub> plants deviating distinctly from the mother variety with regard to colour, structure, stem, leaf, panicle, grain and other characters were counted as mutants.

The frequency of chlorophyll mutations in M<sub>2</sub> generation is presented in Table 1. The chlorophyll deficient types exhibited deficiency in chlorophyll formation in different plant parts which appeared at different stages of development. While some of the chlorophyll mutants were nonviable types and died at the seedling stage, several others were viable chlorophyll mutant, producing normal grains. In this present investigation, various kinds of chlorophyll mutations observed are classified as albina, xantha, alboxantha, striata, viridis and alboviridis which may be lethal or nonlethal (Table 2). Mutagenic efficiency and mutagenic effectiveness were estimated (Table 3). Different types of morphological mutants found were plant height mutants like dwarf, semi-dwarf, semi-tall and tall as well as leaf mutants like rolled leaf, broom stick leaf, grassy leaf and striped leaf and also early flowering and late flowering mutants (Table 4). Similar observations were also made by Sharma *et al.*<sup>4</sup>. The above mentioned mutants

**Table 1.** Frequency of chlorophyll mutations in M<sub>2</sub> generation

Treatment	Total no. of M <sub>2</sub> seedlings investigated	No. of mutant seedlings	Frequency of chlorophyll mutations per 1000 M <sub>2</sub> seedlings
200 Gy	11585	176	15.19
300 Gy	7050	145	20.57

Table 2. Chlorophyll mutations spectra in M<sub>2</sub> generation

Treatment	Albina		Xantha		Viridis		Striata		Alboxantha		Alboviridis		Total no. of mutants of mutant	Percent of mutant
	a	b	a	b	a	b	a	b	a	b	a	b		
	Frequency of different chlorophyll mutant classes													
200 Gy	114(66.47)	1.01	0.00(0)	0.00	11 (6.25)	0.10	0.00 (0)	0.00	26 (14.77)	0.22	22 (12.5)	0.19	176	1.52
300 Gy	109 (75.17)	1.55	13 (8.96)	0.18	0.00 (0)	0.00	6 (4.14)	0.09	17 (11.72)	0.24	0.00 (0)	0.00	145	2.06

a : No. of mutant seedlings recorded in M<sub>2</sub> generationb : Frequency of respective particular chlorophyll mutation per 100 M<sub>2</sub> seedlings

Figures in parenthesis indicates percentage of individual chlorophyll mutation in particular respective dose

were not noticed in M<sub>1</sub> generation, but appeared in M<sub>2</sub> generation reflecting the recessive nature of mutation for the above mentioned characters.

In M<sub>2</sub> generation, the frequency of chlorophyll mutations was high in 300 Gy dose than the 200 Gy dose which were 20.57 and 15.19, respectively, per 1000 M<sub>2</sub> seedlings (Table 1). So, the abundance of induced chlorophyll mutations proportionately increased with the increment of dose. The results are comparable with Chakraborty and Kole<sup>5</sup>. In the mutation breeding programme, the chlorophyll mutants are considered to be the important measure for the mutagenic property<sup>6</sup>.

Among the chlorophyll mutants (Table 2), the frequency of *albina* was highest in both 300 Gy and 200 Gy doses which was, however, higher in the dose of 300 Gy than the dose of 200 Gy. The frequency of *albina* was found to be 1.55 in 300 Gy and 1.01 in 200 Gy. The *xantha* and *striata* mutants appeared in the 300 Gy only with the frequency of 0.18 and 0.09, respectively. The frequency of mutant *alboxantha* was found more in the higher dose of 300 Gy (0.24) than the lower dose of 200 Gy (0.22). Further, *viridis* and *alboviridis* appeared in the lower dose only with a frequency of 0.10 and 0.19, respectively.

While comparing the mutation spectrum of the two doses of gamma rays, it was observed that *albina* was the most frequent chlorophyll mutant in both the doses. Sharma *et al.*<sup>7</sup> and Singh and Singh<sup>8</sup> also observed that the *albina* was the predominant type of mutants. The second next most frequent group was *alboxantha*. Although, *xantha* and *striata* did not appeared in the lower dose, but *viridis* and *alboviridis* were noticed in the lower dose only. So, in general, the frequency of chlorophyll mutation was increased with the increase of dose of gamma ray as also reported by Singh *et al.*<sup>9</sup>. A wide range of variations in the frequency of chlorophyll mutations was observed. So according to their occurrence, they may be placed as *albina*, followed by *alboxantha*, *alboviridis*, *xantha*, *viridis* and *striata*.

To isolate the desirable mutants, it is important to estimate the mutation efficiency and mutagenic effectiveness of a mutagen from a large population. The results indicated that the mutation efficiency (0.06 in 200 Gy and 0.03 in 300 Gy) and mutagenic effectiveness (0.008 in 200 Gy and 0.007 in 300 Gy) of the mutagen is more in

the lower dose of 200 Gy than the higher dose of 300 Gy (Table 3). The low estimates may be due to the amount of damage in the earlier generation that accounted for the mutability of genes<sup>5</sup>.

The frequency of various morphological mutants estimated per 1000 M<sub>2</sub> plants (Table 4) was higher in lower dose of 200 Gy (16.91) than the higher dose of 300 Gy (11.58).

Plant height mutants were primarily characterized by reduction in height and a wide range of variations in this trait was observed. For the sake of convenience, the mutants were classified as dwarf (below 90 cm), semi-dwarf (90 cm to 110 cm), semi-tall (above 90 cm and upto 130 cm), semitall-II (above 130 cm and up to 140 cm) and tall (above 140 cm). Among the plant height mutants, semi-dwarf type was more prevalent followed by dwarf and semitall-I mutants in both the 200 Gy and 300 Gy doses. The frequency of

semi-dwarf, dwarf and semi-tall-I mutants was 5.52, 2.21 and 0.55, respectively, in 200 Gy of dose and 3.68, 2.02 and 1.29, respectively, in 300 Gy of dose. The semitall-II and tall mutants were obtained with a lower frequency of 0.18 and 0.18 respectively, in 200 Gy and 0.92 and 0.37 respectively, in 300 Gy. The number of height mutants is much more in lower dose of 200 Gy than that of higher dose of 300 Gy. Most of the dwarf mutants had higher number of tillers, short panicles with high spikelet sterility. The semi-dwarf mutants had higher number of tillers along with higher number of filled grains and erect leaves. Semi-dwarf and dwarf mutants were also isolated<sup>5,8,10</sup>. Induced mutants with tall habit were also visualized<sup>5,8,11</sup>.

Various other types of morphological mutants were obtained in M<sub>2</sub> generation (Table 4). Among the morphological mutants, a number of

**Table 3.** Efficiency and effectiveness of mutagen in the local aromatic cultivar ‘Tulaipanja’

Treatment	No. of M <sub>2</sub> plants studied	No. of mutants in M <sub>2</sub> generation	Mutation rate (%)	% of Sterility in M <sub>1</sub> generation	Efficiency of mutagen	Effectiveness of mutagen
200 Gy	11585	176	1.52	27.39	0.07	0.008
300 Gy	7050	145	2.06	63.33	0.33	0.007

**Table 4.** Frequency and spectrum of different morphological mutants in M<sub>2</sub> generation of local aromatic cultivar ‘Tulaipanja’

Mutant characters	No. and frequency of morphological mutants					
	200 Gy(3674)		300 Gy(1765)		Total(5439)	
	a	b	a	b	a	b
Dwarf	12	2.21	11	2.02	23	4.23
Semi-dwarf	30	5.52	20	3.68	50	9.19
Semi-tall-I	3	0.55	7	1.29	10	1.84
Semi-tall-II	1	0.18	5	0.92	6	1.10
Tall	1	0.18	2	0.37	3	0.55
Late flowering	3	0.55	6	1.10	9	1.65
Early flowering	16	2.94	0	0.00	16	2.94
Rolled leaf	0	0.00	6	1.10	6	1.10
Broom stick leaf	23	4.23	0	0.00	23	4.23
Grassy leaf	0	0.00	1	0.18	1	0.18
Striped leaf	1	0.18	2	0.37	3	0.55
Sterile mutants	2	0.37	3	0.55	5	0.92
High yield	1	0.18	2	0.37	3	0.55
Total/Frequency	93	17.09	65	11.95	158	29.03

a : No. of respective individual mutant plants

b : Mutant Frequency for respective character per 1000 M<sub>2</sub> plants

Figures in parenthesis indicated total no. of mutant plants evaluated

mutants with broom stick leaf with a frequency of 4.23 were obtained in 200 Gy dose only. Besides this, grassy leaf, rolled leaf, striped leaf mutants were also obtained with a low frequency. The frequency of these mutants varied from 0.18 to 1.1.

The grassy leaf mutant was characterized by typical grassy leaves mostly with profuse and thin tillers. Culms were thin, weak and spreading. Leaves were narrow, droopy and pale green in colour. Single grassy leaf mutant was obtained in the dose of 300 Gy with a frequency of 0.18. The plant was short with a height of 42 cm. The panicles were very short. The grains were awned and smaller than control with high amount of spikelet sterility.

The rolled leaf mutants were characterized with rolled leaves. The rolling of leaves was more in early stage, which become semi-rolled at maturity. The mutants had erect and thin culms with semi-erect, narrow and green leaves. The panicles were short. The frequency of rolled leaf mutants was 1.10 in the dose of 30 Gy, whereas, it was not found in 200 Gy of dose. The height of the mutant plants ranged from 85 to 105 cm.

The striped leaf mutants had leaves with yellowish to white stripes. Three to four leaves were striped and the rest were normal. The mutants were tall with thick, spreading culms and droopy leaves. The frequency of striped leaf mutants obtained in the dose of 300 Gy is 0.37 and it was 0.18 in 200 Gy of dose. The heights of the two plants found in 300 Gy of dose were 141 cm and 137 cm. The height of the striped leaf mutant noticed in 200 Gy of dose was 140 cm. The broom stick leaf mutants were characterized with brush or broom stick or needle like leaves. The mutants were short in height having erect culm. The panicles were short and more than fifty percent of the grains were chaffy. The broom stick like appearance was due to excessive rolling of the leaves. The mutants were obtained in the dose of 200 Gy with a frequency of 4.23. This kind of mutant was not noticed in higher dose. All the mutant plants recovered were dwarf with the height ranging from 53 to 85 cm. except five mutant plants which were semi-dwarf in nature. These kinds of morphological mutants were also noticed earlier<sup>5</sup>.

The other kinds of mutants observed in  $M_2$  generation were early flowering and delayed flowering mutants. The early flowering mutants were characterized by the earliness of heading time, where the date of panicle emergence of

those mutants was around ten days earlier than the control. The date of heading was delayed by one month for delayed mutants as compared to control as well as the panicle exersion were poor and incomplete. Among the other mutants, delayed flowering mutants were obtained with a frequency of 0.55 in 200 Gy of dose and 1.10 in 300 Gy of dose and the early flowering mutants were obtained only in the lower dose of 200 Gy with a frequency of 2.94. The delayed flowering mutants were associated with reduced culm length which were erect and thick. The panicles were short and the grains were chaffy. The height of the mutants ranged from 98 cm to 107 cm. The early flowering mutants obtained in 200 Gy dose were tall and identical with control. The height of the mutants ranged from 125 cm. to 145 cm. Early flowering mutants were also visualized by several researchers<sup>5,8,12,13</sup>. The delayed flowering mutants in rice were also visualized<sup>14</sup>.

The sterile mutants were obtained in both 200 Gy and 300 Gy doses as was also noticed earlier in the  $M_2$  generation<sup>11,15</sup>. These mutants produced one or two grains only and had 99.5% sterile pollen grains. The mutants had semi-erect and thick culms with narrow green leaves. The height of the mutants ranged from 75 cm. to 110 cm.

So in general, the chlorophyll mutants which are of common occurrence in this present investigation have been used as a measure of mutagenic action in the mutation breeding experiments<sup>16</sup>. These are potentially useful in understanding the different physiological functions and effects of specific gene products and have been utilized for the study of mutation frequency and mutation spectrum<sup>17</sup>.

Different classes of height mutants viz., dwarf, semi-dwarf, semi-tall-I, semi-tall-II and tall were recovered. The highest frequency was observed in semi-dwarf followed by dwarf, semi-tall-I and semi-tall-II. Tall mutants were also obtained, but with very low frequency in both the doses. Hence it can be concluded that short culm mutants could be induced in rice rather easily through gamma ray treatment with proper dose. The frequencies of dwarf and semi-dwarf plants were high in lower dose of 200 Gy and low in higher dose of 300 Gy, while the frequency of semi-tall-I, semi-tall-II and tall were more in higher

dose of 300 Gy and small in the lower dose of 200 Gy. The study also reveals that these mutants are phenotypically distinct from each other and form a varied group which could be considered as major class of mutations occurring with a very high frequency indicating that height locus have a number of mutational sites, sensitive to mutagenic treatments. All the short culm mutants were associated with changes in a number of other morphological characters related to yield, indicating that the mutant genes might have pleiotropic effect.

A few high yielding mutants with good performance for yield and yield attributing traits were observed in both the treatments, but it was more in higher dose of 300 Gy. Such kind of high yielding induced mutants in rice were also noticed earlier<sup>5,8,11,13</sup>.

### CONCLUSION

Considering the objective of the present investigation, the quantification of mutation frequency based on M<sub>2</sub> generation provides useful picture which will be helpful for mutation breeding programme of aromatic rice. It is very much effective for the selection of mutagen and ideal dose for the breeders. The genes associated with reduced height and early flowering may play important role to evolve short statured aromatic rice cultivar. The aromatic mutants with important characters like dwarf or semi-dwarf nature of height, early flowering may be utilized directly or for recombination breeding, whereas the high yielding lines may be used directly as aromatic cultivar of rice provided the performance in the later generations is stable.

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