

INTEGRATIVE MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF INDUCED MUTANTS IN *JASMINUM AURICULATUM* USING SSR MARKERS

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ABSTRACT

Mutation breeding is an effective approach for enhancing genetic variability in vegetatively propagated crops such as jasmine. The present study aimed to assess molecular and biochemical variability among putative mutants of *Jasminum auriculatum* derived from the cultivars CO.1 Mullai and Muthu Mullai. Molecular diversity was analyzed using six Simple Sequence Repeat (SSR) markers, of which four markers (Js035, Js041, Js042 and Js063) exhibited polymorphism, while two markers (Js033 and Js055) were monomorphic. The polymorphism revealed moderate genetic variability among the mutants. Biochemical analysis indicated significant variation in chlorophyll content, total phenols, soluble proteins, total soluble sugars and IAA oxidase activity. Notably, early flowering and high-yielding mutants exhibited increased soluble protein and sugar content, suggesting enhanced metabolic activity and growth potential. The integration of molecular marker analysis with biochemical profiling proved effective in identifying genetically diverse and physiologically superior mutants. These mutants can serve as valuable genetic resources for future jasmine breeding and crop improvement programmes.

KEYWORDS: *Jasminum auriculatum*, Mutation breeding, SSR markers, Genetic diversity, Biochemical traits, Induced mutants.

INTRODUCTION

Jasmine flowers hold significant horticultural importance as a popular loose flower and garden plant besides their extensive commercial applications in perfumery and natural cosmetic products (1). In tropical and temperate climates, it is extensively grown as small trees and vines for its fragrant blooms.

Among the 200 species of Jasmine that have been documented earlier, 40 species are distributed in India throughout the tropical and subtropical regions(2). Of the many species, *J. sambac*, *J. grandiflorum* and *J. auriculatum* are extensively cultivated. Notably, *J. auriculatum* is markedly propagated on a commercial scale due to its highly fragrant white flowers, which exhibit a maximum floral flush in the summer (3).

The success of any crop improvement programme depends to a greater extent on the presence of genetic variability within the base population. Due to the limited genetic diversity in *J. auriculatum*, there is a pressing need to enhance its yield and related traits through crop improvement initiatives. This entails implementing programs aimed at increasing genetic variation, thereby facilitating more effective selection processes (4).

Mutagenesis is acknowledged as a pivotal breeding tool in the creation of novel varieties via genetic manipulation. Mutation breeding entails enhancing the genetic base of crop plants and to create desired economic traits through induced mutations (5). Induced mutation has been identified as the most effective technique for creating morphological and genetic variability in jasmine which is a vegetatively propagated crop.

Induced mutation can be done either by physical or chemical means and is highly effective in triggering natural genetic changes and creating mutations in plants (6). These mutations can result in a variety of variations including phenotypic changes, such as extra number of petals, larger floral organs as well as improvement in desirable traits such as yield, quality, resistance or tolerance to biotic and abiotic stresses, etc. Studying these mutants can provide valuable insights into the genetic regulation of flower development in plant species (7).

However, limited studies have integrated molecular marker-based analysis with biochemical profiling to comprehensively evaluate induced mutants in *J. auriculatum*. Such integrative approaches are essential for understanding the genetic and physiological basis of mutation-induced variability and for identifying superior genotypes for breeding programmes. Hence, the objective of this study was to assess the molecular characterization of promising putative mutants derived from CO.1 Mullai and Muthu Mullai and to evaluate their biochemical variability and nutrient analysis for potential use in crop improvement programmes.

MATERIALS AND METHODS

Experimental location and plant material

The experiment was conducted during 2022–2024 at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. Two cultivars of *J. auriculatum*, CO.1 Mullai and Muthu Mullai, which are commercially cultivated in the Coimbatore region, were used as plant material.

Mutagenesis

Semi-hardwood cuttings of Muthu Mullai were treated with different doses of gamma irradiation and Ethyl Methane Sulphonate (EMS). The LD₅₀ dose was determined based on mortality percentage using probit analysis. The M₁V₁ generation was screened for phenotypic variations, and promising putative mutants were advanced to M₁V₂ and M₁V₃ generations through nodal cuttings to confirm the stability and heritability of induced mutations. The mutants exhibited morphological variations highlighting the early flowering, tall and dwarf stature, profuse branching, bolder bud and longer corolla tube were observed (Fig. 1 and Fig. 2).

Genomic DNA isolation

Young leaf samples from putative mutants were collected and stored at –20°C until analysis. Genomic DNA was extracted using a modified CTAB method (Doyle, 1991). Approximately 200 mg of leaf tissue was ground in liquid nitrogen and mixed with CTAB extraction buffer containing β-mercaptoethanol. After incubation at 65°C, the extract was purified using chloroform: isoamyl alcohol (24:1) and DNA was precipitated using ice-cold isopropanol. The DNA pellet was washed with 70% ethanol, air-dried, and resuspended in sterile water.

DNA quantification and quality assessment

The quality and quantity of genomic DNA were initially assessed using 0.8% agarose gel electrophoresis in 1× TBE buffer and visualized under a UV transilluminator. DNA concentration and purity were further determined using a Jenway Genova Nano spectrophotometer by measuring absorbance at 260 and 280 nm. DNA samples with purity ratios between 1.8 and 2.0 were diluted to a working concentration of 100 µg ml⁻¹ for PCR analysis.

SSR marker analysis

A total of six SSR markers were used to assess molecular variation among the mutants. PCR amplification was carried out using a Bio-Rad T100 thermal cycler with an initial denaturation at 94°C for 8 min, followed by 37 cycles of denaturation (94°C for 1 min), annealing (48–52°C for 45 s depending on primer), and extension (72°C for 1 min), with a final extension at 72°C for 10 min. PCR products were separated on 1.5% agarose gel along with 100 bp and 1 kb DNA ladders and visualized using a gel documentation system.

Genetic diversity analysis

Clear and reproducible bands were scored as 1 (presence) or 0 (absence) to generate a binary data matrix. Polymorphic Information Content (PIC) values were calculated (8). Genetic similarity among genotypes was estimated using Jaccard's coefficient, and cluster analysis was performed using the UPGMA method in NTSYS-pc (version 2.02) to generate a dendrogram.

Physiological and biochemical analysis

Chlorophyll content (mg g⁻¹)

Total chlorophyll content was measured using a SPAD-502 chlorophyll meter and calculated according using absorbance values at 645 nm and 663 nm (9) and expressed in mg g⁻¹ of fresh weight.

Total phenols (mg g⁻¹)

Total phenolic content in leaf samples was estimated using the Folin–Ciocalteu method (10). Absorbance was recorded at 660 nm, and results were expressed as mg g⁻¹ fresh weight.

Soluble protein (mg g⁻¹)

Soluble protein content was determined using the Bradford method (11) with Coomassie Brilliant Blue G-250 dye, and absorbance was measured at 595 nm and results were expressed as mg g⁻¹ fresh weight.

Total soluble sugars (mg g⁻¹ DW)

Total soluble sugars were estimated using the anthrone method (12).

IAA oxidase activity (µg of IAA unoxidized g⁻¹h⁻¹)

IAA oxidase activity was determined using the colorimetric method (13), and absorbance was recorded at 540 nm. The activity was expressed as µg of unoxidized IAA g⁻¹ h⁻¹.

Statistical Analysis

Statistical analysis was performed using the statistical package TNAUSTAT.

RESULTS

SSR Polymorphism and Genetic Diversity

The molecular diversity was assessed for the selected putative mutants of CO.1 Mullai and Muthu Mullai using 6 Simple Sequence Repeat (SSR) markers. Among the SSR markers used, Js033 and Js055 markers produced monomorphic bands, whereas Js035, Js041, Js042 and Js063 produced polymorphic bands (Fig. 5 and Fig. 6). The observed polymorphism indicates the effectiveness of SSR markers in detecting genetic variability induced through mutagenesis, thereby facilitating the identification of genetically distinct mutant lines.

Polymorphic information content (PIC) of putative mutants

The results indicated that the polymorphic information content among the genotypes represents the primer efficiency in revealing the genetic diversity. For CO.1 Mullai mutants, the frequency of 0.20 was recorded for the primers, Js035, Js041, Js042 and Js063. The frequency of 0.14 was recorded for the primers, Js035 and Js063,

whereas the frequency of 0.28 was recorded for the primers, Js041 and Js042 for Muthu Mullai mutants. For CO.1 Mullai mutants, the PIC value of 0.96 was recorded for the primers Js035, Js041, Js042 and Js063. The PIC value of 0.98 was recorded for Js035, Js063 and PIC value of 0.91 was recorded for Js041, Js042 for Muthu Mullai mutants.

Cluster analysis of putative mutants

In CO.1 Mullai mutants, the dendrogram was produced by hierarchal clustering and it resulted in the formation of two major clusters namely, Cluster A and Cluster B among the putative mutants. Cluster A was further divided into three sub clusters, namely Sub-cluster I with Parent (CO.1 Mullai) and CM-HYM (CO.1 Mullai-High Yielding Mutant); Sub-cluster II with CM-EFM (CO.1 Mullai-Early Flowering Mutant); Sub-cluster III with CM-DM (CO.1 Mullai-Dwarf Mutant) and Cluster B with CM-PBM (CO.1 Mullai-Profuse Branching Mutant) (Fig. 3).

In Muthu Mullai mutants, the dendrogram was produced by hierarchal clustering and it resulted in the formation of two major cluster namely, Cluster A and Cluster B among the putative mutants. Cluster A was further divided into two sub clusters, namely Sub-cluster I with Parent and MM-EFM (Muthu Mullai-Early Flowering Mutant); Sub-cluster II with MM-TM (Muthu Mullai-Tall Mutant), MM-PBM (Muthu Mullai-Profuse Branching Mutant) and MM-HYM (Muthu Mullai-High Yielding Mutant) and Cluster B was further divided into two sub clusters, namely Sub-cluster I with MM-DM (Muthu Mullai-Dwarf Mutant); Sub-cluster II with MM-BB&LCTM (Muthu Mullai-Bolder Bud & Long Corolla Tube Mutant) (Fig. 4).

Similarity matrix

Based on the genetic distances between the putative mutants, the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm was used. The similarity coefficient ranged from 0.71 to 0.83 for CO.1 Mullai mutants, whereas for Muthu Mullai mutants, similarity coefficient ranged from 0.60 to 1.00. For CO.1 Mullai mutants, the similarity coefficient (0.72) was recorded between Parent and CM-DM, whereas the similarity coefficient (0.75) was recorded between Parent; CM-HYM and CM-EFM (Table 1). For Muthu Mullai mutants, the similarity coefficient (0.66) was recorded between Parent; MM-DM and MM-BB&LCTM. The similarity coefficient (0.84) was recorded between parent and MM-EFM; MM-TM, MM-PBM and MM-HYM (Table 2).

Biochemical variability in mutant Lines

(a) CO. 1 Mullai mutants

Chlorophyll content (mg g⁻¹)

In CO.1 Mullai mutants, the chlorophyll content ranged from 1.47 mg g⁻¹ (25 Gy) to 2.08 mg g⁻¹ (15 Gy) and the maximum variability (5.47 %) was observed in 25 Gy for gamma irradiated mutants. In EMS treated mutants, the mean value ranges from 1.71 mg g⁻¹ (50 mM) to 2.12 mg g⁻¹ (35 mM). The highest variability (6.56 %) was observed in 50 mM (Table 3).

Total phenol (mg g⁻¹)

In CO.1 Mullai mutants, the mean total phenol ranged from 2.20 mg g⁻¹ (25 Gy) to 3.77 mg g⁻¹ (15 Gy) and the maximum variability (7.85 %) was observed in 20 Gy for gamma irradiated mutants, whereas in EMS treated mutants, the total phenol content was ranged from 3.53 mg g⁻¹ (50 mM) to 3.88 mg g⁻¹ (35 mM) and the maximum variability (6.00 %) was observed in 50 mM (Table 3).

Soluble protein (mg g⁻¹)

The early flowering mutants recorded higher soluble protein (15.96 mg g⁻¹) followed by high yielding mutants (15.06 mg g⁻¹) as compared with untreated control (11.37 mg g⁻¹) whereas the maximum variability (3.22 %) was observed in early flowering mutants (Table 4).

IAA oxidase activity (µg oxidised auxin g⁻¹h⁻¹)

The putative mutants with dwarf stature recorded lowest IAA oxidase activity (13.27 µg oxidised auxin g⁻¹ h⁻¹) as compared with untreated control (17.72 µg oxidised auxin g⁻¹ h⁻¹) and the maximum variability (2.48 %) was observed in high yielding mutants (Table 4).

Total soluble sugar (mg g⁻¹)

The total soluble sugar was found to be higher in high yielding mutants (86.07 mg g⁻¹) followed by profuse branching mutants (72.22 mg g⁻¹) as compared with untreated control (63.67 mg g⁻¹). The maximum variability (1.44 %) was observed in early flowering mutants (Table 4).

The variation in biochemical parameters among mutants suggests differential metabolic responses to mutagenic treatments, which may contribute to improved growth, stress tolerance and yield potential.

(b) Muthu Mullai mutants

Chlorophyll content (mg g⁻¹)

The chlorophyll content of MM-AJR-1 (Muthu Mullai-*Aceria jasmini* Resistance-1) (1.72 mg g⁻¹) and MM-AJR-2 (Muthu Mullai-*Aceria jasmini* Resistance-2) (1.78 mg g⁻¹) as compared with control (0.81 mg g⁻¹) and the maximum variability (3.69 %) was observed in control (Table 5).

Soluble protein (mg g⁻¹)

The mean soluble protein of MM-AJR-1 (Muthu Mullai-*Aceria jasmini* Resistance-1) (6.12 mg g⁻¹) and MM-AJR-2 (Muthu Mullai-*Aceria jasmini* Resistance-2) (6.17 mg g⁻¹) as compared with control (11.26 mg g⁻¹) and the maximum variability (1.45 %) was observed in MM-AJR-2 (Table 6).

Total soluble sugars (mg g⁻¹)

The total soluble sugar content of MM-AJR-1 (Muthu Mullai-*Aceria jasmini* Resistance-1) (2.30 mg g⁻¹) and MM-AJR-2 (Muthu Mullai-*Aceria jasmini* Resistance-2) (2.32 mg g⁻¹) as compared with control (1.89 mg g⁻¹) and the maximum variability (2.81 %) was observed in MM-AJR-2 (Table 6).

IAA Oxidase (µg of IAA unoxidized g⁻¹h⁻¹)

The IAA oxidase activity of MM-AJR-1 (Muthu Mullai-*Aceria jasmini* Resistance-1) (0.16 unoxidized g⁻¹h⁻¹) and MM-AJR-2 (Muthu Mullai-*Aceria jasmini* Resistance-2) (0.16 unoxidized g⁻¹h⁻¹) as compared with control (0.17 unoxidized g⁻¹h⁻¹). The highest variability (15.69 %) was observed in MM-AJR-1 (Table 6).

Nutrient analysis of leaves of infested and non-infested mutant lines of Muthu Mullai ecotype

Macro nutrient content (%)

The maximum nitrogen content (1.59 %) was observed in control whereas the minimum nitrogen content was observed in MM-AJR-2 (1.06 %) followed by 1.04 % in MM-AJR-1. The maximum variability in nitrogen content was observed in MM-AJR-1 (2.54 %). The maximum phosphorous content (0.10 % and 0.09 %) was observed in MM-AJR-2 and MM-AJR-1 and the highest variability (25.05 %) was observed in MM-AJR-1. The maximum potassium content (2.68 % and 2.65 %) was observed in MM-AJR-2 and MM-AJR-1 and the highest variability (1.63 %) was observed in control (Table 7).

Secondary nutrient content (%)

The maximum calcium content (0.42 % and 0.41 %) was observed in MM-AJR-2 and MM-AJR-1 whereas the minimum calcium content (0.35 %) was observed in control. The maximum variability in calcium content was observed in MM-AJR-1 (6.76 %). The maximum magnesium content (0.14 % and 0.12 %) was observed in MM-AJR-2 and MM-AJR-1 whereas the minimum magnesium content (0.09 %) was observed in control. The highest variability (16.95 %) was observed in MM-AJR-2 (Table 8).

Micronutrient content (%)

The maximum iron content (22.12 ppm and 21.54 ppm) was observed in MM-AJR-2 and MM-AJR-1 whereas the minimum iron content (7.10 ppm) was recorded in control and the highest variability (3.36 %) was observed in control. The maximum manganese content (11.81 ppm and 11.75 ppm) was observed in MM-AJR-2 and MM-AJR-1 whereas the minimum manganese content (6.25 ppm) was recorded in control and the highest variability (2.33 %) was observed in MM-AJR-2. The maximum copper content (6.75 ppm and 6.69 ppm) was observed in MM-AJR-2 and MM-AJR-1 whereas the minimum copper content (4.89 ppm) was recorded in control and the highest variability (2.25 %) was observed in MM-AJR-2. The maximum zinc content (1.51 ppm and 1.45 ppm) was observed in MM-AJR-2 and MM-AJR-1 whereas the minimum zinc content (0.72 ppm) was recorded in control and the highest variability (2.86 %) was observed in control (Table 9).

DISCUSSION

Genetic divergence is a key focus in any crop improvement program. In addition to conventional methods, molecular diversity analysis offers a more precise understanding of the genetic variability among different genotypes. Simple Sequence Repeats (SSRs), commonly referred to as microsatellites, are short, repeating nucleotide sequences. SSR markers are among the most powerful tools available for this purpose (14, 15). These markers are widely distributed in plant genomes, occurring approximately every 6 kilobases. Due to their high mutation rate, SSRs provide greater polymorphic information compared to other markers like RAPD, ISSR, and SNP (16).

The hierarchical structure of clustering analysis offers insights into the genetic relationship among genotypes. Genotypes that cluster at higher levels within the hierarchy are more closely related than those grouped at lower levels. This analysis can identify subgroups or branches within the broader genetic diversity, shedding insights over the population structure and evolutionary relationships. In CO.1 Mullai mutants, the dendrogram was produced by hierarchical clustering and it resulted in the formation of two major clusters namely, Cluster A and Cluster B among the putative mutants. Cluster A was further divided into three sub clusters, namely Sub-cluster I with Parent and CM-HYM; Sub-cluster II with CM-EFM; Sub-cluster III with CM-DM and Cluster B with CM-PBM. In Muthu Mullai mutants, the dendrogram was produced by hierarchical clustering and it resulted in the formation of two major cluster namely, Cluster A and Cluster B among the putative mutants. Cluster A was further divided into two sub clusters, namely Sub-cluster I with Parent and MM EFM; Sub-cluster II with MM-TM, MM-PBM and MM-HYM and Cluster B was further divided into two sub clusters, namely Sub-cluster I with MM-DM; Sub-cluster II with MM BB&LCTM. The close association among these putative mutants may result from similar genetic profiles or expression patterns. This suggests that they are closely related, potentially sharing a common ancestry or having experienced similar genetic or environmental influences. Such close associations serve as an indicator of genetic relatedness or similarity, which can be valuable for future plant breeding programs. Similar findings were reported in *J. sambac* (17, 18), *J. auriculatum* (19) and in *J. grandiflorum* (20). A similarity matrix offers a quantitative measure of the similarity or dissimilarity between pairs of genotypes based on specific characteristics, such as genetic markers or expression profiles. The matrix values ranged from 0 to 1, where 0 represents complete dissimilarity and 1 indicates complete similarity. This matrix helps reveal relationships and patterns among genotypes, allowing for the identification of clusters or groups of similar genotypes and comparison of overall similarities. High similarity values suggest that genotypes share similar genetic profiles or expression patterns, indicating potential genetic relatedness or common traits, while low values point to significant differences between genotypes. For CO.1 Mullai mutants, the similarity coefficient (0.72)

was recorded between Parent and CM-DM, whereas the similarity coefficient (0.75) was recorded between Parent; CM-HYM and CM-EFM. For Muthu Mullai mutants, the similarity coefficient (0.66) was recorded between Parent; MM-DM and MM-BB&LCTM. The similarity coefficient (0.84) was recorded between parent and MM-EFM; MM-TM, MM-PBM and MM-HYM. The findings of this study demonstrate that SSR analysis (primers - Js033 and Js055) is an effective method for detecting and classifying the genetic diversity of putative mutants of *J. auriculatum*. These matrices are instrumental in uncovering the underlying structure and characteristics of the mutants, enabling deeper analysis and interpretation. Similar findings were reported in *J. sambac* (17), *J. auriculatum* (19) and in *J. grandiflorum* (20).

In the plant system, mutations lead to alterations from the atomic to the organ level through a network of biosynthetic pathways. Analysis of chlorophyll a, chlorophyll b, and total chlorophyll content revealed differential distribution between mutagen-treated and untreated control plants. Plants exhibiting specific morphological and physiological adaptations demonstrated resilience to stress induced by mutagens. Gamma irradiation resulted in the degradation of photosynthetic pigments, leading to a diminished photosynthetic capacity in the mutated plants. The breakdown of the protein complexes containing chlorophyll molecules, particularly through the dephytinization and pheophytinization degradation processes, became progressively evident during the development of the mutated plants (21). The higher concentration of chlorophyll a relative to chlorophyll b in the irradiated samples is attributed to alterations in the biosynthesis or degradation of chlorophyll b precursors (22, 23).

Ionizing radiation at elevated levels causes physiological imbalances through both direct interactions with macromolecular compounds and indirect effects involving reactive oxygen species generated in plants. This leads to increased respiration rates, elevated ethylene production, and enhanced enzymatic activities, particularly affecting the production of secondary metabolites and the accumulation of protein molecules (24). Gamma irradiation has been observed to disrupt cellular metabolism by inducing the accumulation of reactive oxygen species, which can interact with functional (nucleic acids) and structural (proteins, lipids) organic molecules, ultimately resulting in oxidative stress (25).

Mutation can significantly impact various physiological processes in plants. In this study, the assessment of chlorophyll a, b, and total chlorophyll in selected putative mutants revealed an irregular distribution of chlorophyll content among the treated and untreated control plants. This observation aligns with the findings of (26), that chlorophyll is generally insensitive to low doses of gamma irradiation in pigeon pea.

Conversely, (27) noted an increase in chlorophyll a, b, and total chlorophyll levels in *Paulownia tomentosa* plants exposed to gamma irradiation, though higher doses resulted in reduced chlorophyll content compared to controls. Gamma irradiation can damage photosynthetic pigments, leading to a decreased photosynthetic capacity (22). Additionally, (28) demonstrated that soybean exhibits a sensitivity to gamma radiation, with total chlorophyll content decreasing by up to 81.36 % at 400 Gy and 80.91 % at 500 Gy.

Useful mutants were evaluated for various physiological parameters, including total soluble protein content, total soluble sugar and IAA oxidase activity, to determine the basis for the observed positive changes compared to untreated parent plants. In dwarf mutants, IAA oxidase activity was found to be higher than in their untreated counterparts, which is expected as increased IAA oxidase activity typically reducing the auxin production in the growing points, leading to suppressed growth. Conversely, some putative mutants exhibited higher levels of carbohydrate, total soluble sugar, soluble protein content, suggesting enhanced photosynthetic efficiency and potential for increased flower production. The increase in protein content may be attributed to the repression of gene expression in *Amaranthus* species (29, 30). The increased levels of soluble protein serve as a protective mechanism against damage from mutagenic reactions (23). This observation suggests that elevated soluble sugar content induced by gamma rays and EMS may act as protective agents against protein oxidation. These findings align with earlier research in jasmine (31, 32), and similar increases in total soluble sugar content have been reported in Strawberry by (33) and in *Allium cepa* by (34).

The investigation on alterations in nutrient content is crucial for understanding the interaction between mites and their host plants which are essential in addressing and mitigating the deficiencies resulting from mite feeding. This is in accordance with the findings of increased percentage of major nutrients and depletion of secondary nutrients in sugarcane leaf sheath infested by *Aceria sacchari* (35). A similar trend in the micro nutrients depletion in ribbed gourd infested by *Tetranychus pernicis* was reported (36). Meanwhile in *J. auriculatum*, the mite infested leaves showed significant increase in major nutrients and decreased level of secondary and micronutrients compared to healthy leaves as reported (37).

The integration of molecular, biochemical and nutrient analyses in the present study provides a comprehensive understanding of mutation-induced variability. Such combined approaches enhance the efficiency of selection by linking genetic diversity with functional traits, thereby accelerating the identification of superior genotypes for breeding programmes.

CONCLUSION

The present study revealed considerable molecular and biochemical variability among the putative mutants of *Jasminum auriculatum* developed through gamma irradiation and EMS treatments. SSR marker analysis effectively detected genetic polymorphism and distinguished the mutant lines from their parental cultivars. Cluster analysis further indicated the presence of genetic divergence among the mutants. The identified promising mutants may serve as valuable genetic resources for future jasmine breeding and crop improvement programs.

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**CM-EFM – CO.1 Mullai early
flowering mutant
Flowering observed in less than
120 days**



**CM-DM – CO.1 Mullai dwarf mutant
Plant height less than 38-40 cm**



**CM-PBM – CO.1 Mullai profuse branching
mutant
Secondary branches more than 11**

Fig. 1: Mutants exhibiting altered morphological traits observed in the M_1V_3 generation of *Jasminum auriculatum* - CO.1 Mullai mutants



**MM-TM – Muthu Mullai
tall mutant
Plant height more than
80 cm**



**MM-DM – Muthu Mullai dwarf mutant
Plant height less than 40 cm**



**MM-PBM – Muthu Mullai profuse branching mutant
Secondary branches more than 12**



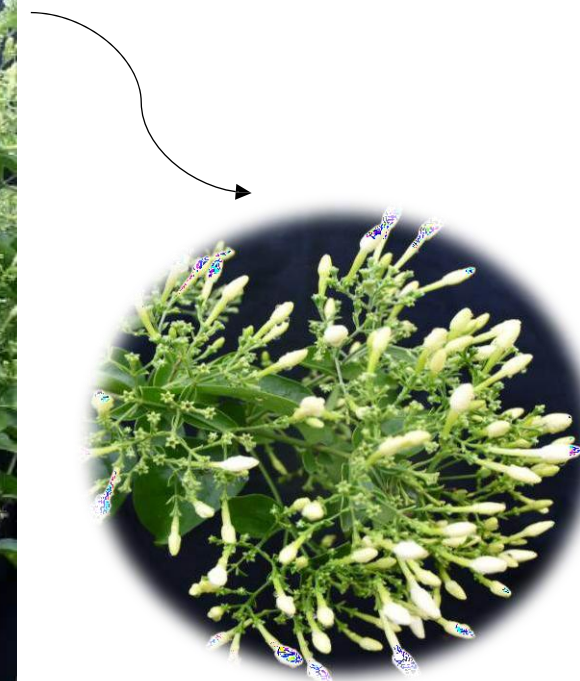
MM-EFM – Muthu Mullai early flowering mutant

Flowering observed in less than 135 days



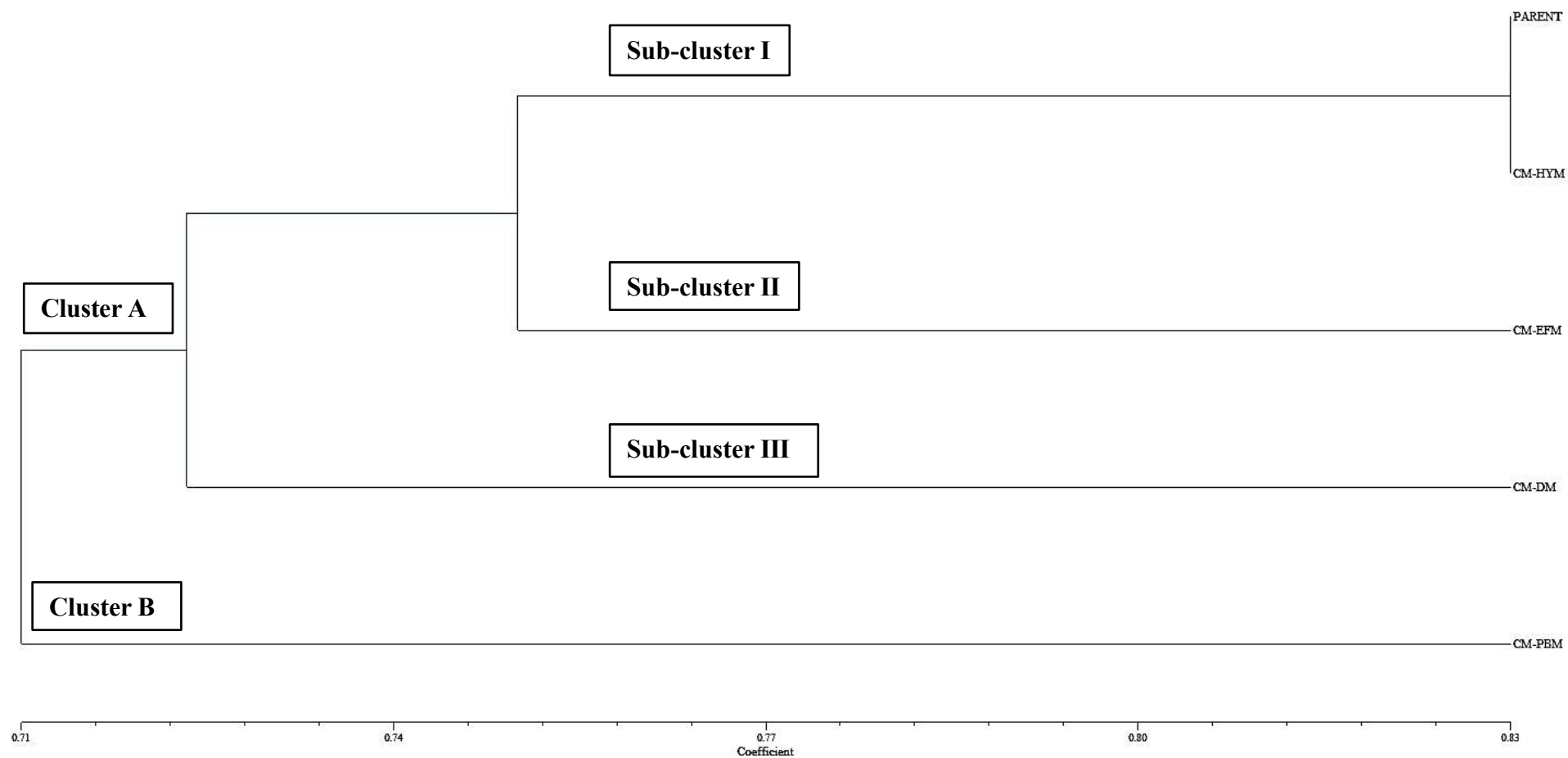
MM-BB&LCTM – Muthu Mullai Bolder bud and long corolla tube mutant

2.25 to 2.75 mm and corolla tube length of more than 2.00 cm



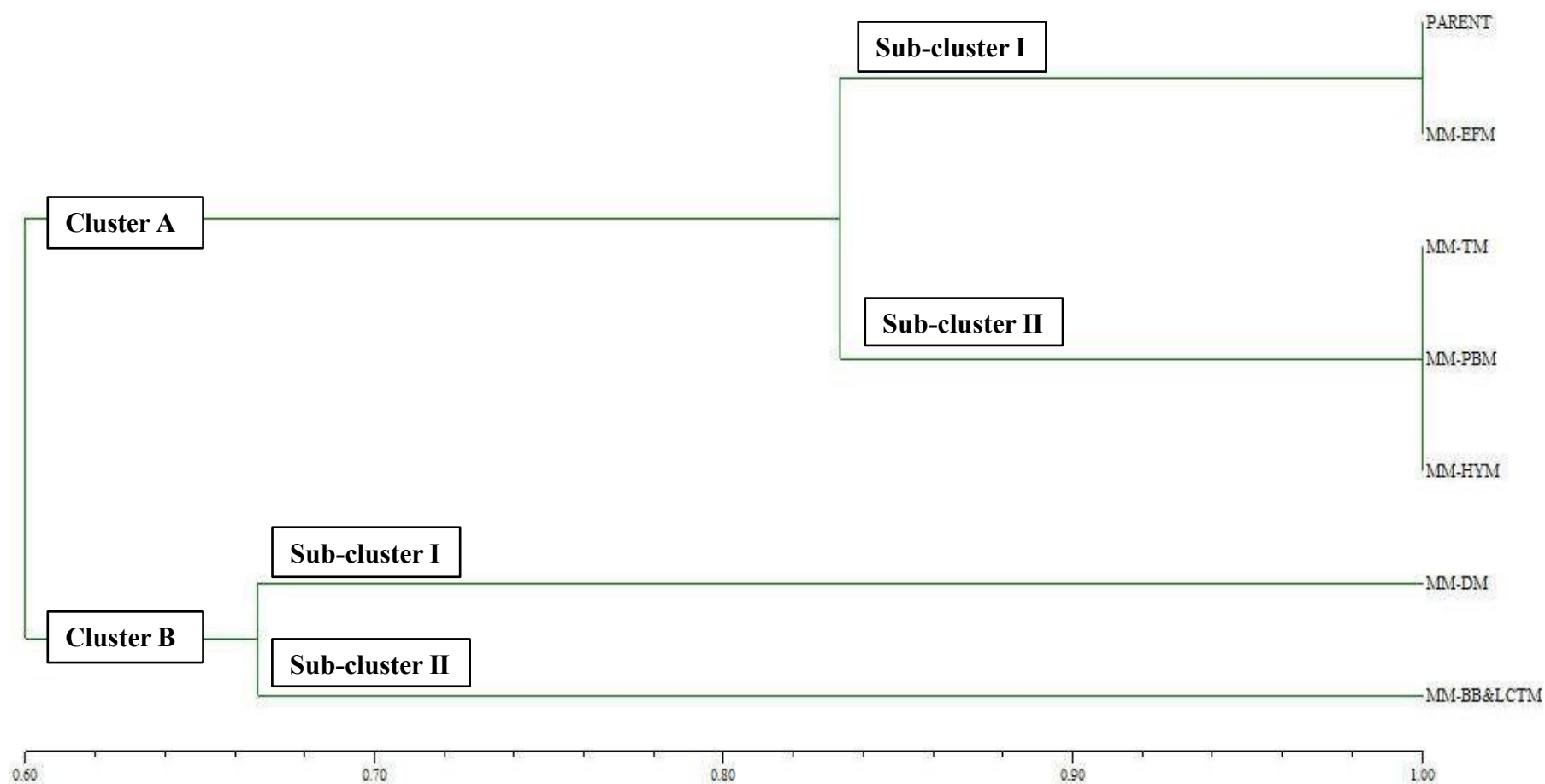
Bud girth more than

Fig. 2: Mutants exhibiting altered morphological traits observed in the M_1V_3 generation of *Jasminum auriculatum* - Muthu Mullai mutants



- CM-DM – CO.1 Mullai - Dwarf Mutant
- CM-EFM – CO.1 Mullai - Early Flowering Mutant
- CM-PBM – CO.1 Mullai - Profuse Branching Mutant
- CM-HYM – CO.1 Mullai - High Yielding Mutant

Fig. 3. Dendrogram for SSR marker-based cluster formation for putative mutants of CO.1 Mullai of *J. auriculatum*



- MM-TM – Muthu Mullai-Tall Mutant
- MM-DM – Muthu Mullai-Dwarf Mutant
- MM-PBM – Muthu Mullai-Profuse Branching Mutant
- MM-EFM – Muthu Mullai-Early Flowering Mutant
- MM-BB&LCTM – Muthu Mullai-Bolder Bud & Long Corolla Tube Mutant
- MM-HYM – Muthu Mullai-High Yielding Mutant

Fig. 4. Dendrogram for SSR marker-based cluster formation for putative mutants of Muthu Mullai of *J. auriculatum*

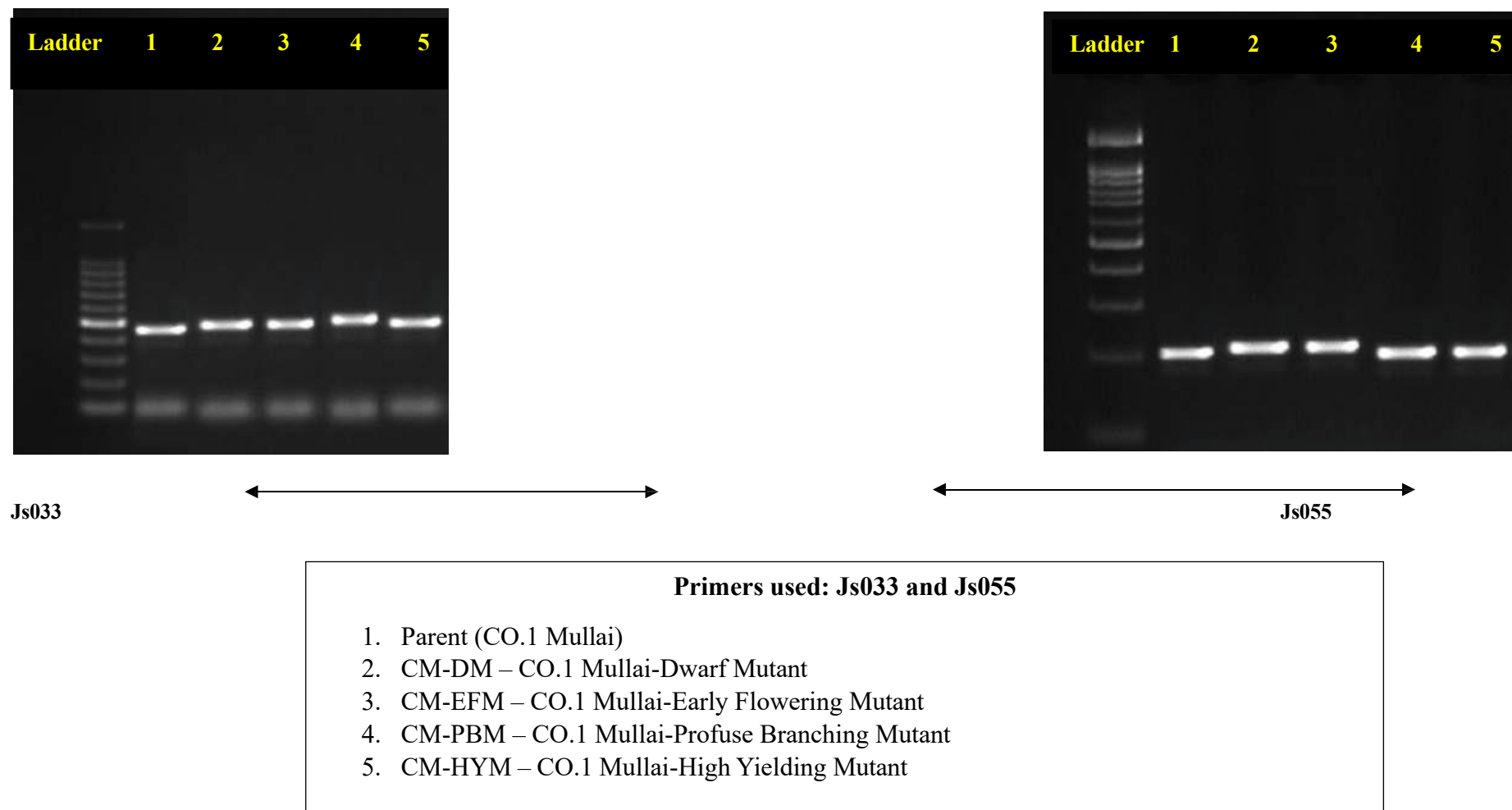


Fig. 5. Molecular characterization of promising putative mutants in *J. auriculatum* using SSR markers - CO.1 Mullai mutants

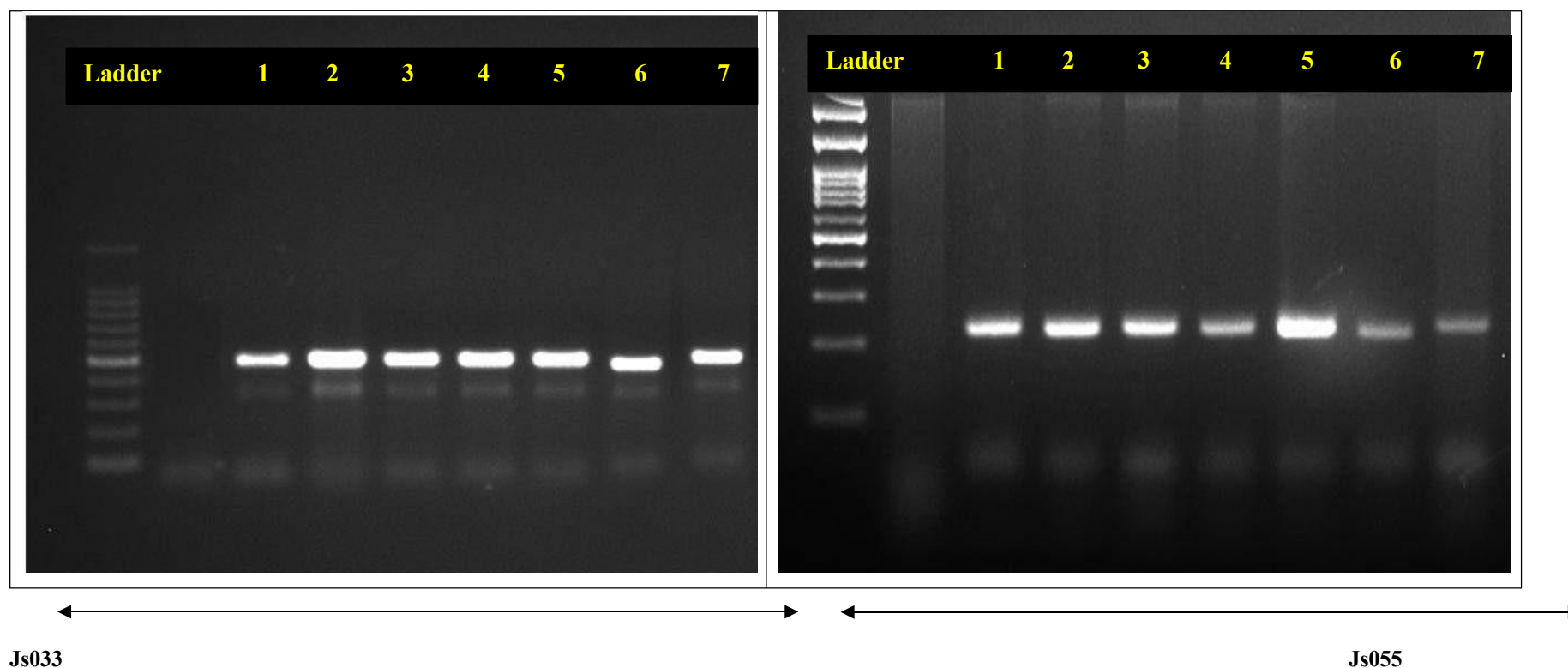


Fig. 6. Molecular characterization of promising putative mutants in *J. auriculatum* using SSR markers - Muthu Mullai mutants

Primers used: Js033 and Js055

1. Parent (Muthu Mullai)
2. MM-TM – Muthu Mullai-Tall Mutant
3. MM-DM – Muthu Mullai-Dwarf Mutant
4. MM-PBM – Muthu Mullai-Profuse Branching Mutant
5. MM-EFM – Muthu Mullai-Early Flowering Mutant
6. MM-BB&LCTM – Muthu Mullai-Bolder Bud & Long Corolla Tube Mutant

	Parent	CM-EFM	CM-DM	CM-PBM	CM-HYM
Parent	1.0000				
CM-EFM	0.6667	1.0000			
CM-DM	0.6667	0.6667	1.0000		
CM-PBM	0.6667	0.6667	0.6667	1.0000	
CM-HYM	0.8333	0.8333	0.8333	0.8333	1.0000

Table 1. Similarity matrix of selected putative mutants of CO.1 Mullai using SSR markers

	Parent	MM-TM	MM-DM	MM-PBM	MM-EFM	MM-BB&LCTM	MM-HYM
Parent	1.0000						
MM-TM	0.8333	1.0000					
MM-DM	0.5000	0.6667	1.0000				
MM-PBM	0.8333	1.0000	0.6667	1.0000			
MM-EFM	1.0000	0.8333	0.5000	0.8333	1.0000		
MM-BB&LCTM	0.5000	0.6667	0.6667	0.6667	0.5000	1.0000	
MM-HYM	0.8333	1.0000	0.6667	1.0000	0.8333	0.6667	1.0000

Table 2. Similarity matrix of selected putative mutants of Muthu Mullai using SSR markers

Treatments	Chlorophyll a (mg/g)		Chlorophyll b (mg/g)		Total chlorophyll (mg/g)		Total phenol (mg/g)	
Gamma Rays (Gy)								
	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Control	1.37 ± 0.10	1.46	0.57 ± 0.03	4.94	1.94 ± 0.01	2.35	3.61 ± 0.02	1.84
10 Gy	1.25 ± 0.02	7.70	0.44 ± 0.02	17.44	1.72 ± 0.01	3.32	3.66 ± 0.03	2.76
15 Gy	1.56 ± 0.03	6.61	0.54 ± 0.02	10.90	2.08 ± 0.02	3.60	3.77 ± 0.04	3.79
20 Gy	1.29 ± 0.01	4.21	0.47 ± 0.02	14.28	1.74 ± 0.02	4.23	2.67 ± 0.05	7.85
25 Gy	1.11 ± 0.03	10.13	0.34 ± 0.01	12.27	1.47 ± 0.02	5.47	2.20 ± 0.03	5.96
EMS (mM)								
Control	1.38 ± 0.02	3.01	0.58 ± 0.04	4.95	1.96 ± 0.02	2.36	3.66 ± 0.03	2.31
35 mM	1.47 ± 0.02	5.09	0.63 ± 0.01	6.58	2.12 ± 0.02	4.45	3.88 ± 0.05	5.40
40 mM	1.33 ± 0.01	3.87	0.56 ± 0.02	10.91	1.85 ± 0.02	3.49	3.74 ± 0.06	5.72
45 mM	1.26 ± 0.02	7.38	0.49 ± 0.01	10.14	1.76 ± 0.02	3.83	3.76 ± 0.04	4.08
50 mM	1.18 ± 0.03	9.21	0.50 ± 0.01	7.48	1.71 ± 0.03	6.56	3.53 ± 0.05	6.00

Table 3. Effect of Gamma rays and EMS on chlorophyll content and total phenol of M1V3 generation mutant population of *J. auriculatum* Cv. CO.1 Mullai

Character	Soluble proteins (mg/g)		IAA oxidase activity (µg of unoxidized auxin/g/h)		Total soluble sugar (mg/g)	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Control (Untreated)	11.37 ± 0.04	1.24	17.72 ± 0.06	1.26	63.67 ± 0.17	1.01
Dwarf stature (2 Nos.)	14.28 ± 0.06	1.60	13.27 ± 0.05	1.45	57.88 ± 0.18	1.18
Early flowering (3 Nos.)	15.96 ± 0.13	3.22	16.42 ± 0.08	1.99	70.41 ± 0.26	1.44
Profuse branching (2 Nos.)	14.67 ± 0.11	2.89	17.52 ± 0.09	1.97	72.22 ± 0.23	1.24
High Yielding (3 Nos.)	15.06 ± 0.06	1.53	14.67 ± 0.09	2.48	86.07 ± 0.31	1.40

Table 4. Effect of Gamma rays and EMS on Soluble protein, IAA oxidase activity and Total soluble sugar of M1V3 generation mutants of *J. auriculatum* Cv. CO.1 Mullai

Dose	Moisture content (%)		Chlorophyll a (mg/g)		Chlorophyll b (mg/g)		Total chlorophyll (mg/g)	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Control	34.68±0.96	1.05	0.55±0.09	6.28	0.26±0.07	10.7	0.81±0.08	3.69
MM-AJR-1 (15 Gy)	31.22±1.15	1.63	1.18±0.05	1.83	0.54±0.06	4.53	1.72±0.08	2.11
MM-AJR-2 (20 mM)	31.35±1.27	1.45	1.21±0.08	2.38	0.57±0.05	2.98	1.78±0.07	1.40

Table 5. Physiological parameters of M1V3 generation *Aceria jasmini* resistant mutants (Muthu Mullai ecotype) and susceptible parent (CO.1 Mullai)

Dose	Soluble Protein (%)		Total Soluble Sugar (%)		IAA Oxidase (µg of unoxidized auxin/g/hr)	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Control	11.26±0.30	1.02	1.89±0.06	1.22	0.17±0.06	13.15
MM-AJR-1 (15 Gy)	6.12±0.14	1.00	2.30±0.12	2.24	0.16±0.06	15.69
MM-AJR-2 (20 mM)	6.17±0.25	1.45	2.32±0.18	2.81	0.16±0.05	10.84

Biochemical parameters of M1V3 generation *Aceria jasmini* resistant mutants (Muthu Mullai ecotype) and susceptible parent (CO.1 Mullai)

6.

Dose	Total Nitrogen (%)		Total Phosphorous (%)		Total Potassium (%)	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Control	1.59±0.10	2.50	0.07±0.04	19.76	2.14±0.09	1.63
MM-AJR-1 (15 Gy)	1.04±0.06	2.54	0.09±0.06	25.05	2.65±0.08	1.39
MM-AJR-2 (20 mM)	1.06±0.05	1.61	0.10±0.05	17.52	2.68±0.09	1.18

Table 7. Macro nutrient content of M1V3 generation *Aceria jasmini* resistant mutants (Muthu Mullai ecotype) and susceptible parent (CO.1 Mullai)

Dose	Calcium (%)		Magnesium (%)	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Control	0.35±0.08	4.07	0.09±0.05	11.55
MM-AJR-1 (15 Gy)	0.41±0.06	6.76	0.12±0.04	12.87
MM-AJR-2 (20 mM)	0.42±0.06	5.19	0.14±0.07	16.95

Table 8. Secondary nutrient content of M1V3 generation *Aceria jasmini* resistant mutants (Muthu Mullai ecotype) and susceptible parent (CO.1 Mullai)

Dose	Iron (ppm)		Manganese (ppm)		Copper (ppm)		Zinc (ppm)	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Control	7.10±0.62	3.36	6.25±0.26	1.62	4.89±0.17	1.32	0.72±0.05	2.86
MM-AJR-1 (15 Gy)	21.54±0.49	1.01	11.75±0.28	1.04	6.69±0.19	1.23	1.45±0.07	2.08
MM-AJR-2 (20 mM)	22.12±1.22	1.96	11.81±0.76	2.33	6.75±0.42	2.25	1.51±0.07	1.70

Table 9. Micronutrient content of M1V3 generation *Aceria jasmini* resistant mutants (Muthu Mullai ecotype) and susceptible parent (CO.1 Mullai)