

Analysis of Genetic Diversity among *Jasminum sambac* (Linn.) Ait. and *J. grandiflorum* Linn. Varieties using Morphological and Molecular Markers

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ABSTRACT

In the present study, genetic relationships among eight varieties of *Jasminum sambac* (Linn.) Ait. and two varieties of *J. grandiflorum* Linn., collected from Southern India were compared by their morphological characters and RAPD (Randomly Amplified Polymorphic DNA) profiles. The morphological data was obtained for their vegetative and reproductive characters. PCR-amplifiable DNA was isolated using the CTAB method and 120 amplified fragments were obtained using 8 random primers. The genetic dissimilarity matrix was calculated based on Squared Euclidian Distances, which revealed a maximum genetic distance of 83% between vars. 'Co-2 Pitchi' and 'Single Mohra', which belong to different species and the minimum genetic distance (21%) was between vars. 'Khoya' and 'Khoya Large' belonging to same species (*J. sambac*). The Ward's method of cluster analysis grouped all the individuals on the dendrogram into two major clusters 'A' and 'B' at 58 linkage distances with varieties of *J. sambac* and *J. grandiflorum*, respectively. Cluster 'A' consisted of two varieties ('Co-1 Pitchi' and 'Co-2 Pitchi') clustered at 33 linkage distances with character differing in corolla tube length and shape, size and number of petal lobes. Cluster 'B' was segregated into two sub-clusters 'B₁' and 'B₂' at 45 linkage distance. Sub-cluster 'B₁' was further divided into two minor clusters at 39 linkage distance with one and six varieties respectively. Sub-cluster 'B₂' with one var. 'Ramanathapuram Mallige' was characterised by short plants. The present study showed moderate to high genetic diversity among the both *Jasminum* spp. RAPD markers combined with morphological analysis proved to be a quick, simple and significant testing method to assess genetic diversity among *Jasminum* spp.

Keywords: cluster analysis, *Jasminum sambac*, *J. grandiflorum*, jasmine, RAPD-PCR, STATISTICA

INTRODUCTION

Jasminum sp., from the olive family (Oleaceae), is a native of tropical and subtropical regions and is one of the oldest traditional crops cultivated for its flowers in India. It is grown commercially in India, Thailand, China, Sri Lanka and the Philippines for its fresh flowers, which are also used to make perfume and as flavouring for tea (Inoue *et al.* 2003). For the past many centuries they adorned the garlands of Central Asia, Afghanistan, Iran, Nepal and many other tropical and sub-tropical countries (Mukundan 2000). The genus *Jasminum* contains more than 200 different species and is mostly tropical in distribution. However, a few species can stand a limited amount of frost. In China the flowers of *J. sambac* are used for flavouring tea, which is well known as fragrant jasmine tea (Ito *et al.* 2002; Inoue *et al.* 2003). Jasmine oil extracted from the flowers is regarded as unique as it blends well with other floral extracts, hence, highly valued throughout the world for producing hair oil, high grade perfumes, used in cosmetics and soap industries, and in flavouring mouth washes (Ramdas *et al.* 1993).

Jasmine grows well in rich loam, or sandy soils with pH 6.0–6.5. Good drainage is essential because water logging kills almost all species of jasmine and moderate irrigation (about 1.5 cm per week) is required (Leonhardt and Teves 2002). Mass propagation for commercial purposes is more feasible using cutting mature stem 7-10 cm (1-2 years old) and inducing rooting with 0.3% naphthyleneacetic acid or indole-3-butyric acid (Panda 2004). Temperature plays an important role in flowering among jasmine. Long days and hot weather favour good flower production with large

flowers. Under shade, the plant does not grow as well and produces fewer flowers (Misra *et al.* 2000). Daytime temperatures of 27-32°C and night time temperatures of 21-27°C are ideal conditions for flowering. If the night temperature decreases below 17°C flowering can be delayed for 1-2 weeks (Leonhardt and Teves 2002). Pruning is essential and usually done between November and January to induce lateral branching and thereby promote flowering (Misra *et al.* 2000; Farooqi and Sreeramu 2001; Panda 2004).

The commercial yields of flowers range widely and depend on many factors, including plant age, planting density, nutrition, irrigation, crop management practices, pest and disease management, and the weather. Farm yield data range from 8000 to 60,000 flower buds harvested per acre per day during peak periods. Jasmine cultivations are affected by a number of diseases such as blight (*Sclerotium rolfsii*), root rot (*Pythium* and *Rhizoctonia* sp.), powdery mildew and infectious chlorosis. The flowers are harvested in the morning while the flowers contain the maximum amount of perfume and could be stored for 4-6 days without loss of fragrance or quality in plastic bag at 4.4-7.2°C (Misra *et al.* 2000; Panda 2005).

The amount of genetic variation within and among populations is strongly influenced by the nature of propagation of the species (Hamrick and Godt 1990). Since, *Jasminum* spp. are vegetatively propagated the estimation of genetic diversity and cultivar identification using phenotypic markers has several limitations, especially in perennial crops (Simon *et al.* 2007). Molecular differences, using DNA and protein-based markers, are more authentic and unaffected by environmental factors (Dhanraj *et al.* 2002).

Hence, the characterisation of genotypes at the genetic level provides the first step towards more efficient conservation, maintenance and utilisation of existing genetic diversity (Prakash *et al.* 2002). Among DNA-based molecular markers, Random Amplified Polymorphic DNAs (RAPDs) provide an excellent tool to study genetic diversity and genetic relationships (Williams *et al.* 1990). RAPD markers have been used successfully to study genetic diversity and relatedness among flowering crops such as *Dianthus caryophyllus* (Scovel *et al.* 1998), *Chrysanthemum* (Huang *et al.*

2000; Lema-Rumińska *et al.* 2004) and *Anthurium andraeanum* (Nowbuth *et al.* 2005). In the present study, morphological and RAPD markers were used to estimate genetic diversity and assess relationships among 10 varieties of jasmine (*Jasminum sambac* and *J. grandiflorum*) collected from different parts of Southern India.

Table 1a Morphological data among varieties of *Jasminum sambac*.

Characters	Ramana-thapuram Mallige	Bale Japani	Single Mohra	Double Mohra	Khoya	Khoya Large	Iruvantige	Butt Mohra
Habit	Erect semi-spreading shrub	Erect semi-spreading shrub	Erect semi-spreading shrub	Erect semi-spreading shrub	Erect semi-spreading shrub	Erect semi-spreading shrub	Erect shrub	Erect semi-spreading shrub
Stem	Woody	Woody	Woody	Woody	Woody	Woody	Woody	Woody
Stem shape	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical
Internode distance (cm)	2.8-4	4-6	3-6	2.8-5.2	4.2	3.3-5.5	4.3-5.8	4.1
Stem girth (cm)	0.2-0.37	0.2-0.5	0.28-0.37	0.25-0.38	0.2-0.5	0.2-0.5	0.38-0.5	0.2-0.5
Stem colour	Green	Green	Green	Green	Green	Green	Green	Green
Stem pubescence	Pubscent	Pubscent	Pubscent	Pubscent	Pubscent	Pubscent	Slightly pubescent	Pubscent
Leaf type	Simple	Simple	Simple	Simple	Simple	Simple	Simple	Simple
Stipule	Exstipulate	Exstipulate	Exstipulate	Exstipulate	Exstipulate	Exstipulate	Exstipulate	Exstipulate
Leaf arrangement	Whorls of three	Whorls of three	Whorls of three	Whorls of three-four	Whorls of three	Whorls of three	Whorls of three	Whorls of three
Leaf shape	Ovate-acute	Ovate	Ovate-acute	Ovate-acute	Ovate-acute	Ovate-elliptic	Elliptic	Ovate-acute
Leaf margin	Slightly wavy	Wavy	Wavy	Wavy	Wavy	Wavy	Slightly wavy	Wavy
Leaf size (cm)	5.3-8.0 × 3.1-5.6	4.2-8.0 × 2.4-5.7	7.2-8.5 × 4.5-5.7	4.2-5.5 × 2.6-3.6	4.0-8.5 × 3.5-5.6	3.8-6.9 × 2.4-5.5	5.2-7.0 × 2.8-3.7	2.4-5.8 × 2.5-3.1
Leaf thickness (mm)	0.37	0.23-0.37	0.23	0.34	0.23-0.37	0.23-0.37	0.3	0.23-0.37
Leaf surface	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Pubscent	Glabrous
Leaf colour	Dark green	Green	Green	Green	Green	Green	Yellowish green	Green
Veins	Prominent benith	Prominent benith	Thick, prominent benith	Prominent benith	Prominent benith	Prominent benith	Thick, prominent benith	Prominent benith
Petiole (cm)	0.45	0.35-0.56	0.56	0.38	0.35-0.56	0.35-0.56	0.35	0.35-0.56
Petiole pubescence	Pubscent	Glabrous	Pubscent	Slightly pubescent	Glabrous	Pubscent	Pubscent	Pubscent
Calyx number	6-8	6-8	6-8	7	8	7	6-8	7
Calyx length (cm)	0.7-1.2	0.7-1.2	1.0-1.2	0.7-1.2	0.7	0.8	0.8	0.8
Calyx arrangement	Linear	Linear	Linear-sunulate	Linear	Linear	Linear	Linear-subulate	Linear
Calyx colour	Green	Green	Green	Green	Green	Green	Green	Green
Inflorescence type	Simple or biparous cyme	Simple	Simple or biparous cyme	Solitary or simple cyme	Solitary	Solitary	Simple or biparous cyme	Solitary
Fragrance	Strongly fragrant	Fragrant	Strongly fragrant	Fragrant	Fragrant	Fragrant	Strongly fragrant	Fragrant
Pedicle length (cm)	0.5-0.8	0.45-1.1	0.98	1.1	0.8	0.7	0.45	0.7
Pedicle pubescence	Slightly pubescent	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Pubescent	Glabrous
Corolla tube (cm)	1.0-1.5	0.7-1.2	0.9-1.2	0.7-1.5	1.1	1.4	1.2-1.3	1.6
Number of whorl	Single	Single	3-5 whorls	3-7 whorls	2-3 whorls	Single	2 whorls	Single
Number of petal lobes	8-11	8-11	22-40	29-130	20-25	7	12-15	6-7
Petal colour	White	Yellowish	White	White	White	White	White and greenish below	White
Petal shape	Broadly ovate	Ovate	Ovate	Broadly ovate	Broadly ovate	Narrowly ovate	Ovate	Ovate-elliptical
Petal size (cm)	1.4 × 1.09	1.1 × 1.42	1.13 × 0.66	1.24 × 0.86	1.1 × 1.42	1.1 × 1.42	1.42 × 0.73	1.1 × 1.42
Petal arrangement	Linear-subulate	Linear	Unfurl	Linear-subulate	Linear	Linear	Linear-subulate	Linear
Petal pubescence	Pubscent	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous
Stamen number	Two	Two	Two	Numerous	Two	Two	Two	Two
Stamen position	Petaliod	Petaliod	Petaliod	Petaliod	Petaliod	Petaliod	Petaliod	Petaliod
Anther colour	Yellow	Yellow	Yellowish white	Yellowish brown	Yellow	Yellow	Yellow	Yellow
Ovary	Bicelled	Bicelled	Malformed	Vestigial	Bicelled	Bicelled	Bicarpellary	Bicelled
Style (cm)	0.37	0.35	0.40	0.35	1.1	1.2	1.0	1.4
Stigma shape	Single and bifid	Bifid	Bifid	Many and bifid	Bifid	Bifid	Distinctly bifid	Bifid
Stigma length (cm)	0.3	0.2-0.5	0.5	0.3	0.3	0.5	0.21	0.4
Fruit	No fruit set	No fruit set	No fruit set	No fruit set	No fruit set	No fruit set	No fruit set	No fruit set

Table 1b Morphological data among varieties of *Jasminum grandiflorum*.

Characters	Co-1 Pitchi	Co-2 Pitchi
Habit (cm)	Semi-spreading shrub, 85-90	Semi-spreading shrub, 90-95
Stem	Woody	Woody
Stem shape	Angular or Grooved	Angular or Grooved
Internode distance (cm)	4-6	4-6
Stem girth (cm)	0.32-0.6	0.32-0.6
Stem colour	Green	Green
Stem pubescence	Pubescent	Pubescent
Leaf type	Pinnatipartite or pinnately comp	Pinnatipartite or pinnately comp
Stipule	Exstipulate	Exstipulate
Leaf arrangement	Whorls of three	Whorls of three
Leaf shape	Ovate-acute, acuminate	Ovate-acute, acuminate
Leaf margin	Wavy	Wavy
Leaf size (cm)	0.7-3.8 × 0.5-1.5	0.7-3.8 × 0.5-1.5
Leaf thickness (mm)	0.35	0.35
Leaf surface	Glabrous	Glabrous
Leaf colour	Green	Green
Veins	Prominent benith	Prominent benith
Petiole	Short	Short
Petiole pubescence	Glabrous	Glabrous
Calyx number	3-5	3-5
Calyx length (mm)	5-10	5-10
Calyx arrangement	Linear-subulate	Linear-subulate
Calyx colour	Green	Green
Inflorescence type	Cymes terminal or axillary	Cymes terminal or axillary
Fragrance	Fragrant	Fragrant
Pedicle length (cm)	0.5-2.5	0.5-2.5
Pedicle pubescence	Glabrous	Glabrous
Corolla tube (cm)	1.3-1.8	2.21
Number of whorl	Single	Single
Number of petal lobes	8-15	13-22
Petal colour	White	White
Petal shape	Salverform	Ovate-elliptical
Petal size (cm)	1.3-2.2	2.05 × 1.2
Petal arrangement	Linear	Linear
Petal pubescence	Glabrous	Glabrous
Stamen number	Two	Two
Stamen position	Middle of corolla tube	Middle of corolla tube
Anther colour	Yellow	Yellow
Ovary	Bicelled	Bicelled
Style (cm)	0.74	0.86
Stigma shape	Bifid	Bifid
Stigma length (cm)	0.3	0.4
Fruit	No fruit set	No fruit set

MATERIALS AND METHODS

Plant materials

Ten varieties of jasmine belonging to two commercially cultivated species were collected from Tamilnadu Agricultural University, Coimbatore, Indian Institute of Horticulture Research, Bangalore, Horticulture College & Research Institute, Periakulam and Agricultural College & Research Institute, Madurai. Eight varieties of *J. sambac* ['Ramanathapuram Mallige' (S1), 'Bale Japani' (S2), 'Single Mohra' (S3), 'Double Mohra' (S4), 'Khoya' (S5), 'Khoya Large' (S6), 'Iruvantige' (S7) and 'Butt Mohra' (S8)] and two varieties of *J. grandiflorum* ['Co-1-Pitchi' (G1) and 'Co-2-Pitchi' (G2)], were used for the analysis. Approximately 50 g of recently matured leaves (15-20 days old) were collected, washed with distilled water, wiped with 70% (v/v) ethanol, then dried in oven at 30-35°C for 20 h and powered by using a 'Remi' mixer for 45 to 60 sec and storage at room temperature in sealed plastic bags prior to DNA extraction.

Morphological characterization

The morphological data was recorded for the characters such as plant habit, stem, stem shape, internodal distance (cm), stem girth (cm), stem colour, stem pubescence, leaf type, stipule, leaf arrangement, leaf shape, leaf margin, leaf size (cm), leaf thickness (mm), leaf surface, leaf colour, veins, petiole length (cm), petiole pubescence, calyx number, calyx length (cm), calyx arrangement, calyx colour, inflorescence type, fragrance of flower, pedicel length (cm), pedicel pubescence, corolla tube length (cm), number of whorl, number of petal lobes, petal colour, petal shape, petal size (cm), petal arrangement, petal pubescence, stamen number, stamen position, anther colour, ovary shape, style length (cm), stigma shape, stigma length (cm) and fruit set. The mean average of 15 individuals of each genotype was selected for the determination of morphological characteristics (**Table 1a, 1b**).

RAPD profiling and statistical analysis

RAPD and statistical analysis were carried out as described in our previous report (Mukundan *et al.* 2007).

RESULTS AND DISCUSSION

Recently matured leaves, preferably light green in colour, of 15-20 days old plants were used to extract DNA, as mature leaves were highly fibrous and rich in polyphenols and polysaccharides that hindered the extraction of PCR quality DNA. The pre-treatment of the leaves removed dust particles and external microbial contaminations. The CTAB method for DNA extraction was found optimal to release the nucleic acid from the cell and to remove RNA and proteinaceous contamination rendering the DNA suitable for PCR amplifications. The average spectrophotometer readings for the DNA samples at 260/280 nm showed OD 1.8, which was found optimal for PCR amplifications. PCR amplification was followed by the standard protocol (Williams *et al.* 1990) with minor modifications, which produced good amplifications with 25 ng of template DNA. The amplifications using 1 unit of *Taq* DNA polymerase and 1.5 mM MgCl₂ produced intense and clear banding patterns. A primary screening of 35 RAPD primers resulted in selection of eight primers that produced clear and reproducible fragment patterns. Screening is essential to save time and cost, and to reject primers not informative for the analysis (Prakash *et al.* 2002).

A total of 120 unambiguous, readable and reproducible RAPD markers were produced using the eight primers selected. The number of bands obtained per primer varied from 10 to 19 with an average of 15 bands per primer and the size ranged from 100 bp to 3.7 kbp. Of the 120 bands, 95 (79.16%) were polymorphic and shared between at least two individuals, 2 (1.67%) was monomorphic and common to all the individuals. 23 (19.17%) were polymorphic and unique. The representative polymorphic gel profiles of primers OPG-13, OPK-19 and OPE-14 is shown in **Fig. 1**. A pair-wise genetic dissimilarity matrix (**Table 2**) was calculated using Squared Euclidian Distance (Sokal and Sneath 1973). The highest genetic dissimilarity of 83% was between vars. 'Co-2 Pitchi' (*J. grandiflorum*) and 'Single

Table 2 Genetic dissimilarity matrix of 10 jasmine varieties based on polymorphism of RAPD markers.

Co-1-Pitchi	0								
Co-2-Pitchi	33	0							
Ramanathapuram Mallige	75	62	0						
Bale Japani	71	78	52	0					
Single Mohra	74	83	65	45	0				
Double Mohra	58	63	43	49	48	0			
Khoya	72	75	57	51	28	46	0		
Khoya Large	73	74	62	56	33	51	21	0	
Iruvantige	72	77	49	51	32	42	26	23	0
Butt Mohra	75	80	48	58	45	61	35	38	27

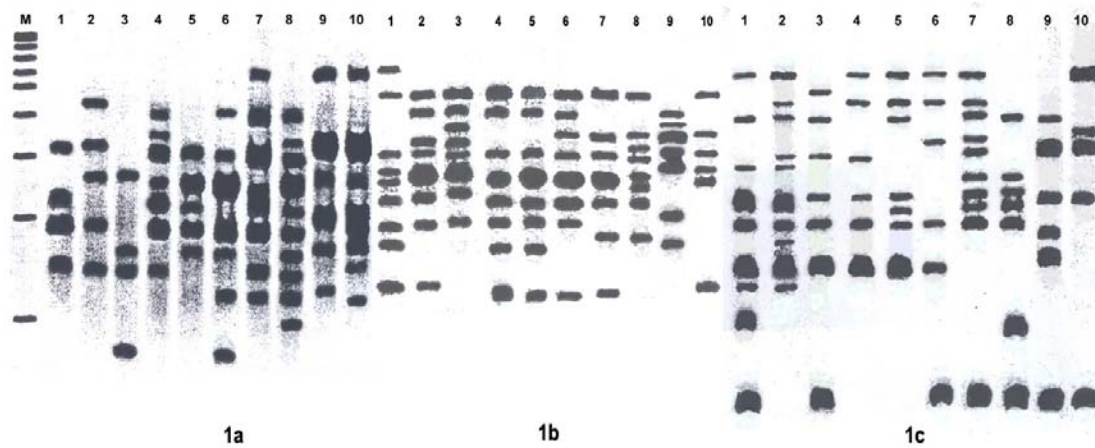


Fig. 1 Gel profile of jasmine varieties obtained using OPG-13 (1a), OPK-19 (1b) and OPE-14 (1c) primers. Lanes 1–10 contain the amplification profile obtained using vars. ‘Ramanathapuram Mallige’, ‘Bale Japoni’, ‘Single Mohra’, ‘Double Mohra’, ‘Khoya’, ‘Khoya Large’, ‘Iruvantige’, ‘Butt Mohra’, ‘Co-1-Pitchi’ and ‘Co-2-Pitchi’. Lane M: 500 bp standard DNA marker.

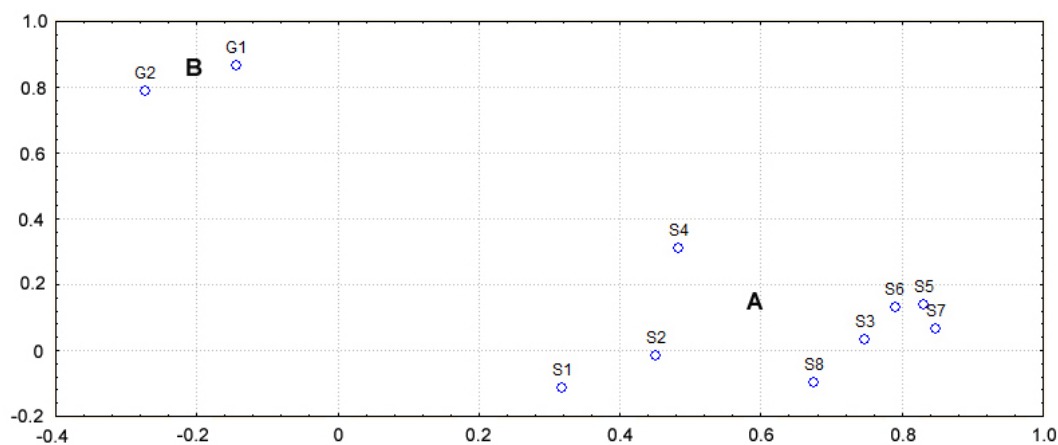


Fig. 2 Principle Component Analysis. Scattered plot showing RAPD-marker-based genetic relationships among 10 jasmine varieties and grouping them into two clusters (‘A’ and ‘B’) based on their species as *J. sambac* (S1, S2, S3, S4, S5, S6, S7 and S8) and *J. grandiflorum* (G1 and G2), respectively.

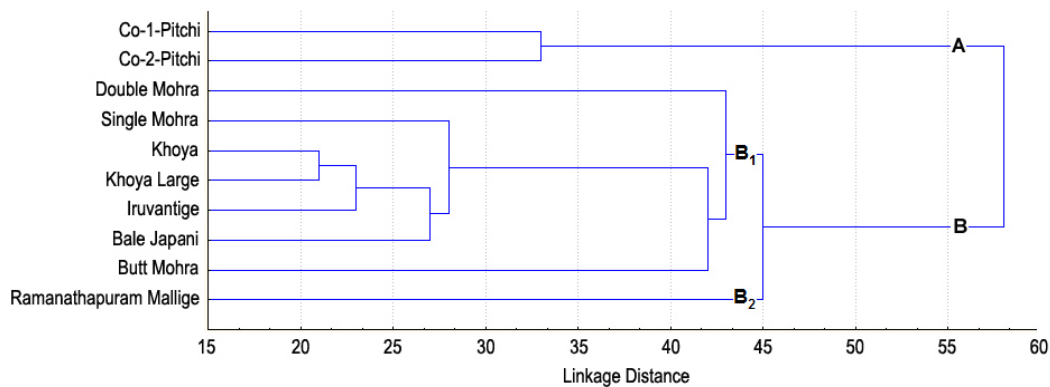


Fig. 3 Cluster analysis. Dendrogram showing RAPD-marker-based genetic relationships among 10 jasmine varieties and grouping them into two clusters ‘A’ with varieties of *J. grandiflorum* and ‘B’ with varieties of *J. sambac*.

Mohra’ (*J. sambac*), while the minimum genetic dissimilarity (23%) was between vars. ‘Khoya’ and ‘Khoya Large’ belonging to same species (*J. sambac*). Principle Component Analysis (PCA) (Fig. 2) distinctly clustered all the varieties into two groups (‘A’ and ‘B’) based on their species as *J. sambac* and *J. grandiflorum*, respectively, similar to the results obtained by Mukundan *et al.* (2007).

In the dendrogram (Fig. 3), all 10 varieties were divided into two major clusters ‘A’ and ‘B’ at 58 linkage distances. Cluster ‘A’ consisted of two varieties (‘Co-1 Pitchi’ and ‘Co-2 Pitchi’) belonging to *J. grandiflorum*, clustered at 33 linkage distances. Both varieties share similar vegetative characters but differ in their corolla tube length (1.3-1.8 and 2.21 cm), number of petal lobes (8-15 and 13-22), petal

shape (salverform and ovate-elliptical), petal size (1.3 × 2.2 and 2.05 × 1.2 cm), style length (0.74 and 0.86 cm) and stigma length (0.3 and 0.4 cm), respectively. Four polymorphic unique bands of size approximately 1020, 900, 630 and 1010 bp were observed in var. ‘CO-1-Pitchi’ with primers OPD-20, OPE-14, OPG-13 and OPK-19, respectively and two polymorphic unique bands of size approximately 780 and 1100 bp were observed in var. ‘CO-2-Pitchi’ with primers OPG-02 and OPE-14, respectively. Cluster ‘B’ consists of 8 varieties of *J. sambac* segregated into two sub-clusters (‘B₁’ and ‘B₂’) at 45 linkage distances. The clusters were differentiated by the banding patterns produced by primers OPA-09, OPA-12, OPE-14 and OPK-07. Sub-cluster ‘B₁’ consisted of seven varieties segregated into two groups

(I and II) at 43 linkage distances. Group I with one variety ('Ramanathapuram Mallige') was characterised by strongly fragrant flowers and distinguished by a long corolla tube and a broad petal shape. Four polymorphic unique bands of approximately 1700 and 1100 bp in size by primer OPA-09, 750 bp by primer OPG-02 and 3000 bp by primer OPK-19 were observed.

Group II with six varieties clustered at 42 linkage distance and var. 'Double Mohra' stood as a separate entity. Var. 'Double Mohra' was characterised by flowers with petals in three to seven whorls and 29 to 130 in number. The stamens were numerous with yellowish-brown anthers. The ovary was vestigial with many styles. Vars. 'Khoya' and 'Khoya large' were closely related among the group at 21 linkage distances. Both varieties share similar morphological features except for calyx number (7 and 8), corolla tube length (1.1 and 1.4 cm), number of whorls (2-3 and single), number of petals (20-25 and seven) and petal shape (broadly and narrowly ovate). Both varieties are closely related to var. 'Iruvantige' at 23 linkage distances which was distinguished by flowers with a shorter pedicle (0.45 cm) and distinct greenish coloured petals in two whorls numbering 12-15. A polymorphic unique band approximately 490 bp in size was produced in var. 'Iruvantige' with primer OPG-13. Var. 'Butt Mohra' was characterised by flowers with the longest corolla tube (1.6 cm) and style (1.4 cm), while var. 'Single Mohra' was characterised by flowers with 22-40 petals in 3-5 whorls and a malformed ovary which were clustered in group II. Three polymorphic unique bands were produced in var. 'Butt Mohra' by primers OPD-20, OPK-19 and OPG-13, and three polymorphic unique bands were produced in var. 'Single Mohra' by primers OPA-12, OPE-14 and OPK-19. Four polymorphic bands specific to group II were produced by primers OPA-12, OPG-02 and OPK-13. Sub-cluster 'B₂' consists of one var. 'Bale Japani' distinguished by fragrant yellowish-white flowers. Two polymorphic unique bands approximately 880 and 2150 bp in size were produced in var. 'Bale Japani' by primers OPE-14 and OPG-13.

The samples in the present study were characterised based on flower morphology and showed moderate to high polymorphism. Cluster analysis helped to group the genotypes based on their species into two major clusters 'A' and 'B' at 58 linkage distances with varieties of *J. sambac* and *J. grandiflorum*, respectively. A similar clustering pattern was observed with PCA similar to the clusters obtained by Mukundan *et al.* (2007). The bands produced by primers OPA-09, OPA-12, OPE-14 and OPK-07 were able to segregate the varieties according to their species. Since, RAPDs are produced from conserved and highly repetitive sequences regions (Paran and Michelmore 1993; Haymer 1994) they are considered to be variable neutral markers. The study also effectively revealed the use of RAPD markers in estimating genetic diversity in jasmine, which could be the first step towards efficient germplasm storage management in the perennial crops where land, time, effort and money could be saved.

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