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ASSESSMENT OF PHYLOGENETIC RELATIONSHIP AMONG *OCIMUM* SPECIES USING RAPD MARKERS

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ABSTRACT: The genetic diversity of 11 accessions of *Ocimum* using random amplified polymorphic DNA (RAPD) markers has been studied. Out of 20 selected RAPD primers 4 primers were amplified in all *Ocimum* species accessions. A total of 54 bands were scored corresponding to an average of 13.5 bands per primer with 52 bands showing polymorphism (96.3%). Two out of four primers gave 100% polymorphism. Jaccard similarity coefficient ranged from 0.195 to 0.695. A dendrogram constructed based on the UPGMA clustering method exhibited two clusters.

Keywords: *Accessions*, *Genetic diversity, Ocimum,*

INTRODUCTION

Ocimum belongs to the family *Lamiaceae* is an important genus of aromatic herbs or shrubs. Earlier, the number of species included in this genus was reported to be about 150 (Anonymous, 1966), but very recently they have been cut down to about 65 species. It is widely distributed in the tropical, sub tropical and warm temperate regions of the world (Paton *et al*., 1999). Nine species are found in India, of which three are exotic (Anonymous, 1966; Willis, 1966). The plants of this genus yield essential oils comprising a number of aromatic chemicals. Genetic diversity has been created at inter- and intraspecific levels in a crop germplasm by evolutionary forces (Stebbins, 1957) and is an important parameter utilized for crop improvement either by selection or applying various breeding methodologies. Information on genetic diversity is also valued for the management of germplasm and for evolving conservation strategies.

Different types of marker systems have been used for genetic analysis and characterization studies. These include morphological, cytological, biochemical and DNA marker systems. Various types of DNA markers are now available which includes variable number of tandem repeats (VNTRs; Nakamura *et al*., 1987), simple sequence repeats (SSRs; Jacob *et al*., 1991), and random amplified polymorphic DNA (RAPD; Williams *et al*., 1990). RAPD technique has gained importance due to its efficiency, relative ease to perform the assay and non-requirement of DNA sequence information (Karp *et al*., 1997; Khanuja *et al*., 1998a). RAPD is convenient to conduct with good polymorphism and can be used in analyzing genetic diversity and the relation between species. It has been used in analyzing the relationships between strains belonging to same genera and genetic diversity in many plants, especially medicinal plants (Lanying *et al*., 2009). Although RAPD is of dominant nature, several strategies have been put forward to minimize the dominance effects on genetic variation analysis (Lynch and Milligan, 1994; Stewart and Excoffier, 1996). RAPD analysis requires only a small amount of genomic DNA and can produce high level of polymorphism and may facilitate more effective diversity analysis in plants. It provides information that can help to define the distinctiveness of species and phylogenetic relationships at molecular level (Arya *et al*., 2011). Use of such techniques for germplasm characterization may facilitate the conservation and utilization of plant genetic resources, permitting the

identification of unique genotypes or sources of genetically diverse genotype. The present study was undertaken to characterize genetic diversity in germplasm of *Ocimum* species using RAPD markers.

MATERIALS AND METHODS

Plant material

Eleven accessions collected from different locations including NBPGR New Delhi (Four), CIMAP Lucknow (Two), local (Four) and wild (One) were maintained at herbal garden of department of Plant Physiology, Jawaharlal Nehru Agricultural University, Jabalpur (Table 1) were used to analyze their phylogenetic relationships.

Table 1: *Ocimum* species accessions used for RAPD analysis

Sl. No.	Accession name	Collection place
1	OL1(*O.basilicum*)	Barela, Jabalpur
2	OL2 (*O.americanum*)	Herbal garden JNKVV, Jabalpur
3	OL3 (*O.kiliman1dscharicum*)	Herbal garden JNKVV, Jabalpur
4	OL4 *(O. gratissimum)*	Herbal garden JNKVV, Jabalpur
5	EC 387377	NBPGR
6	EC 388776	NBPGR
7	IC 369247	NBPGR
8	IC 391924	NBPGR
9	Cim Somya (*O.basilicum*)	CIMAP (Lucknow)
10	Cim Ayu (*O. sanctum*)	CIMAP (Lucknow)
11	OW	Wild

DNA extraction

Fresh leaves were pulverized in liquid nitrogen and DNA was extracted from each plant of *Ocimum* species according to the method described by Doyle and Doyle (1987) at Biotechnology Centre, Jawaharlal Nehru Agricultural University, Jabalpur. Total DNA was quantified by ethidium bromide staining on 0.8% agarose gel electrophoresis using known concentration of λHindIII DNA ladder.

PCR amplification using RAPD primers

Twenty random decamer RAPD primers were screened which were obtained from Operon technologies. The PCR reactions were accomplished in a 20µl reaction mixture containing 10 X assay buffer, one unit of *Taq* DNA polymerase, 200µM of each dNTP, 0.2 µM primers and 50 ng of template DNA. The PCR reaction was carried out in DNA thermal cycler (ESCO). The PCR amplification conditions for RAPD consisted of initial extended step of denaturation at 94°C for 4 minutes followed by 45 cycles of denaturation at 94°C for 1 min, primer annealing at 37°C for 1 min and elongation at 72°C for 4 min. The PCR products were visualized on 1.5% agarose gel.

Statistical analysis

The RAPD bands were scored for presence (1) or absence (0) and each band was regarded as locus. All calculations were done using computer program NTSYS-pc version 2.02 (Rohlf, 1998). Pair wise similarity matrices were generated by Jaccard's coefficient of similarity by using SIMQUAL format of NTSYS-pc software. A dendrogram was constructed by using the UPGMA with SAHN module of NTSYS-pc software to show a phenetic representation of genetic relationship as revealed by the similarity coefficient.

RESULTS

Twenty random decamer primers purchased from Operon Technologies were screened taking DNA of four *Ocimum* samples before performing RAPD analysis in all the genotypes. Out of 20 primers used for screening, 16 did not amplify any fragment while, other 4 primers generated amplicons ranging from 10 (OPAB-07) to 19 (OPC-06). The reproducibility of the bands generated by these 4 primers was confirmed by replicating the amplification twice or thrice. Only the bands showing reproducible amplification were considered for scoring and for further analysis. The number of polymorphic bands ranged from 9 to 19 with range of polymorphisms 90% (OPAB-07) to 100% (OPC-06, OPC-07) (Table 2). The total number of bands generated by four amplifying primers was 54 with an average amplification of 13.5 bands per primer. The average polymorphism generated by these bands was 96.3%. The size of the amplicons generated varied from 350bp to 3200bp. Fig. 1 represented electrophoretic banding pattern of *Ocimum* species.

Table 2. : Details of RAPD primers used in the study

Primer	Sequences 5'-3'	Total alleles	Polymorphic alleles	Monomorphic alleles	% Polymorphism
OPAB-07	GTAAACCGCC	10	9	1	90.0
OPB-18	CCACAGCAGT	13	12	1	92.3
OPC-06	GAACGGACTC	19	19	0	100
OPC-07	GTCCCGACGA	12	12	0	100
Total		**54**	**52**	**2**	**382.3**
Average		**13.5**	**13**	**0.5**	**95.57**

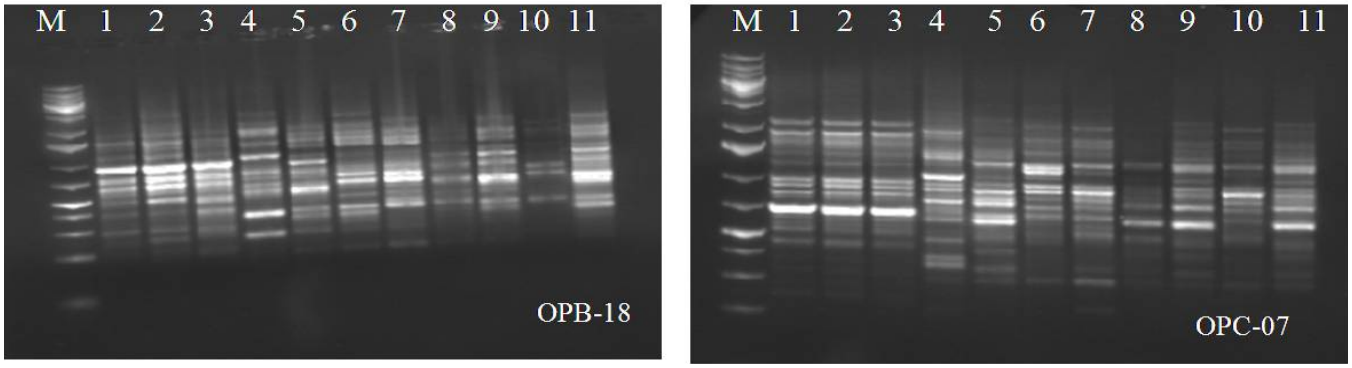

Fig. 1 - Electrophoretic banding pattern analysed by RAPD primers, M - 1Kb DNA ladder, Lane 1-11 *Occimum* accessions as described kin table 1

The Jaccard's pair wise similarity coefficient values ranged from 0.195 (OL1 and OL4) to 0.965 (OL1 and OL2) (Table 3). The clusters constructed through NTSYS-pc (2.02 version) presented in the form of dendrogram has been shown in Fig 2. The dendrogram has put all the genotypes in two groups (group A and B). Cluster-1 comprises of three accessions which were local accessions (OL1, OL2 and OL3) while Cluster–2 includes eight accessions. Cluster-2 further divided into two sub clusters first sub cluster consisted only one accession OL4 and second sub cluster comprised seven accessions namely EC387377, EC388776, IC369247, Cim Somya, OW (Wild accession), IC391924 and Cim Ayu. Principal component analysis (PCA) was carried out using Jaccard's coefficient among *Ocimum* accessions. Two dimensional (Fig. 3) and three dimensional scaling (Fig. 4) illustrated same grouping as presented in dendrogram (Fig. 2).

Table 3. : Jaccard's similarity coefficient among *Ocimum* accessions

	OL1	OL2	OL3	OL4	EC387377	EC388776	IC369247	IC391924	CimSomya	CimAyu	OW
OL1	1.0000000										
OL2	**0.9655172**	1.0000000									
OL3	0.8484848	0.8235294	1.0000000								
OL4	**0.1956522**	0.2173913	0.2500000	1.0000000							
EC387377	0.2765957	0.2978723	0.3541667	0.3720930	1.0000000						
EC388776	0.3863636	0.4090909	0.4347826	0.3953488	0.5476190	1.0000000					
IC369247	0.4000000	0.3913043	0.4782609	0.4418605	0.6341463	0.6585366	1.0000000				
IC391924	0.2500000	0.2432432	0.2820513	0.2571429	0.4411765	0.4285714	0.4857143	1.0000000			
CimSomya	0.4324324	0.4210526	0.4500000	0.4444444	0.5000000	0.5675676	0.6216216	0.5000000	1.0000000		
CimAyu	0.3243243	0.3157895	0.3500000	0.3714286	0.3589744	0.3846154	0.5135135	0.4615385	0.5333333	1.0000000	
OW	0.3902439	0.3809524	0.4090909	0.3658537	0.4878049	0.5897436	0.6842105	0.4838710	0.6875000	0.4705882	1.0000000

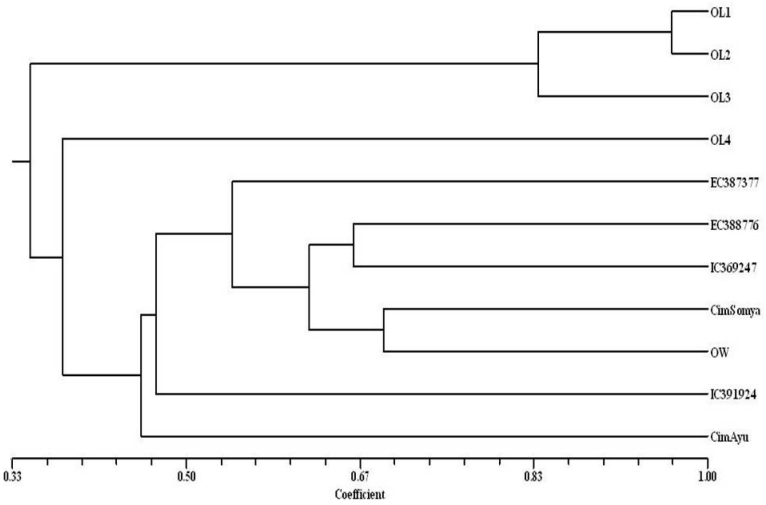

Fig. 2 : Phenetic dendrogram prepared based on genetis similarities among *Ocimum* species germplasm accessions.

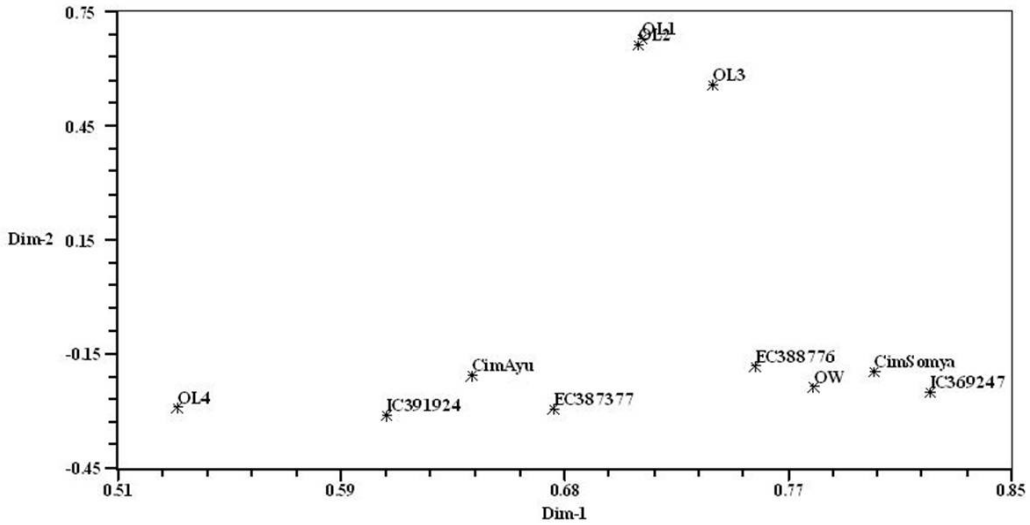

Fig. 3 : Two dimensional principal component analysis based on genetic similarities among *Ocimum* species germplasm accessions.

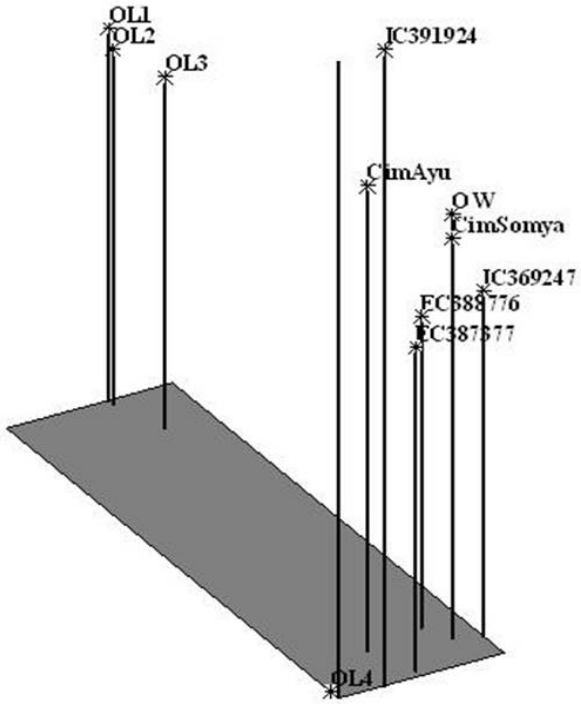

Fig. 4 : There dimensional principal component analysis based on genetic similarities among *Ocimum* species germplasm accessions

DISCUSSION

The intra-population genetic variations observed in heterogenous populations of out breeding plant species complicate the analysis of genetic diversity among populations. The present study has shown RAPD analysis to be a robust and reliable method to detect intra- and inter-specific genetic diversity and study of phylogenetic relationship in the genus *Ocimum*. The number of genetic loci detected with RAPD markers are much higher than detected with morphological and chemical / biochemical (isozyme) markers (Kongkiatngam *et al*., 1995).

RAPD technology is a common and well-proven tool in genetic studies and a convenient procedure for detecting total genetic variation and it's partitioning within and among populations. The RAPD technique had been successfully used in a variety of taxonomic and genetic diversity studies (Vyas *et al*., 2009; Ahmad *et al*., 2010). The technical simplicity of RAPD technique facilitated its use in the analysis of genetic relationship in several genera (Nair *et al*., 1999; Demeke *et al*., 1992).

The major concerns regarding RAPD generated phylogeny include homology of bands showing the same rate of migration, causes of variation in fragment mobility and origin of sequence in the genome. In the present study, the marker technology was used to detect genetic variation within *Ocimum* accessions. In the present study, the medicinal plant *Ocimum* showed a high percentage of genetic polymorphism of 96.3%. Similarly, Singh *et al.* (2004) found 98% polymorphism among *Ocimum* accessions. These studies indicate that RAPD is sufficiently informative and powerful to access genetic variability of natural populations of *Ocimum*. Thus, RAPD markers will provide a useful tool in the future design of collection strategies for germplasm conservation. The genetic diversity of the plants is closely related to their geographic distribution. Species with a wide geographic area generally have more genetic diversity (Wilikie *et al*., 1993).

Ocimum species are valued as spice plants in India. Driven by commercial incentives, the wild population

of this plant has been threatened with depletion in recent years due to excessive harvesting. The present study was preliminary attempt to develop RAPD primers to distinguish the eleven accessions showed that a more difficult screening of primers has to be done before RAPD markers can be developed. This study showed a significant morphological variation and a large genetic diversity within and among accessions.

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