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Phenotypic characterization and genetic variability of Muthu Mullai mutants of *Jasminum auriculatum* for induction of homeotic mutants

S P MIRUNALINI[1], M GANGA[1]*, K RAJAMANI[1], B MEENAKUMARI[1],
M SUGANTHY[1] and N MANIKANDA BOOPATHI[1]

Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641 003, India

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ABSTRACT

An experiment was conducted during 2021–23 at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu to create genetic variation in *Jasminum auriculatum* (Vahl.) by inducing mutation using physical and chemical means, through gamma rays and Ethyl Methane Sulphonate (EMS). The present study was designed to evaluate the M_1V_3 generation putative mutants of *J. auriculatum* ecotype Muthu Mullai generated through physical and chemical mutation with various dosages of gamma radiation and EMS. The study revealed that among the mutagen treated population, the plants subjected to 15 Gy gamma irradiation and 5 mM EMS recorded the maximum vegetative growth parameters including number of primary and secondary branches, internodal length, number of leaves, leaf length and leaf width and flower quality parameters namely days to flowering, number of flowering cymes/branch, flower bud length, corolla tube length, bud girth, flower diameter and 100 flower bud weight than control. Homeotic mutants were identified and isolated from the mutated population and this study further explored the reliability of the observed traits for making effective selection for crop improvement.

Keywords: Elite mutants, Flowering traits, Genetic variability, Homeotic mutants, *Jasminum auriculatum*, Morphological traits

Among the 200 species of jasmine that have been documented, *Jasminum auriculatum* (Vahl.) is one of the most important species of jasmine commercially grown in India (Ganga *et al.* 2022). Tamil Nadu is the leading producer and exporter of jasmine flowers to other countries. This crop is vegetatively propagated and has poor genetic diversity to make effective selection for further improvement. Due to the low genetic diversity and the fact that high yielding varieties are not available for cultivation by the farmers, breeding programme that increases genetic variation is necessary in order to improve yield and contributing traits. The phenotypic variation is largely in response to the environment particularly the temperature which implies on the development of mutants (Chaitanya *et al.* 2020). The success of a crop improvement programme is highly dependent on the genetic diversity of the genotypes available. The most effective technique for the formation of morphological and genotypic variability in plants is induced mutation. The process of induced mutation can be performed either physically or chemically and it is very effective in the production of natural genetic resources for the formation of mutation in plants. In the present study, M_1V_3 generation putative mutants of *J. auriculatum* ecotype Muthu Mullai generated through physical and chemical mutation with various dosages of gamma radiation and EMS were subjected to phenotypic characterization and genetic variability assessment, to be used further under breeding programme for jasmine improvement.

MATERIALS AND METHODS

An experiment was conducted during 2021–23 at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. The M_1V_3 generation of the mutagen treated plants derived from *J. auriculatum* ecotype Muthu Mullai were raised through vegetative propagation of the M_1V_2 generation plants with semi-hardwood cuttings rooted under poly-tunnels with average temperature of 28–30°C and relative humidity 80%. The M_1V_3 generation plants were replanted in larger grow-bags filled with potting mixture after root development where they were maintained for 120 days after which they were transplanted to the field and assessed for growth, flowering and quality characteristics. The analysis was performed using the IBM-SPSS software.

Using Burton's (1953) formula, the coefficient of phenotypic and genotypic changes was computed. In order to determine the degree of genotype-related variation in the

[1]Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. *Corresponding author email: gangasivakumar@yahoo.com

phenotypic variance represented in percentage, heritability in the broadest sense was estimated (Hanson *et al.* 1956). The approach recommended by Johnson *et al.* (1955) was used to compute the predicted genetic progress expressed as a percentage of mean.

Statistical analysis: The statistical programme TNAUSTAT was used to conduct the statistical analysis for this study.

RESULTS AND DISCUSSION

M_1V_3 generation of gamma irradiated plants

Vegetative parameters: The data revealed that number of primary branches (4.2), secondary branches (9.8) and internodal length (6.5 cm) was found higher in 15 Gy than control which expressed number of primary branches 3.0, 7.0 and 4.1 cm, respectively from the above parameters. The leaf traits, such as number of leaves/plant (92.3), leaf length (6.6 cm), leaf width (5.0 cm) were found to be higher in 15 Gy when compared to control which recorded 69.7, 4.5 and 3.3 cm, respectively from the above parameters. Rate of occurrence of abnormal leaves was found to be lower in 15 Gy mutated plants (4.2%) than 25 Gy (10.5%) (Table 1), indicating more the dose rate, higher the abnormal leaves. Chlorophyll variations were also observed in putative mutants (Supplementary Fig. 1). The morphological parameters varied with change in the dose of mutagens. These results are in accordance with the research findings of Hosalli *et al.* (2022) who reported in *Bougainvillea* spp. that 15 Gy of gamma radiation produced the largest co-efficient of variation in internodal length. Meanwhile, the results are in accordance with the observations of Kumari *et al.* (2015) who reported in gladiolus that gamma irradiation showed significant effect in the mutated population with changing dose of radiation and that there is a significant reduction in growth of the plant, viz. number of leaves, leaf length and leaf width, which might be due to the physiological, morphological and cytological disturbances in the irradiated plants resulted in poor growth.

Flowering parameters: Desirable flowering traits including the early flowering (126.0 days), more number of flowering cymes/branch (10.9), more flower bud length (2.9 cm) and long corolla tube length (2.3 cm) were found in 15 Gy when compared to control which showed late flowering (151.2 days), low number of flowering cymes/ branch (5.9), low flower bud length (2.1 cm) and short corolla tube length (1.5 cm). The high bud girth (2.7 mm), 100 flower bud weight (12.2 g) and flower diameter (2.0 cm) were found in 15 Gy when compared to control (1.9 mm, 10.7 g and 1.5 cm, respectively) (Table 2) (Supplementary Fig. 2). The significant improvement in the flowering traits observed might be attributed to the stimulatory effect of gamma irradiation on the physiological process of flowering and this in turn has resulted in early flowering and improved quality traits. These results are in accordance with the earlier findings of Vanmathi *et al.* (2021) who showed that there was a positive correlation in early flowering in cowpea, indicating the stimulatory effect of gamma irradiation in shortening the crop duration.

M_1V_3 generation of chemical mutagen (EMS) treated plants

Vegetative parameters: The height of the plant (90.6 cm), number of primary (4.1) and secondary branches (11.77) and internodal length (8.1 cm) were found to be maximum in the 5 mM treated plants when compared to the control 3.7, 6.1 and 4.8 cm, respectively. For the leaf traits, the number of leaves (85.6), leaf length (6.4 cm) and leaf width (6.5 cm) were found to be maximum in the 5 mM treated plants when compared (60.3, 5.2 and 3.9 cm, respectively). The minimal number of abnormal leaves 6.7% was observed in 5 mM than 20 mM which showed maximum abnormal leaves of 10.2% (Table 3). Leaf variations were also

Table 1 Vegetative growth parameters of M_1V_3 generation of gamma irradiated plants of *J. auriculatum* ecotype Muthu Mullai

Gamma ray dose	No. of primary branches	No. of secondary branches	Internodal length (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Abnormal leaves (%)
Gamma irradiation							
Control	3.0±0.1 (9.7)	7.0±0.1 (2.8)	4.1±0.1 (3.0)	69.7±1.6 (7.2)	4.5±0.1 (9.2)	3.3±0.1 (5.99)	7.9±0.2 (12.1)
5 Gy	3.9±0.1 (18.1)	9.0±0.1 (5.8)	5.4±0.1 (8.6)	84.7±3.1 (14.5)	4.9±0.2 (16.5)	4.8±0.1 (13.0)	8.2±0.2 (11.6)
10 Gy	4.0±0.2 (19.01)	7.1±0.1 (7.5)	6.1±0.1 (9.7)	86.3±3.0 (13.4)	5.9±0.2 (15.5)	4.0±0.1 (10.4)	9.0±0.2 (9.9)
15 Gy	4.2±0.1 (14.9)	9.8±0.1 (5.18)	6.5±0.01 (4.8)	92.3±2.7 (11.7)	6.6±0.2 (13.8)	5.0±0.1 (9.0)	4.2±0.2 (18.0)
20 Gy	3.9±0.1 (17.0)	8.5±0.1 (4.67)	5.2±0.01 (4.5)	83.4±2.6 (12.3)	6.5±0.2 (14.3)	4.4±0.1 (9.2)	9.5±0.2 (10.7)
25 Gy	3.7±0.1 (15.1)	8.6±0.1 (4.7)	4.5±0.01 (6.6)	75.5±2.7 (13.8)	6.7±0.2 (13.1)	4.6±0.1 (9.0)	10.5±0.2 (14.8)

Values are [Mean±Standard Error] (Coefficient of variation).

Table 2 Flowering parameters of M_1V_3 generation of gamma irradiated plants of *J. auriculatum* ecotype Muthu Mullai

Gamma ray dose	Days to flowering	No. of flowering cymes/branch	Flower bud length (cm)	Corolla tube length (cm)	Bud girth (mm)	Flower diameter (cm)	100 flower bud weight (g)
Gamma irradiation							
Control	151.2±2.2 (5.9)	5.9±0.2 (8.9)	2.1±0.1 (9.2)	1.5±0.1 (9.2)	1.9±0.1 (9.1)	1.5±0.1 (9.2)	10.7±0.2 (9.3)
5 Gy	148.5±3.6 (9.0)	6.8±0.2 (17.0)	2.4±0.1 (8.2)	2.2±0.1 (11.5)	2.4±0.1 (13.3)	1.6±0.1 (17.3)	12.0±0.4 (15.4)
10 Gy	141.4±3.0 (8.4)	9.7±0.2 (10.3)	2.4±0.2 (12.7)	2.0±0.1 (17.0)	2.1±0.1 (17.2)	1.9±0.1 (13.6)	11.4±0.5 (15.7)
15 Gy	126.0±3.8 (11.9)	10.9±0.3 (10.6)	2.9±0.1 (11.1)	2.3±0.1 (18.9)	2.7±0.1 (10.9)	2.0±0.1 (20.9)	12.2±0.4 (13.6)
20 Gy	128.8±3.4 (11.71)	9.1±0.2 (11.6)	2.8±0.1 (14.8)	2.2±0.1 (11.6)	2.5±0.1 (11.6)	1.7±0.1 (13.0)	11.3±0.4 (14.7)
25 Gy	146.1±3.8 (10.2)	9.5±0.2 (11.1)	2.4±0.1 (14.7)	1.7±0.2 (17.3)	2.3±0.1 (12.4)	1.9±0.1 (10.5)	11.2±0.4 (16.6)

Values are [Mean±Standard Error] (Coefficient of variation).

observed in putative mutants with EMS dose of 5 mM (Supplementary Fig. 1). It could be observed that EMS mutagen at lower concentrations induces minimal damage to the DNA, resulting in fewer lethal mutations and genetic variations in the mutated population. These results are in accordance with the findings of Purente *et al.* (2020) who reported that the effect of EMS in mutant plants of M_3 generation of *Chrysanthemum indicum* var. *aromaticum* showed significant increase in the leaf traits. The genes which regulate the morphological traits might be mutated to produce the varying range of phenotypes. At a concentration of 0.5% EMS has been reported to induce mutation in the genes which were responsible for the favourable leaf traits in *Dendranthema grandiflora* L. (Topno *et al.* 2023).

Flowering parameters: The chemical mutagen EMS at 5 mM dose produced earlier flowering (130.5 days) mutants when compared to control which took 154.8 days for flowering. Number of flowering cymes/branch (9.3), flower bud length (2.8 cm) and corolla tube length (2.2 cm) were also found to be higher in 5 mM treated plants when compared to control which showed 6.5 flowering cymes/branch, flower bud length of 2.1 cm and corolla tube length of 1.5 cm. Further, the bud girth (3.0 mm), flower diameter (2.3 cm) and 100 flower bud weight (11.6 g) were also maximum in 5 mM treated plants compared to the control which showed minimum bud girth (2.1 mm), flower diameter (1.8 cm) and 100 flower bud weight (10.7 g) (Supplementary Table 1). Variations in flower bud and corolla tube length were also observed in the putative mutants at 15 Gy and 5 mM (Supplementary Fig. 2). It is known that the low and intermediate concentrations of chemical mutagen EMS generally stimulated the growth rate and hastened attainment of the reproductive phase resulting in earlier flowering in specific cases. Differential response of cultivars has also been

Table 3 Vegetative growth parameters of M_1V_3 generation of EMS treated plants of *J. auriculatum* ecotype Muthu Mullai

Dose	No. of primary branches	No. of secondary branches	Internodal length (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Abnormal leaves (%)
Gamma irradiation							
Control	3.7±0.1 (5.9)	6.1±0.2 (11.8)	4.8±0.1 (3.0)	60.3±1.3 (8.9)	5.2±0.1 (9.2)	3.9±0.1 (5.9)	9.6±0.3 (11.7)
5 mM	4.1±0.1 (9.9)	11.7±0.4 (14.9)	8.9±0.1 (3.1)	85.6±2.4 (9.8)	6.4±0.2 (12.6)	6.5±0.1 (6.1)	6.7±0.2 (16.6)
10 mM	4.0±0.1 (12.1)	7.7±0.4 (16.3)	5.9±0.1 (4.8)	82.1±2.2 (10.8)	6.2±0.2 (13.5)	6.2±0.1 (6.1)	8.0±0.3 (14.6)
15 mM	3.7±0.1 (11.9)	10.4±0.5 (18.7)	6.0±0.1 (4.8)	74.3±2.4 (12.6)	6.2±0.2 (14.1)	4.8±0.1 (9.5)	8.7±0.3 (16.2)
20 mM	3.8±0.1 (11.4)	7.6±0.4 (19.3)	5.4±0.1 (5.6)	78.2±2.3 (11.5)	6.1±0.2 (13.9)	5.7±0.1 (7.0)	10.2±0.2 (12.9)
25 mM	3.9±0.1 (11.3)	6.8±0.4 (19.8)	5.1±0.1 (5.8)	71.2±2.4 (13.1)	5.3±0.2 (15.2)	6.2±0.1 (6.5)	7.5±0.2 (15.9)

Values are [Mean±Standard Error] (Coefficient of variation).

reported for earliness and floral traits. Singh *et al.* (2015) reported that among the cultivars of *Polinathes tuberosa,* the cultivar Calcutta Double showed early flowering under the effect of EMS @1%. The early flowering and higher number of flowers/plant in *Dendranthema grandiflora* L. observed by Topno *et al.* (2023) with lower concentration of EMS @0.5% might be attributed to the fact that EMS at low concentrations induces favourable mutation in the genes responsible for the early flowering and number of flowers/plant, specifically in the gene segment which controls the flowering phase.

Variability parameters of M_1V_3 generation of ecotype Muthu Mullai mutants: Progeny selection would be effective as the prevalence of additive gene effect for these traits like Phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and heritability (Rai *et al.* 2021). The genotypic and phenotypic variability of M_1V_3 generation mutants' characteristics revealed that there is an additive gene effects, suggesting that the simple selection method would be applied for enhancing the flowering characters. Though wide range of variations have been recorded in most of the traits in the mutagen treated plants, the dosages of gamma (15 Gy) and EMS (5 mM) treatments have generated the positive effects in the vegetative growth and flowering traits, which had expressed superior traits than the control.

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV): The results depicted in Fig. 1 and 2 shows the variability in the morphological characters and flowering characters of the treated mutants in which the estimates of phenotypic coefficient of variation (PCV) were higher than genotypic coefficient of variation (GCV) estimates for every character under the study, indicating that the variation was not only due to the genotypes but environment had also played a major role in the expression of the traits. These results are in accordance with the findings of Pal *et al.* (2018) in balsam wherein the phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) with respect to all the quantitative traits studied.

The magnitude of moderate PCV and GCV ranging from 10–20% was reported by Thirumalmurugan *et al.* (2020) were observed for morphological characters, viz. number of primary branches at 15 Gy (PCV=14.9, GCV=12.5), number of secondary branches at 5 mM (PCV=14.9, GCV=11.7), leaf length at 15 Gy (PCV=13.8, GCV=11.5) and at 5 mM (PCV=12.6, GCV=10) (Fig. 1) and floral characters, viz. number of flowering cymes/branch at 5 mM (PCV=14.8, GCV=12.1), flower bud length at 5 mM (PCV=16.6, GCV=14.1), corolla tube length at 15 Gy (PCV=18.9, GCV=16.7), flower diameter at 15 Gy (PCV=20.1, GCV=19.3) and 100 flower bud weight at 15 Gy (PCV=13.6, GCV=10.6) and at 5 mM (PCV=13.8, GCV=10.9) (Fig. 2). This is indicative of high scope for plant breeders to utilize the existing variability for future breeding programmes. Moderate PCV and GCV was earlier reported for morphological characters, viz. number of primary branches and floral characters, viz. flower diameter, flower bud weight, number of flowers in *Chrysanthemum morifolium* Ramat by Henny *et al.* (2021).

Heritability (%): In a breeding programme, the choice of any trait is influenced by both the degree of variability present in the population and the degree of heritability

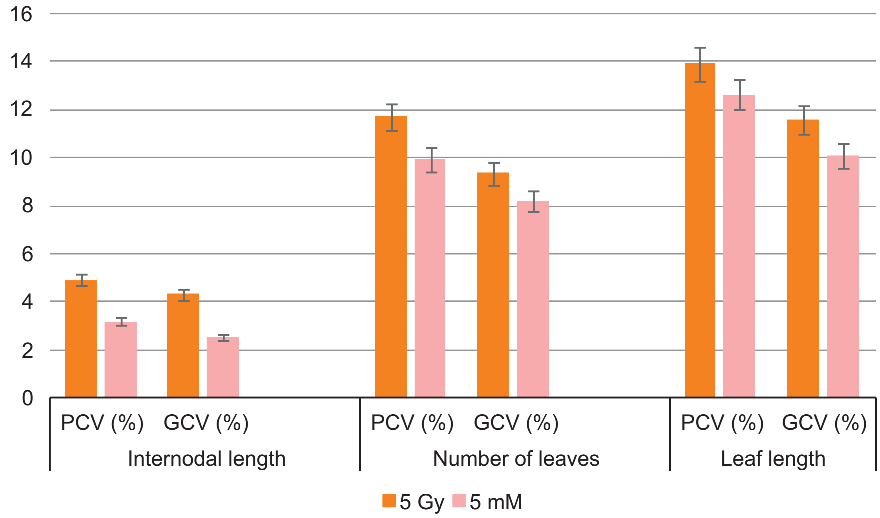

Fig. 1 Estimates of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) on morphological traits of M_1V_3 generation mutants.

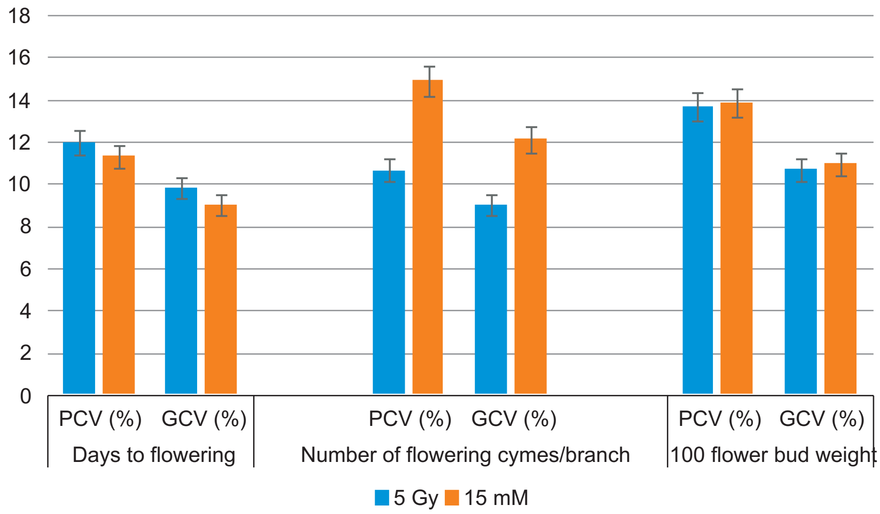

Fig. 2 Estimates of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) on flowering traits of M_1V_3 generation mutants.

of the trait. The most significant component is heritable variability, which is dependent on the genetic makeup of the breeding material and has a direct impact on how the selection is effective for the improvement of the trait. Heritability acts as a gauge for the trait's transmissibility to the following generation (Shravani *et al.* 2024). In the present study, higher estimate of broad sense of variability (more than 60%) was observed for all the morphological and flowering characters, viz. number of primary branches at 15 Gy (70.5%), number of secondary branches at 15 Gy (79.6%), internodal length at 15 Gy (76.9%) and 5 mM (62.8%), leaf width at 5 mM (69.3%), number of flowering cymes/branch at 5 mM (66.1%), flower bud length 5 mM (72.1%), corolla tube length at 15 Gy (77.6%) and flower diameter at 15 Gy (85.7%) in the doses of gamma ray and EMS treatments. These results are in accordance with the findings of Singh *et al.* (2014) in marigold wherein there is an existence of variability and it can be utilized for breeding of novel types with superior traits.

Genetic advance as percent of mean (%): High genetic advance as percent of mean (more than 20%) was observed for morphological character, viz. number of primary branches at 15 Gy (21.7%) and flowering characters, viz. number of flowering cymes/branch at 5 mM (20.2%), flower bud length at 5 mM (24.7%), corolla tube length at 15 Gy (30.3%) and flower diameter at 15 Gy (36.9%). These findings are in accordance with the observations of Henny *et al.* (2021) in *Chrysanthemum morifolium* Ramat wherein high genetic advance was reported for certain morphological characters, viz. number of primary branches and flowering characters, viz. number of flowers/plant and flower diameter, indicating that selection of the above traits among genotypes can be further exploited by breeding programme.

The highest estimates of broad sense heritability (more than 60%) coupled with high genetic advance as percentage of mean (more than 20%) indicate that the heritability is most likely due to additive gene effects and selection may be effective. In the present study, such a trend was observed for the morphological character, viz. number of primary branches at 15 Gy and number of flowering cymes/branch at 5 mM, flower bud length at 5 mM, corolla tube length at 15 Gy and flower diameter at 15 Gy. These results are in accordance with the findings of Bharti *et al.* (2021) in gladiolus wherein number of flowers/plant and flower diameter had high heritability coupled with high genetic advance as percentage of mean, indicating that heritability along with genetic advance in combination with intensity of selection under breeding programme and the amount of variability present in such mutated population influences the genes to be targeted for further improvement.

Homeotic mutants: Due to homeotic mutations, disintegration of cells occurs in the early stage of flower development. Mutants exhibiting alterations in floral morphology were identified after exposure to varying levels of gamma radiation. Changes in the floral organs i.e. number of petals were termed as homeotic mutants. Changes were noticed randomly in 15 Gy and 5 mM treated plants. In 15 Gy mutated plants, one homeotic mutant having 6 petals and another homeotic mutant having 8 petals were observed and isolated (Supplementary Fig. 3) whereas in 5 mM mutated plants, one homeotic mutant having 5 petals and another homeotic mutant having 9 petals were observed and isolated than the control which showed 7 petals (Supplementary Fig. 4). This was earlier confirmed in gladiolus in which homeotic mutants was observed and isolated for the changes in floral organs by Kumari *et al.* (2015). These homeotic mutants can be further utilized to study the phenomenon of development of different flower organs.

In M_1V_3 generation of *J. auriculatum* ecotype Muthu Mullai, 18 putative mutants (10 from gamma irradiated population and 8 from EMS treated population) which expressed distinct mutations in the plant growth and flower yield were selected and compared with control. This study confirmed the reliability of the selection for the investigated traits and those selected mutants can be further exploited for jasmine improvement programme.

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