Received: 01 Jul 2021 | Revised: 05 Aug 2021 | Accepted: 10 Aug 2021

Electronic Journal of Plant Breeding

Research Article

Spectrum and frequency of macro and micro mutations induced through gamma rays in traditional rice landraces of Chhattisgarh

Richa Sao[1], Parmeshwar K. Sahu[1],Suvendu Mondal[2], Vikash Kumar[2], Deepak Sharma*[1] and B.K. Das[2]

[1]Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492012 (C.G.), India
[2]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai-400085 (MS), India
***E-Mail**: deepakigkv@gmail.com

Abstract
Mutation breeding offers a simple and effective means of inducing genetic variation followed by trait selection to improve the traditional rice landraces. Gamma ray induced mutagenesis is most commonly used to obtain chlorophyll mutants and economically useful mutants in many crops. In the present study, gamma ray induced (300Gy) mutagenesis was carried out in the three rice landraces *viz.,* Samundchini, Vishnubhog and Jhilli to study the frequency and spectrum of macro and micro mutations. Macro mutations including four categories of chlorophyll mutations along with many viable mutations *viz*. mutants with reduced height, reduced maturity duration, high tillers, clustered grain type, dark green leaves, strong stem and grassy types were identified in the M_2 population. A total of 81, 98 and 51 putative macro mutants were observed in Samundchini, Vishnubhog and Jhilli with mutation frequency (%) 0.591, 0.796 and 0.434, respectively. Furthermore, 45, 56 and 32 chlorophyll mutants with the 0.382, 0.455 and 0.272 mutation frequency percentages were observed in the M_2 population of Samundchini, Vishnubhog and Jhilli, respectively. In addition, 36, 42 and 19 viable morphological mutants with the mutation frequency percentages (based on M_2 population) 0.263, 0.341 and 0.162 were identified in Samundchini, Vishnubhog and Jhilli, respectively. Also, variations in mean, widening of the range and increase in the coefficient of variation for most of the quantitative traits in mutagen treated populations as compared to control depicts the existence of micro-mutation and release of polygenic variability in all the three mutagenic populations. Many economically important mutants were identified in all three landraces which could be further advanced for varietal development and use in the breeding program.

Key words: Rice landraces, gamma rays, mutation breeding, mutation frequency, macro and micro mutations

INTRODUCTION

Rice (*Oryza sativa* L.) has been considered an important cereal crop for the food security of over half of the world's population (~3.5 billion), with Asia accounting for 90 per cent of global rice consumption (USDA, 2021). India is the second largest rice producing country after China with a 121million metric tonnes of rice in an area of 44 m.ha (USDA, 2021). India is bestowed with the rich heritage of rice biodiversity where more than 40000 rice germplasm have been recorded (Pandey *et al.,* 2010). In India, Chhattisgarh state has enormous and diverse rice biodiversity collections including 23250 accessions conserved at Indira Gandhi Krishi Vishwavidyalaya, Raipur-49012 and therefore the state is popularly known as 'Rice Bowl of India' (Sahu *et al.,* 2017).

The traditional rice landraces are a reservoir of valuable genes/ traits for grain yield, quality, nutritional values, medicinal values, biotic and abiotic stress resistance etc.

These indigenous lines are preserved and maintained by the local farmers which enrich the rice gene pool for developing new rice varieties with wider adaptability to a range of environmental stresses (Whankaew *et al.,* 2020). As most of these landraces are tall and late maturing thus making them prone to lodging and shattering which require more inputs in field operations which may significantly reduce the potential outcomes. Also, they are photoperiod sensitive, susceptible to several pests and diseases, having to spread plant types, bearing spikelets with uneven maturity in the same panicle and poor yielder, which make them unfit for commercial cultivation. Such problems need to be addressed carefully in a sustainable manner.

Consequently, concerted research efforts must be directed to harness their yield potential without altering their inherent grain quality. Incidentally, mutation breeding could offer a simple and effective means of inducing genetic variation followed by trait selection without altering the authentic characters of the plants. Induced mutation can rapidly create variability in quantitatively and qualitatively inherited traits in crop plants which may be exploited for developing an improved genotype over the existing one (Viana *et al*., 2019; Muduli and Mishra, 2007). Interestingly, about 3364 mutant varieties in about 225 crop plants have been developed and registered in the FAO/IAEA Mutant Variety Database (MVD), International Atomic Energy Agency, Vienna, Austria (FAO/IAEA MVD, 2021). Furthermore, the important objectives in improving the rice landraces through induced mutagenesis are to develop short stature, early maturing, high tillering, non-shattering, biotic and abiotic stress resistance, high yielding and new plant type plants.

Mutations in rice crops may be induced by the different types of mutagens *viz.,* physical and chemical mutagens. Of which, gamma ray has been used frequently throughout the world and about 80 per cent of the total rice mutant varieties (852) have been developed through it (FAO/IAEA MVD, 2021). Different doses of gamma rays may produce different frequencies and spectrums of macro and micro mutations in rice genotypes. However, 300 Gy of gamma ray irradiation have been considered as the most significant dose to generate optimum spectrum and frequency of rice mutants with desired traits (Li *et al*., 2019; Kato *et al*., 2020 and Gowthami *et al*., 2017). Estimation of spectrum and frequency of mutant play much significant role in mutation breeding as they are the most reliable and easiest way to estimate the effects of mutagenic treatments in the M_2 population (Gustafsson, 1940).

Irradiations of seeds with desirable mutagens constitute the M_1 population which further on selfing constitute the M_2 population. Dominant types of mutants are obtained in the M_1 generation while most of the mutants being recessive in nature are screened in the M_2 population. Gaul (1964) classified mutations into two groups macro mutations and micro mutations. Macro mutations are phenotypically visible and can be easily detectable in an individual plant. They are qualitatively inherited genetic changes, and occur in major genes or oligogenes. Micro mutations result in a small effect and are evaluated in a group of plants. They are quantitatively inherited, occur in minor genes or polygenes and can be detected only by the help of statistical methods (Sharma and Sharma, 1984). With the aforesaid views, the present study was carried out to identify and measure the spectrum and frequency of macro and micro mutants induced through gamma rays in rice landraces.

MATERIALS AND METHODS

A total of three traditional rice landraces including two aromatic short grain varieties (Vishnubhog, Samundchini) and one super fine grain variety (Jhilli) were taken for the present study (**Table 1**). Samundchini has 165-170 cm plant height and 155-160 days maturity duration; Vishnubhog has 140-145 cm plant height and 140-150 days of maturity duration and Jhilli has a height of 150-155 cm and 145-150 days of maturity duration. Seeds of these landraces were procured from R.H. Richharia Rice Germplasm, Division of Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur, India.

Around 2000 pure, healthy and equal sized seeds of Samundchini, Vishnubhog and Jhilli were irradiated with 300 Gy of gamma rays at Nuclear Agriculture and Biotechnology Division (NA&BTD), Bhabha Atomic Research Centre (BARC), Mumbai, India. The treated seeds were sown as M_1 population during *Rabi,* 2017-18 at research field of Department of Genetics and Plant Breeding, IGKV, Raipur. All the required agronomic and

Table 1. Details of the three rice landraces used in the current study

S. No.	Accession No.	Name of rice landraces	Special feature(s)	Undesirable trait(s)	Signature variety of district
1	IC-125004	Vishnubhog	Aromatic short bold grains	Tall and late maturity, poor yield potential	Sarguja
2	IC-386390	Samundchini	Aromatic short slender grains	Tall and late maturity, poor yield potential	Bilaspur
3	IC-377273	Jhilli	Super fine grains/ long slender	Tall and late maturity, poor yield potential	Mahasamund

cultural practices were adopted as per the standard methodology throughout the crop season. The mother panicle from each M_1 plant was harvested and kept separately in a small envelope to be sown in the next generation.

The seeds obtained from the mother panicle of individual M_1 plants were sown in separate rows by following the panicle to row method at research field of the Department of Genetics and Plant Breeding, IGKV, Raipur during *Kharif,* 2018. The distance maintained between row to row was 20 cm and plant to plant was also 20 cm. For identifying mutants, a total of 13712, 12306 and 11744 plants were maintained in the M_2 generation of Samundchini, Vishnubhog and Jhilli, respectively. The untreated (control) seeds were also sown for comparison along with the M_2 population. All the required agronomic and cultural practices were adopted as per the standard methodology throughout the crop season to obtain good plant growth.

All plants in the M_2 population of all the three genotypes were screened carefully for all possible types of morphological changes which were deviating from the untreated (control) plants. Macro mutations include the chlorophyll mutations and the viable mutations which can be observed an individual plant basis in the M_2 population and M_1 panicle basis. The frequency and spectrum of chlorophyll mutations and all types of viable mutations with the observations for their quantitative traits were recorded during *Kharif,* 2018.

The M_2 population was screened for frequency and spectrum of chlorophyll mutations. Lethal chlorophyll mutations were scored within 10 to 25 days of sowing whereas, viable chlorophyll mutations were scored throughout the life period of plants. The spectrum of chlorophyll mutations was studied and the mutants were classified as per the scheme of Gustafsson (1940).

Following chlorophyll mutants were found in the present study.
1. *Albino*- these mutants were completely white in colour and without chlorophyll in the leaves of seedlings. These mutants cannot survive more than 10-15 days.
2.*Chlorina*- these mutant plants were very light green or pale green which persisted throughout the growth period also few of them reverted to the normal green type.
3. *Viridis*- these mutant plants were having light yellow green colure of leaves, they were lethal mutants.
4. *Xantha*- these mutant plants were yellow to yellowish white, carotenoids present but chlorophyll absent, these mutants did not survive after few days.

Different types of visible mutations were screened and their frequencies were scored up to maturity from the M_2 populations for all three genotypes. Mutants selected for specific morphological features in this generation, were as follows: mutants with reduced maturity duration; reduced height; high tillering and vigorous plant type; Grassy mutants (narrow leaf and dwarf); altered leaf pigmentation; narrow and broad leaves; altered grain shape; clustered grains in a panicle; strong and sturdy stems.

The mutagenic frequency of induced macro mutations in this experiment was estimated as (i) percentage of the M_2 families in which putative mutants were observed, and (ii) percentage of mutants in M_2 population as suggested by Gaul (1964). The latter method is preferred, being independent of variations in progeny size, size of the mutated sector and is also proportional to the initial mutation rate. Formulae for estimating mutation frequency are as follows:

M_2 family basis (percentage of segregating M_2 progenies)

$$\text{Mutation frequency (\%)} = \frac{\text{Number of mutated progenies}}{\text{Total M2 progenies}} \times 100$$

M_2 population basis(percentage of mutated M_2 plants or mutants)

$$\text{Mutation frequency (\%)} = \frac{\text{Number of mutants observed}}{\text{Total number of plants in M2 population}} \times 100$$

For the study of micro-mutations, 30 normal looking plants from the families not segregating in the M_2 population for any kind of macro mutation (excluding the macro-mutants) were randomly selected and properly tagged. The observations for quantitative traits *viz.,* days to 50% flowering, plant height (cm), leaf length (cm), leaf width (cm), panicle length (cm), number of tillers per plant, the effective number of tillers per plant and grain yield per plant (g) were recorded in order to determine the magnitude of induced variability and shift in mean values of these traits after treatment of gamma rays. The data recorded were analysed for descriptive statistics to get the variability parameters by using the software PAST v3.14 (Hammer *et al.,* 2001) by following standard procedure.

RESULTS AND DISCUSSION

Macro-mutants are recognized at a single plant level and it includes all types of chlorophyll mutations and viable morphological changes in plant characters. Observations on macro-mutations were recorded from the 10th day of emergence of seedlings till the plants attained physiological maturity. A total of 13712, 12306, 11744 M_2 plants which included 720, 640, 580 panicle rows of Samundchini, Vishnubhog and Jhilli, respectively

were screened for the identification of chlorophyll and morphological mutations.

In Samundchini, a total of 81 putative macro mutants were identified which included chlorophyll (45) and viable morphological (36) mutants. The mutagenic effects of mutagens on crop plants are measured in different ways. Of which estimating the proportion of mutated plants to normal plants (mutation frequency) is the most reliable and easiest way to estimate treatment effects in M_2 generation (Gustafsson, 1940). In the current study, the mutation frequency of overall macro mutations obtained in Samundchini based on the M_2 population and family rows were 0.591 and 5.00 per cent, respectively (**Table 2**). Moreover, in Vishnubhog, a total of 98 putative macro mutants were obtained which included chlorophyll (56) and viable morphological (42) mutants. The mutation frequency of overall macro mutations obtained in Vishnubhog based on the M_2 population and family rows were 0.796 and 6.563 per cent, respectively being the highest among all the three populations (**Table 3**). Similarly, in Jhilli, a total of 51 total putative macro mutants were obtained which comprises 32 chlorophyll mutants and 19 morphological mutants. The mutation frequency of overall macro mutations obtained in Jhilli based on the M_2 population and family rows were 0.434 and 3.621 per cent, respectively being the lowest among all the three populations (**Table 4**).

In any induction of mutation study, chlorophyll mutations are considered the major group of macro-mutations. Though these mutations are considered to have no agricultural value they are important in horticultural and ornamental crops and for basic research. Chlorophyll mutations are taken as an indicator to judge the effectiveness of mutagen at the phenotypic level. The spectrum and frequency of chlorophyll mutations observed in the gamma ray treated populations of Samundchini, Vishnubhog and Jhilli are presented in **Table 2, Table 3** and **Table 4**, respectively.

In Samundchini, a total of 45 chlorophyll mutants were observed which were distributed in total of 16 panicle rows. Mutation frequency based on the M_2 population and family rows were 0.328 and 2.222 per cent, respectively. The spectrum of chlorophyll mutants of Samundchini M_2 population comprises of 23 *albino,* 13 *xantha,* 7 *chlorina* and 2 *virdis* type of mutants (**Table 2**). Among the three mutant populations, a maximum number of chlorophyll mutants were observed in Vishnubhog *i.e.*, 56 mutants involving total of 19 M_2 families. Mutation frequency based on the M_2 population and family rows were 0.455 and 2.969 per cent, respectively (**Table 3**). The chlorophyll mutants' *viz., albino (*28), *xantha* (16), *chlorina* (9) and *virdis* (3) were observed in the Vishnubhog M_2 population. Furthermore in Jhilli, 32 chlorophyll mutants were observed which were dispersed in total of 16 panicle rows and was least among all three treated genotypes.

Table 2. Mutation frequency and spectrum of chlorophyll and morphological mutants (Macro mutants) observed in M_2 population of Samundchini

Total number of M_2 plants	Total number of M_2 families	Type of macro mutants	Categories of putative mutants	Number of mutants in M_2 population	Number panicle rows where mutants observed	Mutation Frequency (%) based on the M_2 population	Mutation Frequency (%) based on the M_2 families
13712	720	Chlorophyll mutants	Albino	23	9	0.168	1.250
			Xantha	13	4	0.095	0.556
			Chlorina	7	2	0.051	0.278
			Virdis	2	1	0.015	0.139
			Total chlorophyll mutants	**45**	**16**	**0.328**	**2.222**
		Morphological mutants	Grassy mutants	5	2	0.036	0.278
			High tillering mutants	5	2	0.036	0.278
			Broad leaf, high tillering, vigorous mutant	2	2	0.015	0.278
			Narrow leaf mutant	1	1	0.007	0.139
			Purple leaf mutant	3	3	0.022	0.417
			Dark green leaf mutant	1	1	0.007	0.139
			Mid early to early mutants	5	2	0.036	0.278
			Dwarf mutants	4	2	0.029	0.278
			Semi dwarf mid early mutants	4	1	0.029	0.139
			Semi dwarf and late mutants	3	2	0.022	0.278
			Semi tall mutants	3	2	0.022	0.278
			Total morphological mutants	**36**	**20**	**0.263**	**2.778**
			Total putative macro mutants	**81**	**36**	**0.591**	**5.000**

Mutation frequency based on the M_2 population and family rows were 0.272 and 1.897 per cent, respectively. The spectrum of chlorophyll mutants in Jhilli comprises 17 *albino,* 9 *xantha,* 5 *chlorina* and 1 *virdis* type of mutants (**Table 4**).

Among all the chlorophyll mutants, the albino type was the most frequent in all three M_2 populations. Frequent occurrence of albino mutants was earlier reported by Swaminathan *et al.*(1970), Rao and Rao(1983), Reddi and Rao (1988), Chakraborty and Kole (2009), Sivaram *et al*. (2014). In contrast with the above result, the maximum frequency of viridis mutants followed by xantha, albina and other categories were observed in rice by Reddi and Rao (1988); Manikandan and Vanniarajan (2017). A narrow spectrum of chlorophyll mutations was observed in the three rice varieties under the present study. This situation may be viewed on the background stated by Gregory (1967) that the limiting factor for mutation production and its recovery is the genetic constitution of the experimental organism rather than the type of mutagen used. Similar observations on the macro-mutations frequency including chlorophyll and morphological mutations were also reported by Mishra and Singh (2013).

Viable morphological mutations were identified in the M_2 generation through visual screening. Type of viable morphological mutants was mainly emphasized for those mutations which affect the (i) plant height, (ii) flowering time (iii) tillering ability and vigorous growth, (iv) grain shape and its arrangement in the rachis. Moreover, semi-dwarfism and earliness are the characters that are most frequently desired in released rice mutant cultivars, although other traits such as more tiller numbers, improved grain quality and photoperiod insensitivity are also important and should be considered.

In Samundchini, a total of 36 morphological viable mutants were recognized in the M_2 population which were dispersed in 20 panicle rows or M_2 families. The mutation frequency (%) of viable mutations on the basis of the M_2 population and M_1 family were 0.263 and 2.778 per cent, respectively (**Table 2**) and photographs of some of the putative mutants of Vishnubhog are given in **Fig. 1**. Moreover, In Vishnubhog, a total of 42 morphological viable mutants were identified which were maximum among the three genotypes and distributed in 23 panicle rows. The mutation frequency (%) of viable mutations on the basis of the M_2 population and M_1 family were 0.341 and 3.594 per cent, respectively (**Table 3**) and photographs of some of the putative mutants of Vishnubhog are given in **Fig. 2**. In addition, a of total 19 morphological viable mutants were observed which were dispersed in 10 panicle rows. The mutation frequency (%) of viable mutations on the basis of M_2 population and M_1 family were 0.162 and 1.7240 per cent, respectively (**Table 4**) and photographs of some of the putative mutants of Vishnubhog are given in **Fig. 3**. A similar kinds of mutation frequency and spectrum of viable morphological mutations were also reported by Manikandan and Vanniarajan, (2017). Furthermore, a description of the individual classes of viable morphological mutations is given in subsequent paragraphs.

All the three landraces used under study have tall plant stature with an average height of 155 cm which made them prone to lodging and thus having poor grain yield. With the help of induced mutagenesis, reduced plant height was observed in few plants of the M_2 population. Moreover, total of 14, 19 and 11 mutants with reduced plant height were recorded in Samundchini, Vishnubhog and Jhilli, respectively with an average height of about 125 cm. Some of these types of mutants had weak stems whereas some had strong stems with high tillers and thus showed improved canopy and yield over their parents. Significant improvements in plant height through the use of induced mutation was also observed in rice crop by Singh and Singh (2003 b), Elayaraja *et al*. (2005), Bughio *et al.* (2007), Sharma *et al.* (2017 a); Sharma *et al.* (2017 b) and Roy *et al.* (2018).

Moreover, genotypes used under study had a very long maturing duration hence more time, input and field operations are required making the task difficult for farmers. Reducing the crop growth period will ensure early field preparation for the next season of crops. Total, 9, 17 and 11 early maturing mutants have been isolated in Samundchini, Vishnubhog and Jhilli, respectively and the maturity period is reduced by an average of 20 days. Early maturing mutants were also obtained in rice by inducing mutation by Shadakshari *et al.* (2001), Singh and Singh (2003 b) Domingo *et al.* (2007) and Minn *et al.* (2008).

Furthermore, the number of tillers plays a significant role in deciding the plant yield. A total of 7, 2 and 1 mutants with a high number of tillers with vigorous growth were observed in the M_2 population of Samundchini, Vishnubhog and Jhilli, respectively. These types of mutants are important to study the genetics and molecular cause behind the high tillering mechanism in plants and can also be used as donor parents in hybridization programmes to attain the high yielding varieties. High tillering mutants in different rice genotypes were also reported by Manikandan and Vanniarajan, (2017), Sharma *et al.* (2017 a), and Singh and Singh (2003 b).

Mutants with a very high number of tillers, dwarf and narrow leaves were observed which were termed as a grassy types of mutants. Sharma *et al.* (2017 b) also reported grassy mutants in Dubraj. The bushy mutant was also reported in rice by Singh and Singh (2003 b). These types of mutants are important to study the genetic and molecular cause behind the high tillering mechanism in plants and can also be used as a parent in hybridisation programmes to attain the high yielding F_1.

Table 3. Mutation frequency and spectrum of chlorophyll and morphological mutants (Macro mutants) observed in M_2 population of Vishnubhog

Total number of M_2 plants	Total number of M_2 families	Type of macro mutants	Categories of putative mutants	Number of mutants in M_2 population	Number panicle rows where mutants observed	Mutation Frequency (%) based on the M_2 population	Mutation Frequency (%) based on the M_2 families
12306	640	Chlorophyll mutants	Albino	28	9	0.228	1.406
			Xantha	16	5	0.130	0.781
			Chlorina	9	3	0.073	0.469
			Virdis	3	2	0.024	0.313
			Total chlorophyll mutants	**56**	**19**	**0.455**	**2.969**
		Morphological mutants	Grassy mutants	9	5	0.073	0.781
			Broad and dark green leaf mutants	2	1	0.016	0.156
			Narrow and dark green leaf mutants	4	2	0.033	0.313
			High tillering and vigorous mutants	2	2	0.016	0.313
			Erect leaves and sturdy stem mutants	1	1	0.008	0.156
			Strong stem and semi dwarf mutants	1	1	0.008	0.156
			Dwarf and mid early	7	3	0.057	0.469
			Semi dwarf and mid early	8	4	0.065	0.625
			Semitall and mid early mutants	2	1	0.016	0.156
			Semi dwarf and late mutants	2	1	0.016	0.156
			Fine grain mutants	2	1	0.016	0.156
			Clustered grain mutant	2	1	0.016	0.156
			Total	**42**	**23**	**0.341**	**3.594**
			Total putative macro mutants	**98**	**42**	**0.796**	**6.563**

Table 4. Mutation frequency and spectrum of chlorophyll and morphological mutants (Macro mutants) observed in M_2 population of Jhilli

Total number of M_2 plants	Total number of M_2 families	Type of macro mutants	Categories of putative mutants	Number of mutants in M_2 population	Number panicle rows where mutants observed	Mutation Frequency (%) based on the M_2 population	Mutation Frequency (%) based on the M_2 families
11744	580	Chlorophyll mutants	Albino	17	5	0.145	0.862
			Xantha	9	3	0.077	0.517
			Chlorina	5	2	0.043	0.345
			Virdis	1	1	0.009	0.172
			Total chlorophyll mutants	**32**	**11**	**0.272**	**1.897**
		Morphological mutants	Grassy mutant	4	2	0.034	0.345
			Broad leaf mutants	1	1	0.009	0.172
			High tillering and vigorous plant	1	1	0.009	0.172
			Dwarf and mid early	5	2	0.043	0.345
			Semi-dwarf and early	4	2	0.034	0.345
			Semi dwarf and mid early	2	1	0.017	0.172
			Clustered grain	2	1	0.017	0.172
			Total morphological mutants	**19**	**10**	**0.162**	**1.724**
			Total putative macro mutants	**51**	**21**	**0.434**	**3.621**

Samundchini chlorophyll mutant

Samundchini chlorophyll mutant

Samundchini mid early mutant

Samundchini purple leaf mutant

Samundchini semidwarf and late mutant

Samundchini chlorophyll mutant (Albino)

Fig. 1.Various chlorophyll and viable morphological mutants identified in the M2 population of Samundchini.

Apart from the chlorophyll deficient mutants, one and six dark green leaves mutants having new plant type canopy have been isolated in Samundchini and Vishnubhog, respectively. Some of them show vigorous growth and high yield. In Samundchini, the purple margin in leaves is a morphological marker character whereas, in its three mutants a long stripe of purple pigmentation was observed throughout the leaves making these mutants unique in appearance. In a similar way, variation in the intensity of green colour was observed by Sharma *et al.* (2017b). In addition, leaf area is an important factor controlling the photosynthesis in plants also governs the plant architecture. A total of three, six and one mutant with variations in leaf width were identified in Samundchini, Vishnubhog and Jhilli, respectively. Of these, 1, 4 narrow leaf mutants were observed in Samundchini and Vishnubhog, respectively, whereas 2, 2, and 1 broad leaf mutant was observed in Samundchini, Vishnubhog and Jhilli, respectively. Narrow leaf mutants were also reported in rice by Singh *et al*. (1998), Singh and Singh (2003 a) and broad leaf mutants were reported by Chakravarti *et al.* (2012).

Variation in grain width was also observed in two mutants of Vishnubhog which were screened as fine grain mutants. Along with these, mostly grassy mutants showed reduced

Vishnubhog more tillers and narrow leaf (Grassy) mutant

Vishnubhog narrow and dark green leaf mutant

Vishnubhog erect leaves & sturdy stem Mutant

Vishnubhog dwarf and mid early mutant

Fig. 2. Various chlorophyll and viable morphological mutants identified in the M2 population of Vishnubhog.

Jhilli semi dwarf and mid early mutant

Jhilli dwarf and mid early mutant

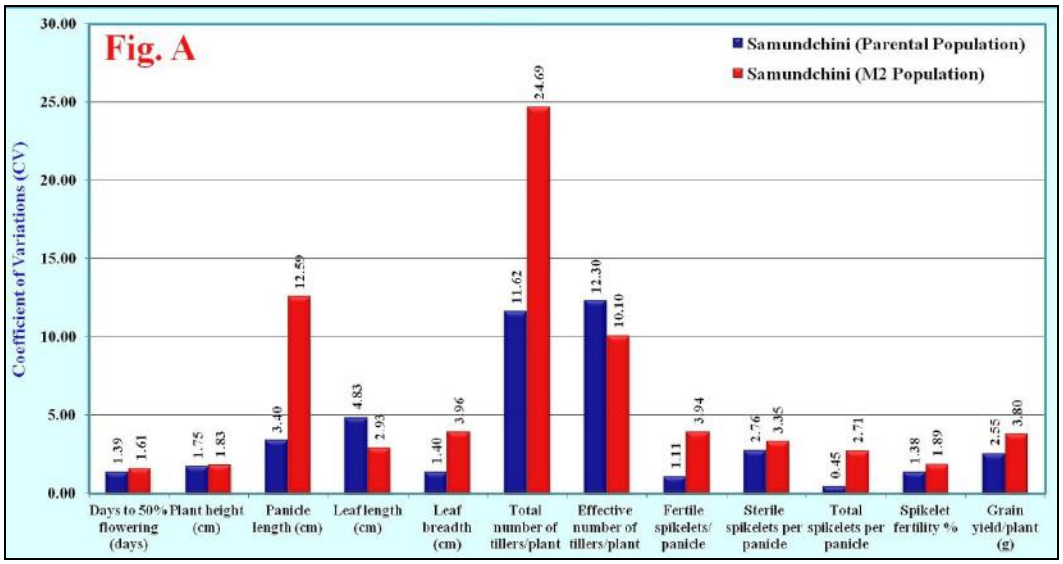
Jhilli clustered grain mutant

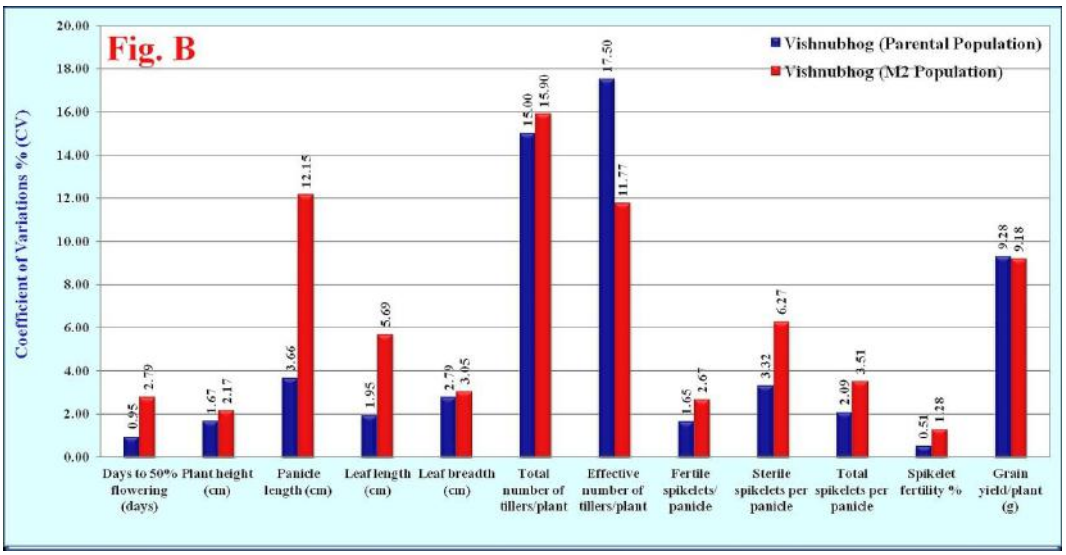
Jhilli dwarf and mid early mutant

Fig. 3. Viable morphological mutants identified in the M_2 population of Jhilli

Table 5. Genetic variability parameters for quantitative traits in M_2 population of Samundchini, Vishnubhog and Jhilli rice landraces

Character	Parameters	Samundchini (Parental Population)	Samundchini (M_2 Population)	Vishnubhog (Parental Population)	Vishnubhog (M_2 Population)	Jhilli (Parental Population)	Jhilli (M_2 Population)
Days to 50% flowering (days)	Minimum	118	117	118	115	114	109
	Maximum	122	122	121	124	117	120
	Mean	120.60	119.20	119.60	120.40	115.40	113.00
	Coeff. var	1.39	1.61	0.95	2.79	0.99	4.01
Plant height (cm)	Minimum	170	165	151	148	168	145
	Maximum	178	172	158	156	174	171
	Mean	173.80	168.00	154.80	151.60	171.04	160.60
	Coeff. var	1.75	1.83	1.67	2.17	1.31	6.55
Panicle length (cm)	Minimum	28.61	24.05	24.11	19.02	26.90	20.50
	Maximum	31.47	32.10	26.30	25.25	28.56	29.50
	Mean	30.07	27.87	25.03	21.94	27.82	24.29
	Coeff. var	3.40	12.59	3.66	12.15	2.46	16.90
Leaf length (cm)	Minimum	27.41	32.14	37.24	30.47	33.42	28.59
	Maximum	31.01	34.52	39.21	35.21	35.61	38.41
	Mean	29.33	33.06	38.18	32.82	34.59	33.74
	Coeff. var	4.83	2.93	1.95	5.69	3.16	12.46
Leaf breadth (cm)	Minimum	1.26	1.28	1.41	1.38	1.47	1.35
	Maximum	1.30	1.40	1.51	1.48	1.49	1.52
	Mean	1.28	1.35	1.48	1.43	1.48	1.43
	Coeff. var	1.40	3.96	2.79	3.05	0.57	4.90
Total number of tillers/plant	Minimum	6	6	6	7	7	8
	Maximum	8	11	9	10	10	14
	Mean	7.20	8.40	7.60	8.20	8.40	11.00
	Coeff. var	11.62	24.69	15.00	15.90	13.57	20.33
Effective number of tillers/plant	Minimum	6	6	6	7	6	8
	Maximum	8	8	9	9	9	13
	Mean	6.80	7.00	7.00	7.60	7.60	10.40
	Coeff. var	12.30	10.10	17.50	11.77	15.00	18.74
Fertile spikelets/ panicle	Minimum	140	142	160	155	150	118
	Maximum	144	155	167	166	156	175
	Mean	142.00	149.00	163.40	160.40	153.20	153.40
	Coeff. var	1.11	3.94	1.65	2.67	1.69	13.98
Sterile spikelets per panicle	Minimum	78	78	60	61	52	40
	Maximum	84	85	65	70	60	64
	Mean	81.00	80.60	62.40	65.20	55.40	53.80
	Coeff. var	2.76	3.35	3.32	6.27	6.20	16.54
Total spikelets per panicle	Minimum	222	220	220	217	207	173
	Maximum	224	236	232	235	210	233
	Mean	223.00	229.60	225.80	225.60	208.60	207.20
	Coeff.Var.	0.45	2.71	2.09	3.51	0.55	10.82
Spikelet fertility %	Minimum	62.50	63.04	71.98	69.96	71.43	68.21
	Maximum	64.86	66.24	72.77	72.40	74.88	80.00
	Mean	63.68	64.88	72.37	71.12	73.45	73.88
	Coeff. var	1.38	1.89	0.51	1.28	2.08	6.30
Grain yield/ plant (g)	Minimum	19.86	21.20	11.45	10.45	16.24	10.52
	Maximum	21.05	23.54	14.44	13.52	18.52	21.41
	Mean	20.50	22.26	12.75	12.09	17.17	15.51
	Coeff. var	2.55	3.80	9.28	9.18	5.27	28.80





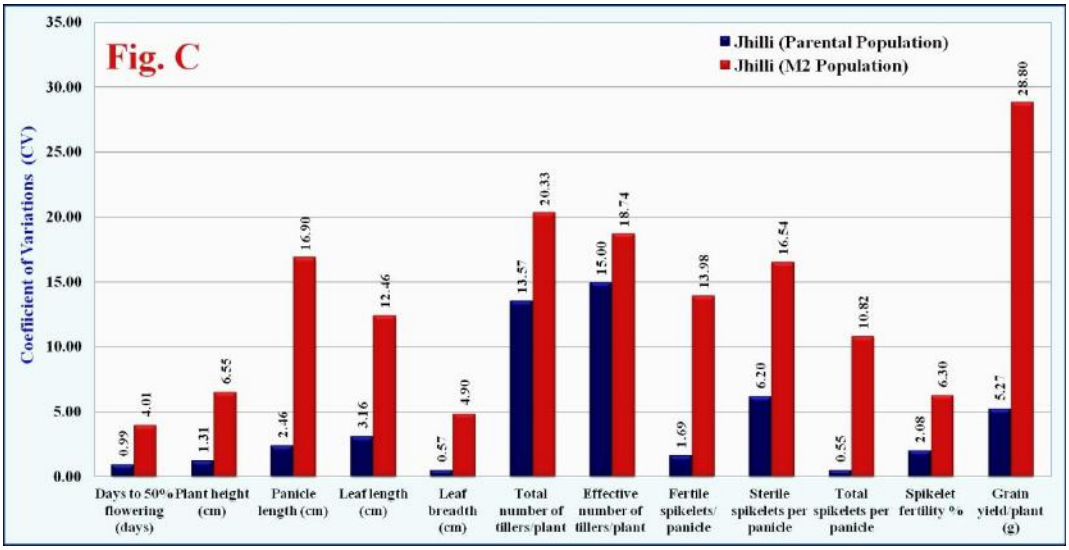


Fig. 4A, 4B & 4C: Changes in coefficient of variation (CV) in mutant populations over parental population of Samundchini (Fig. A), Vishnubhog (Fig. B) and Jhilli (Fig. C)

grain width. Soomro *et al.* (2002) and Chakravarti *et al.* (2012) also reported variation in grain length and width while studying the rice mutants of non-aromatic rice and aromatic rice. On the contrary large grain mutants in rice after mutagenic treatments were reported by Shadakshari *et al.* (2001), Singh *et al.* (2003).

Moreover, plants with strong and sturdy stems are essential to protect the plants from lodging and maintain a good canopy. In Vishnubhog one thick and strong stem mutant was identified making the plants erect and resistant to lodging. Thick, moderate and thin type of stem diameter was observed in Dubraj rice mutants by Sharma *et al.* (2017 b).

In addition, in Vishnubhog and Jhilli, spikelets of two mutants in each, were arranged in clustered fashion on rachis thus imparting a unique type of panicles in plants. Grains of this Vishnubhog mutant were bold, pigmented and more sterile as compared to control.

Induced micro-mutations in various mutagenic treatments can be evaluated based on the deviation in means, range and coefficient of variation of different characters observed in a mutant population and parental population. Hence, in this study we have measured the influence of micro mutations in the M_2 population by estimating the deviation of range, mean and coefficient of variation in respect of different traits of mutants and parental population. For the same, the observations on eight quantitative characters were recorded on randomly chosen 30 plants. The screening procedure used was of course rather arbitrary since the phenotypic effects associated with different mutations are no doubt continuously distributed and in any case will depend on the particular genotype and environment used. The screening merely excluded mutations with gross effects whose presence might otherwise have tended to obscure the presence of the micro-mutations (Lawrence *et al.,* 1968). In view of the importance of induced micro mutations in improving the polygenically controlled characters of economic importance, an attempt was made to explore the possibility of induction of micro mutations of breeding value in the treated populations.

The results showed that the statistical parameters *viz.*, mean range and coefficient of variations for all the characters under study behaved differently in the treated and control populations of all the three landraces. In Samundchini, the mean increased in mutagen treated populations in respect of the characters leaf length, leaf breadth, the total number of tillers/plant, the effective number of tillers/plant, fertile spikelets/panicle, total spikelets/panicle and grain yield whereas it decreased for days to 50% flowering, plant height, panicle length and sterile spikelets/panicle (**Table 5**). In Vishnubhog, mean showed increment in mutagen treated populations in respect of the characters days to 50% flowering, total number of tillers/plant, effective number of tillers/plant and sterile spikelets/panicle whereas, it decreased for plant height, panicle length, leaf length, leaf breadth, fertile spikelets/ panicle, spikelet fertility and grain yield (**Table 5**). In Jhilli, mutagen treated populations showed an increase in mean for the characters like the total number of tillers/plant, an effective number of tillers/plant and fertile spikelets/panicle, spikelet fertility and grain yield. Whereas, it decreased for days to 50% flowering, plant height, panicle length, leaf length, leaf breadth, sterile spikelets/ panicle and total spikelets/ panicle (**Table 5**).

The range of progenies was invariably wider in mutagen treated populations in respect of most of the characters studied in all the three treated populations (**Table 5**). Similarly the coefficient of variations was more in mutagen treated populations of Samundchini than that were in the controls in respect to most of the characters *viz.,* days to 50% flowering, plant height, panicle length, leaf breadth, the total number of tillers/plant, fertile spikelets/panicle, sterile spikelets/ panicle, spikelet fertility and grain yield (**Table 5 and Fig. 4A**). In the case of Vishnubhog, the coefficient of variations was more in mutagen treated populations than that were in the controls in all the characters (**Table 5 and Fig. 4B**) except an effective number of tillers/plants. Similarly in Jhilli, the coefficient of variations was high in the treated population than the control in all the characters under study (**Table 5 and Fig. 4C**).

These variations in mean, widening of the range and increase in the coefficient of variation in mutagen treated populations as compared to control, could be due to variability induced due to micro-mutation in the treated population and release of polygenic variability through them. A study on micro mutation performed by Sharma and Sharma (1984) also described the above similar way by adopting biometrical procedures. These micro-mutations are probably of greater importance than macro-mutations in evolution as variability is the cause behind evolution and mutations are one of the factors responsible for creating variation (Lawrence *et al.,* 1968).

Estimation of spectrum and frequency of macro and micro mutations is essential and are the most reliable parameters to define the irradiation effects of mutagens in the mutagenic populations of any crop. In the current study, many economically important as well as agronomically useful mutants were identified in all three irradiated landraces which could be further advanced for varietal development and use in a breeding program. Furthermore, this study will also be valuable for conducting mutation breeding experiments in other crops to compare the mutation frequency generated through different mutagens and to identify macro and micro mutations.

ACKNOWLEDGEMENT

Board of Research in Nuclear Sciences, Department of Atomic Energy, Government of India and Bhabha Atomic Research Centre, Mumbai-400085 are highly

acknowledged for financial and technical support, respectively.

REFERENCES

Bughio, H. R., Odhano, I. A., Asad, M. A. and Bughio, M. S. 2007. Improvement of grain yield in rice variety Basmati-370 (*Oryza sativa* L.).through mutagenesis. *Pakistan J. Bot.,*39: 2463-66.

Chakravarti, S.K., Kumar, H., Lal, J.P. and Vishwakarma, M.K. 2012. Induced mutation in traditional aromatic rice – Frequency and spectrum of viable mutations and characterizations of economic values. *The Bioscan*.,**7**(4): 739-742.

Chakraborty, N.R. and Kole. P.C. 2009. Gamma ray induced morphological mutations in non-Basmati aromatic rice. *Oryza*, **46**(3): 181-187.

Domingo, C., Andres, F. and Talon, M. 2007. Rice cv. Bahia mutagenized population; a new resource for rice breeding in the Mediterranean Basin. *Spanish J. Agr. Res.,* **5**(3): 341-347. [Cross Ref]

Elayaraja, K., Prakash, M., Kumar, B. S., Saravanan, K. and Ganesan, J. 2005. Effect of physical and chemical mutagens on certain quantitative characters in rice (*Oryza sativa* L.). *J. Crop Res.*, **30**: 309-12.

FAO/IAEA, Mutant Variety Database (MVD). 2021. Available online at: http:// https://mvd.iaea.org/ (accessed on 19th April, 2021).

Gaul, H. 1964. Mutations in plant breeding. *Radiat, Bot,***4**: 155-232. [Cross Ref]

Gowthami, R., Vanniarajan, C., Souframanien, J. and Pillai, M.A. 2017. Comparison of radio-sensitivity of two rice (*Oryza sativa* L.) varieties to gamma rays and electron beam in M_1 generation. *Elect. J. Plant Breed.,* **8**(3): 732-741. [Cross Ref]

Gregory, W.C. 1967. A radiation breeding experiment with peanuts. J. Ser., North Carolina State Univ. Agric. Expt. Station, Raleigh, **81**-215. [Cross Ref]

Gustafsson, A. 1940.The mutation system in chlorophyll apparatus. Lunds, Univ. Arskr.,**36** :1-40.

Hammer, Q., Harper, D.A.T. and Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electronica*, **4**(1): 9.

Kato, H., Li, F. and Shimizu, A. 2020. The selection of gamma-ray irradiated higher yield rice mutants by directed evolution method. *Plants.* **9**: 1-16. [Cross Ref]

Lawrence, C.W. 1968. Radiation-induced polygenic mutation in *Arabidopsis thaliana.Herididty*,**23**(3): 321-337. [Cross Ref]

Li, F., Shimizu, A., Nishio, T., Tsutsumi, N. and Kato, H. 2019. Comparison and characterization of mutations induced by gamma-ray and carbon-ion irradiation in rice (*Oryza sativa* L.) using whole-genome resequencing. G3 (Bethesda, Md.), **9**(11): 3743–3751. [Cross Ref]

Manikandan, V. and Vanniarjan, C. 2017. Induced macromutational spectrum and frequency of viable mutants in M2 generation of rice (*Oryza sativa*L.).*Int. J. Curr. Microbiol. App. Sci.,***6**(7): 1825-1834. [Cross Ref]

Minn, M., Khai, A. A. and Lwin, K. M. 2008. Study on the effect of gamma radiation on rice Sin ThweLatt (IR 53936). GMSARN Proc. Int. Conf. on Sust. Dev.: Issues and Prospects for the GMS. 12-14 Nov. 1-5.

Mishra, D. and Singh, B. 2013.Prediction of M_2 macro and micro-mutation frequency based on m_1 effect in greengram [*Vigna radiata* (L.)Wilczek]. *IOSR J. Agri. Vet. Sci.,***2**(1): 01-04. [Cross Ref]

Muduli, K.C. and R.C. Mishra. 2007. Efficacy of mutagenic treatments in producing useful mutants in finger millet (*Eleusine coracana L.). Indian J. Genet*.,**67***: 232-237.*

Pandey, M.P., Verulkar, S.B. and Sarawgi, A.K. 2010.Status paper on rice for Chhattisgarh. RKMP-DRR, Hyderabad.Pp1-32.

Rao, G. M. and Rao, V. M. 1983.Mutagenic efficiency, effectiveness and factor of effectiveness of physical and chemical mutagens in rice.*Cytologia*,**48**: 427-436. [Cross Ref]

Reddi, T. V. V. S. and Rao, D. R. M. 1988. Relative effectiveness and efficiency of single and combinattion treatments using gamma rays and sodium azide in inducing chlorophyll mutations in rice.*Cytologia*,**53**: 491-498. [Cross Ref]

Roy, B., Kumar, V., Tulsiram, S.D. and Das, B.K. 2018. Development of high yielding aromatic mutants of rice (*Oryza sativa* L.) from a local aromatic cultivar, Tulaipanji by using gamma radiation. *Indian J. Genet*., **78**(4): 409-416.

Sahu, P.K., Mondal, S., Sharma, D., Vishwakarma, G., Kumar, V. and Das, B.K. 2017. InDel marker based genetic differentiation and genetic diversity in traditional rice (*Oryza sativa* L.) landraces of Chhattisgarh, India. *PLoS ONE*, **12**(11): e0188864. [Cross Ref]

Sasikala, R. and Kalaiyarasi, R. 2010. Sensitivity of rice varieties to gamma irradiation. *Elect. J. Plant Breed.,* **1**(4): 885-889.

Shadakshari, Y.G., Chandrappa, H.M., Kulkarni, R.S. and Shashidhar, H.E. 2001. Induction of beneficial mutant in rice (*Oryza sativa* L.). *Indian J.Genet.,***61**(3): 274-276.

Sharma, D., Das, B.K., Kumar, V., Tiwari, A.K. and Sahu, P.K. 2017 a. Development of semidwarf and mid-late maturing jawaphool mutant through the use of gamma radiation. *Int. J. Pure App. Biosci.,***5**(5):228-233. [Cross Ref]

Sharma, D., Das, B.K. Kumar, V., Tiwari, A., Sahu, P.K., Singh, S. and Baghel, S. 2017 b. Identification of semi-dwarf and high yielding mutants in Dubraj rice variety of Chhattisgarh through gamma ray based induced mutagenesis. *Int J. Genet.,* **9**(9): 298-303.

Sharma, S. K. and Sharma, B. 1984.Pattern of induced macro and micro-mutations with gamma rays and NMU in Lentil. *Environ. Experiment. Bot.***24**(4): 343-351. [Cross Ref]

Singh, A. K. and K. P. Singh. 2003 a. Induced quantitative variation for yield and its components in okra *(Abelmoschus esculentus* (L.). Moench). *Adv. Plant Sci.,***16** (II): 519-525.

Singh, S. and Singh, J. 2003 b. Mutation in basmati rice induced by gamma rays, ethyl methane sulphonate and sodium azide. *Oryza,* **40**(1&2): 5-10.

Singh, S., Richharia, A. K. and Joshi, A. K. 1998. An assessment of gamma rays induced mutations in rice (*Oryza sativa* L.).*Indian J. Genet*,**58**(4): 455-463.

Sivaram, A.K., Raveendran, T.S. Kumar, M. and Manonmani, S. 2014. Gamma-ray induced mutations for isolating economic mutants in rice (*Oryza sativa*. L.) cultivars ADT 39 and CR1009. *Int.J.Adv.Biol. Res.,***4**(4): 466-474.

Soomro, A.M., Baloch, A.W., Ahmed, M., Javed, M.A., Bughio, H.R., Bughio, M.S., Muhammad, T. and Mastoi, N.N. 2002. Evaluation of induced mutants of rice for yield and quality characters. *Plan. Sci.,***1**(3): 248-249.

Swaminathan, M. S., Siddiq, E. A., Singh, C. B. and Pai, R. A. 1970. Mutation breeding in rice in India. In: Rice Breeding with Induced Mutations. Vol. II IAEA, Vienna, pp. 25-43.

Whankaew, S., Kaewmanee, S., Ruttajorn, K. and Phongdara, A. 2020. Indel marker analysis of putative stress-related genes reveals genetic diversity and differentiation of rice landraces in peninsular Thailand. *Physiol. Mol. Biol. Plants*,**26**(6): 1237–1247. [Cross Ref]

Viana, V.E., Pegoraro, C., Busanello, C and Oliveira, A.C. 2019. Mutagenesis in rice: the basis for breeding a new super plant. *Front. Plant Sci.,***10**:1326. [Cross Ref]

USDA. 2021. World Agricultural Production. United States Department of Agriculture Foreign Agricultural Service. WAP 5-21.