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Molecular characterization of jasmine genotypes using RAPD and ISSR markers

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ABSTRACT

Understanding the genetic background of existing germplasm represents a value-added component of managing collections. Molecular approaches collectively represent a potential tool for gathering information on genetic improvement. Keeping in view, the present investigation was carried out to explore the molecular diversity among 18 jasmine genotypes by employing two DNA based molecular marker techniques, *viz*., random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR). A total of 33 polymorphic primers (20 RAPD and 13 ISSR) were used. Amplification of genomic DNA of 18 genotypes, using RAPD analysis resulted in a total of 248 products, out of which 234 (94.35 %) products were polymorphic. The polymorphism percentage ranged from 80-100% with an average of 94.24% polymorphism per primer. In ISSR analysis, among thirteen primers tested, a total of 595 products were produced out of which 562 (94.45%) products were polymorphic. The polymorphism ranged from 82.60-100% per cent, with an average of 94.45% polymorphism per primer. Based on the similarity matrix data, dendrograms were generated using UPGMA method. The genotypes were categorized into several groups and subgroups which varied distinctly with *J. primulinum* forming a separate cluster.

Key words: *Jasminum* spp., molecular markers, genetic diversity, similarity index, polymorphism.

INTRODUCTION

Jasmine (*Jasminum* spp.) of family *Oleaceae*, native to South and Southeast Asia is one of the most important traditional flowers of India. Besides being a popular fragrant loose flower and highly preferred garden plant, jasmine is also used for production of concrete, which is used in cosmetic and perfumery industries. The main beauty and uniqueness of jasmine is its fragrance, which cannot be imitated by any known synthetic aromatic chemical and has a unique status in the perfume world. Jasmine flowers are widely used in aromatherapy, since jasmine fragrance is effective in treating depression, nervous exhaustion and stress. Jasmine is also widely used in the medicinal and pharmaceutical industries. In India, Tamil Nadu is the leading producer of jasmine in the country with an annual production of 1,36,901 tonnes from an area of 13,246 ha with a productivity of 11.21 t/ha (Anonymous, 1). Among the eighteen genotypes, cv. Ramnathapuram Gundumalli of *J. sambac*, cvs. CO. 1 Pitchi and CO. 2 Pitchi of *J. grandiflorum* and cvs. CO.1 Mullai and Pari Mullai of *J. auriculatum* are commercially cultivated on large scale for loose flower in Tamil Nadu. Pacha Mullai variety of *J. auriculatum* is grown to a small extent in certain parts of Tamil Nadu. *J. multiflorum* var. White which is popularly known as '*Kakada*' is grown commercially in certain parts of Karnataka and Tamil Nadu during winter season when the other commercial varieties undergo lean season and are pruned. The other genotypes are grown as garden plants for landscaping and in home gardens for loose flowers. Collection of germplasm and assessing the diversity among the genotypes is an essential prerequisite for improvement of any plant species. Characterization can be done by several techniques including morphological, biochemical, genetic, molecular etc. Molecular marker technologies such as random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) are reliable tools for the analysis of genetic diversity in various plant groups (Williams *et al.*, 18). Molecular markers can be used for understanding systematic relationships among species and also for genetic fingerprinting with a specific application towards intellectual property rights. Genetic similarity (or genetic distance) estimates among genotypes are helpful in selecting parental combinations for creating segregating populations so as to maintain genetic diversity in a breeding program and in the classification of germplasm into heterotic groups for hybrid crop breeding. RAPD and ISSR markers have been efficiently used for the study of molecular diversity among genotypes of various ornamental

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crops including tuberose (Kameswari *et al*., 9), mini marguerite (Kapoor *et al*.,10), jasmine (Ghehsareh *et al*., 6), etc. These techniques are known for their relative technical simplicity, level of polymorphism they detect, cost effectiveness and ease of use in any plant species (Kumar *et al*., 11). Therefore, present study was undertaken to explore the genetic diversity in *Jasminum* spp. by employing ISSR and RAPD techniques.

MATERIALS AND METHODS

The present investigation was carried out at Department of Floriculture and Landscape Architecture, Tamil Nadu Agricultural University, Coimbatore during 2016-2018. The experimental material consisted of eighteen cultivars (Table 1) of jasmine belonging to ten species collected from the jasmine germplasm maintained at the Department of Floriculture and Landscape Architecture. Three cultivars each of *J. sambac viz*., Ramnathapuram Gundumalli, Adukkumalli and Iruvatchi; *J. grandiflorum viz*., CO.1 Pitchi, CO. 2 Pitchi, White Pitchi and *J. auriculatum viz*., CO. 1 Mullai, Pari Mullai and Pacha Mullai; *J. multiflorum viz*., Arka Arpan, White (Common name: Kakada) and Pink flowered type along with one cultivar each of these six species *J. nitidum* culture Acc. Jn-1, *J. calophyllum*, *J. flexile*, *J. primulinum*, *J. rigidum* and *J. arborescens* were used for the study. The CTAB extraction method (Doyle, 5) was modified in the present study. Recently matured light green coloured leaves were used for DNA extraction, as mature leaves had high levels of polyphenols and polysaccharides that hinder in extraction of PCR quality DNA (Mukundan *et al*., 12). One gram of fresh leaf tissue was taken in a pestle and mortar and pulverized using liquid N_2. The grinded leaf sample was taken in a test tube to which 500 µL of pre-heated CTAB extraction buffer was added along with 1% β-mercaptoethanol and a pinch of polyvinyl pyrrolidone. The contents of the test tube were shaken gently and placed on a water bath for incubation at 65°C for one hour. The mixture was cooled to room temperature and 500 µL of chloroform/isoamyl alcohol (24:1 v/v) was added to each sample, stirred gently with spiral movement (vortexed) and then centrifuged at 10,000 rpm for 15 minutes. The supernatant was transferred to a new 1.5 mL micro centrifuge tube, and equal volume of ice-cold isopropanol was added and kept at 4°C overnight to enable precipitation of the nucleic acid. The samples were centrifuged at 10,000 rpm for 10 minutes, and the supernatant was discarded. To this, 100 µL of 70% ethanol was added and centrifuged at 10,000 rpm for 10 minutes and the supernatant was discarded. The samples were again washed with ethanol. The pellet was air-dried for 2 h and then re-suspended in 50 µL sterile water.

Table 1. Jasmine genotypes involved in the experiment.

S. No.	Jasmine genotype
1.	*J. sambac* cv. Ramnathapuram Gundumalli
2.	*J. sambac* cv. Adukkumalli
3.	*J. sambac* cv. Iruvatchi
4.	*J. grandiflorum* cv. CO.1 Pitchi
5.	*J. grandiflorum* cv. CO.2 Pitchi
6.	*J. grandiflorum* cv. White Pitchi
7.	*J. auriculatum* cv. CO.1 Mullai
8.	*J. auriculatum* cv. Pari Mullai
9.	*J.auriculatum* cv. Pacha Mullai
10.	*J. nitidum* culture Acc.Jn-1
11.	J. calophyllum
12.	J. flexile
13.	J. primulinum
14.	J. rigidum
15.	J. arborescens
16.	*J. multiflorum* cv. Arka Arpan
17.	*J. multiflorum* cv. White (Common name: Kakada)
18.	*J. multiflorum* Pink flowered type

The extracted DNA samples were quantified at absorbance 260 nm and 280 nm using the Nanodrop (Citizen). The ratio between the readings at 260 nm and 280 nm (OD 260 nm/OD 280 nm) was taken as the estimate of the purity of DNA samples. Pure DNA recorded 260 nm/280 nm OD ratio between 1.73 and 1.99. The DNA samples were further diluted to working concentration of 50-100 ng/µL and stored at -20°C for further use in polymerase chain reaction (PCR) analysis. A set of 40 ISSR primers procured from the University of British Columbia (UBC Set No. 9), and 34 RAPD primers procured from Operon Technologies Inc. (USA) were screened for their repeatable amplification. Out of these primers, 13 ISSR and 20 RAPD primers which produced good bands and amplification profile were selected. For each primer, 25 µl amplification reaction contained 10X Taq DNA polymerase (GeNei™), $MgCl_2$ (20 mM) 50 ng of genomic DNA, 1U of Taq DNA polymerase (GeNei™), dNTP 10 mM, (GeNei™) and 50 picomolar of primer. Amplifications were carried out using a DNA thermal cycler (Applied Biosystems, Bio-Rad) by optimizing the protocol, with the following parameters: initial denaturation for 5 minutes at 95°C followed by 45 cycles, denaturation of 1 minute at

94°C, annealing of 1 minute at 36°C, extension at 72°C for 2 minutes with final extension at 72°C for 5 minutes for RAPD analysis. For ISSR analysis, the thermocycler was programmed with conditions: initial denaturation for 4 minutes at 94°C followed by 35 cycles, denaturation of 45 sec. at 94°C, annealing of 46 at 50-52°C extension at 72°C for 1.15 min., with final extension at 72°C for 8 min. Amplified PCR products were resolved in a 1.2% (w/v) agarose gel, visualized and documented using an Alpha Digidoc system (Alpha Innotech., Sanc Leandro, CA, US). Fragment size was estimated by using a 1 Kb molecular size ladder (Fermentas, Germany).

Reproducible ISSR and RAPD products were manually scored for band presence (1) or absence (0) for each accession and a binary qualitative data matrix was constructed. Initially, the potential of both the markers for estimating genetic variability of *Jasminum* spp. was examined by measuring the marker informativeness through the counting of bands. To distinguish capacity of each primer in order to evaluate genetic profiles of *Jasminum* spp., the performance of the markers was measured using three parameters: polymorphic information content (PIC), marker index (MI) and effective multiplex ratio (EMR). The PIC value for each locus was calculated using formula (Roldan-Ruiz *et al*., 17);

$$PICi = 2f_i (1 - f_i)$$

where PICi is the polymorphic information content of the locus *i*; f_i is the frequency of the amplified fragments and $1 - f_i$ is the frequency of non-amplified fragments. The frequency was calculated as the ratio between the number of amplified fragments at each locus and the total number of accessions (excluding missing data). The PIC of each primer was calculated using the average PIC value from all loci of each primer. Effective multiplex ratio was calculated using the formula;

$$EMR \text{ (effective multiplex ratio)} = n \times \beta$$

where 'n' is the average number of fragments amplified by accession to a specific system marker (multiplex ratio) and β is estimated from the number of polymorphic loci (PB) and the number of non-polymorphic loci (MB);

$$\beta = PB/(PB + MB).$$

Marker index for both the markers was calculated to characterize the capacity of each primer to detect polymorphic loci among the genotypes. Marker index for each primer was calculated as a product of polymorphic information content and effective multiplex ratio (Powell *et al*., 15).

$$MI = EMR \times PIC$$

The percentage of polymorphic loci, PIC, effective multiplex ratio (EMR) and MI were calculated by Microsoft Excel software 2010. The data matrix of both markers was converted into genetic similarity matrix using Jaccard coefficient (Jaccard, 8) using NTSYS-PC 2.02j (Rohlf, 16). The genetic relatedness among the 18 individuals of three populations was analyzed using unweighted pair group method with arithmetic average (UPGMA) based on pair wise (Nei, 13) genetic distance.

RESULTS AND DISCUSSION

Molecular diversity databases prove to be directly useful to develop and analyze intra- as well as inter-specific diversity, since morphological data alone may be limiting or misleading. RAPD and ISSR techniques have proven to be useful in population genetic diversity studies (Jabbarzadeh *et al*., 7). Out of 40 RAPD markers tested, 20 markers produced clear and reproducible bands (Table 2). The number of products generated by these RAPD markers were found to range from 9 to 17, with the marker OPX 02 giving the maximum (17) and the markers OPG 05, OPG 09, OPE 01 giving the minimum number of amplicons (9). Out of 248 products resulted, 234 (94.35 %) products were polymorphic. The percentage of polymorphism ranged from 80.00% for OPX; 01 to 100% for OPG 04, OPG 06, OPG 09, OPE 01, OPE 04, OPE 10, OPX 02 and OPX 05, with an average of 94.24% polymorphism per marker. High PIC value of 0.36 (OPE 01) and low PIC value of 0.17 (OPX 03), with an average PIC value per marker of 0.26 was recorded. PIC has a maximum limit of 0.5 in case of dominant markers (De Riek *et al*., 3). The average value of PIC below 0.5 was also found to be sufficiently informative in discrimination of the individuals in *Alstroemeria* (Aros *et al*., 2). The low PIC values obtained by some RAPD markers may be only due to low number of RAPD loci studied. Similar results have also been reported by other workers (Del Carmen Ramırez-Medeles *et al*., 4). In this study, the highest effective multiplex ratio (EMR) of 17.00 was observed with the marker OPX 02 and the lowest of 6.40 was observed with the marker OPX 01, with an average EMR of 11.07 per marker. The highest MI (marker index) was observed with the marker OPX 02 (4.76) and the lowest in the marker OPG 05 (1.52), with an average MI of 2.98 per marker. Higher EMR values indicated that marker/marker system is effective.

The similarity matrix showed that Jaccard's similarity index ranged from 0.62 to 0.89 (Table 3.) The UPGMA algorithm was used for grouping all accessions based on their genetic distances. The dendrogram (Fig. 1) obtained through cluster analysis revealed that there were two major clusters, Cluster A and Cluster B. Cluster A consisted of *J. primulinum* alone. Cluster B was further divided into

Table 2. Diversity analysis in jasmine genotypes with RAPD markers.

S. No.	Primer	Total alleles	Number of polymorphic loci	Polymorphic percentage (PM%)	Polymorphic information content (PIC)	Effective multiplex ratio (EMR)	Marker Index (MI)
1.	OPG-02	12	11	91.67	0.29	10.08	3.19
2.	OPG-03	15	14	93.33	0.24	13.06	3.36
3.	OPG-04	10	10	100.00	0.32	10.00	3.20
4.	OPG-05	9	8	88.89	0.19	7.11	1.52
5.	OPG-06	11	11	100.00	0.25	11.00	2.75
6.	OPG-07	14	12	85.71	0.18	10.28	2.16
7.	OPG-08	10	9	90.00	0.23	8.10	1.86
8.	OPG-09	9	9	100.00	0.24	9.00	2.16
9.	OPG-10	15	14	93.33	0.25	13.06	3.50
10.	OPE-01	9	9	100.00	0.36	9.00	3.24
11.	OPE-02	12	11	91.67	0.22	10.08	2.42
12.	OPE-03	13	12	92.30	0.27	11.07	3.24
13.	OPE-04	10	10	100.00	0.30	10.00	3.00
14.	OPE-10	16	16	100.00	0.31	16.00	4.96
15.	OPX-01	10	8	80.00	0.22	6.40	1.76
16.	OPX-02	17	17	100.00	0.28	17.00	4.76
17.	OPX-03	16	15	93.75	0.17	14.06	2.55
18.	OPX-04	15	14	93.33	0.24	13.06	3.36
19.	OPX-05	14	14	100.00	0.26	14.00	3.64
20.	OPK-07	11	10	90.91	0.31	9.09	3.10
Total		248	234	1884.89	5.13	221.45	59.73
Avg./primer		12.40	11.70	94.24	0.26	11.07	2.99

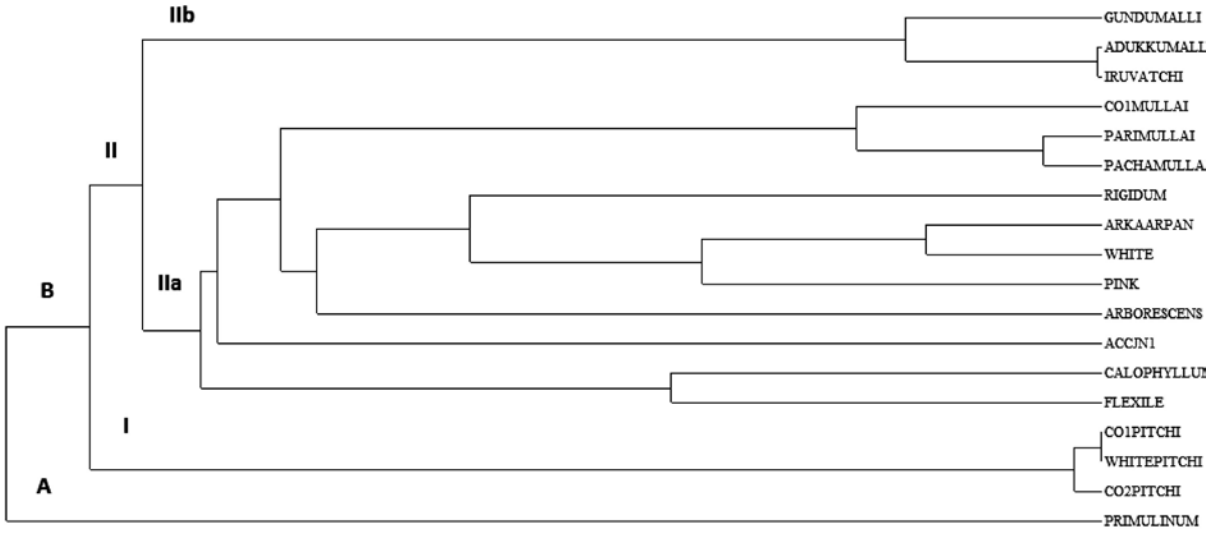

Fig. 1. Dendrogram based on UPGMA of Jaccard's similarity matrix representing the genetic relatedness among 18 jasmine accessions using RAPD primers.

Table 3. Similarity matrix of jasmine genotypes using RAPD markers.

Genotypes	*J. s.* cv. Gundumalli	*J. s.* cv. Adu.	*J. s.* cv. Iru.	*J. g.* cv. CO.1	*J. g.* cv. CO.2	*J. g.* cv. White	*J. a. cv.* CO.1	*J. g.* cv. Pari.	*J .g.* cv. Pacha.	*J. n.* cv. Acc.Jn-1	*J. calophyllum*	*J. flexile*	*J. primulinum*	*J. rigidum*	*J. arborescens*	*J. m.* cv. AA	*J .m.* cv. White	*J. m.* cv. Pink
J.s. cv. Gundumalli	1.00																	
J. s. cv. Adu.	0.85	1.00																
J. s. cv. Iru.	0.83	0.89	1.00															
J. g. cv. CO.1	0.60	0.59	0.63	1.00														
J. g. cv. CO.2	0.59	0.60	0.63	0.89	1.00													
J. g. cv. White	0.62	0.62	0.66	0.89	0.89	1.00												
J. a. cv. CO.1	0.67	0.66	0.68	0.70	0.63	0.69	1.00											
J. g. cv. Pari.	0.65	0.67	0.64	0.68	0.64	0.68	0.84	1.00										
J. g. cv. Pacha.	0.65	0.66	0.66	0.69	0.65	0.68	0.83	0.88	1.00									
J.n. cv. Acc. Jn-1	0.67	0.65	0.65	0.62	0.61	0.64	0.67	0.68	0.68	1.00								
J. calophyllum	0.62	0.61	0.64	0.65	0.63	0.64	0.65	0.65	0.66	0.67	1.00							
J. flexile	0.63	0.63	0.63	0.66	0.63	0.65	0.70	0.72	0.70	0.65	0.79	1.00						
J. primulinum	0.61	0.60	0.59	0.61	0.58	0.61	0.67	0.67	0.64	0.59	0.61	0.66	1.00					
J. rigidum	0.69	0.69	0.67	0.63	0.61	0.64	0.69	0.71	0.66	0.67	0.61	0.67	0.61	1.00				
J. arborescens	0.66	0.64	0.62	0.64	0.60	0.60	0.66	0.67	0.64	0.60	0.58	0.65	0.61	0.69	1.00			
J. m. cv. AA	0.66	0.65	0.69	0.69	0.65	0.68	0.74	0.74	0.69	0.70	0.69	0.72	0.65	0.76	0.73	1.00		
J. m. cv. White	0.66	0.65	0.66	0.65	0.63	0.65	0.71	0.71	0.68	0.68	0.67	0.70	0.60	0.71	0.68	0.84	1.00	
J. m. cv. Pink	0.63	0.63	0.65	0.61	0.61	0.63	0.66	0.69	0.66	0.68	0.65	0.65	0.60	0.74	0.68	0.79	0.79	1.00

two sub-clusters, *viz*., Sub-cluster I and Sub-cluster II, with the *J. grandiflorum* cvs. CO. 1 Pitchi, CO. 2 Pitchi and White Pitchi falling under Sub-cluster I. Sub-cluster II was again subdivided into two groups namely, Sub-cluster IIa and Sub-cluster IIb. Sub-cluster IIa comprised of 11 accessions *i.e*., *J. calophyllum, J. flexile, J. nitidum, J. arborescens, J. rigidum,* three *J. multiflorum* genotypes namely, Arka Arpan, White (Kakada) and Pink flowered type, and three *J. auriculaum* genotypes namely, CO. 1 Mullai, Pari Mullai and Pacha Mullai. Sub-cluster IIb was formed by three cultivars of *J. sambac* namely Ramanathapuram Gundumalli, Adukkumalli and Iruvatchi. The dendrogram obtained through cluster analysis on the basis of data revealed that *J. primulinum* which is a yellow flowered species, formed a separate cluster from rest of the genotypes, which are all white flowered species and some white with pinkish tinge. Based on RAPD data, it was observed that the lowest similarity (59%) was found between *J. grandiflorum* cv. CO. 2 Pitchi and *J. sambac* cv. Adukkumalli, which might be due to the reason that CO. 2 Pitchi has been evolved through mutation and possesses different attributes compared to Adukkumalli. The dendrogram based on RAPD data indicated that in Sub-cluster IIa, the culture Acc.Jn-1 (*J. nitidum*) formed one separate group comprising of this culture alone, which might be due to its unique characters such as dark pink tinged flower buds, year-round flowering habit, relative freeness from pests and diseases, *etc.* The main changes observed from RAPD profiles were the presence and absence of different bands as well as variations in their intensities (Fig. 3). The genetic distances indicated that the cultivar Iruvatchi recorded 89% similarity with Adukkumalli. The cultivar Arka Arpan recorded 72% similarity with *J. flexile*. The lowest similarity of 58% was found between *J. primulinum* and *J. grandiflorum* cv. CO. 2 Pitchi.

Out of 21 ISSR markers tested, 13 markers produced clear and reproducible bands (Table 4). The number of products generated by these ISSR

Table 4. Diversity analysis in jasmine genotypes with ISSR markers.

S. No.	Primer	Total alleles	Annealing temperature (°C)	Number of polymorphic loci	Polymorphic percentage (PM%)	Polymorphic information content (PIC)	Effective multiplex ratio (EMR)	Marker Index (MI)
1.	UBC 841	10	50	8	87.5	0.25	6.40	2.00
2.	UBC842	35	50	35	100	0.35	35.00	12.25
3.	UBC 880	45	50	40	88.88	0.27	35.55	10.80
4.	UBC 817	46	50	46	100	0.31	46.00	14.26
5.	UBC 821	56	52	56	100	0.23	56.00	12.88
6.	UBC 852	55	51	52	94.54	0.30	49.16	15.60
7.	UBC 853	47	51	41	87.23	0.24	35.76	9.84
8.	(AC)8GA	46	52	38	82.60	0.24	31.39	9.12
9.	(GT)8CC	52	52	50	96.15	0.34	48.07	17.00
10.	(AG)8CG	55	52	51	92.72	0.31	47.29	15.81
11.	(GA)8T	53	50	53	100	0.26	53.00	13.78
12.	(AG)8CT	45	50	42	93.33	0.31	42.00	13.02
13.	GA(9A)	50	50	50	100	0.28	50.00	14.00
Total		595	-	562		1222.95 3.69	535.62	160.36
Mean/primer		45.77	-	43.23	94.07	0.28	41.20	12.34

markers ranged from 10 to 56 with marker UBC 821 giving the maximum (56) and marker UBC 841 giving the minimum number of amplicons (10). A total of 595 products were produced with the different markers, out of which 562 (94.45%) products were polymorphic. The percentage of polymorphism ranged from 82.60% for (AC)8GA to 100% for UBC 842, UBC 817, UBC 821, (GA)8T and (GA)9A, with an average of 94.07% polymorphism per marker. The highest PIC value of 0.35 (UBC 842) and the lowest of 0.24 [UBC 853 and (AC) 8GA], with an average PIC value per marker of 0.28 were obtained. Average value of PIC showed a good efficiency of the used primers in discrimination of the individuals. Although the low PIC values obtained by some ISSR markers may be only due to low number of ISSR loci studied. Similar results have been reported by other workers (Del Carmen Ramırez-Medeles *et al*., 4). The effective multiplex ratio depends on the fraction of polymorphic fragments (b). In this study, the highest effective multiplex ratio (EMR) of 56.00 was observed with the marker UBC 821 and the lowest EMR of 6.40 was observed with the marker UBC 841, with an average EMR of 41.20 per marker. The highest MI (marker index) was observed with the marker (GT)8CC (17.00) and lowest in the marker UBC 841 (2.00), with an average MI of 12.34 per marker. Marker index is used to calculate the overall utility of a marker system, and the higher value represents the merit of the technique (Perez de la Torre *et al*., 14).

The similarity matrix developed using the NTSYS-PC 2.02 software showed that Jaccard's similarity index ranged from 0.63 to 0.95 (Table 5). The UPGMA algorithm was used for grouping all accessions based on their genetic distances. The dendrogram (Fig. 2) obtained through cluster analysis revealed that there were two main groups, Cluster A and Cluster B. Cluster A consisted of *J. primulinum* alone. Cluster B was further divided into two sub-clusters, namely Sub-cluster I and Sub-cluster II. Sub-cluster I was further divided into two groups with the cultivars of *J. grandiflorum* namely, CO.1 Pitchi, CO.2 Pitchi and White Pitchi forming one group (Ia) and *J. calophyllum* and *J. flexile* forming another group (IIb). Sub-cluster II was also further subdivided into two groups namely IIa and IIb. Group IIa comprised of *J. auriculatum* cv. CO.1 Mullai, Pari Mullai, Pacha Mullai and Acc. Jn-1 (*J. nitidum*). Group IIb was grouped into two subgroups wherein Sub-group 1 comprised of *J. arborescens, J. rigidum* and the three *J. multiflorum* genotypes, namely Arka Arpan, White (Kakada) and Pink flowered type. Sub-group II consisted of *J. sambac* cvs. Gundumalli, Adukkumalli and Iruvatchi. Ecotypes of *J. sambac* showed close association among each other, which might be due to the similar genetic makeup.

The genetic distances indicated that the *J. grandiflorum* cultivars CO.2 Pitchi, White Pitchi and CO.1 Pitchi were most closely related genotypes with 89% similarity. Lowest similarity (59%) was

Table 5. Similarity matrix of jasmine genotypes using ISSR markers.

Genotypes	*J.s.* cv. Gundumalli	*J.s.* cv. Adu.	*J.s.* cv. Iru.	*J. g.* cv. CO.1	*J. g.* cv. CO.2	*J. g.* cv. White	*J. a. cv.* CO.1	*J. g.* cv. Pari.	*J .g.* cv. Pacha.	*J. n.* cv. Acc.Jn-1	*J. calophyllum*	*J. flexile*	*J. primulinum*	*J. rigidum*	*J. arborescens*	*J. m.* cv. AA	*J .m.* cv. White	*J. m.* cv. Pink
J.s. cv. Gundumalli	1.00																	
J.s. cv. Adu.	0.87	1.00																
J.s. cv. Iru.	0.84	0.95	1.00															
J. g. cv. CO.1	0.65	0.67	0.66	1.00														
J. g. cv. CO.2	0.71	0.72	0.70	0.85	1.00													
J. g. cv. White	0.63	0.66	0.66	0.81	0.84	1.00												
J. a. cv. CO.1	0.63	0.68	0.66	0.60	0.67	0.68	1.00											
J. g. cv. Pari.	0.69	0.77	0.75	0.70	0.70	0.70	0.84	1.00										
J .g. cv. Pacha.	0.64	0.66	0.63	0.63	0.64	0.64	0.79	0.77	1.00									
J. n. cv. Acc. Jn-1	0.66	0.68	0.68	0.65	0.67	0.68	0.71	0.76	0.75	1.00								
J. calophyllum	0.61	0.63	0.65	0.70	0.73	0.74	0.65	0.67	0.62	0.68	1.00							
J. flexile	0.60	0.65	0.67	0.65	0.67	0.71	0.70	0.72	0.64	0.68	0.88	1.00						
J. primulinum	0.63	0.60	0.59	0.59	0.65	0.63	0.64	0.63	0.62	0.61	0.67	0.65	1.00					
J. rigidum	0.70	0.71	0.68	0.68	0.67	0.68	0.67	0.74	0.62	0.63	0.67	0.73	0.60	1.00				
J. arborescens	0.63	0.64	0.62	0.64	0.63	0.63	0.68	0.67	0.65	0.63	0.66	0.69	0.61	0.74	1.00			
J. m. cv. AA	0.66	0.72	0.69	0.65	0.66	0.67	0.69	0.72	0.65	0.73	0.68	0.71	0.67	0.79	0.74	1.00		
J .m. cv. White	0.68	0.72	0.72	0.62	0.64	0.65	0.67	0.72	0.62	0.74	0.66	0.69	0.64	0.79	0.69	0.83	1.00	
J. m. cv. Pink	0.63	0.68	0.69	0.59	0.63	0.60	0.65	0.66	0.64	0.68	0.60	0.64	0.62	0.69	0.68	0.82	0.76	1.00

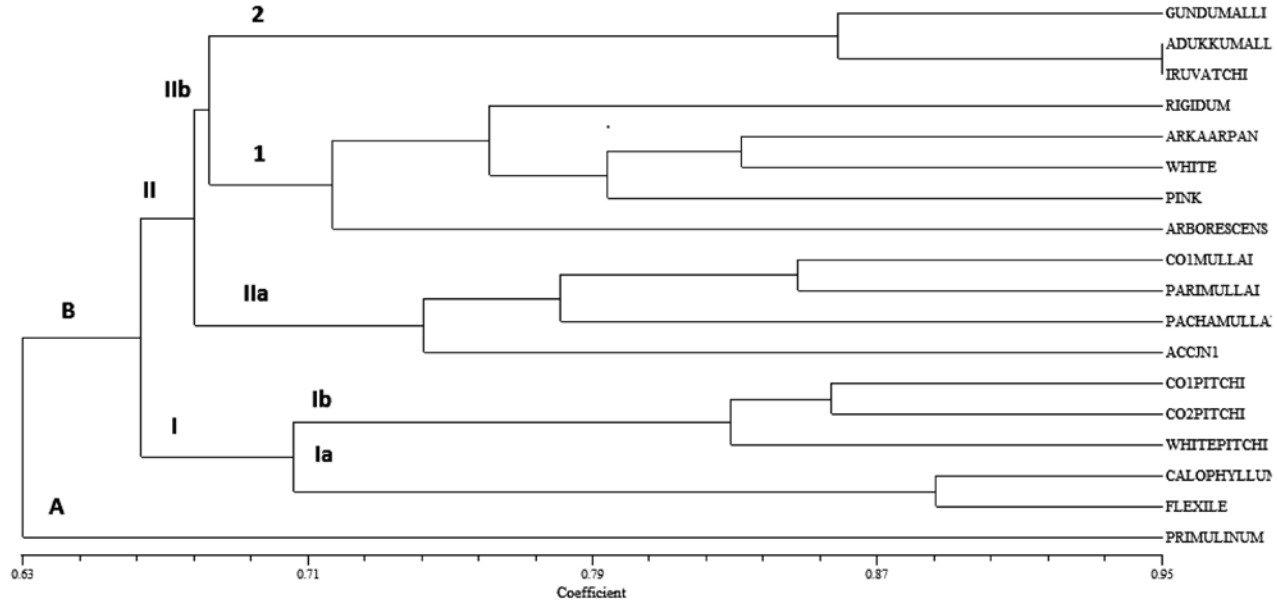

Fig. 2. Dendrogram based on UPGMA of Jaccard's similarity matrix representing the genetic relatedness among 18 jasmine accessions using ISSR primers.

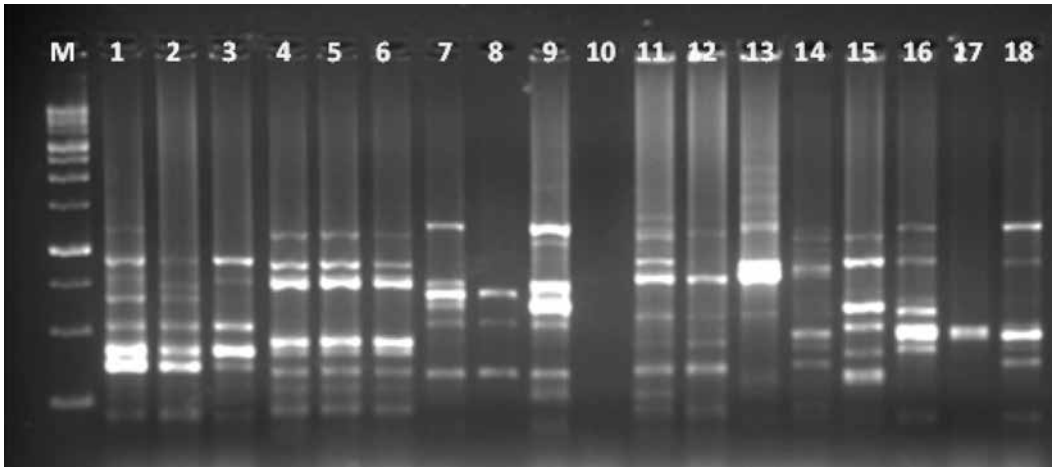

Fig. 3. ISSR profile of jasmine genotypes generated by primer UBC 817. Lane M is 1 Kb DNA ladder and lanes 1–18 represent different jasmine genotypes as listed in Table 1.

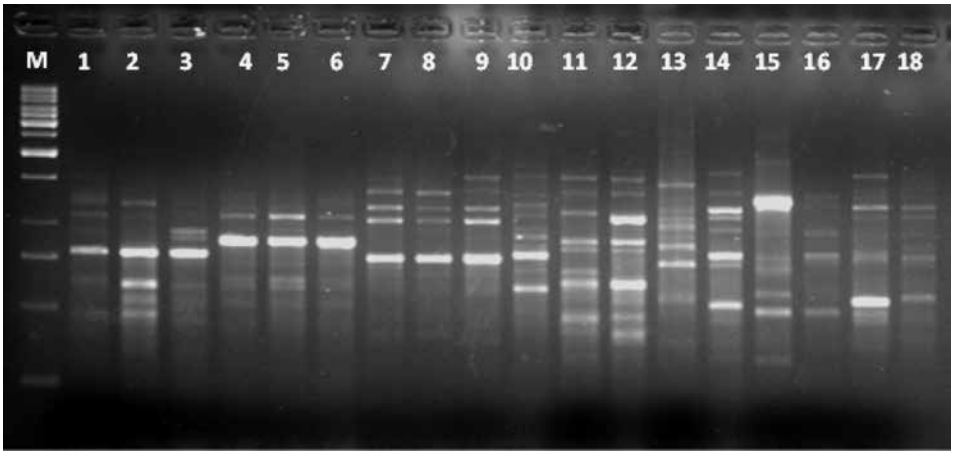

Fig. 4. RAPD profile of jasmine genotypes generated by primer OPX 02. Lane M is 1 Kb DNA ladder and lanes 1–18 represent different jasmine genotypes as listed in Table 1.

found between CO.1 Pitchi (*J. grandiflorum*) and Adukkumalli (*J. sambac*) and also between CO.2 Pitchi (*J. grandiflorum*) and Ramanathapuram Gundumalli (*J. sambac*). The main changes observed from ISSR profiles were the presence and absence of different bands as well as variations in their intensities (Fig. 4). Further, the identification of cultivars or breeding lines is very important in all horticultural and agricultural species in order to protect the rights of plant breeders. The results of the present study pertaining to molecular characterization can be helpful in identifying or differentiating between different jasmine varieties/cultivars.

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