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GENETIC DIVERSITY ASSESSMENT IN SIX MEDICINALLY IMPORTANT SPECIES OF *OCIMUM* FROM CENTRAL GUJARAT (INDIA) UTILIZING RAPD, ISSR AND SSR MARKERS

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ABSTRACT
Six different species of *Ocimum* were taken for studying genetic relationship using RAPD, ISSR and SSR markers. Eleven RAPD markers collectively amplified 536 bands, comprised in 329 loci, all bands were polymorphic. Three ISSR markers collectively amplified total 209 bands with 112 loci; all were polymorphic. Two SSR markers generated total 71 bands with 41 loci. Analysis of all three markers expressed 100% polymorphism. Through RAPD and SSR analysis, it was revealed that *O. basillicum* and *O. polystachyon* are the most similar with highest similarity index of 0.28000 and 0.61538, respectively and through ISSR analysis, the highest similarity index of 0.32258 was observed between *O. americanum* and *O. basillicum*. The combined analysis of RAPD, ISSR and SSR revealed that *O. basillicum* and *O. americanum* were the most similar with highest similarity index (0.2892) and the least similarity index of 0.01123 was observed between *O. viride* and *O. americanum.*

KEYWORDS: Genetic similarity; *Ocimum;* Dendogram; RAPD; ISSR; SSR.

INTRODUCTION

Various species of *Ocimum* belonging to family Lamiaceae are very important for their therapeutic potentials and medicinal properties. Genus *Ocimum* contains more than 150 species (Anonymous, 1966). *Ocimum sanctum* L. (holy basil), *Ocimum gratissimum* (African basil), *Ocimum basillicum* (sweet basil), *Ocimum ammericanum are* some examples of important species of genus *Ocimum*. Plants of this genus are widely distributed in warm temperate, tropical and sub tropical regions of the world (Paton *et al.* 1999). The plants of this genus are grown for the essential oils in leaves and stems. Essential oils from the plant have been reported to possess an interesting spectrum of antifungal properties (Dubey *et al.* 2000; Lemos *et al.,* 2005), antinociceptive property (Rabelo *et al.* 2003), anticonvulsant (Raj *et al.,* 2003), antioxidant (Devi *et al.,* 2001; Javanmardi *et al.,* 2003), germicidal (Holetz *et al.,* 2003; Pessoa *et al.,* 2002) and antimalarial activity (Ezekwesili *et al.,* 2004). The presence of many pharmacologically active compounds in *Ocimum* species provides them protection against free radical induced oxidative damage of cellular components. The genus *Ocimum* is very variable and possesses wide range of genetic diversity. Genetic diversity can be seen at intra specific and inter specific levels (Stebbins, 1957). Analysis of genetic diversity in crop species is an important component of crop improvement programs; also it is helpful in constructing genetic maps. Study of genetic diversity is the process by which variation among individuals or groups of individuals or populations is analyzed by a specific method or a combination of methods (Mohammadi and Prasanna, 2003). Genetic diversity studies can be done at different levels such as morphological, cytological, biochemical and DNA marker systems. Assessment of genetic diversity at the molecular level is more meaningful than at the phenotypic level as the later involves data on morphological traits which are environmental dependent. Though, they significantly contribute towards phenotypic variation but cannot be accurately phenotyped. So the study of polymorphism is best done at the level of arrangement of nucleotide bases in DNA, the primary source of all biological information (Mukharib *et al.,* 2010).

Several novel DNA-markers: Variable Number of Tandem Repeats (VNTRs, Nakamura *et al.,* 1987), Random Amplified Polymorphic DNA (RAPD, Welsh and McClelland, 1990 and Williams *et al.,* 1990), Restriction Fragment Length Polymorphism (RFLP, Sambrook *et al.,* 1989), Simple Sequence Repeats (SSRs, Jacob *et al.,* 1991), Inter Simple Sequence Repeats (ISSR, Zietkiewicz *et al.,* 1994) etc. are available for genome analysis.

RAPD markers amplify products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers. RAPD has found a wide range of applications in many areas of biology because of its simplicity as it does not require prior knowledge of a DNA sequence. RAPD markers can detect a large number of genetic polymorphism (Williams *et at*., 1990). They can also be used in assessing diversity within plant populations (Dawson *et al.,* 1993, Hu and Quiros, 1991), for constructing linkage maps and for tracking hybrid species origins (Crawford *et al.,* 1993). Low expense, efficiency in developing large number of DNA markers in a short time and requirement for less sophisticated equipment has made the RAPD technique valuable (Bardakci, 2001).

Among various molecular markers, inter-simple sequence repeats (ISSRs) use repeat-anchored primers to amplify DNA sequences between two inverted SSRs (Zietkiewicz *et al.,* 1994). Because of high annealing temperature and longer sequence of ISSR primers, they can yield reliable and reproducible bands, and the cost of the analysis is relatively lower than that of some other markers, that is, AFLP (amplified fragment length polymorphism). Therefore, ISSRs have established wide applications in genetic diversity studies in a wide range of medicinal plant species (Yao *et al.,* 2008).

Simple sequence Repeats (SSRs) are stretches of DNA containing tandem repeating di-, tri-, or tetra- nucleotide units ubiquitously distributed throughout the eukaryotic genomes (Pearson and Sinden, 1998). SSRs are the markers of choice in many plant breeding programs because they are transferable, multi-allelic co dominant markers, PCR-based, easily reproducible, randomly and widely distributed along the genome, and their analysis may be automated. Microsatellite polymorphism is based on the different numbers of a short repeated motif at a given locus (Rafalski *et al.,* 1995).

The present study was carried out with the objective to analyse the genetic distance among six *Ocimum* s*pp.* using RAPD, ISSR and SSR markers. The results of this study can provide strong base for crop improvement and breeding programmes.

MATERIALS AND METHODS

Young leaves of six species namely, *O. sanctum*, *O. polystachyon*, *O. basillicum*, *O. viride*, *O. americanum* and *O. gratissimum* were collected from nearby areas of Anand, Gujarat, India (Table-1) for isolation of genomic DNA or they were stored at -20^{o}C for further experiment.

Genomic DNA extraction

Genomic DNA from tender leaves of all six species was isolated according to the method described by Doyle and Doyle (1990) with slight modifications. The use of an additional detergent SDS and potassium acetate was incorporated in order to remove protein impurities (Waterhouse and Glover, 1993). Also the RNaseA treatment to remove RNA was given before precipitating DNA. The final purified DNA was checked in 0.8% agarose gel electrophoresis and the purity and concentration of DNA was checked by Nanodrop 1000 (Thermo Fisher Scientific, USA).

TABLE-1: Name, Images and Place of collection of different *Ocimum spp*.

Sr. No.	Species name	Location	Image
1	*O. sanctum*	Indukaka Ipcowala College of Pharmacy botanical garden, New Vallabh Vidyanagar, Anand	
2	*O. polystachyon*	Herbal garden of Dept. of Pharmacology and toxicology, Anand Agricultural University (AAU), Anand.	
3	*O. basillicum*	Herbal garden of Dept. of Pharmacology and toxicology, Anand Agricultural University (AAU), Anand.	
4	*O. viride*	National Research Centre for Medicinal & Aromatic plants (ICAR), Boriavi, Anand.	

5	*O. americanum*	National Research Centre for Medicinal & Aromatic plants (ICAR), Boriavi, Anand.	
6	*O. gratissimum*	National Research Centre for Medicinal & Aromatic plants (ICAR), Boriavi, Anand.	

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD), SIMPLE SEQUENCE REPEATS (SSR) AND INTER SIMPLE SEQUENCE REPEATS (ISSR) ANALYSIS

In the present study total twenty one RAPD primers, six ISSR primers and six SSR primers were used. Polymerase chain reactions were performed in 25µl system containing 2.5µl of 10 X assay buffer A (10 mM Tris-Cl; pH 9.0, 1.5mM $MgCl_2$, 50mM KCl and 0.01% gelatin), 2.5 µl of each dNTPs (dATP, dTTP, dCTP and dGTP; 2.5mM; Bangalore Genei Pvt. Ltd, Bangalore, India), 1µl of primer (40µM, Sigma Aldrich, Bangalore, India), 0.5µl of Taq DNA polymerase (5U/µl; Bangalore Genei Pvt. Ltd, Bangalore, India),1µl of template DNA (20ng-320ng) and 17.5µl of deionized water. The reaction was carried out using thermo cycler (Corbett research gradient automatic, UK). Reaction was carried out in three steps. The first step of initial denaturation was performed at 94ºC for 5 min. Following the initial denaturation step, PCR was carried out for 45 cycles (in case of RAPD) and 40 cycles (in case of ISSR and SSR). Each cycle consisted of a denaturation step of 1 min at 94ºC, followed by primer annealing step for 1 min and an extension step of 2 min at 72ºC. The last cycle was followed by final extension step of 5 min (in case of RAPD) and 7 min (in case of ISSR and SSR) at 72ºC. The holding temperature was 4ºC.

Agarose gel electrophoresis

Amplified PCR products were checked on agarose gel electrophoresis (1.5% agarose gel for RAPD and 2% for ISSR and SSR), performed for four hours at a constant voltage of 100 volts. Ladder of 100bp was used to determine the size of the amplicons. The bands were visualized under U.V transilluminator and the photography was done by gel documentation system (Alpha Innotech, Alpha Imager EP, USA).

RAPD, ISSR AND SSR DATA SCORING AND STATISTICAL ANALYSIS

The photographic plate was transformed into binary matrix using '1' and '0' characters. The band was scored '1' for its presence and '0' for its absence. The polymorphism information content (PIC) value was calculated using the formula, PIC= $1-\sum p_i^2$, where p_i is the frequency of the i^{th} allele (Smith *et al.,* 1997). Further analysis was done using the NTSYS-pc (Numerical taxonomy system, applied biostatistics, Inc., New York, USA, software version 2.02e) (Rohlf, 1997), which included the construction of similarity matrix among the six species of genus *Ocimum* using the Jaccard's coefficient (Jaccard, 1908). The dendogram was constructed using the unweighted pair group method using arithmetic means (UPGMA) (Sneath and Sokal, 1973) and SAHN clustering (Sequential Agglomerative Hierarchical Nesting). Groupings of cultivars were also evaluated by principle coordinate analysis (PCA) as reported by Thomas *et al.* (2006). PCA was performed by extracting Eigen value and Eigen vectors from a correlation matrix which was generated using a standardized data matrix. 2-D and 3-D plots were constructed to evaluate the groupings of *Ocimum spp.*

RESULT AND DISCUSSION

Random amplified polymorphic DNA (RAPD)

Out of twenty one RAPD primers, eleven primers gave satisfactory amplification, from which two primers, OPO 15 and OPC 12 gave amplification in *O. viride* species only. Banding pattern of four RAPD primers OPO 15, OPN 15, OPT 20 and OPD 20 is shown in Figure 1(A), Figure 1(B), Figure 1(C) and Figure 1(D). Total 536 scorable bands were obtained; all were polymorphic which resulted in 100% polymorphism. Primer OPAF 05 gave maximum bands (65) comprised in 45 loci and minimum bands (38) were observed in two primers OPO 09 and OPO 18 comprised in 22 and 25 loci respectively. The PIC values were calculated for each primer. Highest PIC value (0.9732) was observed in OPAF 05 primer and the least PIC value (0.9418) was observed in OPO 09 primer. The average PIC value obtained was 0.9549. The amplicons were observed ranging from 148-3022bp. The largest amplicon (3022bp) was amplified by OPD 20 primer, while the shortest amplicon (148bp) was amplified by OPO 18 primer. Details regarding RAPD primers and banding patterns are shown in table-2. Similarity matrix amongst all six species of genus *Ocimum* was plotted using the Jaccard's coefficient. According to the matrix, the similarity index was ranging from 0.0097–0.2800 with mean similarity index of 0.3690. The highest similarity index, 0.2800 was observed between *O. polystachyon* and *O. basillicum* and least similarity index, 0.0097 was observed between *O. sanctum* and *O. viride* (data not shown). The dendogram constructed using UPGMA and SAHN clustering method is shown in Figure 1(E). This dendogram also showed that *O. polystachyon* and *O. basillicum* are nearest in genetic distance and *O. viride* is the farthest from all the other five species.

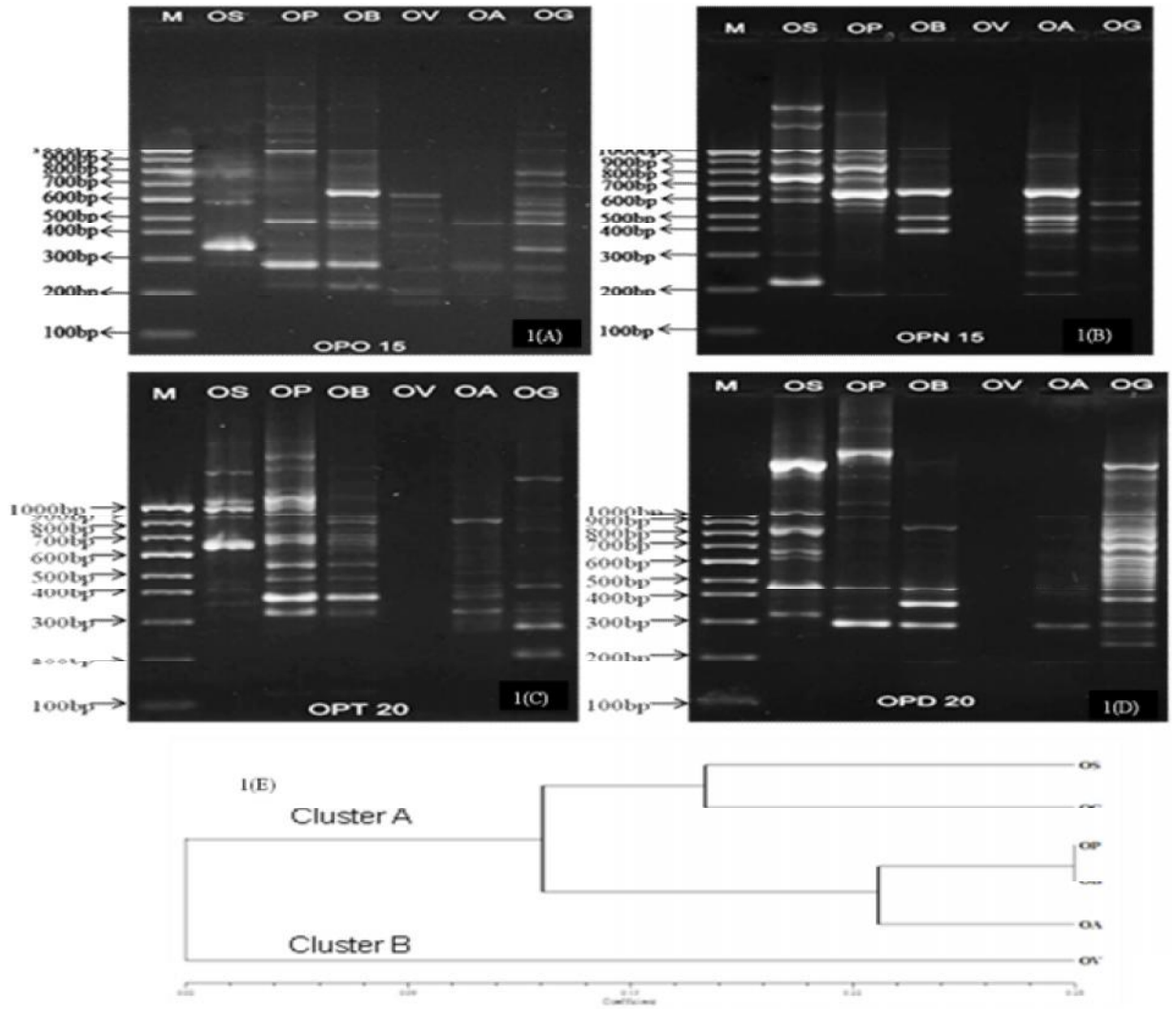

FIGURE 1. Banding pattern and dendogram generated among six *Ocimum spp*. utilizing RAPD markers; **(A)** amplification with OPO 15 primer, **(B)** amplification with OPN 15 primer, **(C)** amplification with OPT 20, **(D)** amplification with OPD 20 and **(E)** Dendogram based on UPGMA and SAHN clustering method showing genetic relationship (M=100bp ladder, OS=*Ocimum sanctum*; OG=*Ocimum gratissimum*; OP=*Ocimum polystachyon*; OB=*Ocimum basillicum*; OA=*Ocimum americanum*; OV=*Ocimum viride*)

TABLE-2: Details of RAPD primers, annealing temperature and banding patterns.

Primer	Sequence	Annealing Temperature (^oC)	Range of Amplicons (bp)	Loci	Scorable Bands	PIC Value
OPA 03	AGTCAGCCAC	35	161 - 1284	35	59	0.962942
OPAF 05	CCCGATCAGA	35	178 - 2198	45	65	0.973254
OPAF 15	CACGAACCTC	35	173 - 2088	30	46	0.955577
OPC 12	TGTCATCCCC	35	199 - 1385	32	47	0.956994
OPD 20	AACCCGGTCA	35	176 - 3022	29	54	0.949931
OPN 15	CAGCGACTGT	35	194 - 1942	31	47	0.958805
OPO 03	CTGTTGCTAC	35	134 - 1560	28	43	0.957274
OPO 09	TCCCACGCAA	35	183 - 2040	22	38	0.941828
OPO 15	TGGCGTCCTT	35	162 - 1840	25	47	0.946129
OPO 18	CTCGCTATCC	35	148 - 1736	25	38	0.945983
OPT 20	GACCAATGCC	35	206 - 2209	27	52	0.955621
Total			**148 - 3022**	**329**	**536**	**Avg PIC: 0.954942**

INTER SIMPLE SEQUENCE REPEATS (ISSR) ANALYSIS

Out of six ISSR primers, three primers gave satisfactory amplification. DNA of *O. viride* was not amplified with any of the six ISSR primers. Banding pattern of ISSR primer (GA)9T is shown in Figure 2(A). All three primers generated total 209 scorable bands with 112 loci; all were polymorphic giving 100% polymorphism. Primer (GA)9T gave maximum 79 bands comprised in 43 loci and (AGG)6 gave minimum bands of 60 comprised in 35 loci. The highest PIC value (0.9713) was observed with (GA)9T primer and the lowest PIC value (0.9620) with (GACA)4 primer, with an average of 0.9653. ISSR primers tested in present investigation produced fragments of different size. The minimum (119bp) sized fragment was amplified by primer (AGG)6, whereas maximum (1587bp) sized fragment was amplified by primer (GA)9T. Details regarding ISSR primers and banding patterns are shown in table-3. Similarity matrix amongst all six species of genus *Ocimum* was plotted using the Jaccard's coefficient. According to the matrix, the highest similarity index of 0.3225 was observed between *O. americanum* and *O. basillicum* and negligible similarity index was observed in *O. viride* with all the other five species. The mean similarity index of 0.3868 was observed (data not shown). The dendogram constructed using UPGMA and SAHN clustering method is shown in Figure 2(B). Two major clustering groups were observed in this dendogram, cluster A and cluster B. Cluster A comprised of *O. sanctum*, *O. polystachyon*, *O. basillicum*, *O. americanum* and *O. gratissimum* and cluster B comprised of *O. viride*.

TABLE-3: Details of ISSR primers, annealing temperature and banding patterns.

Primer	Annealing Temperature (°C)	Range of Amplicons (bp)	Loci	Scorable Bands	PIC Value
(GACA)4	49	325 - 1336	34	70	0.962041
(GA)9T	53.1	138 - 1587	43	79	0.971319
(AGG)6	45	119 - 1509	35	60	0.962778
Total		**119 - 1587**	**112**	**209**	**Avg PIC: 0.965379**

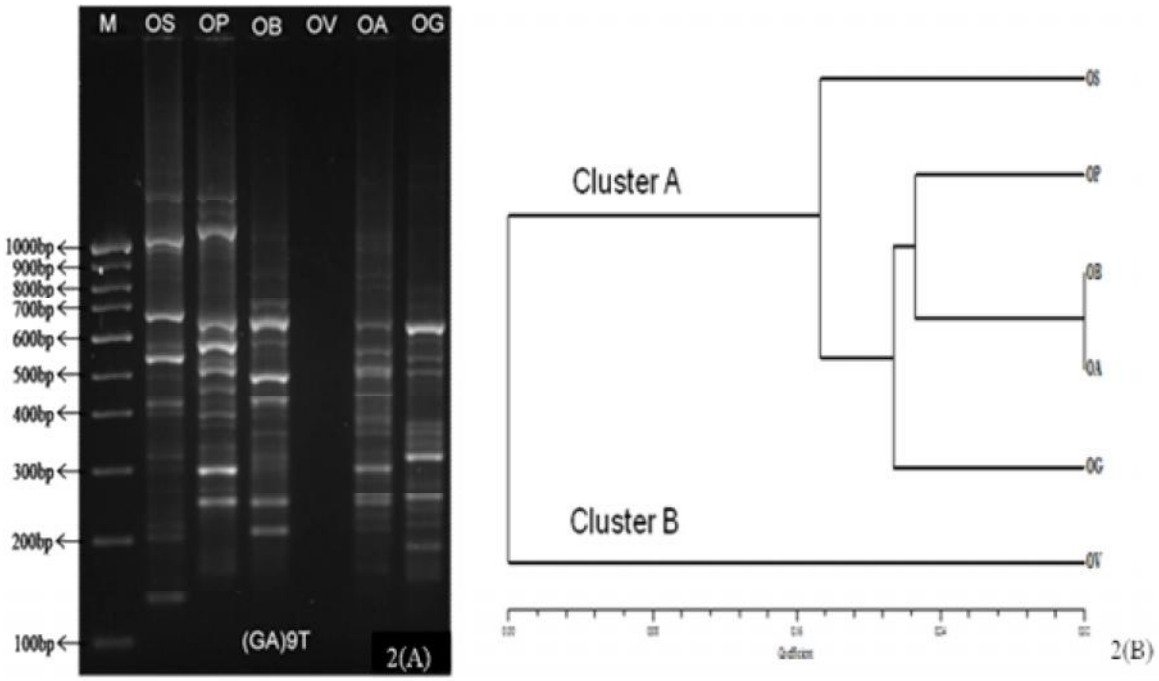

FIGURE 2 Banding pattern and dendogram generated among six *Ocimum spp*. utilizing ISSR markers; **(A)** amplification with (GA)9T primer and **(B)** Dendogram based on UPGMA and SAHN clustering method showing genetic relationship (M=100bp ladder, OS=*Ocimum sanctum*; OG=*Ocimum gratissimum*; OP=*Ocimum polystachyon*; OB=*Ocimum basillicum*; OA=*Ocimum americanum*; OV=*Ocimum viride*)

SIMPLE SEQUENCE REPEATS (SSR)

Out of six, two SSR primers gave satisfactory amplification. DNA of *O. viride* was not amplified with (GAA)7 primer, whereas (AAGC)3 primer amplified only DNA of *O. sanctum* and *O. viride*. Banding pattern of SSR primer (GAA)7 is shown in Figure 3(A). Both the primers generated total 71 scorable bands with 41 loci, all were polymorphic giving 100% polymorphism. The average polymorphic information content (PIC) value of both primers was 0.9336. Details of both the SSR primers are shown in table-4. Similarity matrix amongst all six species of genus *Ocimum* was plotted using the Jaccard's coefficient. According to the matrix, the highest similarity index (0.6153) was observed between *O. polystachyon* and *O. basillicum* and negligible similarity index was observed

in *O. viride* with other species. The mean similarity index of 0.4070 was observed (data not shown). The dendogram constructed using UPGMA and SAHN clustering method is shown in Figure 3(B). Two major clustering groups were observed in this dendogram, cluster A and cluster B. Cluster A comprised of *O. sanctum*, *O. polystachyon*, *O. basillicum*, *O. americanum* and *O. gratissimum* and cluster B comprised of *O. viride*.

TABLE-4: Details of SSR primers, annealing temperature and banding patterns.

Primer	Annealing Temperature (°C)	Range of Amplicons (bp)	Loci	Scorable Bands	PIC Value
(GAA)7	54	325 - 1336	29	58	0.956005
(AAGC) 3	60	216 - 1100	12	13	0.911243
Total		216 - 1336	41	71	Avg PIC: 0.933624

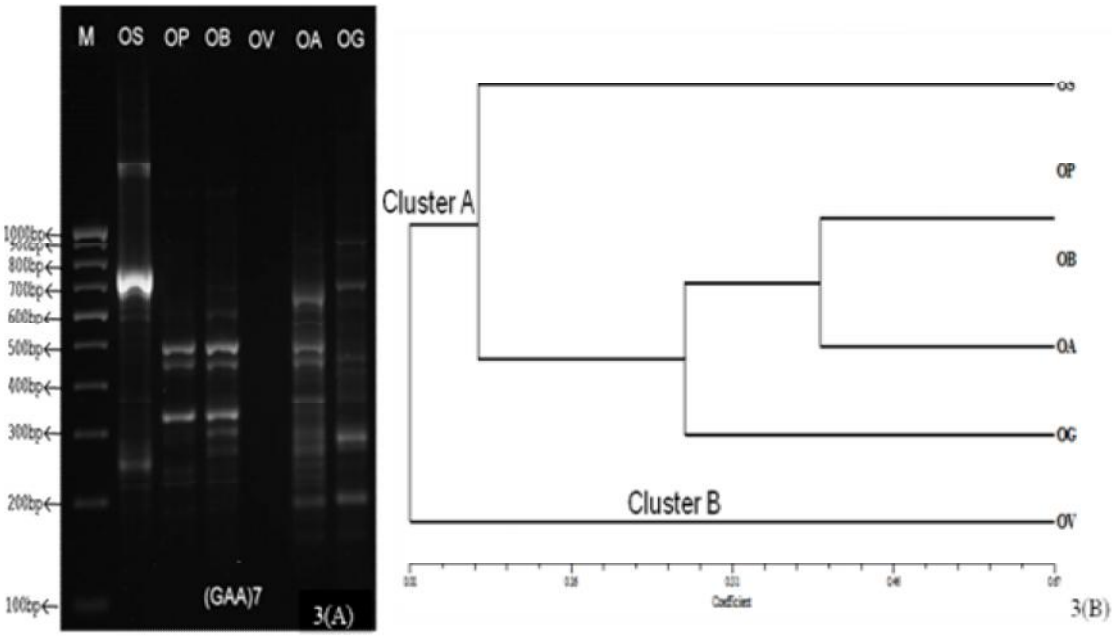

FIGURE 3. Banding pattern and dendogram generated among six *Ocimum spp*. utilizing SSR markers; **(A)** amplification with (GAA)7 primer and **(B)** Dendogram based on UPGMA and SAHN clustering method showing genetic relationship (M=100bp ladder, OS=*Ocimum sanctum*; OG=*Ocimum gratissimum*; OP=*Ocimum polystachyon*; OB=*Ocimum basillicum*; OA=*Ocimum americanum*; OV=*Ocimum viride*)

The combined RAPD, ISSR and SSR analysis detected high degree of genetic variations among the six *Ocimum spp*. The highest similarity index (0.2892) was observed between *O. basillicum* and *O. americanum* while the least similarity index (0.0112) was observed between *O. viride* and *O. americanum*. The mean similarity index was observed (0.3757), as shown in table-5. This indicates high genetic variation among six *Ocimum* species. Similar variations were observed in dendogram constructed using UPGMA method based on RAPD, ISSR and SSR markers, shown in Figure 4. Overall through this study, *O. basillicum* and *O. americanum* were observed to be the most similar among the six *Ocimum* species. However, it was observed that *O. sanctum, O. basillicum, O. polystachyon, O. americanum* and *O. gratissimum* were clustered in one group (cluster A) in phylogenetic dendogram. *O. viride* did not fall in any group and was seen the farthest in the dendogram (cluster B). Similar results can be seen in 2-D and 3-D plots as shown in Figure 5 and Figure 6 respectively.

TABLE-5: Similarity matrix based on Jaccard's coefficient revealed by RAPD, ISSR and SSR markers.

	OS	OP	OB	OV	OA	OG
OS	1.0000000					
OP	0.1438849	1.0000000				
OB	0.1235955	0.2880000	1.0000000			
OV	0.0120482	0.0160428	0.0117647	1.0000000		
OA	0.1119134	0.2043796	0.2892562	0.0112360	1.0000000	
OG	0.1580882	0.1541096	0.1814815	0.0218579	0.1637011	1.0000000

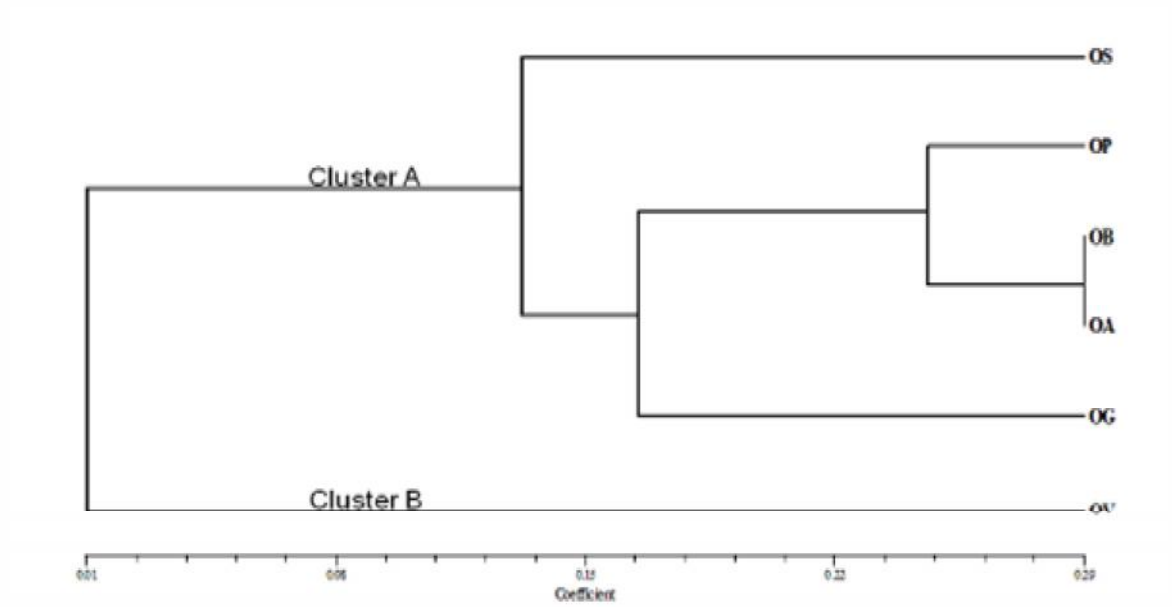

FIGURE 4. Dendogram based on UPGMA method showing genetic relationship revealed by RAPD, ISSR and SSR markers among six *Ocimum spp.* (OS=*Ocimum sanctum*; OG=*Ocimum gratissimum*; OP=*Ocimum polystachyon*; OB=*Ocimum basillicum*; OA=*Ocimum americanum*; OV=*Ocimum viride*)

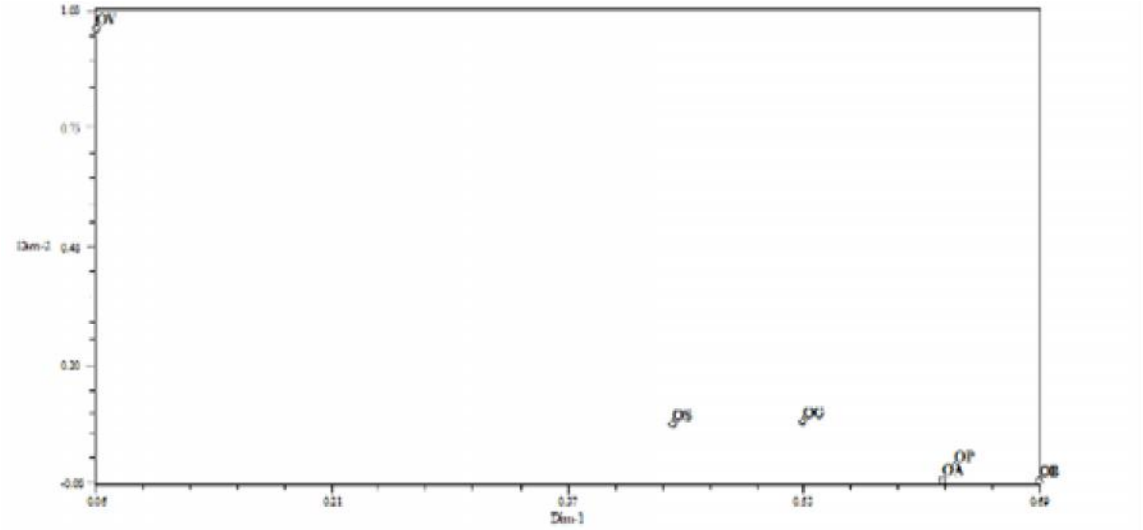

FIGURE 5. 2-D plot showing phylogenetic relationship among six *Ocimum spp*. revealed by RAPD, ISSR and SSR primers (OS=*Ocimum sanctum*; OG=*Ocimum gratissimum*; OP=*Ocimum polystachyon*; OB=*Ocimum basillicum*; OA=*Ocimum americanum*; OV=*Ocimum viride*)

Through this study it was investigated that all the three markers successfully differentiated six selected *Ocimum spp*. from each other. Harisaranraj *et al.* (2008) estimated genetic inter-relationship of seven *Ocimum* species using RAPD markers. Three *Ocimum* species (*O. basillicum, O americanum* and *O. gratissimum*) were common in their study and our study. According to their study, *O. basillicum* was found to be more similar to *O. gratissimum* than *O. americanum*. Also *O. basillicum* and *O. gratissimum* fall in one group in dendogram. However, in our present study *O. basillicum* and *O. gratissimum* were not found similar to fall in one group.

Ajibade *et al.* (2000) and Galvan *et al.* (2003) concluded that ISSR would be a better tool than RAPD for phylogenetic studies. Nagaoka and Ogihara (1997) have also reported that the ISSR primers produced several times more information than RAPD markers in wheat. However in our investigation, the high discriminating power of all three markers (RAPD, ISSR and SSR) among selected six *Ocimum* species was observed. Similar results were obtained by Shaw *et al.* (2008). They studied the genetic variation in cultivars of *C. roseus* using RAPD and ISSR markers. They concluded that both markers are equally potential to differentiate the closely related cultivars of *C. roseus.*

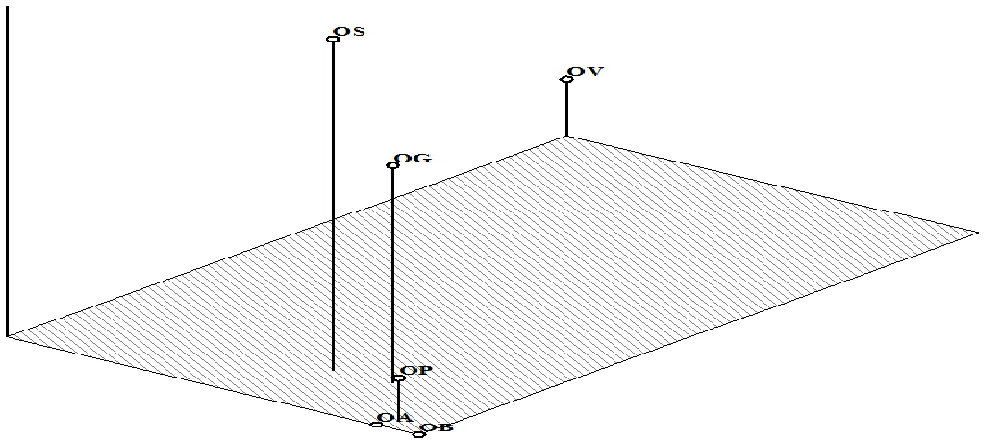

FIGURE 6. 3-D plot showing phylogenetic relationship among six *Ocimum spp*. revealed by RAPD, ISSR and SSR primers (OS=*Ocimum sanctum*; OG=*Ocimum gratissimum*; OP=*Ocimum polystachyon*; OB=*Ocimum basillicum*; OA=*Ocimum americanum*; OV=*Ocimum viride*)

Lalhruaitluanga and Prasad (2009) studied comparative results of RAPD and ISSR markers for genetic diversity assessment in *Melocanna baccifera* Roxb. growing in Mizoram State of India. They observed higher polymorphism, 98.02% and 84.1% in RAPD and ISSR markers respectively. Moreover, Lal *et al.* (2010) checked the efficiency of three PCR based markers (ISSR, RAPD and SSR) in *Cicer arietinum* L. and *Cajanus cajan* L. Millspaugh and reported higher polymorphism in all three markers. Singh *et al.* (2004) examined genetic relationships among thirty germplasm accessions belonging to five *Ocimum* species using RAPD markers. They observed very high degree of polymorphism (98.20%). Similarly in our present study, higher polymorphism (100%) was obtained among the selected six species of *Ocimum* in all the three markers.

Posselt *et al.* (2006) did the comparative analysis of genetic similarity between perennial Ryegrass genotypes using AFLP, ISSR, RAPD and SSR primers. They found lower mean similarity index mean (0.15) in SSR compared to RAPD (0.23), ISSR (0.43) and AFLP (0.31). However in our present study, the mean similarity index of 0.3690, 0.3868, 0.4070 and 0.3757 were obtained for RAPD, ISSR, SSR and pooled RAPD, ISSR and SSR analysis, respectively. Least mean similarity index was obtained in RAPD primers which indicate that RAPD primers have slight higher discriminating power among the species compared to ISSR and SSR. Similar results were obtained by Ponnusamy *et al.* (2008). They evaluated genetic variation among 10 landraces of rice bean using RAPD and ISSR markers and obtained lower mean similarity index value of 0.677 in RAPD primer compared to ISSR primer (0.729) and combined RAPD and ISSR data (0.694). Lal *et al.* (2011) assessed genetic diversity in nine cultivars of *C. roseus* from central Gujarat (India) through RAPD, ISSR and SSR markers. They also observed lower mean similarity index (0.56) in RAPD markers compared to ISSR (0.76) and SSR (0.68) markers.

To best of our knowledge, no earlier results have been reported related to the genetic diversity studies among *Ocimum spp.* using ISSR and SSR markers to compare our results with.

From our overall study, it can be concluded that all the three markers can be used to design a strategy to maintain or enhance the genetic diversity of future varieties. The polymorphism data generated can be used for plant breeding, crop improvement programs and also might be helpful in future strategies for evaluation of desired genotypes and further development of new species of *Ocimum*.

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Abbreviations: Random amplified polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR), Simple Sequence Repeats (SSR), basepair (bp)