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Research Article

GENETIC DIVERSITY OF *OCIMUM SPECIES* THROUGH BIOCHEMICAL TECHNIQUE AND UPGMA CLUSTER ANALYSIS

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ABSTRACT

Total eleven *Ocimum spp.* samples were collected from northern region of India. All the samples were used to isolate leaf proteins. For the study of genetic diversity various techniques based on the DNA and protein which includes RFLP's, AFLP's, RAPD's micro satellite DNA fingerprinting and SDS-PAGE were in used according to literature survey. Present study SDS-PAGE was utilized to the determination of the variability in different genotypes of *Ocimum spp.* Isolated protein samples were subjected to separation through Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE). 17% Separating gel and 5% stacking gel were used. SDS-PAGE analysis results were shown in table 1a, 1b & 1c. Molecular weights of the proteins were determined according to the marker protein. Similarity matrix was analyzed by UPGMA method. The data was analyzed by using computer software. Euclidean dissimilarity coefficients ranged between 0.00 and 1.00. The lowest similarity and the highest similarity was observed in the samples and the observations were shown in a dendrogram. Considering vast medicinal uses of *Ocimum*, it is essential to study this plat at genetic and molecular levels to get potential uses for industrial purposes and to develop conservation and management studies.

Keywords: *Ocimum*, SDS-PAGE, UPGMA method, Genetic diversity, Similarity matrix, Dendrogram.

INTRODUCTION

Plant gene pools are reservoirs of variations, which provide the raw material for crop improvement (Rehana Asghar *et.al*.; 2004). Most species display a complex of genetic variations along their range of distribution. Genetic conservation strategies are initially concerned with understanding of the genetic variation within species and then by the geographical distribution of

genetic variation. (Reda Sammour *et.al.* 2007). During the last decade several novel DNA-markers (RAPD, RFLP, SSR, ISSR etc.) have been rapidly integrated into the tools available for genome analysis, has been used for DNA fingerprinting and assessing genetic diversity. Molecular markers are the best tools for determining genetic relationships. The electrophoresis of seed storage proteins is a method to investigate genetic variation and to classify plant varieties. Isozyme analysis offers a rapid and more reliable means for producing genetic profiles (fingerprints) and elucidation of genetic relationships within and different taxa. (Rahman Md. *et.al*.2004). Electrophoresis patterns of the protein fractions are directly related to the genetic makeup. SDS-PAGE is used to describe the genetic structure of crop germplasm identification. (Rehana Asghar *et.al.* 2004). UPGMA cluster analysis of genetic similarity indices grouped all the accessions into two major clusters corresponding to proviously reported botanical sections. Intra-clustering within the two clusters precisely grouped the accessions belonging to one species in one sub – cluster as expected from their genetic background (Ajay Pratap Singh *et.al*. 2004).

The traditional uses of *Ocimum* are very similar among the species, despite the wide diversity of terpenes and phenylpropanoids for which these the plants are so well recognized. Basil essential oils have long been used to flavour foods, dental and oral products, in gragrances and in traditional rituals and medicines. (Roberto F. vicira and James E. Simon. 2000).The juice of *Ocimum* exhibited potent anti viral activity. (Joshi, C.G. *et.al*.1952).The essential oils found in leaves, seeds, flowers, and roots of *Ocimum* species are used as medicine. (Lexa G. Matasyoh *et.al*. 2008). The essential oils of basil are widely used in the cosmetic, pharmaceutical, food, and flavoring industries.(Andhrea Copette *et.al.* 2006)

Considering vast medicinal uses of the plat, it is essential to study this plat at genetic and molecular levels to get potential uses for industrial purposes and to develop conservation and management studies. For above multifaceted utilities of the plant, it is necessary to find out first, the protein content of the plant, and second, to assess genetic variability of proteins using Sodium Dodecyl Sulphate – Poly Acrylamide Gel Electrophoresis (SDSPAGE) in different natural population of *Ocimum*. In this study our aim results toward the exploitation of the natural genetic diversity of *Ocimum* species.

MATERIALS AND METHODS

Sample Collection

Total eleven samples of *Ocimum spp.* were collected from northern region of India. The areas of collection of samples (with local name) are listed below.

Sample 1: Jungli Tulsi, Sikandra, Uttarpradesh (1,361km)

Sample 2: Ram Tulsi, Bharatpur, Rajasthan (1,613km)

Sample 3: Syam Tulsi, Bodla, Agra, Uttarpradesh (1,447km)

Sample 4: Ram Tulsi, Fatehpur, Uttarpradesh (1,278km)

Sample 5: Syam Tulsi, Faridabad, Haryana (1,752km)

Sample 6: Ram Tulsi, Shahganj, Agra (1,449km)

Sample 7: Syam Tulsi, Bichupuri, Agra (1,501km)

Sample 8: Syam Tulsi, New Delhi (1,778km)

Sample 9: Ram Tulsi, Ram Bagh, Agra (1,445km)

Sample 10: Ram Tulsi, Ashram, Agra, U.P. (1,163km)

Sample 11: Syam Tulsi, Ashram, Agra, U. P.(1,163km)

Isolation of proteins

All samples were processed for the isolation of proteins. 1gm. of tissue was grinded in a chilled pestle mortar maintaining 4ºC temperature. 10ml per gram of tissue of Protein extraction buffer (100mM tris HCL) was added. Acid washed sand was added as an abberasive and the samples were grinded little more, and then incubated at 4oC for 1/2hr. The homogenate was transfered in a sterilized test tube and centrifuged at 15,000 rpm for 15 min. The supernatant was collected into another eppendorf tube and store at 4°C till further use.

SDS-Page

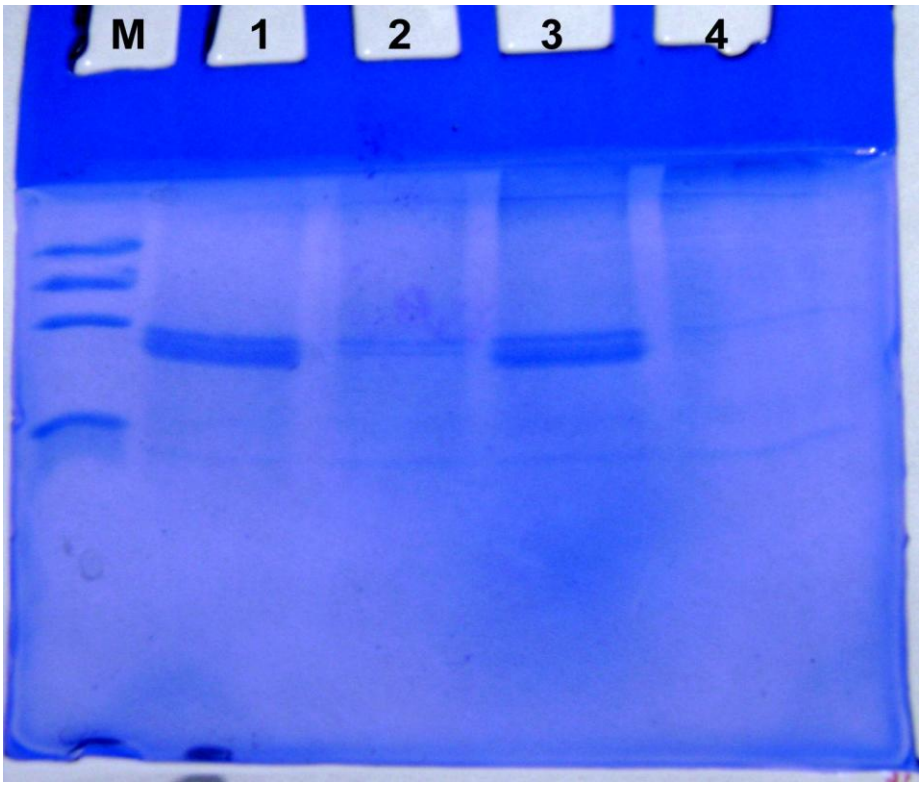
The isolated proteins were subjected to separation through Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE). 17% Separating gel and 5% stacking gel were used. Protein samples were mixed with loading dye in 4:1 (protein : loading dye) and

heated for 5 min. in boiling water. 10µl of protein marker was mixed with 15µl loading dye and was heated for 5 min in boiling water. 50µl heat treated samples and 20µl of protein marker were loaded in the wells of stacking gel. Electrophoretic unit was connected with a power supply and was run at 50v till the dye reached 1cm above the bottom of the gel. The gel setup was dissembled and the gel was stained with comassie Blue stain for overnight and then destained to observe the separated protein bands.

RESULTS AND DISCUSSION

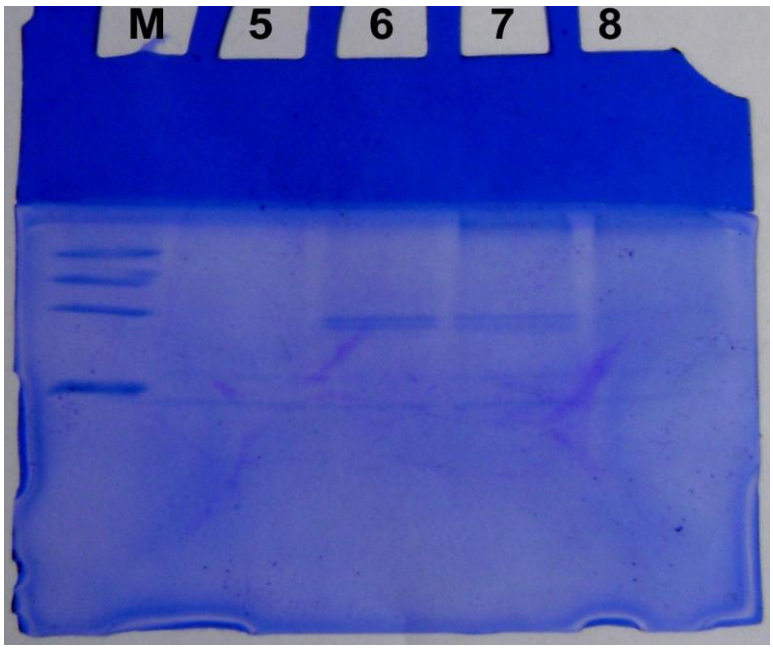
The results of SDS-PAGE of all the eleven samples are shown in Photograph 1, 2 & 3:-

Photograph 1



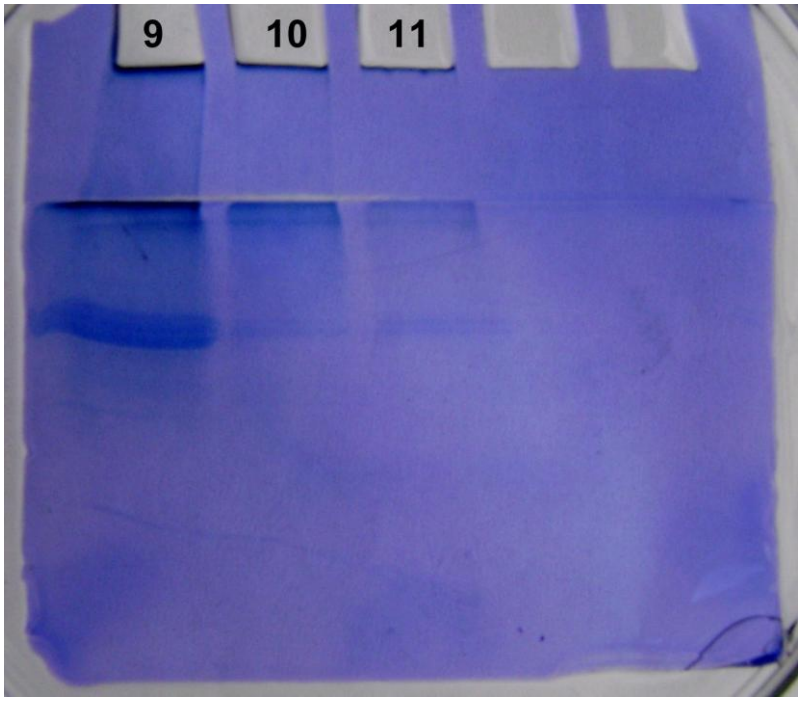
Lane M : Marker. Lane 1 : Jungli Tulsi, Sikandra, Uttarpradesh (1,361km) Lane 2: Ram Tulsi, Bharatpur, Rajasthan (1,613km) Lane 3: Syam Tulsi, Bodla, Agra, Uttarpradesh (1,447km) Lane 4: Ram Tulsi, Fatehpur, Uttarpradesh (1,278km)

Photograph 2



Lane M : Marker. Lane 5: Syam Tulsi, Faridabad, Haryana (1,752km) Lane 6: Ram Tulsi, Shahganj, Agra (1,449km) Lane 7: Syam Tulsi, Bichupuri, Agra (1,501km) Lane 8: Syam Tulsi, New Delhi (1,778km)

Photograph 3



Lane 9: Ram Tulsi, Ram Bagh, Agra (1,445km) Lane 10: Ram Tulsi, Ashram, Agra, U.P. (1,163km) Lane 11: Syam Tulsi, Ashram, Agra, U. P.(1,163km)

Table 1: SDS-PAGE Analysis Results: Showing the Rf values and the molecular weights of the observed protein bands.

Table 1a:

Marker		Sample 1		Sample 2		Sample 3		Sample 4	
Rf	**MW**	**Rf**	**MW**	**Rf**	**MW**	**Rf**	**MW**	**Rf**	**MW**
0.12	**66.0**	-	-	-	-	-	-	-	-
0.18	**43.0**	0.26	26.0	0.26	26.0	0.26	26.0	0.26	26.0
0.24	**29.0**	0.28	24.3	0.28	24.3	0.28	24.3	0.28	24.3
0.42	**14.3**	0.32	22.3	-	-	0.32	22.3	-	-
		0.46	11.3	0.46	11.3	0.46	11.3	0.46	11.3

Table 1b:

Sample 5		Sample 6		Sample 7		Sample 8	
Rf	**MW**	**Rf**	**MW**	**Rf**	**MW**	**Rf**	**MW**
-	-	-	-	-	-	-	-
-	-	0.26	26.0	0.26	26.0	-	-
-	-	0.28	24.3	0.28	24.3	-	-
-	-	-	-	-	-	-	-
0.46	11.3	0.46	11.3	0.46	11.3	0.46	11.3

Table 1c:

Sample 9		Sample 10		Sample 11	
Rf	**MW**	**Rf**	**MW**	**Rf**	**MW**
0.22	**33.3**	-	-	-	-
0.26	26.0	0.26	26.0	0.26	26.0
-	-	0.28	24.3	0.28	24.3
0.32	22.3	-	-	-	-
-	-	-	-	-	-

Table 2: Similarity matrix based on the observed leaf protein banding pattern between all possible pairs of all the 11 samples of different varities of *Ocimum sanctum.*

	1	2	3	4	5	6	7	8	9	10	11
1		0.75000	1.00000	0.75000	0.25000	0.75000	0.75000	0.25000	0.40000	0.50000	0.50000
2			0.75000	1.00000	0.33333	1.00000	1.00000	0.33333	0.20000	0.66667	0.66667
3				0.75000	0.25000	0.75000	0.75000	0.25000	0.40000	0.50000	0.50000
4					0.33333	1.00000	1.00000	0.33333	0.20000	0.66667	0.66667
5						0.33333	0.33333	1.00000	0.00000	0.00000	0.00000
6							1.00000	0.33333	0.20000	0.66667	0.66667
7								0.33333	0.20000	0.66667	0.66667
8									0.00000	0.00000	0.00000
9										0.25000	0.25000
10											1.00000
11											

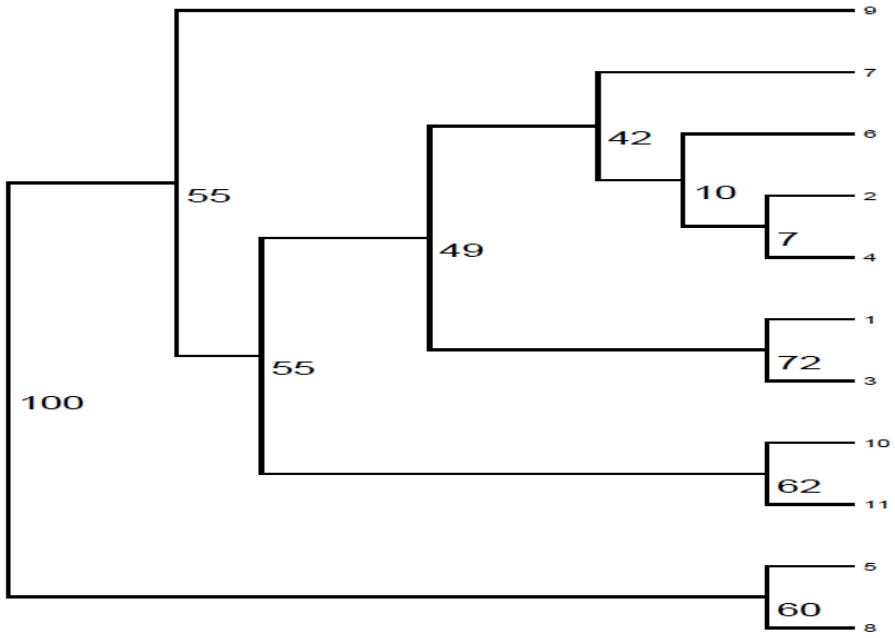


Fig. 1: Dendogram showing relationship between all the 11 samples of different varities of *Ocimum sanctum* based on the observed leaf protein banding pattern.

Morphological traits can be used for assessing genetic diversity but are often influenced by the environment. The use of biochemical/molecular markers for the evaluation of genetic diversity has received much attention in recent years. A large number of germplasm lines can be characterized for biochemical markers in a short period of time. In addition the data reflects more truly the genetic variability as biochemical markers are direct product of genes and the environment does not influence their expression (Perry and McIntosh, 1991; Masood *et al*., 2004). For an effective breeding program, information regarding the extent and nature of genetic

diversity within a crop species is essential. It is particularly useful for characterizing individual accession and as a guide in the selection of parents for hybridization. Protein electrophoresis is a useful method for describing the genetic structure of crop germplasm (Kaleem Ahmed *et.al*. 2008). Among biochemical techniques SDS-PAGE is widely used due to its simplicity and effectiveness for describing the genetic structure of crop germplasm. (Murphy *et al.,* 1990; Javaid *et al.,* 2004; Anwar *et al.,* 2003.).

Ocimum sanctum has immense medicinal value against malaria, gastric diseases, blood and heart diseases, cough, bronchitis, asthma, chronic fever, liver disorder, earache, ringworm and skin diseases. (Ahmad, S.D. and Khaliq, I. 2002) Leaf samples of two varities of *O. sanctum* (Ram Tulsi, with green leaves and stem; and Shyam Tulsi, with purple leaves and stem) were collected from different locations in Agra District, Haryana, Rajasthan and Delhi. Total leaf proteins in SDS-PAGE produced diverse banding pattern among the genotypes

compared **(Photograph: 1 a, b & c)**. The data obtained from SDS-PAGE was scored for the presence (1) and absence (0) of the bands and entered in a binary data matrix. Based on the results of electrophoretic band spectra, similarity index was calculated for all possible pair of electrophoregrams. The similarity matrix thus generated was converted to a dissimilarity matrix and used to construct the dendrogram by the unweighted pair group average method (UPGMA). The data was analyzed using TREEVIEW and FREETREE computer software. Euclidean dissimilarity coefficients ranged between 0.00 and 1.00 **(Table 2).** The lowest similarity was exhibited sample numbers (5 & 9), (5 & 10), (5 & 11), (8 & 9), (8 & 10) and (8 & 11), whereas the highest similarity was observed between (1 & 3), (2, 4, 6 & 7), (5 & 8), (6 & 7) and (10 & 11). The observation of the dendrogram **(Figure 1)** also appears to be in two major clusters, where cluster 1 include sample numbers 1, 2, 3, 4, 6, 7, 9, 10 & 11 and cluster 2 include sample numbers 5& 8, which also support the areas of sample collections where cluster 1 represent Agra District & Rajasthan and cluster 2 represents Haryana & New Delhi. Due to the larger genetic diversity in germplasm of *Ocimum sanctum* and its suitability for commercial cultivation in the area under small land holdings, the investigation suggests it's genetic as well as biochemical investigation on larger scale for the production of commercial varieties and exploitation of the plant for economic benefits of the local communities. (Ahmad, S.D. and Khaliq, I., 2002).

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