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

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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 M₂ population. A total of 81, 98 and 51 putative macro mutations 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 M₂ population of Samundchini, Vishnubhog and Jhilli, respectively. In addition, 36, 42 and 19 viable morphological mutants with the mutation frequency percentages (based on M₂ 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 photoresponsive 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<sub>2</sub> population (Gustafsson, 1940).

Irradiation of seeds with desirable mutagens constitute the M<sub>1</sub> population which further on selfing constitute the M<sub>2</sub> population. Dominant types of mutants are obtained in the M<sub>1</sub> generation while most of the mutants being recessive in nature are screened in the M<sub>2</sub> 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&BT), Bhabha Atomic Research Centre (BARC), Mumbai, India. The treated seeds were sown as M<sub>1</sub> population during Rabi, 2017-18 at research field of Department of Genetics and Plant Breeding, IGKV, Raipur. All the required agronomic and

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**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 M₂ 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 M₂ plants were sown in separate rows by following the panicle to row method at research fields of the Department of Genetics and Plant Breeding, IKGV, 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 M₂ generation of Samundchini, Vishnubhog and Jhilli, respectively. The untreated (control) plants were also sown for comparison along with the M₂ 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 M₂ 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 M₂ population and M₁ 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 M₂ 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 mutations 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 colour 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 M₂ 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 M₂ families in which putative mutants were observed, and (ii) percentage of mutants in M₂ 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:

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

\[
\text{M₂ population basis(percentage of mutated M₂ plants or mutants)}
\]

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

For the study of micro-mutations, 30 normal looking plants from the families not segregating in the M₂ 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 M₂ 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$_1$ generation (Gustafsson, 1940). In the current study, the mutation frequency of overall macro mutations obtained in Samundchini based on the M$_1$ 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$_1$ 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$_1$ 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$_1$ population and family rows were 0.328 and 2.222 per cent, respectively. The spectrum of chlorophyll mutants of Samundchini M$_1$ 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$_1$ families. Mutation frequency based on the M$_1$ 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$_1$ 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

| Type of macro mutants | Categories of putative mutants | Number of mutants in M$_1$ population | Number of mutants observed | Mutation Frequency (%) based on the M$_2$ population | Mutation Frequency (%) based on the M$_2$ families |
|-----------------------|--------------------------------|--------------------------------------|---------------------------|------------------------------------------------------|--------------------------------------------------|
| 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                                             |
|                       | 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                                             |

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Mutation frequency based on the M<sub>1</sub> 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<sub>1</sub> 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 virdis 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<sub>1</sub> 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<sub>2</sub> population which were dispersed in 20 panicle rows or M<sub>1</sub> families. The mutation frequency (%) of viable mutations on the basis of the M<sub>2</sub> population and M<sub>1</sub> family were 0.283 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<sub>2</sub> population and M<sub>1</sub> 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<sub>2</sub> population and M<sub>1</sub> 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<sub>1</sub> 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<sub>2</sub> 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<sub>1</sub>.
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                                          |
|                             |                               |                       | 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                                          |
|                             |                               |                       | Semi tall 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                                          |
|                             |                               |                       | 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                                          |
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 Jhili 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 Jhili, respectively. Narrow leaf mutants were also reported in rice by Singh et al. (1998), Singh and Singh (2003a) 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
Fig. 2. Various chlorophyll and viable morphological mutants identified in the M2 population of Vishnubhog.
Fig. 3: Viable morphological mutants identified in the $M_2$ population of Jhilli.
Table 5. Genetic variability parameters for quantitative traits in M₀ population of Samundchini, Vishnubhog and Jhilli rice landraces

| Character                        | Parameters | Samundchini (Parental Population) | Samundchini (M₀ Population) | Vishnubhog (Parental Population) | Vishnubhog (M₀ Population) | Jhilli (Parental Population) | Jhilli (M₀ 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                     |
|                                 | 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                     |
|                                 | 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                    |
|                                 | Minimum    | 27.41                              | 32.14                       | 37.24                            | 32.82                      | 33.42                       | 28.59                    |
|                                 | Mean       | 31.01                              | 34.52                       | 39.21                            | 35.21                      | 35.61                       | 38.41                    |
|                                 | Coeff. var | 29.33                              | 33.06                       | 38.18                            | 32.82                      | 34.59                       | 33.74                    |
|                                 | Minimum    | 4.83                               | 2.93                        | 1.95                             | 5.69                       | 3.16                        | 12.46                    |
|                                 | Mean       | 1.26                               | 1.28                        | 1.41                             | 1.38                       | 1.47                        | 1.35                     |
|                                 | Coeff. var | 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                     |
|                                 | Minimum    | 6.6                                 | 6                           | 6                                | 7                          | 7                           | 8                        |
| Total number of tillers/plant   | 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                    |
|                                 | 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                    |
|                                 | Minimum    | 78                                   | 78                          | 60                                | 61                         | 52                          | 40                       |
| Fertile spikelets/panicle       | 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                    |
|                                 | 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                    |
| Sterile spikelets per panicle   | 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                     |
|                                 | 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                    |
| Spikelet fertility %            | Coeff. var | 2.55                                | 3.80                        | 9.28                             | 9.18                       | 5.27                        | 28.80                    |

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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 M₂ 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 grain yield whereas it decreased for days to 50% flowering, plant height, panicle length, 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.

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