Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **2 (3):** 312-317 (2014)

Research Article

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Detection of Genetic diversity in *Jasmine* species by DNA Fingerprinting using RAPD Molecular Markers

Sushant Shekhar[2]*, Prasad M.P[1] and Sujatha Sriram[1]

[2]Research Scholar, Department of Microbiology, Tumkur University, Tumkur
[1]Sangenomics Research Labs, Bangalore- 560071
*Corresponding Author E-mail: shekharsushant21@gmail.com

ABSTRACT

In the following study genomic DNA was extracted from eight Jasmine species. DNA fingerprinting was performed by the random amplified polymorphic DNA (RAPD)-PCR. RAPD has been one of the most commonly used molecular techniques to develop DNA markers since it does not require prior knowledge of a DNA sequence. RAPD-PCR produced a spectrum of amplification products which are characteristics of the selected Jasmine DNA. From the DNA fingerprinting, Dendrogram was constructed and genetic similarity matrix were estimated which revealed variations between selected species of Jasmine. A total of 10 random primers were used for conducting the RAPD analysis. The primers were OPU5, OPU6, OPU7, OPU8, OPU9, OPU10, OPU11, OPU12, OPU13, OPU14 and OPU15. Of these selected random primers OPU8, OPU9, OPU10, OPU11, OPU12, produced clear banding patterns which were used for constructing Dendrogram. OPU8 produced a total 55 bands ranging from 6-8, OPU9 produced a total 44 bands ranging from 2-8, OPU10 produced a total 33 bands ranging from 3-6, OPU11 produced a total 82 bands ranging from 9-12, OPU12 produced a total 61 bands ranging from 5-9. Most of the bands were monomorphic with some polymorphic bands which can be used for marker development for these jasmine species. The described approach holds great promise for genetic diversity polymorphism, cultivar characterization and genetic population conservation of Jasmine species.

Keywords: *Jasmine, DNA Fingerprinting, Dendrogram, RAPD, Genetic Diversity.*

INTRODUCTION

Natural sources have important and many useful bioactive compounds and they have been discovered using bioactivity directed fractionation and isolation (BDFl). The research of pharmacognosy or isolation of natural products facilitated by newly development of new bioassay methods. It has been found that the bioactive compounds are mostly plant secondary metabolites, which become medicine after processing to pure compounds; some are very useful dietary supplements, and many useful commercial products. Further modification of the active compounds lead to enhance the biological profiles and a large number of such compounds which are approved or undergoing clinical trials for clinical uses against different diseases like pulmonary diseases, cancer, HIV/AIDS, malaria, Alzheimer's and other diseases[2,6]. Crude herbs are used as drugs in different country of the world and therefore it take a basic part of many traditional medicines worldwide. In Asia, traditional Chinese medicine (TCM), Korean Chinese medicine, Japanese Chinese medicine (kampo), ayurvedic medicine (India) and jamu (Indonesia), phytotherapy and homeopathy in Europe. Alternative medicines are typically named when herbal therapies use with various other traditional remedies in America.

In India, Jasmine is extensively used in manufacturing high grade aromatherapy, cheaper synthetic oil obtained by blending a few constituents are used incenses, room fresheners and soaps etc. Juices from the leaves of *J. sambac* are applied to treat ulcers, remove corns, effecting in expelling worms, regulating

menstrual flow, to clean kidney waste, inflamed and blood-shot eyes. It is useful in treating diseases of the mouth and teeth, especially for toothache[4]. The leaves of Jasminum grandiflorum is used in folk medicine for treating ulcerative stomatitis, toothache, skin diseases, ulcers, wounds, corms and also as gargles. The plant is reported to possess spasmolytic, anti-inflammatory, Anti-microbial[7], antioxidant, anti-ulcer, cyto-protective, chemo preventive, wound healing[5] and anti-acne activities[8].

Although more than 2,000 species are known, 40 species have been identified in India and 20 are cultivated in South India[1]. Jasmine plants are of great economic value as a field crop for the florist, landscape, medicinal and pharmaceutical industries[3]. Jasmine can be grown in a variety of climate and soils. Generally, it prefers mid tropical climate for proper growth and flowering. Mostly, jasmine plants are grown in houses and gardens for ornamental purposes, and are sometimes also used for cut flowers to make garlands. However, there are a few species with fragrant flowers. Among these species, *J. grandiflorum, J. auriculatum* and *J. sambac* are commercially cultivated for oil extraction[3].

MATERIAL AND METHODS

Plant material

The plant material of *Jasminum species* were obtained from University of agriculture, Bangalore India. The different species of Jasmine that were collected are *Jasminum grandiflorum, J. sambac, J. humile, J. sambac wild, J. arborescens, J. angustifolium, J. auriculatum, J. officinale, J sambac* L

DNA Isolation and PCR Amplification

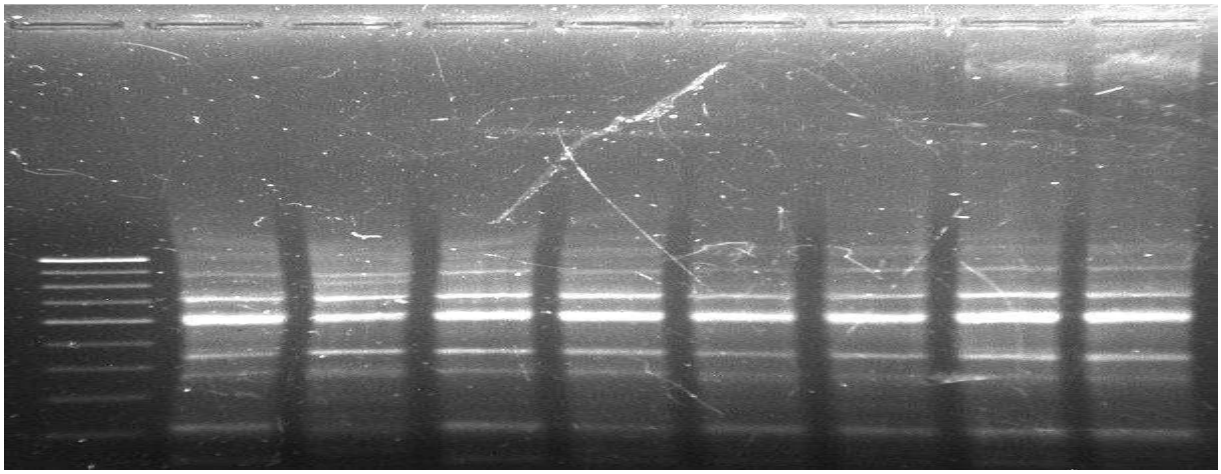
DNA was isolated from fresh leaf tissues as per the procedure described previously. The polymerase chain reaction was carried out in final volume of 25 µl containing 100 ng DNA, 1 U of Taq DNA polymerase (Chromous Biotech, Bangalore), 2.5 mM MgCl (Chromous Biotech, Bangalore), 2.5 mM each dNTPs (Chromous Biotech, Bangalore) and 100 p mol of primers (GeNei, Bangalore). The DNA amplification was performed in the Corbett RG 6000 thermo cycler using the following conditions: complete denaturation (94°C for 5 min), 10 cycles of amplification (94°C for 45 sec, 35°C for 1 min and 72°C for 1.5 min) followed by 30 cycles of amplification (94°C for 45 sec, 38°C for 1 min and 72°C for 1 min) and the final elongation step (72°C for 5 min). All PCR products were separated on 1.5% (w/v) Agarose gel containing ethidium bromide (0.5 µg / ml). The gel was photographed with HP Alpha-imager.

Data Analysis

The RAPD profiles were analyzed based on the presence or absence of individual RAPD bands. The genetic distance was calculated by the coefficient of frequency similarity. The matrix of genetic distance was used for grouping the lemongrass cultivars based on the Dendrogram constructed by UPGMA (unweighed pair group method with Arithmetic averages)

RESULTS

Fig.1: DNA fingerprint, Dendrogram and Matrix for OPU 8



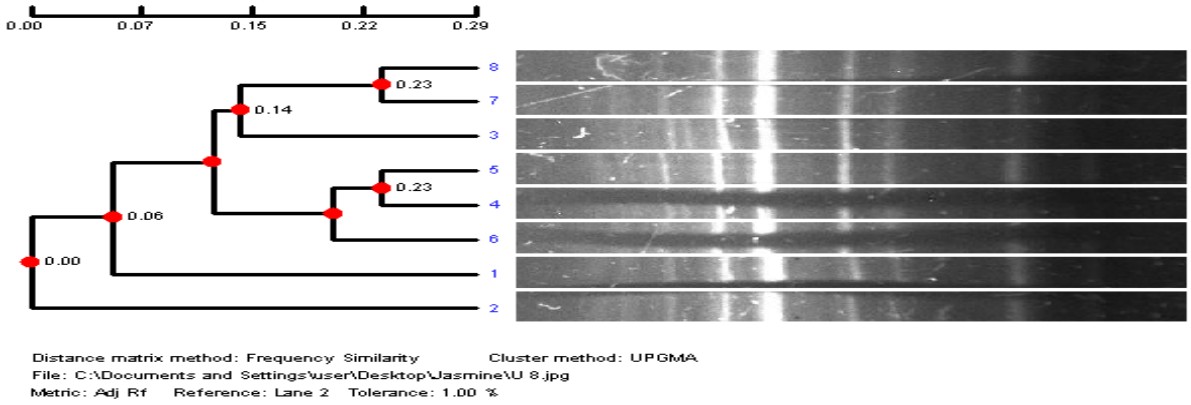


Fig.2: DNA fingerprint, Dendrogram and Matrix for OPU 9

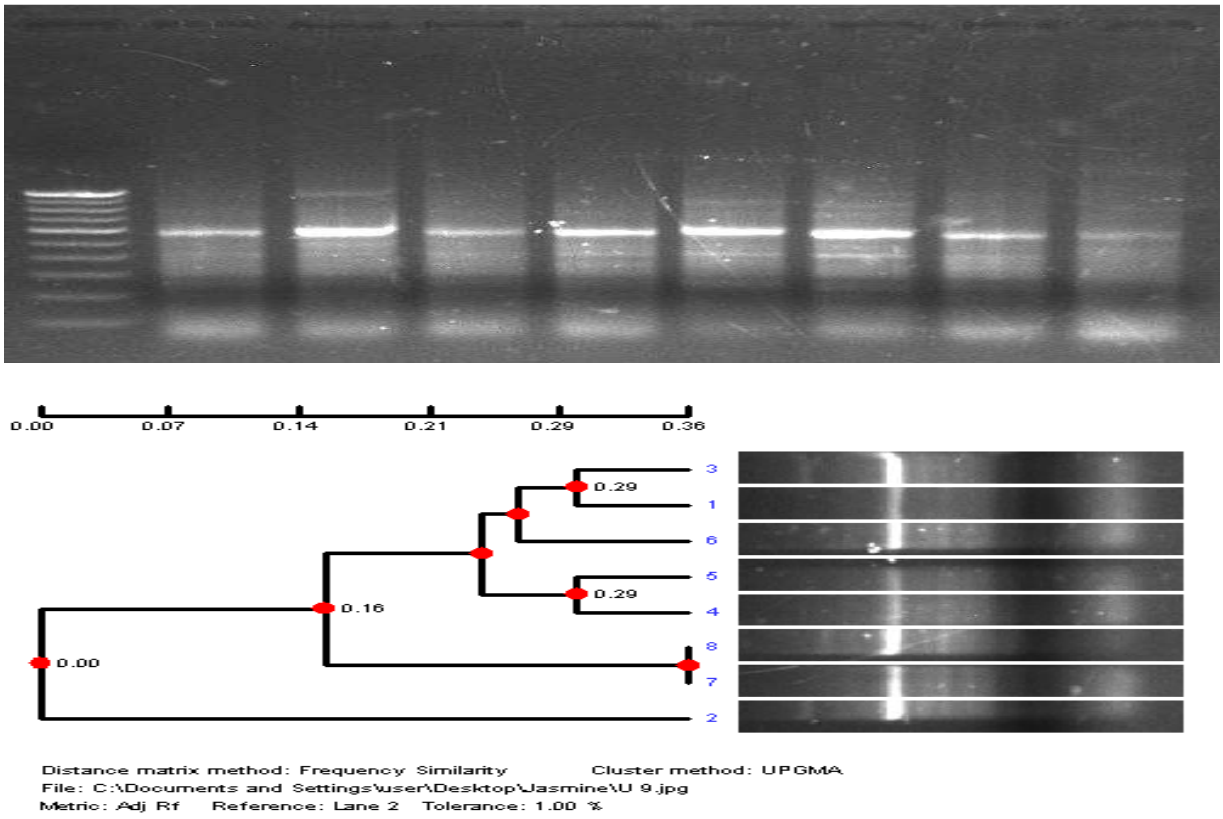
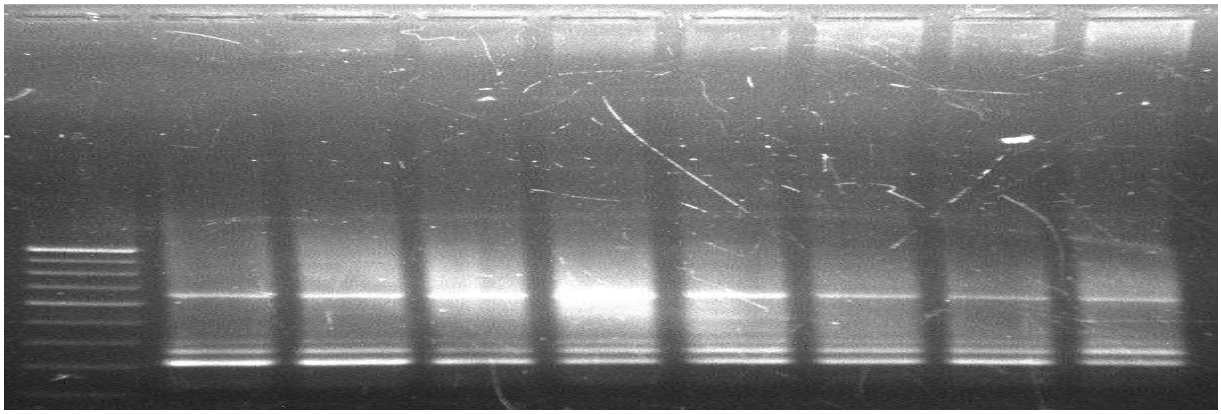


Fig.3: DNA fingerprint, Dendrogram and Matrix for OPU 10



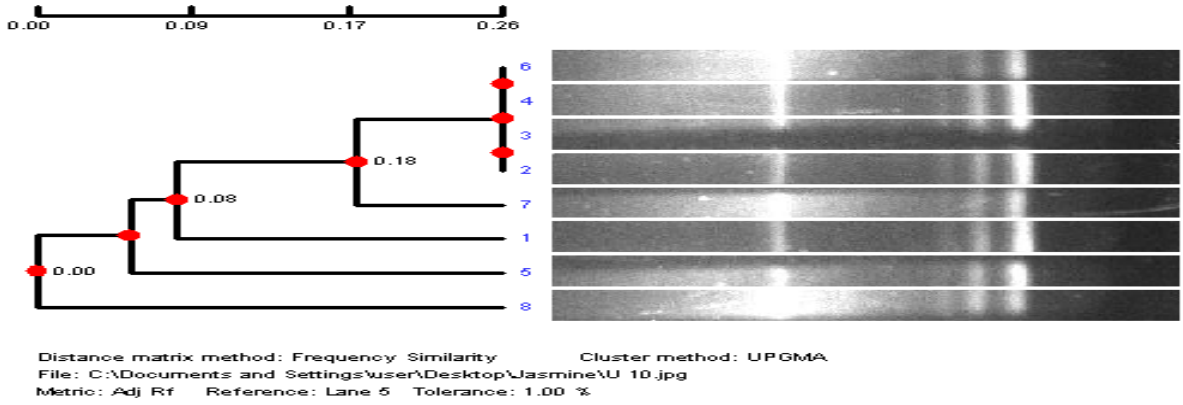


Fig.4: DNA fingerprint, Dendrogram and Matrix for OPU 11

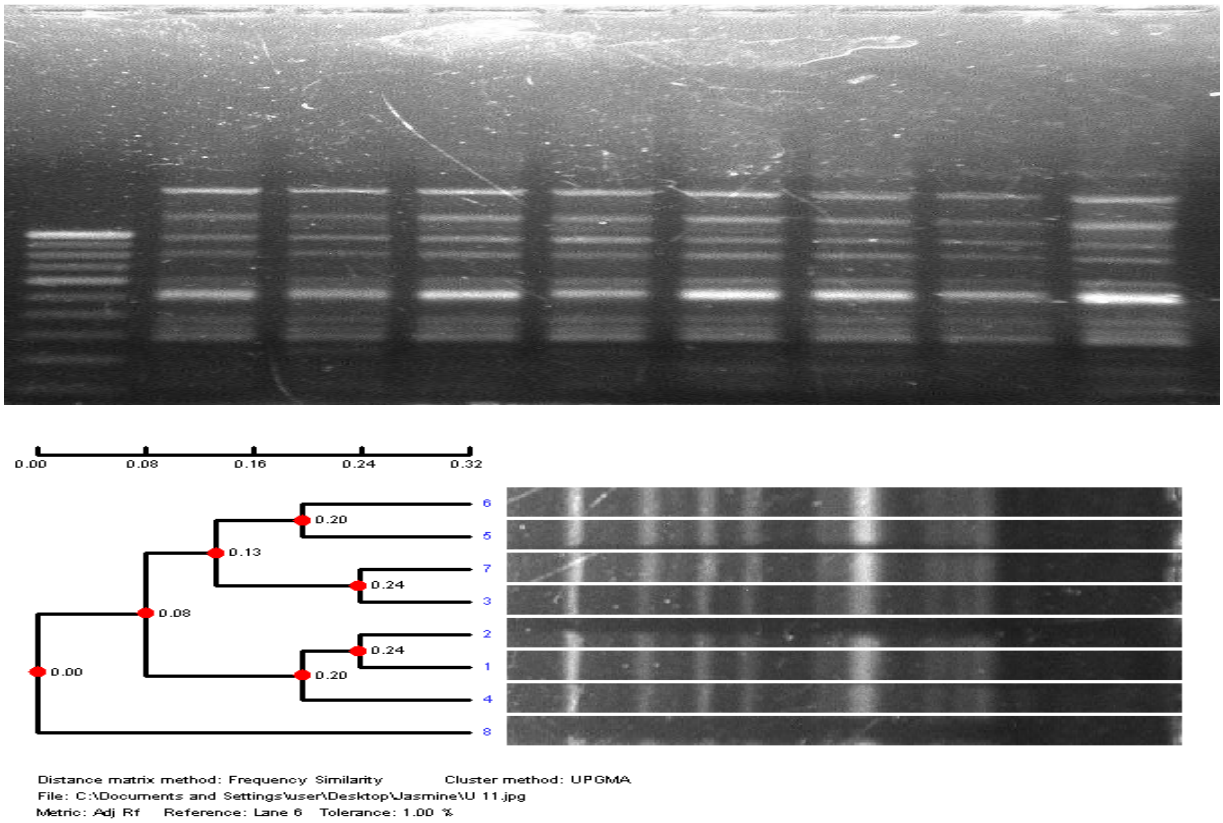
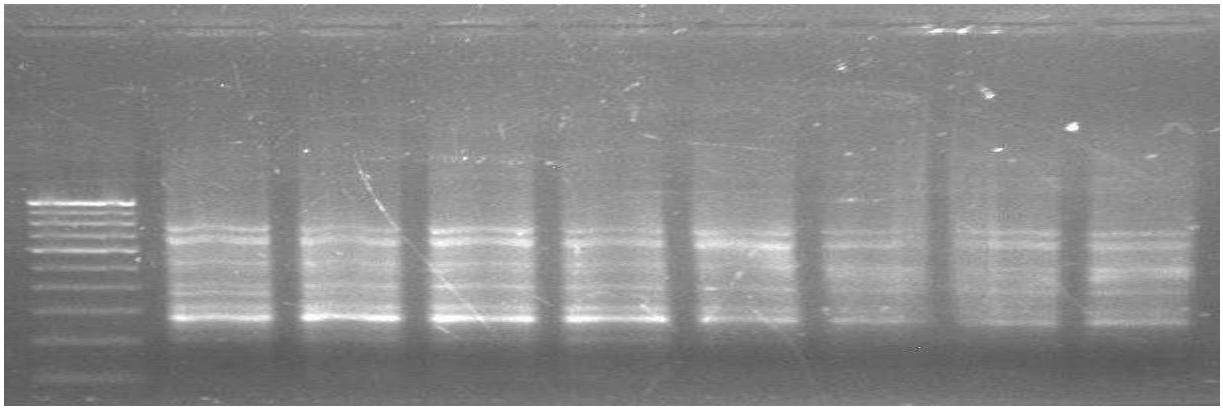


Fig.5: DNA fingerprint, Dendrogram and Matrix for OPU 12



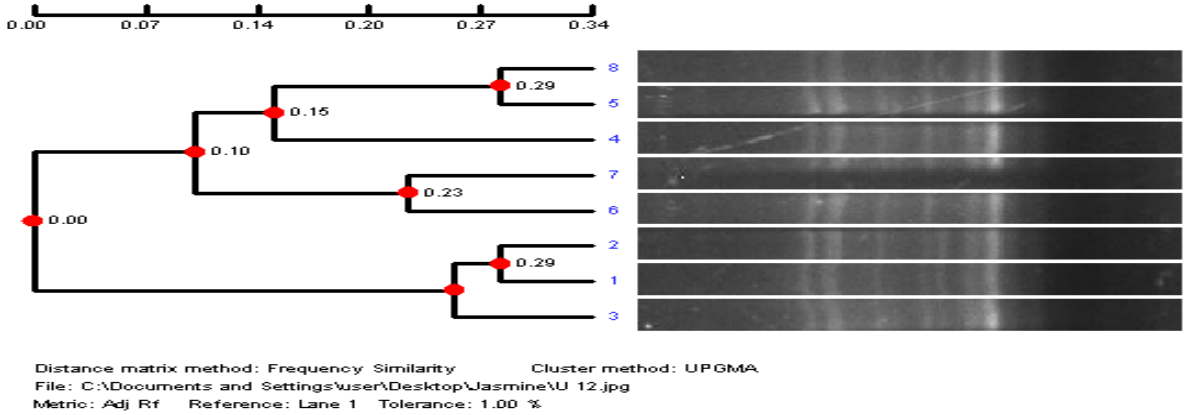


Table 1: RAPD Band pattern of different primers

Pattern of polymorphism	OPU 8	OPU 9	OPU 10	OPU 11	OPU 12	TOTAL
Total No. of bands	55	44	33	82	61	275
Total No. of polymorphic bands	14	6	3	4	12	39
Total No. of monomorphic bands	40	15	24	56	46	181
Total No. of unique bands	2	1	2	1	0	6
Polymorphism, %	25.5	13.6	9.1	4.9	19.7	14.2
Monomorphism, %	72.7	34.1	72.7	68.3	75.4	65.8
Uniqueness, %	3.6	2.3	6.1	1.2	0.0	2.2

Here, the quantitative estimation of genomic DNA was done by Thermo Scientific Nanodrop 1000 spectrophotometer. The genomic DNA were obtained in high concentration for all the samples and they showed a good 260/280 ratio (i.e. between 1.8 and 2.0) indicating absence of any protein or RNA contaminants.

Ten random primers (OPU8, OPU9, OPU10, OPU11, OPU12, OPX6, OPX9, OPZ8, OPZ9 and OPZ 10) produced clear banding patterns which were used for dendrogram construction. The amplification product generated by each RAPD primer were scored as ‘‘1’’ or ‘‘0’’ for presence or absence of specific band. OPU8 produced a total 55 bands ranging from 6-8, OPU9 produced a total 44 bands ranging from 2-8, OPU10 produced a total 33 bands ranging from 3-6, OPU11 produced a total 82 bands ranging from 9-12, OPU12 produced a total 61 bands ranging from 5-9.

DISCUSSION

Plants are potent biochemical factories for bioactive components for medicinal use. Plant constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds etc. Jasmine plants are of great economic value as a field crop for the florist, landscape, medicinal and pharmaceutical industries. The present study deals with determining genetic diversity and phylogenetic relationship among eight *jasmine* species by RAPD markers. The isolated genomic DNA gave single, sharp and distinct bands devoid of any smear on 0.8% agarose gel. Thus, genomic DNA of good quality without any degradation was successfully isolated from all the eleven samples.

To estimate the similarity and genetics distance among different jasmine, cluster analysis based on frequency similarity with weighted pair-group with arithmetic average (UPGMA) was performed using the Alpha Imager HP and dendrogram was constructed.

The investigation on diversity analysis among jasmine samples revealed vast genetic variations among the samples. The investigation revealed good polymorphism among jasmine species. In the present study, 8 different types of jasmine samples were subjected to diversity analysis. 39 polymorphic bands were obtained from 5 different random primers (listed in table no 1 above) out of 275 total bands. The different primers produced different number of bands in PCR. Even though all the varieties of jasmine have the same DNA profile, there were somehow some bands that were different from the other.

Similar observations were made in rose by Galligo and Martinez[10] and Matsumoto and Fukui[11]. Further Wolff *et al.*[12] and Scott *et al.*[13] studied genetic variations in chrysanthemum using RAPD technology and observed that the variation between cultivars was high. A wide variation was noticed among the genotypes studied in the present investigation with respect to morphological characters such as plant habit, leaf and floral characters. Similar study revealing differences in morphological characters in *Heliconia sp.* with RAPD markers was reported by Prakash *et al.*[14] which also listed triploid cultivars of *Heliconia psittacorum*. These showed identical RAPD profile with 10 different primers. Renou *et al.* (1997) reported varietal identification through similar kind of study in *Pelargonium sp.*

CONCLUSION

The results of present study showed that RAPD analysis provides a good tool to detect and classify the genetic diversity of *Jasminum* spp. Cluster analysis based on RAPD primers clearly classified the different species of *jasmine* into different clusters or groups. Wide genetic diversity as detected in this study has provided a solid plat form for further improvement of jasmine flower as aromatic floricultural crop. Genetic mapping of the *jasmine* genome will help in understanding their complex traits such as yield, size, colour, flavour and shelf-life. Data obtained from this experiment demonstrated that morphological analyses together with RAPD markers are useful for classification and indication of relationships among different *Jasmine* spp and can be useful for *Jasmine* breeding program.

REFERENCE

1. Bhattacharjee, R., Taxonomic studies in Stachys II: A new ingfrageneric classification of Stachys L – Notes Royal Bot. Garden Edinburgh, **38**: 65-96 (1980)
2. Butler, M.S., The role of natural products in drug discovery. *J Nat Prod*, **67**: 2141–2153 (2004)
3. Green, P., & Miller, D. The genus Jasminum in cultivation. Royal Botanic Gardens, (2009)
4. Kirtikar, K.R. Basu, B.D., Indian Medicinal Plants. Allahabad, India. 2nd Ed, **2**: 1523 (1993)
5. Nayak, B.S. Mohan, K. I., *J. Physiol Pharmacol*, **51(2)**: 189-194 (2007)
6. Newman, D.J. Cragg, G.M. Snader, K.M., Natural products as sources of new drugs over the period 1981–2002. *J Nat Prod*, **66**: 1022–1037 (2003)
7. Sandeep, Paarakh, P.M. Gavani, U., *J. Pharm Res.* **2(7)**: 1206-1207 (2009)
8. Sharma, P.C. Yelne, M.B. Dennis, T.J., Database on Medicinal Plants Used in Ayurveda. CCRAS, New Delhi 2005; 332-345.
9. Shekhar, S., Sriram, S., & Prasad, M. P. Genetic Diversity Determination of Jasmine Species by DNA Fingerprinting Using Molecular Markers. International Journal of Biotechnology and Bioengineering Research. Volume 4, Number 4, 2013; pp. 335-340.
10. Gallego, F. J. And Martinez, I., Molecular typing of rose cultivars using RAPDs. *J. Hort. Sci.*, **71(6)**: 901-908 (1996)
11. Matsumoto, S. And Fakui, H., Identification of rose cultivars and clonal plants by random amplified polymorphic DNA. *Sceintia Hort.*, **67**: 49-52 (1996)
12. Wolff, K., Schoen, E.D., Peters, J., Van Rijn, Optimizing the generation of random amplified polymorphic DNAs in Chrysanthemum, *Theor. Appl. Genet.*, **86**: 1033-1037 (1993)
13. Scott, M.C., Caetano, A.G. And Trigiand, R.N., DNA amplification finger printing identifies closely related chysanthemum cultivars. *J. Amer. Hort. Sci.*, **121**: 1047-1048 (1996)
14. Prakash, N. Govindranath sing, K. Bahorun, T. And Venkatasamy, S., Assessing genetic diversity of some Anthurium andraeanum Hort. Cut-flower cultivars using RAPD Markers. *African J. Biotech.*, **4**: 1189-1194 (2005)