Indian J. Genet., 78(4): 409-416 (2018)
DOI: 10.31742/IJGPB.78.4.2

Development of high yielding aromatic mutants of rice (*Oryza sativa* L.) from a local aromatic cultivar, Tulaipanji by using gama radiation

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(Received: July 2018; Revised: November 2018; Accepted: November 2018)

Abstract

Mutation induced variability is an important method in plant breeding to create non-existing desirable genotype(s) for crop improvement. In this endeavour, gamma radiation was used to create variability for improving plant type and yield attributing traits in a local aromatic cultivar, Tulaipanji. Eight desirable mutants were identified from M_4 generation. All those eight mutants induced from Tulpanji were photoperiod-insensitive, non-lodging, semi-dwarf with high yield potential and retained the aroma. Mutants TP3-2, TP3-4 and TP3-6 showed high increase in yield (>89%) over the control cultivar, Tulpanji. As the mutants were photoperiod insensitive, they may be recommended for cultivation for both *boro* and *kharif* seasons after necessary trials and demonstrations.

Key words: Aromatic rice, mutation breeding, high yielding aromatic mutants, gamma radiation

Introduction

Mutation is an alternative method of plant breeding, in which genes are permanently altered under environmental conditions while being transferred between generations (Tüylü et al. 2009). Term 'Mutation Breeding' was firstly used in 1944 by Freisleben and Lein to explain the use of induction for product development on plants and development of mutant lines (Forstera et al. 2012). Classical breeding methods are still being used effectively for the development of new crop varieties and to obtain improved plant types, yet, use of current populations leads to a contraction in genes. This situation inspires the breeders to adopt new breeding technologies. Mutation breeding have been one of the alternative methods for breeders as it provides the chance of obtaining some desired features that do not exist in the nature or got lost during the evolution. Mutation has been successfully employed in creating genetic variability for desirable traits and used these variants for breeding several crops including cereals, pulses and oilseeds (Mohamad et al. 2005; Singh and Balyan 2009; Rampure et al. 2017; Mahla et al. 2018). Induced mutation is an important tool in rice breeding worldwide (Maluszynski 1998). The observed photoperiod insensitive mutants were proposed for higher yield per unit area and taking offseason crop for farm economization. Grain quality is second only to yield as the major breeding objective (Juliano et al. 1964).

Assam and adjoining states in India and some neighbouring south Asian countries are the primary centre (The Hindustan Centre of Origin, which includes Myanmar, Assam, Malaya Archipelago, Java, Borneo, Sumatra and Philippines) of origin of rice. Traditional land races are important reservoirs of valuable traits which need special attention for future conservation. Some varieties are being extensively grown in specific problem oriented areas of West Bengal and Assam by tribal farmers, such as deep water rice, submergence and drought tolerant genotypes etc. Landraces and wild species possess immense potential of most valuable gene which can be efficiently utilize in the breeding programmes to develop high yielding rice varieties with quality (Trikey et al. 2013). Most of the Farmers' Varieties grown in these areas are highly photoperiod-sensitive. A number of aromatic traditional rice cultivars are available in

*Corresponding author's e-mail: bcroy10@yahoo.com
Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by indianjournals.com; www.isgpb.org

the northern part of West Bengal (Roy et al. 1985; Talukdar et al. 2012; Chakravorty and Ghosh 2013). Tulaipanji is a popular aromatic Farmers' Variety of rice grown in Dakshin Dinajpur and Uttar Dinajpur districts of West Bengal, India. Its quality is at par with the Basmati rice. However, this cultivar has some drawbacks, like highly photoperiod-sensitive, long duration, weak culm, lodging susceptible and low yield. The present investigation was therefore, aimed at developing photoperiod-insensitive mutants, short or medium duration, dwarf or semi-dwarf plant stature, non-lodging type with comparatively high yield potential along with other desirable traits such as slender grains and aroma retention of a Farmers' Variety of rice, "Tulaipanji" through mutation breeding.

Materials and methods

Plant material and field experimentation

The rice var. Tulaipanji was used as the experimental material in this study. Pure and healthy dry seeds of Tulaipanji were treated with gamma rays from ^{60}Co source at the doses of 250 Gy, in gamma radiation chamber GC5000 Bhabha Atomic Research Centre, Trombay, Mumbai, India. The treated seeds were sown in *kharif* season of 2014. The four weeks old M_1 seedlings were transplanted to the main field using spacing of 30 cm × 20 cm with one seedling per hill. The population of M_1 generation was more than 3000 plants/treatment. Since mutants are mostly recessive, selection was not done in M_1 generation. The main panicle in each plant was harvested separately as suggested by Aung et al. (2016) and kept for raising M_2 generation. In *boro* season (dry season) of 2014-15, the cultivar Tulaipanji and harvest of M_1 generation were grown as plant to row progenies. Mutants, which were able to flower early in *boro* season displaying photoperiod-insensitivity were identified and tagged. Photoperiod-insensitive mutants were continuously screened for important agronomic traits. Mutants plants isolated from the population were harvested individually and seeds were raised in M_3 generation during *kharif* 2015.

The selected mutants were grown in M_4 generation during *boro* 2015-16 in the Experimental Research Farm of Uttar Banga Krishi Viswavidyaaya, Pundibari, Cooch Behar. Twenty-five days old seedlings were transplanted (singles seedling per hill) in plots measuring 3 x 1.5 m area in Randomized Block Design with three replications. Standard agronomic practices compatible to the humid tropic of *Terai* zone were followed to obtain good crop stand. Observations recorded on days to 50% flowering, plant height, number of panicles per plant, panicle length, flag leaf length, flag leaf width, stem thickness, number of filled grain per plant, 100-grain weight, grain length, grain width, decorticated grain length, decorticated grain width, grain type and grain yield per plant. Guidelines described by the PPV & FRA (2007) were followed to characterize the genotypes for DUS traits. The mean performance of individual genotypes was employed for statistical analysis.

The experimental plan used was randomized blocks design with 8 treatments (genotypes) per replication and three replications, in a total of 24 experimental units. Statistical analyses of data were conducted with absolute values. The data were analysed using AgRes Statistical Software, (c) 1994 Pascal Intl Software Solutions, Version 3.01 to find out the variability of the experimental treatments.

Results

The data recorded for different agronomic traits of all the eight mutants of Tulapanji rice variety in M_4 generation was used for analysis during *boro* season of 2015-16. The induced mutants namely, TP3-1, TP3-2, TP3-3, TP3-4, TP3-5, TP3-6, TP3-7 and TP3-8 were found photoperiod-insensitive. The control variety, 'Tulaipanji' is a highly photoperiod-sensitive and can be grown during *kharif* as well as *boro* seasons. Photoperiod-sensitivity is an understandable trait and easy to be selected in *boro* season because the Tulaipanji is photoperiod-sensitive and could be grown during *kharif* season only. Hence the mutant generations were continuously screened for selecting photoperiod-insensitive, short to medium duration, dwarf and non-lodging mutants with aroma. Analysis of variation showed that all the yield attributing and grain characters, such as days to 50% flowering, plant height, panicles per plant, panicle length, flag leaf length, flag leaf width, stem thickness, number of filled grains per panicle, number of chaffy grains per panicle, grain length, grain width, grain thickness, decorticated grain width, 1000 grain weight and grain yield per plant had highly significant differences among the mutants (Tables 1A and 1B). However, decorticated grain length showed non-significant difference among the mutants and control variety Tualipanji.

The agronomic data were compared with the values obtained for control variety. Mean data of yield attributing characters of the eight mutants are

Tables 1A and 1B. Analysis of variation among eight mutants of Tulaipanji, a local aromatic rice cultivar of West Bengal

1A.

Sources	d.f.	Sum of square							
		Days to 50% flowering	Plant height	Panicles/ plant	Panicle length	Flag leaf length	Flag leaf width	Stem thickness	No. of filled grain
Total	26	63.564	523.000	16.525	2.130	1.349	0.0028	1.2022	25.227
Treatment	8	204.083**	1698.343**	41.428**	6.015**	2.381**	0.0076**	3.9031**	56.075**
Error	16	1.027	0.688	6.042	0.316	0.786	0.0006	0.0017	3.690

**denote significance at P= 0.01

1B.

Sources	d.f.	Sum of square							
		No. of chaffy	Grain length	Grain width	Decorticated grain length	Decorticated grain width	100-seed weight	Yield (kg/ha)	
								Boro	*Kharif*
Total	26	3.361	0.057	0.030	0.037	0.012	0.0136	194688.02	153046.28
Treatment	8	10.365**	0.182**	0.097**	0.060^{NS}	0.035**	0.0437**	632968.66**	498056.08**
Error	16	0.260	0.001	0.001	0.027	0.002	0.0003	3094.50	2317.64

**denote significance at P= 0.01, NS: Non-significant

presented in Tables 2A and B. Days to 50% flowering showed narrow range of variation from 117 to 143 days but it showed significant difference among the mutants. Plant height ranged from 73.60 to 146.15 cm (Table 2A). Significant differences among the mutants were observed for plant height. Shortest plant height was observed for TP3-5 (73.60 cm) followed by TP3-6 (77.00 cm) and TP3-1 (77.66 cm). Tallest plant among the mutants was in the population of TP3-3 (116.60 cm) followed by TP3-8 (95.40 cm) and TP3-2 (92.40 cm). Number of panicles per plant in the mutants varied from 18 to 29 (Table 2A). The significant variation in number of panicles per plant indicated that all the mutants have high tillering ability. Highest number of tillers per plant was observed for TP3-2 followed by TP3-4 and TP3-6.

Panicle length varied from 19.60 to 23.60 cm among the mutants (Table 2A). As per the DUS guidelines, the panicle length in TP3-1, TP3-2, TP3-3, TP3-4, TP3-5 and TP3-8 mutants fell under medium category (21-25 cm). However, the mutants TP3-6 and TP3-7 showed short panicles (16-20 cm). Flag leaf length varied from 24.94 to 27.50 mm among the mutants. Maximum flag leaf was recorded for TP3-7 (27.50 cm) followed by TP3-6 (27.00 cm) and TP3-4 (26.00 cm), while flag leaf width ranged from 1.25 to 1.40 mm among the induced mutants (Table 2A). Broadest flag leaf was found in TP3-8 followed by TP3-7 and TP3-5. Narrowest leaf was recorded for TP3-1. Observation on stem thickness was recorded at milk development stage (formation of white milky sap within the spikelets) considering a number of individual stems thickness. Stem thickness is an important character to decide the tolerance towards lodging. Stem thickness varied from 1.34 to 5.00 mm among the Tulaipanji mutants (Table 2A). Stem thickness of all the Tulaipanji mutants were categorized under medium category (0.40-0.55 mm). Since the mutants were dwarf and having medium stem thickness, these were characterized lodging tolerant as compared to stem thickness of Tulaipanji (1.34 mm). The control variety Tuaipanji produces very weak culm and hence lodging susceptible.

The number of filled grains per panicle in the mutants varied from 107.40 to 115.80. The range of variation in number of filled grains per panicle is not wide; however, statistically it showed significant difference among the mutants. Highest number of filled grains per panicle was observed in TP3-6 which is at par with TP3-3 (Table 2A). Most of the mutants showed higher number of filled grains as compared to the mother cultivar, Tulaipanji. The number of chaffy grains per panicle in the mutants varied from 17.80 to 22.60 (Table 2A). Lowest number of chaffy grains per panicle

Tables 2A and B. Yield attributing characters of isolated mutants of Tulaipanji, a local aromatic rice cultivar of West Bengal (*boro* 2015-16)

2A.

Mutants	Days to 50% flowering	Plant height (cm)	No. of panicles/ plant	Panicle length (cm)	Flag leaf length (cm)	Flag leaf width (mm)	Stem thick-ness	No. of filled grain	No. of chaffy grain	Sterility (%)
TP3-1	118	77.66	18.00	23.40	25.14	1.25	4.52	120.40	17.80	12.88
TP3-2	118	92.40	29.00	22.40	24.94	1.29	4.39	110.60	22.20	16.72
TP3-3	117	116.6	25.00	23.60	25.36	1.33	4.57	115.60	21.60	15.74
TP3-4	122	82.20	28.00	23.00	26.00	1.28	4.61	107.40	17.80	14.22
TP3-5	120	73.60	25.00	21.00	25.22	1.37	5.00	112.00	18.80	14.37
TP3-6	120	77.00	27.00	19.60	27.00	1.35	4.87	115.80	21.00	15.35
TP3-7	117	80.40	23.00	20.20	27.50	1.37	4.69	107.60	20.40	15.94
TP3-8	118	95.40	20.00	22.40	25.70	1.40	4.98	111.80	22.60	16.82
Tulaipanji*	143	146.15	21.65	22.45	26.43	1.29	1.34	108.91	21.70	16.61
Range	117-143	73.60-146.15	18-29	19.60-23.60	24.94-27.50	1.25-1.40	1.34-5.00	107.40-115.80	17.80-22.60	12.88-16.82
Mean	118.75	86.90	24.37	21.95	25.85	1.33	4.70	112.65	20.27	15.41
CD (5%)	1.75	1.43	4.25	0.97	1.53	0.04	0.07	3.32	0.88	-
CD (1%)	2.41	1.97	5.86	1.34	2.11	0.06	0.10	4.58	1.21	-

*Data recorded from *kharif* season, because it is higly photo-period sensitive and can be grown only during *kharif* season

2B.

Mutants	Grain width (mm)	Grain wt (g)	100-grains grain (mm)	Decorticated length (mm)			Grain type	Aroma
				Length	Breadth	L:B ratio		
TP3-1	8.32	2.52	17.1	6.45	2.13	3.03	LS	SA
TP3-2	8.42	2.56	17.5	6.52	2.19	2.98	LB	SA
TP3-3	8.23	2.43	15.9	6.27	2.21	2.84	LB	SA
TP3-4	8.30	2.52	17.6	6.31	2.25	2.80	LB	SA
TP3-5	8.21	2.55	17.3	6.24	2.19	2.85	LB	SA
TP3-6	8.11	2.51	17.2	6.17	2.15	2.87	LB	SA
TP3-7	8.04	2.52	17.7	6.20	2.25	2.76	LB	MA
TP3-8	8.28	2.54	16.8	6.36	2.17	2.93	LB	SA
Tulaipanji	7.58	1.99	13.9	6.06	1.89	3.21	LS	SA
Range	7.58-8.42	1.99-2.56	13.9-17.7	6.17-6.52	2.13-2.25	-	-	-
Mean	8.23	2.51	17.1	6.31	2.19	-	-	-
CD (5%)	0.28	0.07	0.03	NS	0.08	-	-	-
CD (1%)	0.39	0.09	0.04	NS	0.11	-	-	-

LS = Long slender; LB = Long bold; SA = Strong aroma; MA = Mild aroma; NS = Non-significant

was recorded for TP3-1 and TP3-4, whereas maximum number of chaffy grains per panicle was recorded for TP3-8. The spikelet sterility of all the mutants was comparatively low including Tulaipanji. A minimum of variation was observed for grain length ranging from 8.04 to 8.42 mm among the mutants, the lowest by

Fig. 1. Tulaipanji mutants. A) Mutant-TP3-7 at maturity, it is semi dwarf, early as compared to Tulaipanji, yield advantage over Tulaipanji is 58%, mild aromatic; B) Mutant-TP3-5 at maturity, it is semi dwarf, early as compared to Tulaipanji, yield advantage over Tulaipanji is 74.81%, strong aromatic; C) Mutants at maturity while the Tulaipanji still at vegetative stage

the TP3-7 (8.04mm) but higher than the Tulpanji (7.58mm) (Table 2B). Based on the grain length all the mutants were classified under short (6.1-8.5 mm) category. TP3-2 produced longest grains (8.42 mm). The grain breadth of the mutants ranged from 1.99 to 2.56 mm. As per the guidelines of PPV&FR (2007), all the mutants had fallen under narrow (2.1-2.5 mm) class. The grains of Tulaipanji were found to be narrowest.

Observation on grain dimension was taken when the caryopsis turned to hard stage (can be no longer be dented by thumb nail and over 90% spikelets ripened) stage. Longitudinal dimension measured from 10 well-developed decorticated grains as the distance from the base to the tip of the grain was considered for the grain length. Decorticated grain length of Tulaipanji mutants varied from 6.17 to 6.52 mm (Table 2B). All the mutants were classified under short category. The decorticated grain breadth varied from 2.13 to 2.25 mm (Table 2B). Based on decorticated grain length and L:B (Length x Breadth) ratio the mutant TP3-1 and Tulaipanji fall under long slender group. Rest all the mutants were classified under long bold group (Table 2B). Volumetric weight such as test weight is another useful varietal characteristic. Observation on 1000-grain weight was taken at caryopsis hard (can be no longer be dented by thumb nail and over 90% spikelets ripened) stage and observation was taken by measuring 100 fully developed grains dried to 13% moisture content (PPV & FRA 2007).

Grain yielding ability is also used as a varietal characteristic. Grain yield of mutants varied from 2087 to 3396 kg/ha during *boro* season and from 2168 to 3268 kg/ha during *kharif* season (Table 3). Highest yield was recorded for TP3-2 during *boro* (3396 kg/ha) followed by TP3-4 (3283 and 3164 kg/ha during *boro* and *kharif* seasons. Lowest yield was recorded in Tulaipanji, the control variety during *kharif* season. Among the mutants, TP3-1 was found to have lowest yield potential. Increased in mean yield over Tulaipanji was highest in TP3-2 (99.40%) followed by TP3-4 (92.91%), TP3-6 (89.05%), TP3-5 (82.95%) and TP3-3 (61.07%). All the selected mutants mutants were found to have considerable higher yield than the mother cultivar, Tulaipnji.

Discussion

Aromatic rice varieties have poor combining ability, and cross-breeding with non-aromatic varieties will lead to a decrease in aroma and quality (Bourgis et al. 2008; Rutger and Bryant 2004). Under this situation, mutation breeding is the next alternative method of creating variability followed by selection for the desired traits. Ideally, high-yielding, aromatic and photoperiod-insensitive varieties can be bred to serve increasing demands of customers in domestic and the world markets and to increase the farmers' income.

All the mutants were categorized under medium duration genotypes (Table 2A; Fig. 1). The control variety, Tulaipanji is late maturing variety taking about 140 days to attain days to 50% flowering and it is highly photoperiod-sensitive. It can be cultivated suitably during *kharif* season but the induced mutants selected from mutagenized Tulaipanji could be cultivated both in *kharif* (winter rice) as well as *boro* (summer rice) seasons. The isolated mutants showed photoperiod-insensitive short or medium duration combining with aroma. Aung et al. (2016) also developed photoperiod-insensitive mutants from photoperiod-sensitive traditional cultivar. Le (2006) selected three photoperiod-insensitivity mutants from local rice cultivar. *Hd1* to *Hd5* are the dominant loci

Table 3. Yield of mutants during *kharif* and *boro* seasons

Mutants	Grain yield (kg/ha)		Mean	Increased in yield over Tulaipanji (%)		Mean
	Boro	*Kharif*		*Boro**	*Kharif*	
TP3-1	2087	2168	2127.50	24.90	29.74	27.32
TP3-2	3396	3268	3332.00	103.23	95.57	99.40
TP3-3	2773	2610	2691.50	65.95	56.19	61.07
TP3-4	3283	3164	3223.50	96.47	89.35	92.91
TP3-5	3088	3026	3057.00	84.80	81.09	82.95
TP3-6	3211	3107	3159.00	92.16	85.94	89.05
TP3-7	2576	2519	2547.50	54.16	50.75	52.46
TP3-8	2473	2409	2441.00	48.00	44.17	46.09
Tulaipanji	-	1671	-	-	-	-
Range	2087-3396	2168-3268	2127.50-3332.00	-	-	-
Mean	2860.88	2783.88	2822.38	71.21	66.60	68.90
CD (5%)	97.42	84.31	-	-	-	-
CD (1%)	135.21	117.02	-	-	-	-

*Increase in yield over Tulaipanji (%) during *boro* season was calculated considering its yield during *kharif* season as Tulaipanji is photoperiod sensitive and can be grown only in *kharif*

regulating photoperiodic flowering responses which act as single Mendelian factors in a segregating F_2 population (Yamamoto et al. 2000). The Nipponbare alleles of *Hd1* and *Hd2* were shown to be short day flowering promoters with the largest effects on phenotype, acting independently of one another (Yano et al. 1997). *Hd3a* genetically interacts with *Hd1* and *Hd2* to inhibit flowering under short day conditions (Gowda et al. 2003). The Kasalath alleles of *Hd4* and *Hd5* delayed flowering under long day conditions (Brambilla and Fornara, 2013). The genetic effect of *Hd4* identified to be additive to that of *Hd1* and *Hd2*, whereas an epistatic relationship was detected among *Hd5* and *Hd1* (Wang et al. 2013).

The plant height of the mutants was reduced drastically. The control variety is tall (146.15 cm) with weak culm, which leads to lodging susceptibility. All the selected mutants fall under dwarf category and lodging resistant having aroma. The mutants developed and reported earlier by different workers (Bughio et al. 2007; Aung et al. 2016; Schiocchet et al. 2014) possessed significantly shorter height displaying lodging tolerance as compared to the control variety. One of the objectives of this endeavour was to develop short or medium duration mutants with aroma. Tulaipanji was found to be the tallest (146.15 cm) and lodging susceptible. It is interesting to note that all the mutants fallen under dwarf category which also led to non-lodging type of plants. The genotypes having low spikelet sterility is desirable, generally it has negative correlation with yield. The production of chaffy grains in panicles was observed in some mutants.

The decorticated grain length and breadth is important dimension to decide the gain type, as per the preference of the farmers. The long slender grains are also being preferred by most of the consumers. Some of the mutants possessed desirable traits.Under comparable growth conditions, each variety shows a fairly consistent composition of grain yield in terms of the number of panicles per unit area, number of well-developed grains per panicle and grain weight (Chang et al. 1965). The differences in respect of 1000-grains weight (TGW) (15.9 to 17.7 g) among the mutants was very narrow, however, it showed significant variation among the mutants. Based on the TGW, it was found that all the mutants could be classified under low TGW category. Thousand grains weight is an important yield attributing character and this character showed positive and significant correlation with grain yield (Singh et al. 2006; Senapati et al. 2009; Roy, 2010; Rangare et al. 2012).

Schiocchet et al. (2014) also reported high yield potential in rice mutants. In general, it is to be believed that the Farmers' Varieties are low grain yielders.

However, all the mutants of Tulaipanji recorded high yield potential showing yield advantage over the control variety. A mutant TP3-2 was adjudged to be the best among all the mutants in respect yield increase. The mutant line TP3-2 also produced higher number of panicle per plant, higher number of filled grains per panicle and long bold grains. It showed comparatively short stature with high grain yield as compared to control variety, which is tall with low grain yielding ability. The present findings are well supported by the results of Bughio et al. (2007). Based on the enhanced yield and aroma, mutants may be recommended for cultivation after necessary trials conducted across the locations in similar conditions.

The desirable mutants isolated in the present study are characterized as dwarf plant type, early maturation, having slender grain and higher yield with desired level of aroma. Le et al. (2006) also selected three mutants from local rice cultivar with several desirable characteristics, such as photoperiod-insensitivity, shortened plant type, early maturity, extra-long and large grain type with high yield potential. Sivaram et al. (2014) also reported similar findings from their study. The results of the present study demonstrated that there are great possibilities for improving qualitative and quantitative traits by use of appropriate mutation breeding approaches. The present study on mutagenesis has generated a good amount of genetic variability for economically and agronomically important characters. Mutation breeding is one of the most effective non-conventional methods of plant breeding to induce desirable character in locally adapted varieties. If these mutants are releasedfor general cultivation, they may replace the low yielding local cultivars like Tulpanji and thereby promoting better socio-economic conditions of farmers.

Authors' contribution

Conceptualization of research (BR, BK); Designing of the experiments (BR, BK); Contribution of experimental materials (BR); Execution of field/lab experiments and data collection (BR, SDT); Analysis of data and interpretation (BR, BK, SDT, BKD); Preparation of manuscript (BR, BK, SDT, BKD).

Declaration

The authors declare no conflict of interest.

Acknowledgement

The authors duly acknowledge the research grant from Board of Research in Nuclear Sciences (BRNS), Bhabha Atomic Research Centre, Department of Atomic Energy, Trombay, Maharashtra, India.

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