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EVALUATION OF GENETIC DIVERSITY USING RAPD MARKERS *in Ocimum*

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ABSTRACT : Sixteen germplasm accessions belonging *Ocinum* (nine *Ocimum basilicum* and seven *Ocimum sanctum*) were subjected to Random Amplified Polymorphic DNA (RAPD) analysis in relation to morphometric parameters for estimating the extent of diversity within and between species. Morphological evaluation of the 16 accessions for selected characters showed qualitative variation among the accessions studied. The RAPD analysis revealed comparable inter and intra species variation. A total of 144 bands amplified, 90.79% was polymorphic and 9.02% was unique to a particular accession which made it distinct from all other accessions. Maximum similarity (0.71) was measured between accession IC-369247 and IC-201233 and least similarity (0.34) was measured in IC-387837 and EC-388896. The RAPD profiles would be useful in genetic improvement and authentication of *species* and genotypes of this medicinally and economically important genus.

Keywords : *Ocimum, RAPD, cluster analysis, genetic similarity.*

Ocimum belonging to the family Lamiaceae, is genus of herbaceous and medicinal plant cultivated extensively in France, Egypt, Hungary, Indonesia, Morocco, and the United States, Greece and Isreal. In India, it is grown in about 400 ha (Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan and Uttar Pradesh). Although about 160 species of *Ocimum* have been reported (Balyan and Pushpangadan, 1) Basil is the popular name given to any aromatic herb belonging to the genus *Ocimum.* Holy Basil, also called 'Tulsi' is highly revealed in *Hinduism* and also has religious significance in the Greek Orthodox Church, where it is used to prepare holy water.

Various molecular markers have been developed as powerful tools for diversity analysis and establishing relationships between species and cultivars. The assessment of genotypic indentity among individuals of a species is central to making valid biological interpretations of a species about population structure, breeding systems, reproductive biology and micro evolutionary processes within and among the species. Among various molecular markers, the Random Amplified Polymorphic DNA (RAPD) is most widely, used because it allows a rapid and inexpensive assay with different primers (Williams *et al.,* 8) Due to the technical simplicity and speed of RAPD methodology, it has been successfully used for the assessment of genetic structure and phytogenetic analysis (Gepts, 2). It has been successfully applied to studies of genetic differentiation in some genera-like *Mangifera* (Karihaloo *et al.,* 3); Eucalyptus (Keil and Griffin, 4) and *Gossypium* (Multani and Lyon, 6) etc.

In recent years, DNA-based molecular markers have been used for cultivar identification and assessment of the genetic relationships between germplasm individuals and species, and contributing on evolutionary and ecological studies (Gepts, 2)

MATERIALS AND METHODS

Sixteen accessions of *Ocimum* species were taken from NBPGR, New Delhi for the study. Seven were Exotic Collections (EC) from (USA, Russia, Philippines and Taiwan) and 9 were Indigenous Collections (IC) from different parts of the country. The indigenous accessions were randomly selected to include the maximum variability by virtue of their place of collections. representing 4 states of India (Madhya Pradesh, Gujarat, Andhra Pradesh, Jharkhand and Uttar Pradesh).

Plant DNA Isolation

Genomic DNA was extracted from 2 g of the fresh tender leaf samples using CTAB (Cetyl Trimethyl Ammonium Bromide) method with modifications as described by Khanuja *et al.* (5) and dissolved in 200 µl of high salt TE buffer (pH 8.0). For the removal of RNA, 10 µg of RNAse A was added to the DNA solutions and incubated for 10 minutes at 37°C. The DNA was then purified by phenol : chloroform extraction and ethanol precipitation. The quality and purity of the DNA were assessed, checked for quality and purity of the DNA were assessed and checked for quality by

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electrophoresis in agarose gel in 1× TAE buffer (Sambrook *et al.,* 7). Quantification of DNA was done in UV spectrophotometer by measuring optical densities at 260 nm and the ratio of OD 260/280 nm, respectively. Agarose gel electrphoresis was also carried out to check quantity and quality of extracted DNA. Finally all DNA samples were diluted to get 50 ng μl/l solutions and were stored at – 20°C for use in RAPD assay.

RAPD Assay

A set of ten deca nucleotide random RAPD primers of Operon series (OPB, OPC, OPE and OPF) were employed for PCR amplification. For RAPD analysis four were tested. Out of which most reproducible primers were used for the fingerprinting. In the present study, fifteen RAPD primers were used for the fingerprinting, four each from OPB, OPC and OPE series and three form OPE series. The sequence and the GC content of primers are given in Table 1.

Table 1 : Sequences of GC content of random 10-mer primers used in the analysis.

Primer	Sequence 5′ to 3′	GC Content (%)
OPB-01	5′GTTTCGCTCC3′	60
OPB-02	5′TGATCCCTGG3′	60
OPB-03	5′CATCCCCCTG3′	70
OPB-04	5′GGACTGGAGT3′	60
OPC-01	5′TTCGAGCCAG3′	60
OPC-02	5′GTGAGGCGTC3′	70
OPC-03	5′GGGGGTCTTT3′	60
OPC-04	5′CCGCATCTAC3′	60
OPE-01	5′CCCAAGGTTCC3′	70
OPE-02	5′GGTGCGGGAA3′	70
OPE-03	5′CCAGATGCAC3′	60
OPE-04	5′GTGACATGCC3′	60
OPF-01	5′ACGGATCCTG3′	60
OPF-02	5′GAGGATCCCT3′	60
OPF-03	5′CCTGATCACC3′	60

DNA amplification was performed in 25 μl of reaction volume containing 25 ng of genomic DNA, 0.1 nM of each dNTPs, 1 mM $MgCl_2$, 13 ng of each primer 0.4 unit of Taq μl in each tube) was gently mixed. The PCR amplification was achieved in a thermal cycler (Biometra, USA) and the cycling conditions were : Initial denaturation at 94°C for 5 min followed by 40 cycle of 72°C for 7 min. 12 μl aliqots of amplification products were photographed using an Alpha Image Gel Documentation System. Amplified fragments were scored as presence (1) or absence (0) of individuals. Initially a genetic similarity matrix was constructed using Jaccard's Similarity Coefficient. The similarity matrix was subsequently used to construct a tree for cluster analysis by UPGMA (Unweighted Pair Group Method with Arithmetic average) method using the computer package NTSYS 2.1

RESULTS AND DISCUSSION

The RAPDs generated were used to determine the genetic diversity among 16 accessions of *Ocimum* as depicted in Table 1. Among all primers three (OPB-2, OPB-4 and OPC-4) yielded maximum amplification products with all *Ocimum* accessions. The primers amplified DNA products from each *Ocimum* accession generated reproducible band patterns. The remaining primers gave patterns that were identical or had differences too small to provide information on the genetic diversity. Analysis of sixteen accessions of *Ocimum* revealed 91% of polymorphism. The total 144 bands were scored for the 14 RAPD primers out of which 13 bands were monomorphic. A total of 144 bands were amplified with an average of 9.6 bands per primer and these were in the size range from 800 pb–2800 bp. The individual primers generated bands ranged from 6 (with the primer OPF-02) to a maximum of 14 (with primers OPB-04 and OPC-04). Out of 144 bands, 131 (91%) were found to be polymorphic for one or more accessions. About ten primers showed the highest polymorphism of 100% (Table 2). Results of the present study revealed an average of 8.7% polymorphic bands per primer. OPC-02 amplified the least polymorphism of 25% Amplification profiles observed with two primers (OPB-04 and OPC-04) are shown in Fig. 1 a and b, respectively. 13 unique (specific to an accession) bands (9%) were also indentified. OPE-03 amplified maximum number of accessions specific bands of 10. Among all the 15 primers tested only five produced at least one unique band while rest of the 10 produced no unique band (Table 2). Significant inter and intra specific variations could be visualized as evident from the similarity coefficients (Table 3) developed on the basis of relative indices among all possible pairs. Similarity of 0.71 was observed between accession IC-369247 and IC-201233 while least similarity of 0.34 was measured in IC-387837 and EC-387837 and EC-388896 (Table 3). Cluster analysis using UPGMA (Fig. 2) generated from this matrix classified these accessions into two major clusters. The second major group sub divided into two sub groups. The first sub group (includes IC-388895 and IC-75730) showed similarity with EC-388892. The correlation coefficient calculated between RAPD when using the similarity

Table 2 : List of RAPD primers along with percentage of polymorphism and unique bands detected.

Primer	Sequence 5′ to 3′	No. of scored bands	No. of polymorphic bands	Polymorphism (%)	No. of unique bands	Unique rate (%)
OPB-01	5′GTTTCGCTCC3′	10	10	100	0	0
OPB-02	5′TGATCCCTGG3′	12	10	83.3	2	16.7
OPB-03	5′CATCCCCCTG3′	10	10	100	0	0
OPB-04	5′GGACTGGAGT3′	14	14	100	0	0
OPC-01	5′TTCGAGCCAG3′	10	10	100	0	0
OPC-02	5′GTGAGGCGTC3′	8	2	25	6	75
OPC-03	5′GGGGGTCTTT3′	10	10	100	0	0
OPC-04	5′CCGCATCTAC3′	14	14	100	0	0
OPE-01	5′CCCAAGGTTCC3′	9	9	100	0	0
OPE-02	5′GGTGCGGGAA3′	8	8	100	0	0
OPE-03	5′CCAGATGCAC3′	12	8	66.6	4	33.4
OPE-04	5′GTGACATGCC3′	8	8	100	0	0
OPF-01	5′ACGGATCCTG3′	9	9	100	0	0
OPF-02	5′GAGGATCCCT3′	6	5	83.3	1	16.7
OPF-03	5′CCTGATCACC3′	14	4	28.5	10	71.5
Total		144	131	91	13	9
Average		9.6	8.7	6.06	0.8	0.6
Range		6-14	2-14	28.5-100	0-10	0-71.5

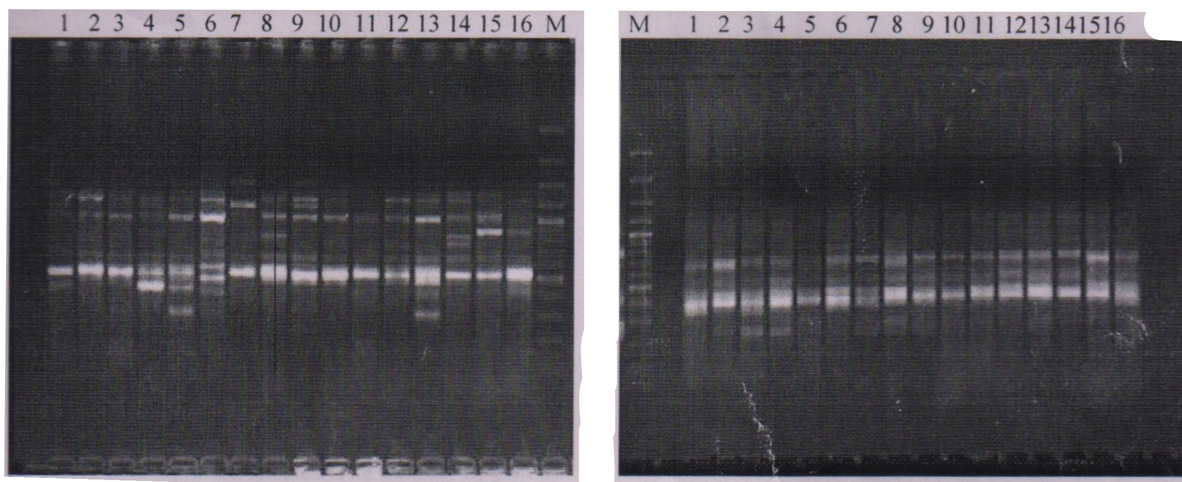

Fig. 1 : RAPD profiles generated from 16 *Ocimum* accessions with primer OPB-04 (A) and primer OPC-04 (B). Lanes 1-16 are EC38892, 2-IC7530, 3-IC326735, 4-IC388895, 5-IC201233, 6-EC344638, 7-EC 388896, 8-IC326732, 9-EC388889, 10-IC344681, 11-IC434653, 12-IC434663, 13-EC11248, 14-IC387837, 15-EC3888788, 16-388990

90.97% when using the dendogrames. The similarity as differences of accessions among these groups is expected because of their genetic resemblace or genetic divergence. A result of this study suggests that the molecular diagnoses of cultivars of *Ocimum* differ very little among themselves. Observations suggest that the genetic base may be utilized in their breeding programme and these observations may be considered for utilization in plant improvement programme.

Genetic similarity measured through analysis of RAPD data of sixteen accessions of belonging to two *Ocimum* species revealed varying degree of genetic relatedness among accessions belonging to different species. The highest similarity (0.71) was measured between accession IC-369247 and IC-201233 and

Table 3 : Similarity indices of RAPD markers of *Ocimum* species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.00															
2	0.63	1.00														
3	0.54	0.51	1.00													
4	0.56	0.62	0.40	1.00												
5	0.53	0.59	0.49	0.58	1.00											
6	0.50	0.54	0.43	0.59	0.61	1.00										
7	0.47	0.48	0.39	0.58	0.53	0.60	1.00									
8	0.50	0.58	0.45	0.62	0.57	0.57	0.66	1.00								
9	0.49	0.75	0.41	0.60	0.58	0.62	0.64	0.67	1.00							
10	0.46	0.50	0.41	0.51	0.55	0.65	0.54	0.56	0.63	1.00						
11	0.44	0.51	0.41	0.53	0.71	0.69	0.55	0.53	0.59	0.69	1.00					
12	0.47	0.52	0.38	0.53	0.57	0.63	0.45	0.53	0.59	0.55	0.62	1.00				
13	0.44	0.50	0.42	0.51	0.56	0.56	0.58	0.58	0.70	0.54	0.56	0.53	1.00			
14	0.45	0.52	0.44	0.48	0.50	0.52	0.34	0.54	0.60	0.51	0.55	0.70	0.58	1.00		
15	0.52	0.54	0.51	0.55	0.63	0.53	0.49	0.61	0.55	0.52	0.60	0.59	0.50	0.55	1.00	
16	0.53	0.59	0.46	0.63	0.55	0.68	0.54	0.54	0.65	0.53	0.53	0.64	0.54	0.57	0.60	1.00

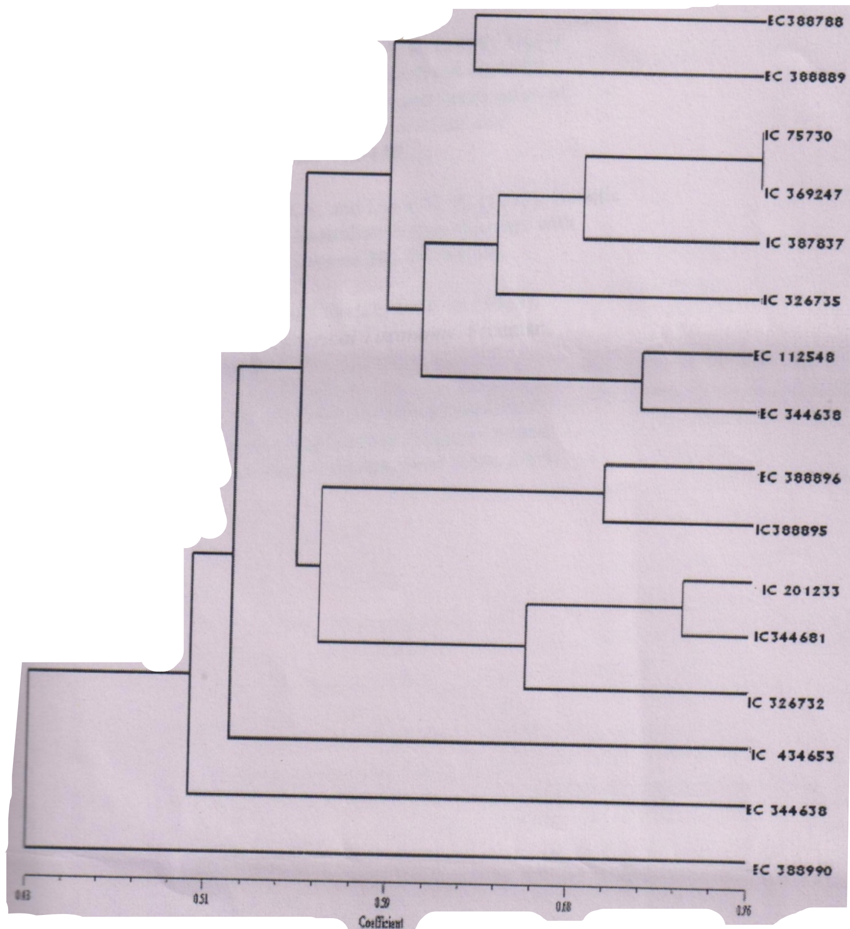

Fig. 2 : UPGMA cluser analysis-based dendrogram depicting genetic relationships among 16 accessions of *Ocimum.*

least similarity (0.34) was measured in IC-387837 and IC-388896, and the phytogenetic tree comprising a

total of sixteen accessions of *Ocimum.* RAPD markers was constructed as shown in Fig.2, Cluster analysis clearly branched out *Ocimum* accessions into two groups suggesting that these two are more divergent from other accessions. Hence, phenotypic characters combined with RAPD analysis provided a better relationship to identify these species. The number of genetic loci detected with RAPD markers are much higher than detected with morphological and chemical/biochemical markers.

In the dendogram, the sixteen accessions were divided into the major groups. The second major sub divided into two sub groups. The first sub group includes IC-388895 and IC-75730 showed similarity with EC-388892. The second sub group was further sub divided into three sub groups, where the first sub group EC-388990 and EC-388788, the second sub group include IC-344681 with two genetic similar accessions IC-364247 and IC-201233, IC-344638 accession was also similar with these accessions. IC-434653 and IC-387831 was also seems to be similar with accessions of second sub group, the third sub group includes IC-326735 and IC-326732 with EC-388889 and EC-112548. The first major group include EC-388896 accession only. The similarity and differences of accessions among these groups is expected because of their genetic resemblance or genetic divergence.

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