

**CLUSTER ANALYSIS OF RICE (*Oryza sativa* L.) MUTANT LINES OF USING MORPHOLOGICAL PARAMETERS**

**Abstract**

Mutation breeding has been used to develop numerous crop varieties in recent decades. This study describes the scope for using gamma irradiation to create genetic variation in indica rice cultivar, Rajendra Mahsuri 1, using analysis of morphological traits to identify candidate mutants. The M<sub>3</sub> mutant lines were screened using 11 morphological traits. Morphological analyses showed that maturation time ranged between 105 and 135 days and height ranged from 45 to 160 cm which showed that there was enough variation to create promising mutant lines for breeding programs. The results obtained using morphological analysis and have considerable potential for detecting useful mutants.

Keywords: Rice (*Oryza sativa* L.), Mutation breeding, morphological analysis

**Introduction**

Rice (*Oryza sativa* L.) belongs to the family Graminae and is the staple food source for one third of the world's population. After wheat, rice is the second largest source of calories in the human diet and provides approximately 20% of the total calories consumed worldwide. It is a particularly important food source in Asia and provides more than two billion people with 60-70% of their daily energy requirements (FAO, 2021). Since the 1960s, several collections of mutant lines from different species have been isolated and successfully used in many different areas of plant biology and crop breeding (Domingo et al., 2007; Fu et al., 2008). Of the more than 2700 mutant varieties that have been released worldwide, 64% were created through exposure to gamma rays, 22% through exposure to x-rays and the rest by other mutagenic treatments (Ahloowalia et al., 2004; Shu and Lagoda, 2007). Gamma ( $\gamma$ ) rays are physical mutagens; gamma irradiation has proven to be a useful method for introducing new trait variations that may result in crop improvement and can be used as a complementary tool in plant breeding (Babaei et al., 2010). Induced rice mutants have been useful research tools for genetic and physiological assessments of yield-limiting factors in rice. Mutants have made it possible to identify critical elements for developing high yield potential varieties exhibiting desirable traits such as semi-dwarfism, early maturity, a greater number of panicles/plant and increased fertility. By 2003, 440 mutant rice varieties had been developed. Of these, 264 were produced by the direct application of mutagens and 176 were created by cross-breeding with induced mutants

(Shehata et al., 2009). Mutants were traditionally identified on the basis of their morphological properties. Breeders therefore often adopt germplasm selection criteria that emphasize the importance of genetic diversity (Agrama and Eizenga 2008; Kiula et al., 2008; Agrama et al., 2009).

The main objective of the study described in this paper was to use morphological trait analysis to evaluate the level of genetic variation induced by gamma irradiation in the M3 populations of ten mutant lines.

## **Materials and methods**

### **Plant material and mutagenic treatment**

Rice seeds (*Oryza sativa* L.) were exposed to dose of  $\gamma$  radiation of 450 Gy at 13% moisture using a Co60 source at the Department of Bhabha Atomic Nuclear Research Institute, Mumbai. The duration of the gamma irradiation treatment was between 0.5 and 2 hours, depending on the  $\gamma$ ray dose. The radiated seeds were grown through to the M1, M2 and M3 generations during 2014, 2015 and 2016.

### **Morphological study**

Treated seeds, were planted in a seed nursery and then transferred to a paddy field after 28 days. The morphological characteristics of the M3 generation plants were measured and recorded; the specific traits measured were the plants' heights (cm), Total number tiller per plant, Spikelt fertility, number of grain per panicle, yield per plant number of fertile tillers, percentage of sterile panicles, panicle length (cm), number of spikelets per panicle, 1000 grain weight (gr), yield per plant (gr), stem color and the condition of the seed awn.

### **Data analysis**

Variation in the morphological traits of the M3 population was analyzed using a randomized complete block design (RCBD) with three replicates and mean separation by the least significant difference (LSD) method (Yazdi Samadi et al., 2008). Results obtained by Analysis clustering using the NTSyPc ver.17 software package.

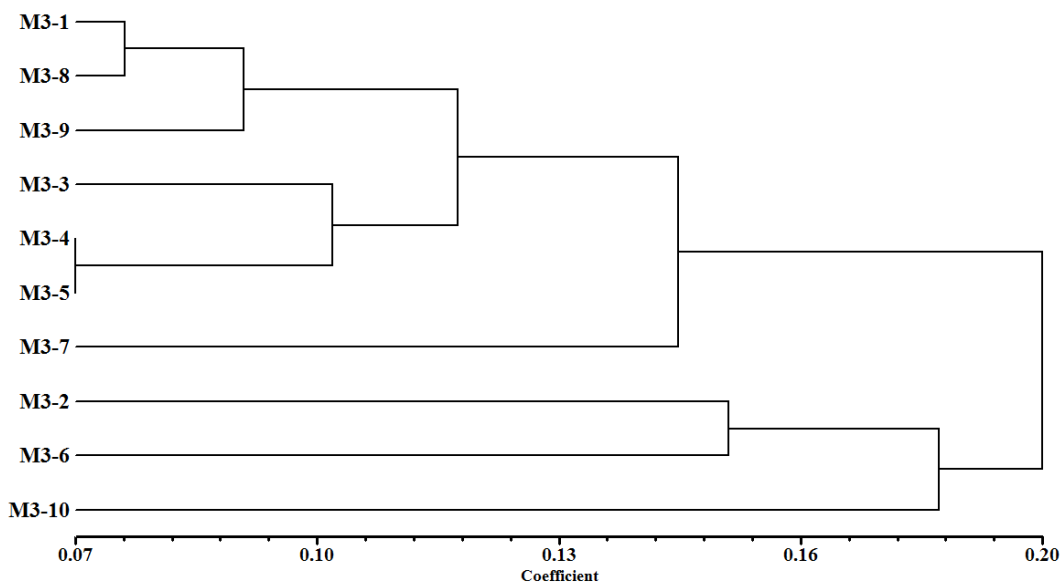
## **Results and Discussion**

### **Morphological analysis**

Cluster analysis of the M3 cultivar indicated significant differences in plant height between the different  $\gamma$ ray doses relative to the control. A similar result was obtained for the proportion of unfilled seeds in the panicle and the yield per plant at the and also for total number of tillers, 1000-grain weight and the number of fertile tillers. No significant differences were found in the panicle length or the number of filled seeds per panicle between any of the gamma doses. (Cheema and Atta, 2003; Domingo et al., 2007; Shereen et al., 2009)

## Divergence analysis

The divergence study revealed significant differences among genotypes for eleven characters indicating presence of an acceptable amount of genetic variability. Assessment of clusters revealed that clusters were heterogeneous within themselves and between each other based on major character relations. The results cleared that the inter cluster distance in most treatments were larger than intra cluster distance suggesting wider diversity among the mutant of different groups (Shereen et al., 2009; Babaei 2010; Shehata et al. 2009). These genotypes were grouped into five clusters, displaying cluster V with maximum number of genotypes (6 genotypes) accounting for 60.00 per cent of total genotypes followed by cluster I, II, III and IV (1genotypes) offering 10 per cent of total genotypes monogenic as given in Figure 1. The inter cluster distances were more than intra cluster distance stating wide genetic diversity among lentil genotypes. The highest intra-cluster distance among genotypes from these clusters could be exploited as parental mutant lines in rice breeding programmes owing to their mean performance within their group (Luzi-Kihupi et al. 2009).



**Figure 1.** Cluster analysis of 10 M3 Mutant line genotypes based on 11 morphological traits

**Table 1.** Estimates of eleven morphological parameters based similarity coefficients among ten mutant lines rice used in the present study

|       | M3-1 | M3-2 | M3-3 | M3-4 | M3-5 | M3-6 | M3-7 | M3-8 | M3-9 |
|-------|------|------|------|------|------|------|------|------|------|
| M3-2  | 0.15 |      |      |      |      |      |      |      |      |
| M3-3  | 0.11 | 0.13 |      |      |      |      |      |      |      |
| M3-4  | 0.11 | 0.17 | 0.09 |      |      |      |      |      |      |
| M3-5  | 0.11 | 0.17 | 0.12 | 0.07 |      |      |      |      |      |
| M3-6  | 0.28 | 0.16 | 0.22 | 0.26 | 0.24 |      |      |      |      |
| M3-7  | 0.6  | 0.19 | 0.19 | 0.13 | 0.11 | 0.27 |      |      |      |
| M3-8  | 0.08 | 0.14 | 0.11 | 0.13 | 0.10 | 0.24 | 0.17 |      |      |
| M3-9  | 0.11 | 0.14 | 0.16 | 0.15 | 0.11 | 0.23 | 0.14 | 0.08 |      |
| M3-10 | 0.21 | 0.17 | 0.23 | 0.22 | 0.17 | 0.19 | 0.18 | 0.17 | 0.11 |

## Reference

Shereen A, Ansari R, Mumtaz S, Bughio HR, Mujtaba SM, Shirazi MU, Khan MA (2009) Impact of gamma irradiation induced changes on growth and physiological responses of rice under saline conditions. *Pak J Bot* 41(5): 2487-2495.

Luzi-Kihupi A, Zakayo JA, Tusekelege H, Mkuya M, Kibanda N, Khatib KJ, Maerere A (2009) Mutation breeding for rice improvement in Tanzania. *FAO, Rome Book*.

Shehata SM, Ammar MH, Abdelkalik AF, Zayed BA (2009) Morphological, molecular and biochemical evaluation of Egyptian jasmine rice variety and its M5 derived mutants. *Afr J Biotechnol* 8(22): 6110-6116.

Babaei A (2010) Genetic diversity analysis of Nemat rice mutant (*Oryza sativa* L.) via RAPD marker. *Am-Euras J Agric & Environ Sci* 8(4): 452-456.

Babaei A, Nematzadeh GA, Avagyan V, Hashemi H (2010) Radio sensitivity studies of morpho-physiological characteristics in some Iranian rice varieties (*Oryza sativa* L.) in M1 generation. *Afr J Agric Res* 5(16): 2124-2130.

Domingo C, Andrés F, Talón M (2007) Rice cv. Bahia mutagenized population: a new resource for rice breeding in the Mediterranean basin. *Span J Agric Res* 5(3): 341347.

Cheema A, Atta B (2003) Radio sensitivity studies in basmati rice. *Pak J Bot* 35 (2): 197-207.

Yazdi Samadi B, Rezaei A, Valyzadeh M (2008) *Statistical designs in agricultural research*, University of Tehran Press

Rohlf FJ (1998) *NTSYSpc: Numerical taxonomy and multivariate analysis system, Version 2.02*. Exeter Software, NY, USA.

Fu HW, Li YF, Shu QY (2008) A revisit of mutation induction by gamma rays in rice: implications of microsatellite markers for quality control. *Mol Breeding J* 22(2): 281-288

Ahloowalia, BS, Maluszynski M, Nichterlein K (2004) Global impact of mutation-derived varieties. *Euphytica* 135: 187-204

Agrama HA, Eizenga GC (2008) Molecular diversity and genome-wide linkage disequilibrium patterns in a worldwide collection of *Oryza sativa* and its wild relatives. *Euphytica* 160: 339-355

Kiula BA, Lyimo NG, Botha AM (2008) Association between AFLP-based genetic distance and hybrid performance in tropical maize. *Plant Breeding J* 127: 140144

UNDER PEER REVIEW