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Comparative Analysis and Phylogenetic Relationships between Populations of Commercially Important *Jasminum* sp. by Using RAPD Markers

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ABSTRACT

In the present paper, genetic relationships of 32 varieties of jasmine belonging to four species, [*Jasminum sambac* (Linn.) Ait., *J. grandiflorum* Linn., *J. auriculatum* Vahl., and *J. multiflorum* (Burm.f.) Andr.] obtained from different locations in southern India are described on the basis of RAPD (Randomly Amplified Polymorphic DNA) markers. PCR-amplifiable DNA was isolated using the CTAB method and 169 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 56% between varieties, 'Bale Japani' and 'Manamadhurai Mullai', while the minimum genetic distance (8%) was between the varieties 'Rameswaram Mallige' and 'Nilakottai Mallige', belonging to the 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 52 linkage distances. Cluster 'A' consisted of two varieties belonging to species *J. multiflorum*, clustered at 18 linkage distances. Cluster 'B' was segregated into two sub-clusters at 44 linkage distances with varieties of *J. auriculatum* in sub-cluster 'B_1', and varieties of *J. sambac* and *J. grandiflorum* in sub-cluster 'B_2' at 39 linkage distances. The present study showed low to moderate genetic diversity among the *Jasminum* sp. Thus RAPD has the potential for use in species identification and genetic relationships between taxa and species of jasmine for breeding programs.

Keywords: genetic variability, jasmine, phylogenetic relationships, RAPD analysis

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 flower 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. For the past several centuries they adorned the garlands of Central Asia, Afghanistan, Iran, Nepal and many other tropical and sub-tropical countries. India exports flowers to countries like Singapore, Malaysia, Japan, UK, USA, Eastern European and Gulf countries (Mukundan 2000). The characteristic dark green foliage, elegant star-like flowers with a rich fragrance facilitate it as a highly valued ornamental plant for home gardens and commercial cultivation as well.

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. It is a fragrant-flowered shrub, 2-3 feet wide and up to 6 feet tall, that is fairly hardy and drought resistant (Mukundan 2000). Flowers and buds are used for making garlands, bouquets, as hair adornment, welcome guests and for religious offerings. 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 (Seemanthini *et al*. 1995). In India, oil of jasmine is obtained from *J. multiflorum* and *J. sambac*, and in Southern China the flowers of this latter species are used for flavouring tea, which is well known as fragrant jasmine tea (Ito *et al*. 2002; Inoue *et al.* 2003). The flowers are also used for decorating lampshades, buildings during religious ceremonies and for scenting food, wine and drinks.

The fresh juice of the leaves are used as an application to soften corns, and the use of an oil prepared with it to be chewed by those who suffer from ulceration of the mucous membrane of the mouth (Umamaheswari *et al*. 2007). The leaves are used as a remedy for skin diseases and given internally in decoction for fevers, diarrhoea, etc. They are used in oil to exude a balsam, which is used in anointing the head for eye-complaints and strengthen vision. It is also used as a remedy in cases of insanity (Shiva *et al*. 2002: Sun *et al*. 2007; Umamaheswari *et al*. 2007). The dried leaves, soaked in water and made into a poultice, is applied to indolent ulcers. The flowers are used as an antispasmodic and for calming and sedative infusion, taken to relieve tension. Jasmine oil is considered antidepressant and relaxing. The flavanone glucosides isolated from the plant and essential oil showed antioxidant activity (Sun *et al*. 2007; Wei and Shibamoto 2007). It is used externally to soothe dry or sensitive skin. Jasmine is used extensively in India to control fertility in women. The leaves and flowers are used as a valuable lactifuge that are effectual in arresting the secretion of milk in the puerperal state in cases of threatened abscess (Abraham *et al*. 1979; Acharya 1987; Shrivastav *et al*. 1988). The decoction of the roots or infusion of the flowers has pectoral properties, and is employed in bronchitis and pulmonary catarrh, and also in asthma. The roots are said to be very poisonous but a tincture made from them is said to have very powerful sedative, anaesthetic, and vulnerary properties. The root along with the leaves is used to make an eye-lotion that is given fresh for venereal diseases (Lis-Balchin *et al*. 2002; Kulkarni and Shahida 2004).

Cultivar identification and estimation of genetic diversity using phenotypic markers have several limitations, especially in perennial crops. Molecular differences, using DNA and protein-based markers, are more authentic and unaffected by environmental factors (Dhanraj *et al*. 2002).

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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, RAPDs provide an excellent tool to study genetic diversity and genetic relationships (Williams *et al*. 1990). They are simple, versatile, relatively inexpensive, and can detect slight genetic differences and help in identifying duplicates in the population. 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; Ruminska *et al*. 2004) *Anthurium andraeanum* (Nowbuth *et al*. 2005). To the, best of our knowledge no work on molecular analysis of genetic variability of different species and varieties of jasmine has been done despite its manifold importance in tropic and subtropical regions of the world. In the present study, RAPD markers were used to estimate genetic diversity and assess relationships among 32 varieties of four species of jasmine (*Jasminum sambac*, *J. grandiflorum*, *J. auriculatum*, and *J. multiflorum*) collected from different parts of Southern India.

MATERIALS AND METHODS

Plant materials

Thirty two varieties of jasmine belonging to four commercially cultivated species were collected from Tamilnadu Agricultural University, Coimbatore, Indian Institute of Horticulture Research, Bangalore, Horticulture College and Research Institute, Periakulam, Agricultural College and Research Institute, Madurai and farmers' fields of Nilakottai in Tamilnadu, and Hoovinahadagali and Mysore in Karnataka. Eleven varieties of *J. grandiflorum* ['Co-1-Pitchi' (G1), 'Co-2-Pitchi' (G2), 'Bangalore Pitchi' (G3), 'Coimbatore Pitchi' (G4), 'Lucknow Pitchi' (G5), 'Nilakottai Pitchi' (G6), 'Thimmapuram Pitchi' (G7), 'Triploid' (G8), 'Periakulam Pitchi' (G9), 'Tenkasi white Pitchi' (G10) and 'Arka Surabhi' (G11)], eleven varieties of *J. sambac* ['Ramnathapuram Mallige' (S1), 'Bale Japani' (S2), 'Single Mohra' (S3), 'Double Mohra' (S4), 'Khoya' (S5), 'Khoya Large' (S6), 'Iruvantige' (S7), 'Butt Mohra' (S8), 'Mysore Mallige' (S9), 'Rameswaram Mallige' (S10) and 'Nilakottai Mallige'(S11)], two varieties of *J. multiflorum* ['Mysore Kakada' (M1) and 'Arka Arpan' (M2)] and eight varieties of *J. auriculatum* ['Co-1-Mullai' (A1), 'Co-1-Mullai' (A2), 'Pari Mullai' (A3), 'Manamadhurai Mullai' (A4), 'Long Point' (A5), 'Short Point' (A6), 'Nilakottai Mullai' (A7) and 'Periakulam Mullai'(A8)] were used for the analysis. Approximately 50 g of recently matured leaves (15-20 days) old were collected, washed using distilled water, wiped with 70% (v/v) ethanol, then dried in oven at 30-35ºC for 20 hours and powered by using a 'Remi' mixer for 45 to 60 seconds, prior to storage at room temperature in sealed plastic bags.

DNA extraction and purification

DNA was extracted from the dried leaf powder of jasmine by the cetyl trimethyl ammonium bromide (CTAB) method according to a modified protocol of Porebski *et al*. (1997). Half a gram of leaf powder was mixed with 20 ml extraction buffer, preheated to 65°C, which contained 100 mM Tris-Base, pH 8.0, 20 mM EDTA, pH 8.0, 1.4 M NaCl, 3% (w/v) CTAB, 2% polyvinyl pyrrolidone and 1% β-mercaptoethanol, then incubated at 65°C for 30 minutes with gentle shaking. The mixture was cooled to room temperature, 10 ml cold 24:1 (v/v) chloroform:isoamylalcohol was added, and the contents were mixed well. After centrifugation at 6000 × *g* for 20 min at 4°C, the supernatant was transferred to a fresh tube and the chloroform:isoamylalcohol step was repeated until a clear supernatant was obtained. 5 M NaCl was added to the supernatant (0.5 v/v) and mixed gently followed by the addition of 0.8 volume of cold isopropanol to precipitate the DNA. The mixture was incubated overnight at 4°C, and then centrifuged at 8,000 × *g* for 20 min. The resulting pellet was washed with 70% (v/v) ethanol, air dried, and dissolved in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). Two µg RNase (bovine pancreatic ribonuclease, Bangalore Genei, Bangalore, India) was added to each sample which was incubated for 3 h at 37°C, mixed with an equal volume of phenol and centrifuged at 6000 × *g* for 20 min at room temperature. This step was followed by a wash with an equal volume (1:1 (v/v)) of phenol:chloroform then with chloroform alone. DNA was precipitated overnight at 4°C with 0.5 vol of 5 M NaCl and 1 vol cold isopropanol, and the resulting pellet obtained after centrifugation was dissolved in TE buffer, analysed on an agarose gel and quantified using a spectrophotometer (NanoDrop Technologies, Wilmington, USA).

PCR amplification

PCR amplification followed the protocol of Williams *et al*. (1990) with minor modifications. Of the 35 primers screened using the genotype 'Triploid', 8 showing clear and distinguishable bands was selected for RAPD-PCR analysis (**Table 1**). Reproducibility of the primers was tested by repeating the PCR amplification three times under similar conditions. PCR reactions were carried out in a volume of 25 µl containing 25 ng template DNA, 150 µM each dNTP, 1.5 mM $MgCl_2$, 1 unit *Taq* DNA polymerase (Sigma Aldrich Chemicals, Bangalore, India), 5 pmol primer (OPA, OPB, OPC, OPD, OPE, OPF, OPG, OPH, OPI, OPJ and OPK series, Operon Technologies, Alameda, CA, US) in PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 9.0, 0.05% (v/v) NP40 and 0.05% (v/v) Triton X-100). Amplifications were performed in a MJ Research PTC-100 Thermocycler (Bio-Rad Laboratories, Bangalore, India), programmed for an initial denaturation at 95°C for 5 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 35°C for 1 min, primer extension at 72°C for 2 min, and a final extension at 72°C for 5 min. PCR products were resolved in a 1.2% (w/v) agarose gel, visualised and documented using an Alpha Digidoc system (Alpha Innotech, San Leandro, CA, US).

RAPD profile analysis

Amplified fragments from each primer were manually scored for their presence (1) or absence (0) and a matrix of the different RAPD phenotypes of all eight primers were assembled for statistical analysis. The sizes of the fragments were estimated using 500 bp standard DNA markers (Sigma Aldrich Chemicals, Bangalore, India), co-electrophoresised with the PCR products. A genetic dissimilarity matrix was developed using Squared Euclidean Distances, which estimates all pair-wise differences in the amplification products (Sokal and Sneath 1973) and a cluster analysis was based on Wards method using a minimum variance algorithm (Ward 1963). Principal Component Analysis (PCA) was used to make a multivariate statistical analysis of the RAPD data (Sokal and Sneath 1973).

Table 1 RAPD-PCR Primers. The sequence and level of polymorphism of primers.

S. No	Primer	Sequence	Total number of bands	Number of polymorphic shared bands	Number of polymorphic unique bands	Number of monomorphic bands
1	OPA-09	GGGTAACGCC	25	23	2	0
2	OPA-12	TCGGCGATAG	23	23	0	0
3	OPD-20	ACCCGGTCAC	29	26	3	0
4	OPE-14	TGCGGCTGAG	26	26	0	0
5	OPG-02	GGCACTGAGG	15	14	1	0
6	OPG-13	CTCTCCGCCA	21	19	2	0
7	OPK-07	AGCGAGCAAG	13	13	0	0
8	OPK-19	CACAGGCGGA	17	14	2	1

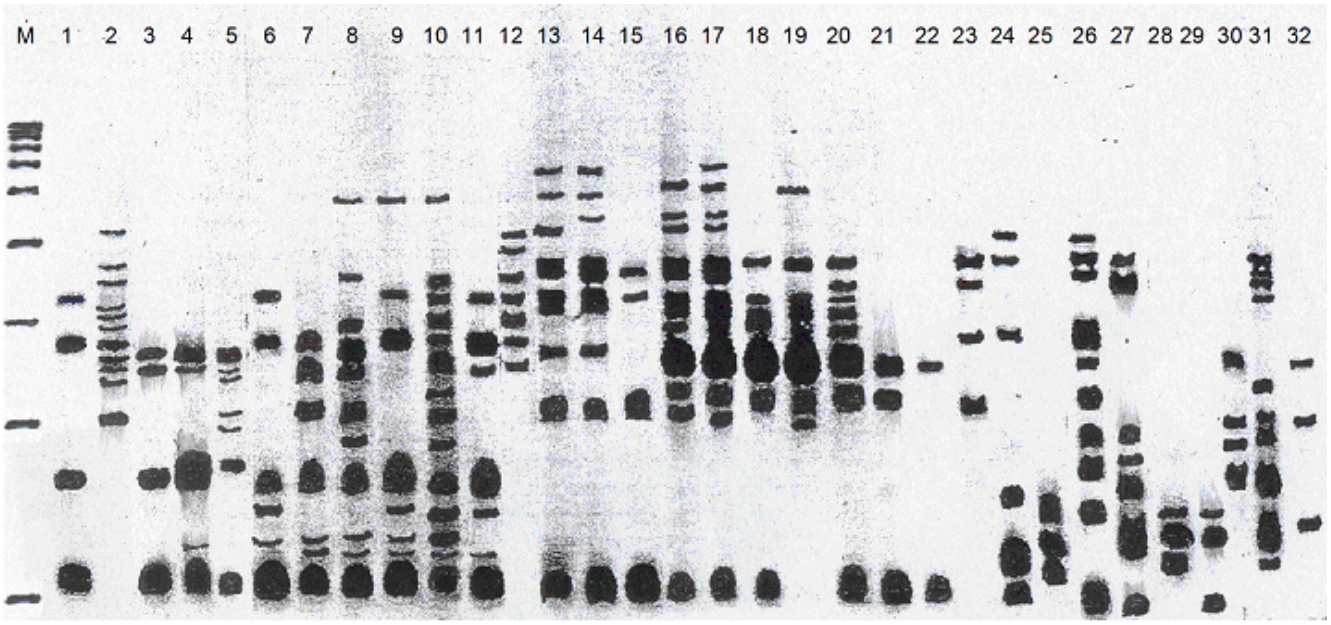

Fig. 1 Gel profile of Jasmine using RAPD-PCR primers OPD-20. Lanes 1-32 contain the amplification profile obtained using the varieties G1, G2, G2, G4, G5, G6, G7, G8, G9, G10, G11, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, M1, M2, A1, A2, A3, A4, A5, A6, A7 and A8. Lane M contains 500 bp standard DNA markers.

Table 2 Genetic dissimilarity matrix of 32 Jasmine varieties based on polymorphism of RAPD markers.

Co-1-Pitchi	0																														
Co-2-Pitchi	22	0																													
Bangalore Pitchi	18	17	0																												
Coimbatore Pitchi	22	20	16	0																											
Lucknow Pitchi	20	14	14	19	0																										
Nilakottai Pitchi	14	26	21	24	21	0																									
Thimmapuram Pitchi	20	24	16	17	17	17	0																								
Triploid	26	24	20	20	17	19	17	0																							
Periakulam Pitchi	19	32	25	25	29	12	24	24	0																						
Tenkasi White Pitchi	31	29	23	25	24	24	17	14	27	0																					
Arka Surabhi	27	31	26	24	30	17	21	18	19	19	0																				
Ramanathapuram Mallige	39	36	37	39	39	36	40	38	38	41	33	0																			
Bale Japani	37	43	40	44	42	38	38	39	41	39	34	17	0																		
Single Mohra	36	45	38	40	40	36	38	41	36	40	36	22	13	0																	
Double Mohra	28	31	26	33	26	30	26	29	37	35	32	25	23	25	0																
Khoya	36	40	38	40	39	39	39	42	38	42	35	17	12	14	24	0															
Khoya Large	36	39	40	41	40	36	39	40	41	40	33	20	16	17	26	11	0														
Iruvantige	36	40	36	38	38	31	34	37	36	39	33	16	16	16	21	13	12	0													
Butt Mohra	38	41	43	42	43	36	42	42	36	39	37	17	21	23	31	18	19	14	0												
Mysore Mallaige	31	32	30	35	29	32	27	30	40	35	33	21	22	28	16	25	24	18	25	0											
Rameswaram Mallige	29	31	25	30	22	30	28	31	39	38	36	25	29	30	17	31	32	25	31	16	0										
Nilakottai Mallige	30	26	23	30	20	33	28	32	40	38	36	28	31	31	18	29	30	26	32	20	8	0									
Mysore Kakada	32	33	29	31	28	33	27	33	36	34	33	44	43	41	33	45	43	39	40	30	30	29	0								
Arka Arpan	34	33	34	35	32	37	32	36	41	38	39	51	48	44	36	49	45	42	46	36	35	34	9	0							
C0-1-Mullai	34	30	31	36	28	40	35	35	44	43	41	48	49	47	33	47	48	43	49	39	31	28	32	36	0						
Co-2-Mullai	37	37	37	41	31	39	33	40	47	45	43	50	52	50	40	51	48	45	53	41	35	32	36	35	23	0					
Pari Mullai	37	35	36	41	31	42	37	40	46	43	44	50	55	53	33	51	51	48	53	41	33	31	34	37	17	24	0				
Manamadurai Mullai	38	36	39	40	33	42	37	42	46	45	45	52	56	51	37	55	52	49	50	44	38	34	29	32	18	27	11	0			
Long Point	32	29	29	38	23	36	31	36	40	41	39	45	49	48	30	47	46	43	50	38	28	24	32	35	15	21	12	13	0		
Short Point	33	31	29	33	23	34	29	33	39	36	37	46	46	45	33	45	44	41	45	34	29	25	34	46	24	23	25	24	17	0	
Nilakottai Mullai	46	39	43	42	36	43	41	43	47	42	44	51	54	52	40	53	53	51	55	46	40	38	34	36	27	28	19	24	24	31	0
Periaculam Mullai	41	32	35	39	26	41	37	39	48	42	46	51	55	55	39	55	53	50	52	42	33	32	37	40	26	28	23	24	19	18	31	0

RESULTS AND DISCUSSION

Recently matured leaves preferably light green coloured, 15-20 days old 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. The concentration of DNA obtained at 260 nm varied from 800-8400 ng g^{-1} of leaf tissue. Intact DNA was obtained when analysed by 0.7 % agarose gel electrophoresis.

PCR amplification was followed by a 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 $MgCl_2$ produced intense and clear banding patterns. A primary screening of 35 RAPD primers resulted in the selection of 8 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).

Approximately 169 unambiguous, readable and reproducible RAPD markers were produced using the 8 primers selected. The number of bands obtained per primer (**Table 1**) varied from 13 to 29 with an average of 21.13 bands per primer and the size ranged from 100 bp to 3.7 kbp. Of the 169 bands, 158 (93.49%) were polymorphic and shared between at least two individuals, 1 (0.59%) was monomorphic and common to all the individuals. Only ten (5.92%) were polymorphic and unique. Similarly, high polymorphism using RAPD have been reported in *Oryza sativa* (Raghunathachari *et al*. 2000), *Actinidia* sp. (Huang *et al*. 2002) and *Bambusa* sp. (Nayak *et al*. 2003). The representative polymorphic gel profiles of primer OPD-20 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 56% was between varieties 'Bale Japani' (*J. sambac*) and 'Manamadhurai Mullai' (*J. auriculatum*), while the least genetic dissimilarity (8%) was between the varieties 'Rameswaram Mallige' and 'Nilakottai Mallige', belonging to the same species (*J. sambac*). The PCA (**Fig. 2**) distinctly clustered all the varieties into four groups ('A', 'B', 'C' and 'D') based on their species as *J. multiflorum*, *J. auriculatum*, *J. sambac* and *J. grandiflorum*, respectively.

In the dendrogram (**Fig. 3**), all 32 varieties were clearly divided into two major clusters 'A' and 'B' at 52 linkage distances. Cluster 'A' consisted of two varieties ('Arka Arpan' and 'Mysore Kakada') belonging to *J. multiflorum*, clustered at 18 linkage distances and showed a dissimilarity of 9%. Primers OPA-09, OPE-14, OPG-02 and OPK-02 produced unique bands specific to both varieties. Cluster 'B' consist of two sub-clusters, 'B_1' with eight varieties ('Co-1-Mullai', 'Co-1-Mullai', 'Pari Mullai', 'Manamadhurai Mullai', 'Long Point', 'Short Point', 'Nilakottai Mullai', 'Periakulam Mullai') belonging to *J. auriculatum*. Primer OPK-07 produced a band of size approximately 1250 bp that was specific to all varieties of sub-cluster 'B_1'. The varieties 'Pari Mullai' and 'Manamadhurai Mullai' are closely related among the group linked at 22 distances. The maximum dissimilarity among the cluster (31%) was between the varieties 'Short Point' and 'Nilakottai Mullai', and 'Nilakottai Mullai' and 'Periakulam Mullai'. The minimum dissimilarity (11%) was noticed between the varieties 'Pari Mullai' and 'Manamadhurai Mullai'.

The sub-cluster 'B_2' is segregated into two minor clusters 'B_{2a}' and 'B_{2b}' clustered with varieties of *J. sambac* and *J. grandiflorum* respectively. The minor cluster 'B_{2a}' grouped the varieties of *J. sambac* into two groups (I and II) with four and seven varieties respectively. Among the four varieties ('Rameswaram Mullai', 'Nilakottai Mullai', 'Mysore Mallige' and 'Double Mohra') of group I 'Rameswaram Mullai' and 'Nilakottai Mullai' are closely related at 11 linkage distance. The varieties 'Mysore Mallige' and 'Double Mohra' are linked together at 31 linkage distances in the group. Among the seven varieties of group II ('Ramnathapuram Mallige', 'Bale Japani', 'Single Mohra', 'Khoya', 'Khoya Large', 'Iruvantige' and 'Butt Mohra') the varieties 'Khoya' and 'Khoya Large' are closely related at 21 linkage distance and closely linked to the variety 'Iruvantige' at 23 linkage distance. The variety 'Ramnathapuram Mallige' stands as a separate variety among the group II. Among the varieties of *J. sambac* a maximum dissimilarity (32%) was noticed between the varieties 'Butt Mohra' and 'Nilakottai Mallige', and 'Khoya Large' and 'Rameshwaram Mallige' and a minimum dissimilarity of (8%) was noticed between the varieties 'Rameswaram Mullai' and 'Nilakottai Mullai'.

The minor cluster 'B_{2b}' consisted of 11 varieties belonging to *J. grandiflorum* segregated into three groups at 34 linkage distances. The group I consist of two varieties ('Tenkasi white Pitchi' and 'Triploid') clustered at 28 linkage distance. The group II consist of five varieties ('Co-2-Pitchi', 'Bangalore Pitchi', 'Coimbatore Pitchi', 'Lucknow Pitchi' and 'Thimmapuram Pitchi') clustered at 31 linkage

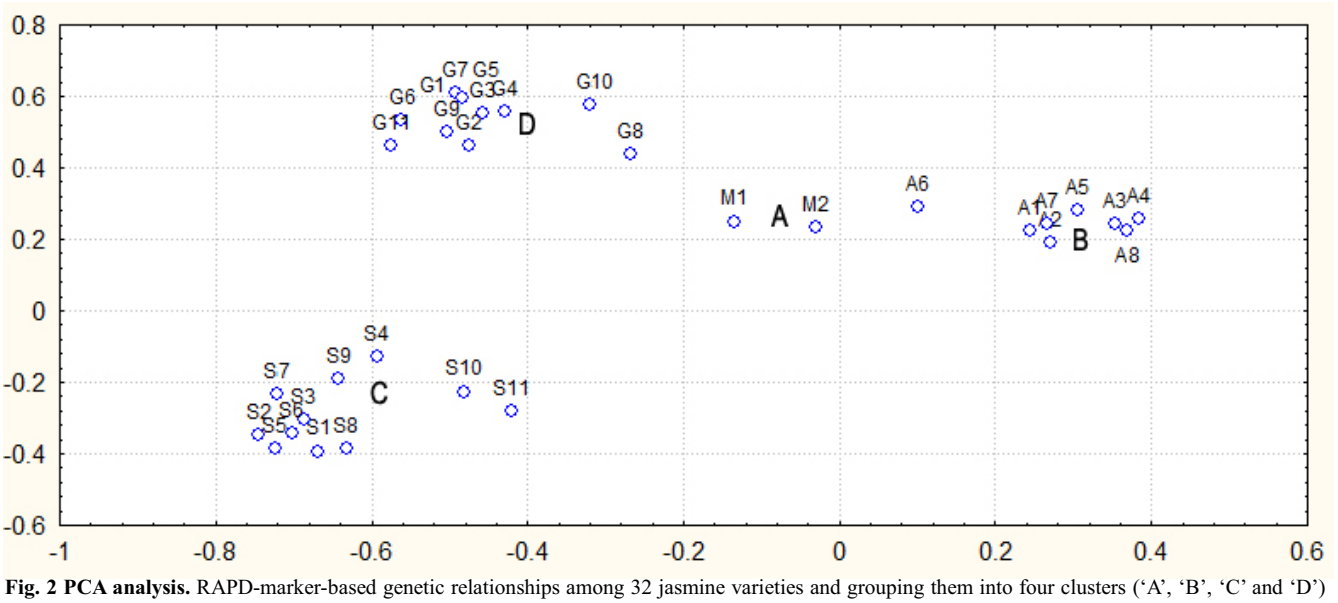

Fig. 2 PCA analysis. RAPD-marker-based genetic relationships among 32 jasmine varieties and grouping them into four clusters ('A', 'B', 'C' and 'D') based on their species as *J. multiflorum, J. auriculatum*, *J. sambac* and *J. grandiflorum* respectively.

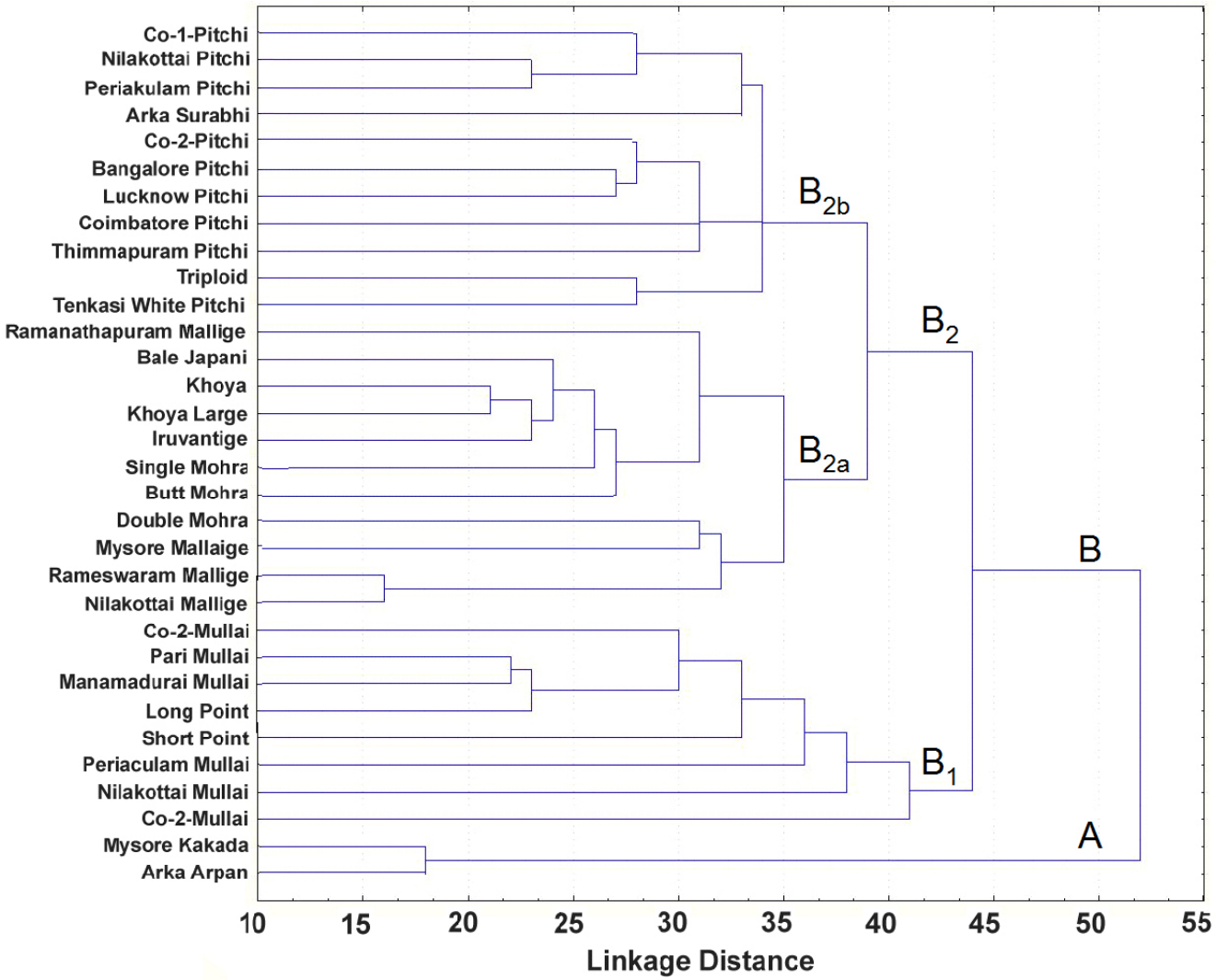

Fig. 3 Cluster analysis. Dendrogram showing RAPD-marker-based genetic relationships among 32 jasmine varieties and grouping them into two clusters 'A' with varieties of *J. multiflorum* and 'B' with varieties of *J. auriculatum, J. sambac* and *J. grandiflorum* in 'B_1', 'B_{2a}' and 'B_{2b}' respectively.

distance and segregated into three minor groups. The varieties 'Bangalore Pitchi' and 'Lucknow Pitchi' are clustered at 27 linkage distance and are closely linked to the variety 'Co-2-Pitchi'. The variety 'Coimbatore Pitchi' and 'Thimmapuram Pitchi' stands as separate entity among the varieties of group II. The group III consists of four varieties ('Periakulam Pitchi', 'Arka Surabhi', 'Co-1-Pitchi' and 'Nilakottai Pitchi') clustered at 33 linkage distance. The varieties 'Periakulam Pitchi' and 'Nilakottai Pitchi' are clustered at 23 linkage distance and are closely linked to the variety 'Co-1-Pitchi'. The variety 'Arka Surabhi' stands as a single entity among the group III. Among the varieties of *J. grandiflorum* a maximum dissimilarity of (32%) was noticed between the varieties 'Co-2-Pitchi' and 'Periakulam Pitchi', and a minimum dissimilarity of (12%) was noticed between the varieties 'Nilakottai Pitchi' and 'Periakulam Pitchi'. The banding pattern produced by primers OPE-14 and OPG-13 could distinguish both the minor clusters 'B_{2a}' and 'B_{2b}'.

RAPDs are produced by the annealing of random primers to sites distributed along the genome flanking both conserved and highly variable regions. The amplified sequences may fall into the low copy gene category or into the high repetitive sequences, but the probability that the priming sites are found in repetitive sequences is high (Devos and Gale 1992; Paran and Michelmore 1993; Haymer 1994) and are assumed to be neutral markers. Intra- and interspecific genetic differentiation can be driven by ecological, evolutionary and historical factors, and the knowledge of the effects of genetic variability in species of jasmine is important for an understanding of how diversity is distributed among populations. The species *J. sambac* and *J. grandiflorum,* which appear genetically close, suggest that a common ancestor separated in ancient times to form different species with a modified genome.

The varieties grouped under the clusters 'A', 'B_1', 'B_{2a}' and 'B_{2b}' belong to *Jasminum* species *J. multiflorum*, *J. auriculatum*, *J*. sambac and *J. grandiflorum*, respectively. The appearance of four different cluster groups was due to their genetic difference at species level. A high genetic dissimilarity (56%) was achieved among the species level but a low genetic dissimilarity was obtained among the varieties of individual species [*J. grandiflorum* (12-32%), *J. sambac* (8-32%), *J. multiflorum* (9%) and *J. auriculatum* (11-31%)]. This could be attributed to the vegetative method of propagation among the species, due to no fruit set. Predominantly, out-crossing species show higher levels of variability within populations than others (Hamrick and Godt 1990; Schoen and Brown 1991). The low level of genetic variation can also be correlated with limited population size, in which the stochastic processes of genetic drift might have led the genetic composition toward allele fixation at most loci with high probability (Hartl and Clark 1997) and populations diverged recently had no sufficient time to differentiate through drift (Elena *et al*. 2003).

All the individuals in the present studies which were collected from different geographical locations of Southern India showed low to moderate polymorphism, regardless of the locations from where they were obtained. The RAPD marker analysis clustered the varieties based on their spe-

cies. In summary, the results from this study indicate that the RAPD technique is a useful tool for the identification of germplasm and genetic relationships between and within the Jasmine species. The banding pattern produced by primer OPE-14 could differentiate all four jasmine species. The relatively large number of polymorphisms obtained seems due to large phylogenetic distance among these taxa. It would allow a more quantitative assessment of genetic distances between species. Such an analysis, together with data from other classical methods, could thus be used to make a more accurate reconstruction of evolution (Nayak *et al*. 2003). Understanding the spatial organization of genetic diversity within and among populations is of critical importance for the development of strategies designed to preserve genetic variation (Brown and Briggs 1991; Hamrick *et al*. 1991). Furthermore, such an approach might be helpful in identifying taxa of potential value in genetic improvement programmes.

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