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Induced macro mutational spectrum and frequency of viable mutants in m_2 generation of non-basmati aromatic rice

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Abstract
This experiment was aimed at generate genetic variability in a non-basmati aromatic rice using the most potent physical mutagen i.e. gamma rays. The seeds were irradiated with four different doses of gamma rays viz 250 Gyh^{-1}, 300 Gyh^{-1}, 350 Gyh^{-1} and 400 Gyh^{-1}. In M_2 generation, large numbers of morphological mutants were identified. Observations were recorded in each of selected putative mutants in M_2 generation. Several viable mutants were observed with respect to plant type, increased tillering, stem colour, grain colour, rapid elongation, complete spikelet sterility, stigma colour, grain type, broom stick appearance, awned grains and early maturing. The plant yield of many mutants were reduced with respect to the parents, the ideotype of the some mutants were good with respect to plant height, the early maturing and double spikelet at mid and tip region of panicle. The gene which is responsible for dwarfism and early maturity in non-basmati aromatic rice can play significant role to develop short stature rice cultivar with retaining original quality.

Keywords: Gamma rays, M_2 generation, viable mutants, non-basmati aromatic rice

Introduction
In present time, crop improvement programme in cereals is undertaken through mutation breeding all over the world. Among the cereals, rice is one of the most important crop being grown in maximum regions of India. The innovation of new rice cultivar was expected to increase the productivity of rice production. The desired changes in genotypes of crop species are achieved by a series of interrelated and largely interdependent activities viz., creation of variation, selection, evaluation, multiplication and distribution, out of which creation of variation is important for effective selection many attempts in the field of mutation research have been made by different scientists to get desirable traits in cultivated rice and in determining the most effective mutagenic treatment (Reddy and Rao 1988, Bansal *et al.*, 1990, Pillai *et al.*, 1993) [11, 1, 10]. The mutagens may cause genetic changes in an organism, break the linkage and produce many new promising traits for the improvement of crop plants (Shah *et al.*, 2008) [13]. Gamma ray is one of the potent mutagens because it has the ability to penetrate in deep to plant tissue. Mutation induction was directed to rectify one or more important characters while other original characters are retained. Viable mutations are those which affecting the morphology of different parts of the plants such as habit, stature, leaf, stem, pod and seed. Wide spectrum of viable morphological mutations was isolated in M_2 generation (Wani, 2011) [15]. Mutations are phenotypically classified into two groups (Gaul, 1964) [4]; macro mutations: easily detectable in individual plants, phenotypically visible and morphologically distinct and they are qualitatively inherited genetic changes, and control by major genes or oligogenes; and micro mutations can be detected only by help of statistical methods and quantitatively inherited genetic changes, and control by minor genes or polygenes, phenotypically not visible.

Materials and Methods
Dry, uniform, bold seeds of aromatic cultivar Badshahbhog each weighing 250g were taken in five packets for the experiment. Four packets were irradiated by ^{60}Co gamma rays four different doses viz. 250 Gyh^{-1}, 300 Gyh^{-1}, 350 Gyh^{-1} and 400 Gyh^{-1} at Bidhan Chandra Krishi Viswa vidyalaya (BCKV), West Bengal. The unexposed seed packet was consider as control. In M_1 generation mother and daughter panicle harvested seperately. In M_2 generation these panicle seeds are sown to raise M_2 seedlings. Thirty-day-old seedlings were transplanted in puddled field as progeny-row with one seedling per hill. Total number of M_2 families was 111,108,69 and 57 in 250Gyh^{-1}, 300Gyh^{-1}, 350Gyh^{-1} and 400Gyh^{-1} doses respectively.

Frequency and spectrum of viable mutants

The frequency and spectrum of different types of viable mutants were scored at various developmental stages of M_2 plants particularly from flowering to maturity period. The frequency and spectrum of viable mutants were calculated on M_1 plant basis and M_2 seedling basis.

$$\text{Mutation Frequency (MF) based on } M_1 \text{ plant basis (\%)} = \frac{\text{Number of viable mutant } M_1 \text{ families}}{\text{Total number of } M_1 \text{families}} \times 100$$

$$\text{Mutation Frequency (MF) based on } M_2 \text{ seedling basis (\%)} = \frac{\text{Number of viable mutant } M_2 \text{ seedlings}}{\text{Total number of } M_2 \text{ seedlings}} \times 100$$

Results

Viable mutation frequency in M_2 generation (M_1 plants & M_2 seedlings basis)

The observed data of viable mutations were given in Table-1.The frequency of viable mutants ranged from 34.78 to 42.10 on M_1 plants and 3.26 to 11.95 on M_2 seedlings basis. The viable mutants were observed in all the doses. The maximum and minimum frequencies were observed in 400 Gyh^{-1} and 350 Gyh^{-1} for M_1 plant basis and 300 Gyh^{-1} and 350 Gyh^{-1} for M_2 seedling basis. In the present observation, the viable mutation frequency was high on M_1 plant basis than M_2 seedlings basis.

Spectrum and frequency of viable mutations in M_2 generation (M_2 plants basis)

A total of 11 types of morphological mutations were identified in non-basmati aromatic rice; there frequencies of morphological deviants observed on segregating progeny basis and individual plant basis (Table; 2)

1. Plant type

a) Tall (>140 cm) - The frequency of viable mutants of M_2 plants ranged from 26.6 to 28.59 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 250 Gyh^{-1} and 400 Gyh^{-1}. Sharma (1985) [14] reported similar tall variety.

b) Semi dwarf (110-140 cm) - The frequency of viable mutants of M_2 plants ranged from 1.9 to 3 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 250 $Gyh^{-1}$and 400 Gyh^{-1}.

c) Dwarf (<110 cm) - The frequency of viable mutants of M_2 plants ranged from 0.05 to 0.13 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 400 Gyh^{-1} and 250 Gyh^{-1}.

2. Increased tillering - The frequency of viable mutants of M_2 plants ranged from 7.7 to 27.7 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 250 Gyh^{-1} and 300 Gyh^{-1}.

3. Stem colour

a) Dark green - The frequency of M_2 plants for dark green stem ranged from 6.2 to 19 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 250 $Gyh^{-1}$and 300 Gyh^{-1}.

b) Light green - The frequency of M_2 plantsfor light green stem ranged from 24.43 to 28 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 250 $Gyh^{-1}$and 400 Gyh^{-1}.

4. Grain colour

a) Black- The frequency of viable mutants of M_2 plants ranged from 0.15 to 1.46 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 400 Gyh^{-1} and 300 Gyh^{-1}.

b) Purple- The frequency of viable mutants of M_2 plants ranged from 0.5 to 0.93 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 400 Gyh^{-1} and 300 Gyh^{-1}.

5. Rapid elongation- The frequency of M_2 plants for rapid elongation recorded from 1.07 to 2.91 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 250 Gyh^{-1} and 400 Gyh^{-1}.

6. Complete spikelet sterility - The frequency of viable mutants of M_2 plants ranged from 10.5 to 13.49 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 400 Gyh^{-1} and 35 Gyh^{-1}.

7. Stigma colour

a) Black - The frequency of M_2 plants for black stigma colour observed from 0.08 to 1.52 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 400 Gyh^{-1} and 250 Gyh^{-1}.

b) Purple - The frequency of M_2 plants for Purple stigma colourobserved from 0.27 to 1.02 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 25 Gyh^{-1} and 40 Gyh^{-1}.

8. Grain type

a) Long slender grain - The frequency of viable mutants of M_2 plants ranged from 0.8 to 3.64 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 300 $Gyh^{-1}$and 250 Gyh^{-1}.

b) Bold grain - The frequency of viable mutants of M_2 plants ranged from 6.35 to 8.2 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 250 Gyh^{-1} and 350 Gyh^{-1}.

c) Double spikelet at mid and tip region of panicle - This type of viable mutants only appear in single progeny in all treatments except 300 Gyh^{-1}. The frequency of viable mutants of M_2 plants in non-basmati aromatic rice maximum and minimum were observed in two doses 400 Gyh^{-1} (0.06) and 250 Gyh^{-1} (0.03).

9. Broom stick appearance - The frequency of viable mutants of M_2 plants ranged from 7.1 to 8.1 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 300 Gyh^{-1} and 350 Gyh^{-1}.

10. Awned grains

a) Partially awned- The frequency of viable mutants of M_2 plants ranged from 0.06 to 0.43 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 400 Gyh^{-1} and 350 Gyh^{-1}.

b) Completely awned- The frequency of viable mutants of M_2 plants ranged from 0.1 to 0.34 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 400 Gyh^{-1} and 250 Gyh^{-1}.

11. Early maturing (10-15 days)- The frequency of viable mutants of M_2 plants ranged from 0.06 to 0.16 in non-basmati aromatic following gamma rays treatment. The maximum and minimum frequencies were observed in two doses 400 Gyh^{-1} and 250 Gyh^{-1}.

Discussions

The viable mutation frequency was high on M_1 plant basis than M_2 seedlings basIs also found by Manikandan and Vanniarajan (2017) [7]. According to Blixt (1972) [2], morphological changes are either due to pleotropic gene action or of cryptic chromosomal deletions. Mutated plants displayed obvious phenotypic changes, in the color, shape, and size, flowering time, panicle variation, tillering habit, which distinguished them from non-irradiated plants, as previously reported by Haris *et al.* (2013) [5], Manikandan and Vanniarajan (2017) [7]. According to the statements of Luo *et al.* (2012) [6], these morphological traits may be controlled by recessive genes or susceptible to environment. Dwarfness was mostly caused by recessive major gene mutations (Mikaelsen, 1980). *Shadakshari et al.* (2001) [12] reported a higher frequency of dwarf/semi-dwarf non-lodging mutants in five rice varieties treated with gamma rays. Early maturing mutants had less number of productive tillers, recorded higher grain and straw yield, and matured earlier by 10 days. Similar observations were recorded by Sharma (1985) [14]. Micke (1999) [8] viewed that pleotropy is a typical attribute of induced mutations. Mutations affecting pleotropic genes governing several characters were also reported by Deshmukh *et al.* (1972) [3].

Table 1: Viable mutation frequency in M_2 generation

Mutagens (Dose)	No. of M_1 plants		No. of M_2 plants		Mutation frequency (%)	
	Plants forwarded	Segregating	Studied	Chlorophyll mutants	M_1 plant basis	M_2 seedling basis
Control	10	-	100	-	-	-
250 Gyh^{-1}	111	44	6811	572	39.64	8.39
300 Gyh^{-1}	108	42	5958	712	38.89	11.95
350 Gyh^{-1}	69	24	3343	109	34.78	3.26
400 Gyh^{-1}	57	24	2845	208	42.10	7.31

Table 2: Spectrum and frequency of viable mutations in M_2 generation

Dose	Total No. of M_2 progenies studied	Total No. of M_2 seedling studied	Plant Type						Increased Tillering	
			Tall		Semi dwarf		Dwarf			
			No. of Progenies segregating*	No. of Mutant**	No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant
250 Gyh^{-1}	111	6333	108(97.30)	1811(28.59)	34(30.63)	189 (3)	3(2.70)	3(0.05)	107(96.39)	487(7.7)
300 Gyh^{-1}	108	6255	104(96.30)	1667(26.6)	35(32.40)	150(2.4)	5(4.62)	6(0.1)	104(96.30)	512(27.7)
350 Gyh^{-1}	69	4017	65(94.20)	1095(27.25)	22(31.88)	76(1.9)	3(4.34)	3(0.07)	65(94.20)	288(25.57)
400 Gyh^{-1}	57	3225	52(91.22)	955 (29.6)	14(24.56)	43(1.34)	3(5.26)	4(0.13)	53(92.98)	240(24.43)

Dose	Total No. of M_2 progenies studied	Total No. of M_2 seedling studied	Stem Colour				Grain Colour			
			Dark Green		Light Green		Black		Purple	
			No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant
250 Gyh^{-1}	111	6333	82(73.87)	1227 (19)	106(95.49)	1790 (28)	5(4.50)	15(0.2)	12(10.81)	36(0.6)
300 Gyh^{-1}	108	6255	59(54.62)	391(6.2)	101(93.51)	1754(27.7)	4(3.70)	10(0.15)	10(9.26)	30(0.5)
350 Gyh^{-1}	69	4017	47(68.11)	419(10.43)	63(91.30)	1027((25.57)	4(5.80)	11(0.27)	9(13.04)	32(0.8)
400 Gyh^{-1}	57	3225	51(89.47)	325(10.08)	52(91.22)	788(24.43)	16(28.07)	47(1.46)	14(24.56)	30(0.93)

Dose	Total No. of M_2 progenies studied	Total No. of M_2 seedling studied	Rapid Elongation		Complete Spikelet Sterility		Stigma Colour			
							Black		Purple	
			No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant
250 Gyh^{-1}	111	6333	46(41.44)	68(1)	91(81.98)	1897 (12)	2(1.80)	5(0.08)	6(5.40)	17(0.27)
300 Gyh^{-1}	108	6255	62(57.40)	101(1.6)	101(93.51)	743(11.8)	4(3.70)	10(0.15)	11(10.19)	33(0.5)
350 Gyh^{-1}	69	4017	40(57.97)	58(1.44)	60(86.96)	422(10.5)	4(5.80)	10(0.25)	9(13.04)	35(0.87)
400 Gyh^{-1}	57	3225	54(94.73)	94(2.91)	52(91.22)	435(13.49)	19(33.34)	49(1.52)	15(26.31)	33(1.02)

Table 2: Continued…..

Dose	Total No. of M_2 progenies studied	Total No. of M_2 seedling studied	Grain Type						Broom Stick Appearance	
			Long Slender Grain		Bold Grain		Double spikelet at mid and tip region of panicle			
			No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant
250 Gyh^{-1}	111	6333	21(18.91)	52(0.8)	98(88.29)	521(8.2)	1(0.90)	2(0.03)	98(88.29)	519(8)
300 Gyh^{-1}	108	6255	36(33.34)	199(3.19)	97(89.82)	444(7)	0(0)	0(0)	95(87.96)	507(8.1)
350 Gyh^{-1}	69	4017	14(20.29)	146(3.64)	60(86.96)	255(6.35)	1(0.93)	2(0.05)	60(86.96)	285(7.1)
400 Gyh^{-1}	57	3225	6(10.53)	23(0.71)	51(89.47)	224(6.95)	1(1.75)	2(0.06))	56(98.25)	251(7.78)

Dose	Total No. of M_2 progenies studied	Total No. of M_2 seedling studied	Awned Grains				Early Maturing	
			Partially awned		Completely awned			
			No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant	No. of Progenies segregating	No. of Mutant
250 Gyh^{-1}	111	6333	2(1.80)	4(0.06)	2(1.80)	7(0.1)	4(3.60)	4(0.06)
300 Gyh^{-1}	108	6255	7(6.48)	16(0.3)	7(6.48)	17(0.27)	6(5.56)	6(0.1)
350 Gyh^{-1}	69	4017	5(7.25)	9(0.22)	7(10.15)	10(0.25)	4(5.80)	4(0.1)
400 Gyh^{-1}	57	3225	8(14.03)	14(0.43)	5(8.77)	11(0.34)	5(8.77)	5(0.16)

* values in parantheses indicate the frequency of segregating progenies in M_2 generation

** values in parantheses indicate the frequency of mutants over total seedling studied in M_2 generation

Conclusion

The present study revealed that the importance of gamma rays inducing genetic variability in rice crop. The mutants varied in different morphological characters which are generally absent in available germplasm. The gene which is responsible for dwarfism and early maturity in non-basmati aromatic rice can play significant role to develop short stature rice cultivar with retaining original quality.

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