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RESEARCH ARTICLE

# Assessment of genetic divergence in multi-whorled Tuscan jasmine (*Jasminum sambac*) using microsatellite markers

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## Abstract

The genus *Jasminum* comprises nearly 200 species native to Southeast Asia. Among them, *Jasminum sambac,* known for its intensely fragrant flowers hold significant value in perfumery, traditional medicine and cultural practices. Due to its wide morphological diversity and adaptability, molecular characterisation is essential to understand its genetic structure, precise identification and management of germplasm. A total of 24 alleles were detected with two alleles per locus indicating moderate allelic polymorphism. The Polymorphic Information Content (PIC) values ranged from 0.30 to 0.40 averaging 0.40 which reflects a moderate level of marker effectiveness and ability to distinguish accessions. Primers JS035, JS042, JS055 and JS079 were recognised as the most effective markers because of their strong polymorphic potential. The effective number of alleles (Ne = 1.808) suggested that alleles were distributed with relatively even frequencies which further indicated that no single allele had dominated. Analysis of Molecular Variance (AMOVA) revealed that 96 % of the total genetic variation was within populations while only 4 % occurred among populations. A PhiPT of 0.035 showed that *J. sambac* populations were nearly genetically indistinguishable indicating very little differentiation among them. SSR markers have proven highly effective in revealing this genetic unity emphasising their importance in identifying distinct varieties. The revealed genetic diversity provides a strong basis for effective germplasm conservation, management and targeted breeding of superior *J. sambac* cultivars supporting advancements in horticulture, perfumery and pharmaceutical industries.

**Keywords:** AMOVA; Arabian jasmine; genetic variability; PIC; repetitive DNA motifs

## Introduction

The Oleaceae family includes about 27 genera and close to 900 species, with the *Jasminum* genus contributing nearly 200 species. Among the various species, *Jasminum sambac,* commonly referred to as 'Belle of India', 'Arabian jasmine' or 'Tuscan jasmine' is particularly valued for its ornamental beauty and intensely fragrant blooms (1). Classifying species within the diverse *Jasminum* genus is challenging for taxonomists because of overlapping morphological traits and the presence of multiple synonymous names. Some sources report nearly 89 recognised species while others suggest the number may be closer to 64 species (2). This variation is attributed to genetic and morphological diversity as *Jasminum* species can be evergreen or deciduous shrubs with scented or unscented white flowers. Morphological characters such as calyx size, petal lobe number and hairiness are commonly used for species identification. Environmental factors and phenotypic flexibility make it difficult for scientists to classify species accurately. The challenge grows when regional variants though morphologically similar come from different geographic areas (3).

The flowers of *J. sambac* are typically classified into three types based on petal arrangement: Single-Petal (SP), Double-Petal (DP) and Multi-Petal (MP). SP varieties are fragrant but less prolific and stress sensitive. DP types with higher yield and adaptability are preferred in commercial cultivation (4). MP forms like the Grand Duke of Tuscany are aesthetically prized offer longer fragrance retention and produce more essential oil (5). Despite the visible differences in flower forms the genetic diversity of *J. sambac* is very limited. This is mainly due to its triploid chromosome structure (2n = 3x = 39) which restricts normal sexual reproduction and results in poor seed development (6). As a result, the plant is mostly vegetatively propagated leading to widespread clonal populations with little opportunity for natural gene recombination. Although the species shows some traits that could encourage cross-pollination

such as protogyny where the stigma becomes receptive before pollen is released successful outcrossing rarely occurs. This is largely because of low pollen viability, sterility in most cultivars and the absence of reliable pollinators (7). Consequently, inbreeding dominates resulting in high genetic uniformity among cultivated varieties.

The development of PCR revolutionised DNA marker systems leading to the creation of various markers such as RAPD, SCAR, AFLP, STS, SSRs, ISSRs, CAPS, SSCP, TGGE, SRAP, TRAP and SCoT. Among these SSRs or microsatellites have gained popularity due to their high polymorphism, reproducibility and codominance (8). Molecular markers are increasingly used in plant research to explore genetic diversity and population structure. Various molecular marker techniques like RAPD, SSR, ISSR, AFLP and SARP have been used to study plants in the Oleaceae family including olives (9-13).

This study addresses a critical research gap by applying highly informative SSR markers to assess genetic diversity among multi-petal *J. sambac* genotypes. While MP forms are commercially valued for their superior fragrance, essential oil yield and prolonged freshness, there is a lack of molecular-level studies that explore their genetic distinctiveness. Limited genetic information hampers precise variety identification, conservation strategies and the development of improved cultivars. Therefore, it is hypothesized that multi-whorled *J. sambac* genotypes possess considerable genetic variability detectable through SSR markers and that this variability is associated with morphological differences in floral traits enabling precise varietal discrimination and selection of elite lines for commercial cultivation.

## Materials and Methods

A comprehensive study was conducted on *J. sambac* genotypes preserved in the gene bank of the Botanical Garden, Tamil Nadu Agricultural University (TNAU). The genotypes were evaluated for the floral morphological trait and 27 genotypes exhibited multi-whorled floral structure. The details of 27 genotypes are mentioned in Table 1. Fig. 1, 2 describes the morphological traits of flowers and leaves of different accessions. The selected genotypes were subjected to detailed characterization based on qualitative floral attributes such as flower colour, fragrance intensity, corolla shape and petal arrangement and quantitative traits including flower bud length, flower bud diameter, number of petals per flower, number of whorls, flower weight and blooming duration. In addition to morphological evaluation, molecular diversity analysis was performed to assess the genetic variability among the selected multi-whorled *J. sambac* genotypes.

### PCR analysis and electrophoresis

Young, tender leaves were sectioned to retain only the laminar tissue between the midrib and lateral veins and approximately 100 mg of this tissue was transferred into pre-labelled 2 mL sterile Eppendorf tubes for DNA extraction using a magnetic bead-based protocol (14,15). In total 12 SSR primers developed in jasmine were screened to detect the polymorphism in 27 *J. sambac* genotypes. The DNA was purified with ethanol-based wash buffers and eluted in nuclease-free water or TE buffer. Concentration and purity were measured spectrophotometrically (260/270 ratios of 1.6-1.9). Twelve reproducible SSR primers were used and they are depicted in Table 2. Genomic DNA was subjected to polymorphism analysis using Simple Sequence Repeat (SSR) markers to elucidate the genetic diversity and molecular variation among the studied *J. sambac* genotypes. PCR amplification for SSR markers was carried out. PCR amplification component details are provided in the Table 3. The following program Table 4 was used to carry out DNA amplification in a thermal cycler (T100 Thermal Cycler from Bio-Rad).

The amplified PCR products were resolved on a 1 % agarose gel in 1× TAE buffer with samples mixed with 6X loading dye and a 1kb DNA ladder included as a size reference. Electrophoresis was conducted at 70V for 30 min, bands were visualized and documented under UV illumination using a gel documentation system. For molecular data, the presence and absence of bands were scored in a binary format (1/0) to generate a data matrix.

**Table 1.** Collection sites and key morphological features of multi-whorled *Jasminum sambac*

| S. no. | Accession no. | Collection sites | Latitude | Longitude | Morphological features |
|---|---|---|---|---|---|
| 1. | Acc no 1 | CBE - 2 | 10.9974° N | 76.9589° E | White flowers with unifoliate opposite leaves |
| 2. | Acc no 3 | Thangachimadam | 9.2829° N | 79.2446° E | White flowers with unifoliate opposite leaves |
| 3. | Acc no 4 | MTP - 1 | 10.9041° N | 76.9983° E | White flowers with unifoliate opposite leaves |
| 4. | Acc no 5 | Madurai Malli | 9.9252° N | 78.1198° E | White flowers with unifoliate opposite leaves |
| 5. | Acc no 7 | Adukkumalli (CBE) | 10.9974° N | 76.9589° E | White flowers with unifoliate opposite leaves |
| 6. | Acc no 8 | Adukumalli (PKM) | 10.1239° N | 77.5475° E | White flowers with unifoliate opposite leaves |
| 7. | Acc no 9 | Karamadai | 11.2428° N | 76.9587° E | White flowers with unifoliate opposite leaves |
| 8. | Acc no 10 | Iruvatchi (MDU) | 9.9252° N | 78.1198° E | White flowers with unifoliate opposite leaves |
| 9. | Acc no 11 | Double Mohra | 11.3028° N | 76.9383° E | White flowers with unifoliate opposite leaves |
| 10. | Acc no 12 | Star-flowered type | 9.3639° N | 78.8395° E | White flowers with unifoliate opposite leaves |
| 11. | Acc no 13 | Rajamalli | 9.35° N | 77.56° E | White flowers with unifoliate opposite leaves |
| 12. | Acc no 16 | Dindgul | 10.3620° N | 77.9736° E | White flowers with unifoliate opposite leaves |
| 13. | Acc no 18 | *J.sambac* Unknown 1 | 11.5034° N | 77.2444° E | White flowers with unifoliate opposite leaves |
| 14. | Acc no 19 | Kanyakumari - 1 | 8.0844° N | 77.5495° E | White flowers with unifoliate opposite leaves |
| 15. | Acc no 21 | PMK - 1 | 10.1239° N | 77.5475° E | White flowers with unifoliate opposite leaves |
| 16. | Acc no 23 | Satyamangalam | 11.5034° N | 77.2444° E | White flowers with unifoliate opposite leaves |
| 17. | Acc no 25 | MTP - 2 | 11.3028° N | 76.9383° E | White flowers with unifoliate opposite leaves |
| 18. | Acc no 26 | Kanyakumari - 2 | 8.0844° N | 77.5495° E | White flowers with unifoliate opposite leaves |
| 19. | Acc no 27 | Nagercoil | 8.1833° N | 77.4119° E | White flowers with unifoliate opposite leaves |
| 20. | Acc no 28 | Kanyakumari - 3 | 8.0844° N | 77.5495° E | White flowers with unifoliate opposite leaves |
| 21. | Acc no 30 | Sangagiri | 11.4745° N, | 77.8691° E | White flowers with unifoliate opposite leaves |
| 22. | Acc no 31 | Thovalai | 8.2312° N | 77.5060° E | White flowers with unifoliate opposite leaves |
| 23. | Acc no 32 | Double Mohra (PMK) | 10.1239° N | 77.5475° E | White flowers with unifoliate opposite leaves |
| 24. | Acc no 33 | Mandapam Gundumalli | 9.2886° N | 79.1329° E | White flowers with unifoliate opposite leaves |
| 25. | Acc no 34 | *J.sambac* Unknown 3 | 9.2770° N | 79.1252° E | White flowers with unifoliate opposite leaves |
| 26. | Acc no 35 | CBE - 1 | 10.9974° N | 76.9589° E | White flowers with unifoliate opposite leaves |
| 27. | Acc no 36 | PMK - 2 | 10.1239° N | 77.5475° E | White flowers with unifoliate opposite leaves |


Geno -1 CBE 2


Geno -3 Thangachimadam


Geno -4 MTP-1


Geno -5 Madurai malli


Geno -7 Adukumalli (CBE)


Geno -8 Adukumalli (PKM)


Geno -9 Karamadai


Geno -10 Iruvatchi (MDU)


Geno -11 Double Mohra


Geno 12- Star flowered Jasmine


Geno -13 Rajamalli


Geno -16 Dindgul


Geno -18 *J. sambac* unknown 1


Geno -19 Kanyakumari-1


Geno -21 PKM 1


Geno -23 Sathya Mangalam


Geno -25 MTAP -2


Geno-26 Kanyakumari -2


Geno -31 Thovalai


Geno -32 Double mohra


Geno33 Mandapam gundumalli


Geno-34 *J.sambac* unknown-3


Geno -35 CBE-1

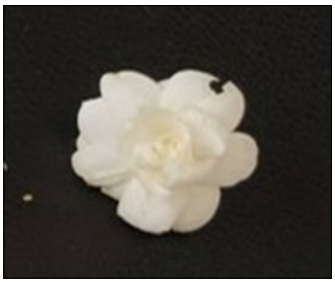
Geno -36 PKM 2

**Fig. 1.** Floral morphological traits of different accessions.

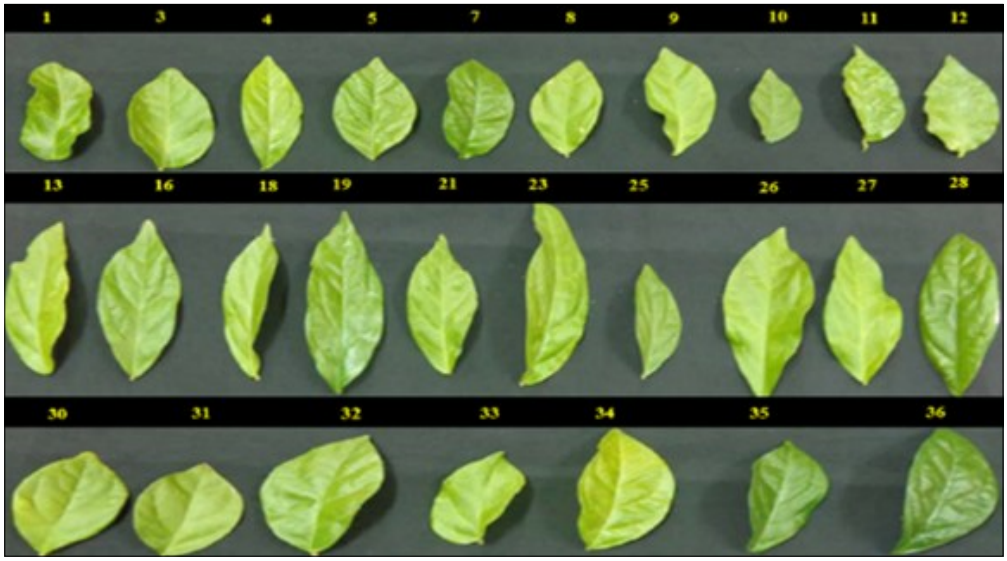


**Fig. 2.** Morphological traits of leaves of different accessions.

**Table 2.** Details of 12 primers that were screened to discriminate the 27 genotypes of *J. sambac* (13)

| S. No | Primer | Forward Sequence (5'-3') | Reverse Sequence (3'-5') | Repeat motif |
|---|---|---|---|---|
| 1 | JS021 | F:GAAGAGGGAACTACCCGC | R:ATGAGAGCAAAGAGGGGA | $(GT)_6$ |
| 2 | JS030 | F:AACTCCGTGTACTCCGCAG | R:GCGAAAATCAAACTGCCAC | $(GA)_7$ |
| 3 | JS033 | F:GAAATCTGATGCTGCAAA | R:AAAGAGTTCATCCATTCGG | $(AT)_7$ |
| 4 | JS035 | F:GACTTTGCGAGGGAAAAG | R:CCAACCCTTCGACTCCTACA | $(TGG)_6$ |
| 5 | JS040 | F:GTAGATTCGGCGTTACTCG | R:CTTTTCTTCATAGCCCGACG | $(AAG)_6$ |
| 6 | JS041 | F:GGAATTGTGGATGGCTCT | R:TGAGAGTTGGATGGGCTTTT | $(CAAA)_5$ |
| 7 | JS042 | F:AGAAATTTTTCCGGCTACG | R:CCCATGACTAACCCGGTAA | $(CCG)_5$ |
| 8 | JS055 | F:TGTTGCTCCTTCACATCAC | R:GCCCCATCGTAGGGTAAAT | $(CTC)_6$ |
| 9 | JS064 | F:TCAACGCCTTAAATTGCTG | R:CCACAAACCTTCGAGGAGC | $(CT)_6$ |
| 10 | JS076 | F:TGTAACGCGAAACGGAAT | R:ACCAACCACGGTGTTTCTTC | $(TGG)_6$ |
| 11 | JS079 | F: CAAGAAAATTGACCCCAG | R:GACTTGGTCGCCATTGTTTT | $(TA)_7$ |
| 12 | JSO85 | F:CCATGGACAAACATTGTA | R:TTTCAAAGAGCGGAACCAT | $(AAC)_5(AGC)_5$ |

**Table 3.** Composition of PCR reaction mixture for SSR amplification

| PCR components | Volume for one reaction (µL) |
|---|---|
| Taq buffer with $Mgcl_2$ | 1.5 µL |
| dNTP's | 0.5 µL |
| Primer | 2.0 µL |
| Template DNA (20-30 ng/100 µL) | 2.0 µL |
| Taq DNA polymerase | 0.2 µL |
| Sterile distilled water | 3.8 µL |
| Total reaction volume(µL) | 10 µL |

**Table 4.** Thermal cycling conditions for PCR amplification

| Sl. No. | Step | Temperature (°C) | Time | No. of cycles |
|---|---|---|---|---|
| 1. | Initial denaturation | 94 | 3-5 min | |
| 2. | Final denaturation | 94 | 30-60 sec | |
| 3. | Primer annealing | 55 | 30-60 sec | 35 |
| 4. | Initial extension | 72 | 6 sec | |
| 5. | Final extension | 72 | 5-10 min | |

Genetic similarity coefficients were computed using Jaccard's similarity index and a UPGMA dendrogram was constructed to infer genetic relationships among genotypes. Principal Co-ordinate Analysis (PCoA) was performed to assess the spatial distribution of accessions and PIC values were calculated to evaluate the informativeness of each SSR marker. For morphological traits, Analysis of Variance (ANOVA) was carried out to determine significant differences among genotypes and mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at 5 % significance level. All statistical analyses were performed using SPSS, NTSYSpc and GenAlEx software packages.

## Results

### SSR marker amplification and polymorphism

A total of 27 genotypes of multi-whorled *J. sambac* genotypes were characterized using 12 SSR primers. The markers exhibited distinct and scorable banding patterns and polymorphism. This led to the identification of 24 polymorphic alleles with two alleles amplified per primer resulting in an average of 2 alleles per locus. All SSR markers showed polymorphisms among the tested genotypes. The distinctive polymorphism has been found in the JS035, JS042, JS055 and JS079 as represented in Fig. 3. The allele size of the markers ranged from 142-275 bp.

### Heterozygosity analysis

The observed heterozygosity ($H_0$) ranged from 0.30 to 0.49 with a mean of 0.2. The observed heterozygosity ($H_0$) and expected heterozygosity ($H_e$) were calculated by using the GenAlEx v.6.5 program based on Microsoft Excel Office (16). The effective number of alleles ($N_e$) ranges from 1.4 (JS 055) to 1.9 (JS 021, 035 and JS040) with an average of 1.7. The values from the study indicate a moderately balanced allele frequency distribution across loci. Expected heterozygosity ($H_e$) reflects the probability of two randomly selected alleles at a locus being different ranged between 0.3 to 0.4 with a mean value of 0.4.

### Polymorphism Information Content (PIC)

Power Marker software version 3.25 was used in this study to calculate the PIC values for each SSR marker (17). Similarly, PIC values ranged from 0.3 to 0.4 with a mean of 0.4 (Table 5). The highest PIC values (0.4) were observed for primers JS021, JS035 and JS040 indicating their marker efficiency.

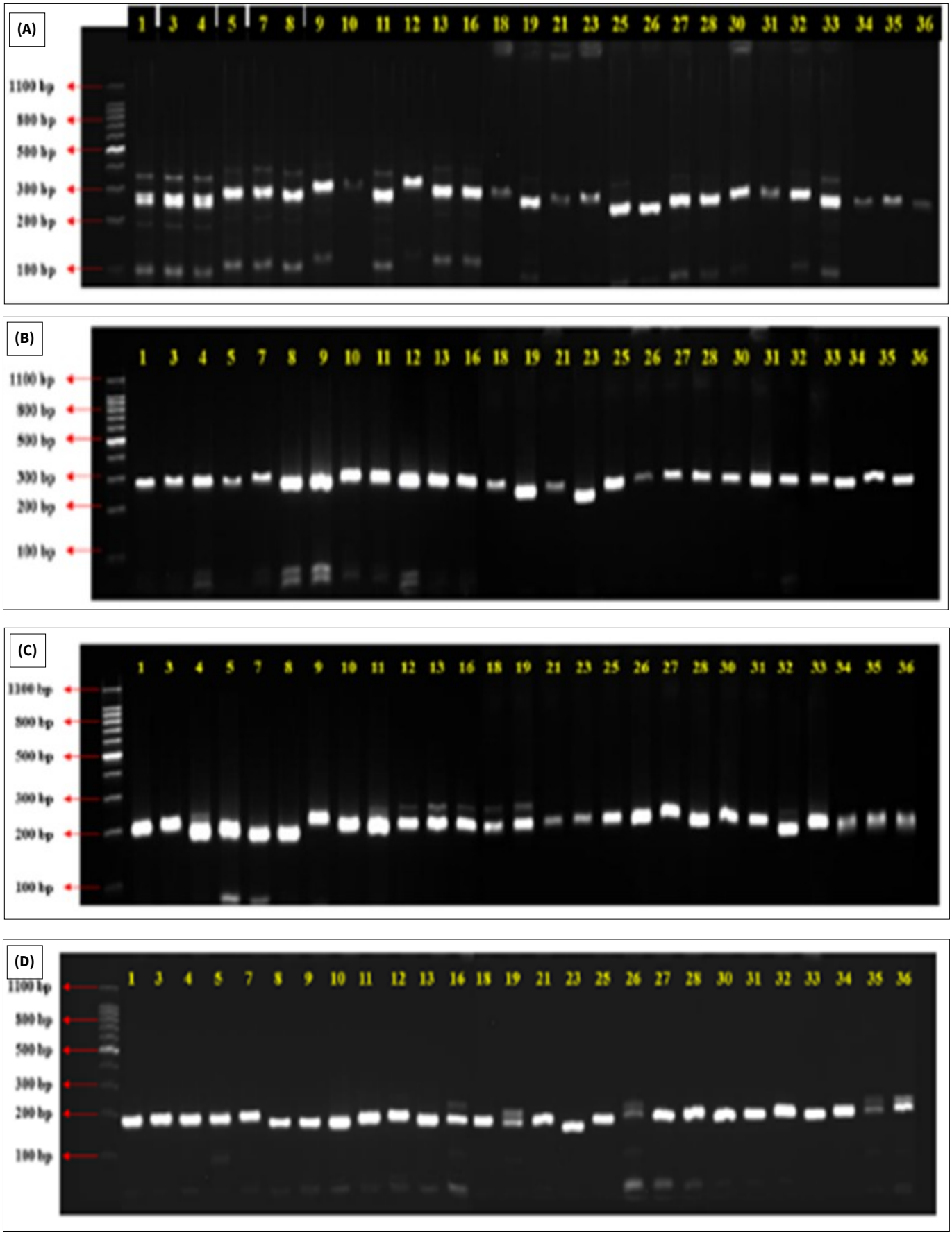


**Fig. 3.** PCR amplification of SSR primers.

(A) Primer JS035; (B) Primer JS042; (C) Primer JS055; (D) Primer JS079

1-Geno.1;2-Geno.3;3-Geno.4;4-Geno.5;5-Geno.7;6- Geno.8;7- Geno.9;8-Geno.10; 9-Geno.11; 10-Geno.12; 11- Geno.13; 12- Geno.16; 13- Geno.18; 14-Geno.19; 15- Geno.21; 16- Geno.23; 17-Geno.25; 18- Geno.26; 19-Geno.27; 20-Geno.28; 21-Geno.30; 22-Geno.31; 23-Geno.32; 24- Geno.33; 25-Geno.34; 26- Geno.35; 27-Geno.36

**Table 5.** Summary of microsatellite allele data, including number of alleles, major allele frequency, observed heterozygosity, polymorphism information content and number of effective alleles

| S. No | Primer | Product size (bp) | N | $M_{AF}$ | $H_O$ | PIC | $N_e$ | I |
|---|---|---|---|---|---|---|---|---|
| 1 | JS021 | 275-275 | 2 | 0.55 | 0.49 | 0.4 | 1.9 | 0.68 |
| 2 | JS030 | 245-251 | 2 | 0.62 | 0.46 | 0.4 | 1.8 | 0.65 |
| 3 | JS033 | 256-262 | 2 | 0.62 | 0.46 | 0.4 | 1.8 | 0.65 |
| 4 | JS035 | 252-254 | 2 | 0.5 | 0.49 | 0.4 | 1.9 | 0.68 |
| 5 | JS040 | 204-213 | 2 | 0.5 | 0.49 | 0.4 | 1.9 | 0.68 |
| 6 | JS041 | 136-142 | 2 | 0.6 | 0.44 | 0.4 | 1.8 | 0.63 |
| 7 | JS042 | 212-218 | 2 | 0.6 | 0.46 | 0.4 | 1.8 | 0.65 |
| 8 | JS055 | 171-180 | 2 | 0.8 | 0.30 | 0.3 | 1.4 | 0.47 |
| 9 | JS064 | 275-271 | 2 | 0.6 | 0.44 | 0.4 | 1.8 | 0.63 |
| 10 | JS076 | 163-172 | 2 | 0.7 | 0.38 | 0.3 | 1.6 | 0.57 |
| 11 | JS079 | 157-163 | 2 | 0.6 | 0.44 | 0.4 | 1.8 | 0.63 |
| 12 | JSO85 | 204-207 | 2 | 0.7 | 0.41 | 0.4 | 1.7 | 0.60 |

N = No. of alleles; $M_{AF=}$ Maximum Allele Frequency; $H_O$= Observed Heterozygosity; PIC= Polymorphism Information Content; Ne= Number of Effective Alleles; I = Shannon index

## Effective number of alleles (Ne)

The effective number of alleles (Ne) ranged from 1.432 (JS055) to 1.975 (JS021, JS035 and JS040) with an average of 1.808. These values indicate a moderately balanced allele frequency distribution across loci.

## Marker informativeness

None of the markers surpassed the 0.50 value generally recognised as the standard for high informativeness, moderately informative. This confirms that the selected SSR markers are effective for genotype discrimination, fingerprinting and diversity analysis. These results align with previous studies on SSR efficiency in ornamental and aromatic crops (18-20). The major allele frequency ($M_{AF}$) ranged from 0.5 to 0.8 with an average of 0.55 per locus (Table 2).

## Analysis of genetic relationships between the genotypes

Cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Jaccard's dissimilarity coefficient calculated based on a previous study (21). The genetic relationships of *J. sambac* genotypes were evaluated. Jaccard's dissimilarity coefficient for all the tested SSR markers ranged from 0.0 to 0.8 (Fig. 4). In cluster I, grouped with five genotypes comprising Acc 10, Acc 11, Acc 13, Acc 31 and Acc 34. Cluster II emerged as the largest including Acc 1, Acc 3, Acc 4, Acc 5, Acc 7, Acc 9, Acc 12, Acc 16, Acc 18, Acc 19, Acc 21, Acc 23, Acc *27*, Acc 30 and Acc 35. Cluster III consisted of Acc 25, Acc 26, Acc 27, Acc 32, Acc 33 and Acc 36. Cluster IV was represented by a single genetically distinct Acc 8 appearing unique from all other clusters. PCoA based on Jaccard's dissimilarity matrix effectively revealed the underlying genetic structure among the *J. sambac* genotypes. The distribution of genotypes across the two major coordinate axes illustrated clear differentiation into four genetic clusters (Fig. 5).

## AMOVA analysis of population

The distribution of genetic variation among the tested genotypes is presented in Table 6. According to the results of the AMOVA, 96 % of the total genetic variation occurred within the population and 4 % of the total genetic variation occurred between the populations (Table 6). The highest genetic variation was realised between individuals within in population and the least genetic variation was realised between the populations.

# Discussion

## Morphological implications inferred using SSR markers

Neighbor-joining analysis from SSR markers resulted in the grouping of 27 accessions into four major clusters. The 1st cluster (4) consists of a single accession that is Acc 8. This accession is characterized by pinkish, multi-whorled flowers. The 2nd major cluster (1) consists of 5 accessions with the same flower morphological trait with unifoliate opposite leaves. The 3rd cluster (2) consists of 15 genotypes with the same morphological and unifoliate leaves. The success of crop improvement programs depends on the extent of genetic variability existing in the germplasm or population. Greater genetic variation allows plant breeders to improve crops more quickly and effectively by selecting and crossing them by choosing the best population.

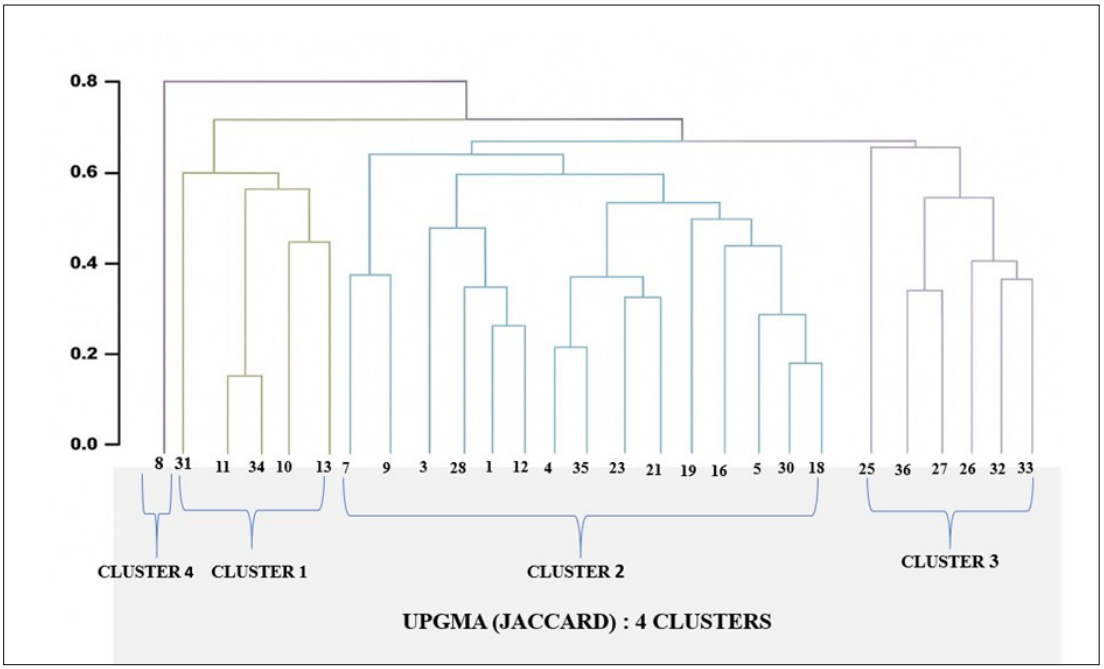


**Fig. 4.** UPGMA dendrogram of the Jaccard dissimilarity matrix representing the genetic relationships among the 27 *Jasminum sambac* genotypes indicated.

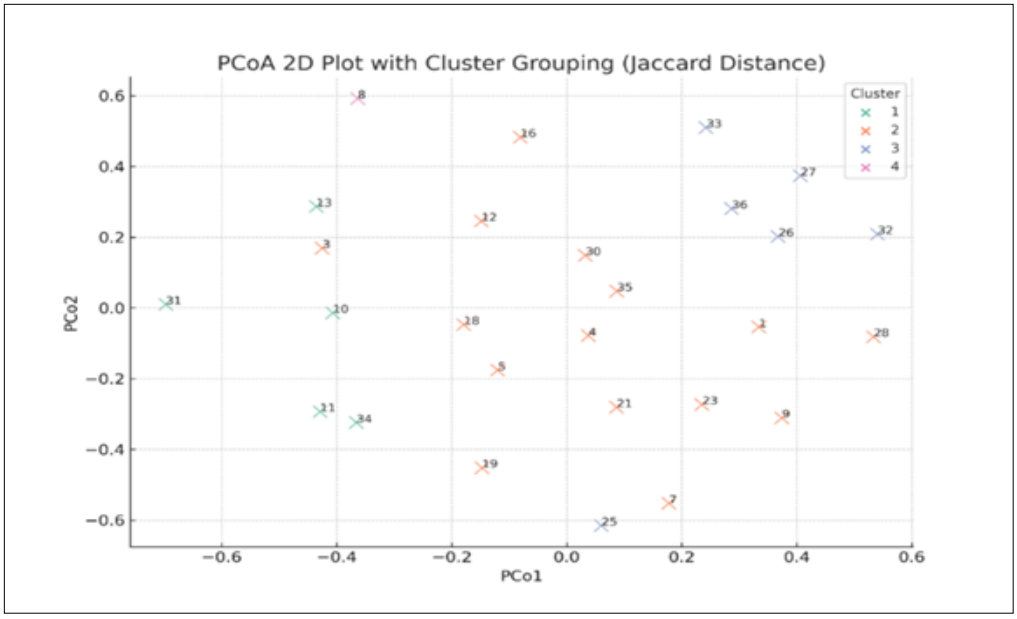


**Fig. 5.** SSR- data based PCoA of the genetic distance matrix in the studied *Jasminum sambac* accessions.

**Table 6.** Molecular Analysis of Variance (AMOVA) of *Jasminum sambac*

| Source | Degrees of freedom (df) | Total square (SS) | Mean square (MS) | Variance (%) |
|---|---|---|---|---|
| Among pop | 1 | 4.036 | 4.036 | 4 % |
| Within pop | 25 | 67.742 | 2.710 | 96 % |
| Total | 26 | 71.778 | - | 100 % |

## Polymorphism degree of SSR markers in *Jasminum sambac* accessions

SSR markers also known as microsatellites act as genetic fingerprints made of short repeating DNA sequences. SSR markers developed for one species often work well in closely related species because the DNA sequences adjacent to SSR regions remain conserved. This allows primers designed for one organism to reliably amplify equivalent loci in closely related species especially within the same genus or family (22). Numerous studies have applied molecular markers such as RAPD, AFLP, ISSR and SRAP to examine genetic diversity and relationships within *Jasminum* species (23-25).

In this study 27 genotypes were evaluated using 12 SSR markers producing a total of 24 alleles with an average of two alleles per locus. All 12 markers showed polymorphism with primers JS035, JS042, JS055 and JS079 displaying the highest level of polymorphic amplification. A maximum of two alleles per locus was observed for all SSR markers. Gene diversity among all genotypes ranged from 0.30 to 0.40 while allelic frequencies ranged from 0.80 for SSR marker JS055 to 0.45 for SSR marker JS021 averaging 0.60 across all markers.

Moderate PIC values (0.30-0.40) show that SSR markers can distinguish genotypes to some extent, but the overall allelic diversity remains limited reflecting the clonal propagation and narrow genetic base of *J. sambac*. In contrast, a study on 148 rose genotypes including both wild and cultivated varieties reported SSR PIC values ranging from 0.08 to 0.80 with a mean of 0.50 ± 0.20 indicating moderate-to-high diversity. Similarly, chrysanthemums showed PIC values of around 0.52 (26) while roses reported values between 0.50 and 0.56 (27). Compared to these ornamentals the SSR markers in this *J. sambac* panel reveal only moderate diversity highlighting its relatively narrow genetic foundation.

In population genetics, allele frequency reflects genetic diversity at the individual, population or species level. Changes in allelic frequencies over time can indicate genetic drift or the introduction of new mutations (28,29). Another measure of diversity is the PIC of markers. In this study, the average PIC for all SSR markers was 0.42 suggesting only moderate genetic diversity among the *J. sambac* genotype. Highly polymorphic SSR markers are preferred for diversity studies and molecular characterization, as they better reveal variations within germplasm and among individual genotypes (29). The average expected heterozygosity (He) was 0.38 while the average observed heterozygosity was slightly lower. For comparison, previous studies reported Ho and He values ranging from 0.35 to 0.86 and 0.54 to 0.80 respectively, with an average of 0.64 and 0.66 (10).

The use of SSR markers remains a highly effective approach for identifying cultivars and genotypes particularly in studies that focused on genetic diversity, molecular characterization and early-stage selection in plant breeding programs (30). However, the number of SSR markers available for the Oleaceae family is limited leaving fewer options for selecting highly polymorphic markers. Since most SSRs were not developed from a broad genetic base their polymorphism levels cannot always be predicted with certainty.

## Cluster analysis among *J. sambac* accessions

UPGMA is a clustering method used to group genotypes based on genetic similarity producing a dendrogram that reflects their phylogenetic relationships under a relatively uniform rate of genetic change (31). In this study four distinct clusters were identified. Cluster I revealed a high degree of genetic uniformity suggesting limited variability while cluster II being the largest exhibited the highest genetic diversity within the population. Cluster III showed moderate variation with genotypes 32 and 33 forming a unique subset. Cluster IV contained only Acc 8, with pinkish multi petal flowers and minimal similarity (0.20) to other genotypes. This indicates it carries rare or divergent alleles making it a valuable resource for hybridization, genetic improvement and conservation.

PCoA confirmed these findings by simplifying SSR-derived genetic distances into a reduced-dimensional space (32). Fig. 5 shows clear cluster separation and the outlier status of Acc 8, confirming genetic diversity and analysis effectiveness.

AMOVA revealed that most genetic variation exists within populations showing that multi-petaled *J. sambac* genotypes remain genetically similar despite their diverse geographic origins. Other studies related to *Jasminum* genotypes from Pakistan showed 79 % of variation among populations and 21 % within populations (23). In contrast, wild olive trees showed 43 % among populations and 57 % within indicating species-specific genetic structuring (12). This finding suggests hypothesis of a narrow genetic base resulting from clonal propagation and limited sexual reproduction. Similar trends are seen in roses and chrysanthemums where most variation is within cultivars emphasizing the role of SSR markers in breeding and conservation of the germplasm (27,33).

## Conclusion

This study demonstrated the potential of SSR markers for the molecular characterization of *J. sambac* genotypes at Tamil Nadu Agricultural University, Coimbatore. The application of 12 primers across 27 genotypes revealed notable polymorphism with JS035, JS042, JS055 and JS079 identified as the most informative markers. However, the overall level of variation detected was limited indicating the need for broader and more powerful molecular tools. Future research on *J. sambac* should therefore incorporate a wider set of highly polymorphic SSRs including recently developed genic SSRs (eSSRs) as well as high-density markers such as SNPs which have proven effective in ornamentals like roses. Expanding the germplasm collection to include material from diverse geographic regions beyond clonal varieties will not only provide deeper insights into allelic variation but also enhance the identification of rare genetic traits. Such efforts will be crucial in guiding effective breeding and conservation strategies within the Oleaceae family ultimately supporting the development of improved flower varieties with superior qualities and greater resilience to environmental stress.

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## Authors' contributions

The investigation and original draft preparation were carried out by PSG. Conceptualization was performed by STBS and methodology was developed by MG. Funding acquisition was managed by AG. Resources were provided by ATH and KD. Software, validation and formal analysis were handled by DLN and VK. Writing, review and editing were contributed by PSG and ATH. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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