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Short Communication

Evaluation of genetic diversity in five species of *Ocimum* by SDS-PAGE

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In India *Ocimum* (family : Lamiaceae; commonly known as basil and rich source of essential oil) is represented by only 6 species [1] and the species are classified into two broad groups *viz*., *Basilicum* and *Sanctum* on the basis of morphological and cytological characters [2]. However, standard morphological markers may not always be adequate because of the wide spectrum of phenotypic variation and their interaction with environment [3]. Further, interspecific hybridization and polyploidy, common occurrences within the genus, have created taxonomic confusion for proper identification of the species [4]. In such instances electrophoretic banding patterns of seed protein can be used effectively to decipher interrelationships between genotypes [5]. SDS-PAGE of seed protein has also been successfully used to assess the genetic diversity [6], breeding endeavour [7] and hybrid identification [8]. In the present study, five species of *Ocimum* were characterized using seed protein polymorphism derived from electrophoretic banding patterns.

Five *Ocimum* species (*O. basilicum* L., sweet basil, collected from medicinal plant garden, Narendrapur, Ramkrishna Mission; *O. canum* Sims, hoary basil, collected from Kalyani University campus; *O. kilimandscharicum* Guerke, camphor basil, collected from NBPGR- Accession No. P-2086; *O. gratissimum* L., shrubby basil, NBPGR- Acc. No. EC 213933 and *O. tenuiflorum* L., holy basil, collected from medicinal plant garden, Narendrapur) were used to study one dimensional SDS-PAGE (10% separating gel: stock acrylamide 30%, bisacrylamide 0.8%, water 100ml, separating gel buffer - 1.875 M Tris-HCl, water up to 100ml, TEMED 25µl and 0.2ml Ammonium per sulphate, P^H 8.8; 4.5% stacking gel: 0.6 M Tris-HCl, water upto 100 ml, 20 µl TEMED, 0.04 ml 10% ammonium persulphate, P^H-6.8) following Laemmli [9] in a vertical gel system (BIOTECH, Yercaud-Salem). For the purpose, total seed protein was extracted in 0.2 M Tris buffer (P^H-8.5), suspended overnight (0-4^{o}C) and centrifuged at 15,000 rpm (–4^{o}C) for 30 minutes. The protein samples along with sample buffer containing bromophenol blue were denatured in boiling water (1mins), cooled and loaded in lanes with micropipette (8µl/lane; 0.3220 µg/µl). A protein molecular weight marker (GENEI Bangalore, Cat No. PMW-M) was also incorporated into the gel (as marker lane) as reference to detect molecular weights of the bands. The gel was run at 33mA (3mA/lane) for 2 hours, stained in Coomassie Brilliant Blue R250 for overnight, destained and stored in 7% acetic acid (10). Gels were scored and analyzed in gel documentation unit (Ultra Lum, USA) using the software Total Lab. (Version 2.01).

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, dissimilarity matrix was generated for all possible pair of elctrophoregrams from squared Euclidean Distance and used to construct the dendrogram by the unweighted pair group average method (UPGMA). The data were analyzed using software SPSS (Ver. 1.3).

SDS-PAGE of seed protein (Fig. 1) of five *Ocimum* species led to the detection of 22 polypeptide bands (Table 1) with diverse molecular weight (16.6 kD to 50.4 kD). Mostly the polypeptide bands detected in these five species of *Ocimum* were of medium (25.0 kD to 39.9 kD) to low (<25.0 kD) molecular weights (Table

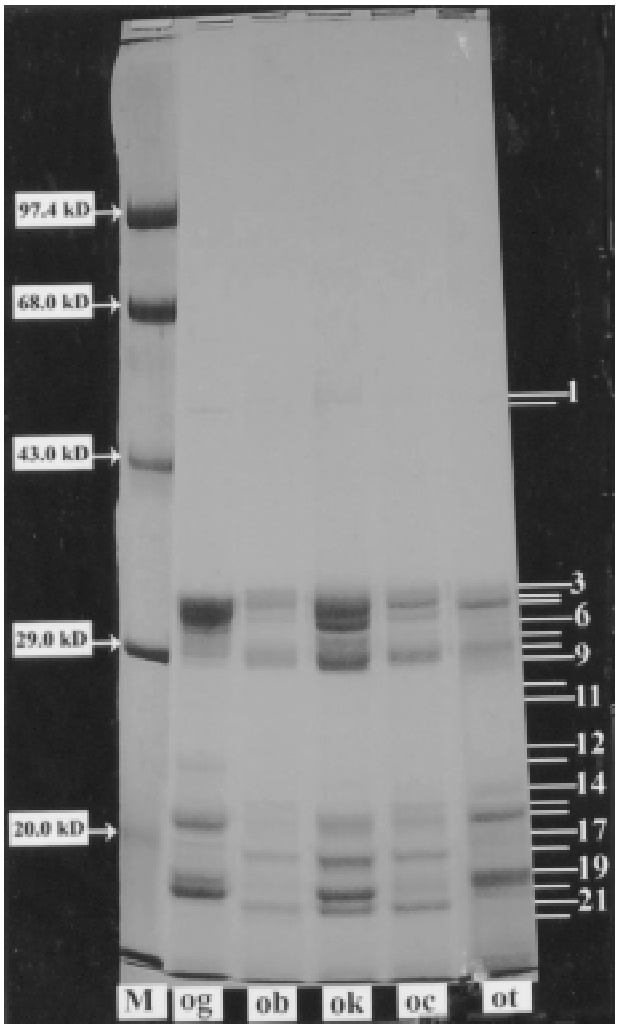

Fig 1. SDS-PAGE electrophoretic pattern of seed storage proteins from five species of *Ocimum*. og: *O. gratissimum*; ob: *O. basilicum*; ok: *O. kilimandscharicum*; oc: *O. canum*; ot: *O. tenuiflorum*; M: Molecular weight marker; Molecular weights of the bands on the marker lane on the left

Table 1. SDS-PAGE banding pattern of seed storage proteins in different *Ocimum* species

Band no.	Mole-wt(kD)	Genotypes				
		O. basilicum	*O. canum*	*O.kilimandscharicum*	*O.gratissimum*	*O. tenuiflorum*
1	50.4	+	+	+	+	+
2	48.8	(+)	-	-	-	-
3	32.0	+	+	+	+	+
4	31.8	-	-	-	+	+
5	31.1	+	+	+	-	-
6	30.4	-	-	-	+	+
7	30.0	-	+	+	-	-
8	29.0	+	+	+	+	+
9	28.4	-	-	-	(+)	+
10	27.4	-	-	-	(+)	-
11	26.2	-	-	-	(+)	-
12	23.7	-	-	-	(+)	-
13	22.8	-	-	-	(+)	-
14	22.3	-	-	-	-	(+)
15	21.8	+	+	-	-	-
16	21.0	+	+	+	+	+
17	20.2	-	-	-	+	+
18	19.3	+	+	+	+	+
19	18.1	+	+	+	+	+
20	17.6	-	-	-	+	+
21	17.0	+	+	+	-	-
22	16.6	(+)	-	-	-	-
Total		11	10	9	14	11

*( ) - Species specific

2). *O. basilicum* had two (48.8 kD to 50.4 kD) high molecular weight bands, while the other species had only one (50.4 kD). Out of 22 polypeptide bands, 11 in *O. basilicum* and *O. tenuiflorum,* 10 in *O. canum,* 9 in *O. kilimandscharicum* and 14 in *O. gratissimum* were recorded. Band number 1 (50.4 kD), 8 (29.0 kD), 16 (21.0 kD), 18 (19.3 kD) and 19 (18.1 kD) were present in all the species. Polypeptide band no. 5 (31.1 kD) and 21 (17.0 kD) were common among members of Basilicum group (*O. basilicum*, *O. canum*, and *O. kilimandscharicum)*; while 4 (31.8 kD), 6 (30.4 kD), 9 (28.4 kD),17 (20.2 kD) and 20 (17.6 kD) were specific to Sanctum group (*O. gratissimum* and *O. tenuiflorum*).Band no. 2 (48.8 kD) and 22 (16.6 kD) for *O. basilicum,* 10 (27.4kD), 11 (26.2 kD), 12 (23.7 kD) and 13 (22.8 kD) for *O. gratissimum* and 14 (22.3kD) for *O. tenuiflorum* were specific. Polypeptide band No. 7 (30.0 kD) was only detected from *O. canum* and *O. kilimandscharicum* and 15 (21.8 kD) from *O. basilicum* and *O. canum*. Mostly the polyopeptide bands were very faint, faint and medium types (Table 2). *O. basilicum* and *O. canum* had no intense bands; however, three (32 kD, 29.0 kD and 18.1 kD), two (31.8 kD and 17.6 kD) and one (18.1 kD) intense bands appeared in *O. kilimandscharicum*, *O. gratissimum* and *O. tenuiflorum*, respectively.

The cluster analysis perfomed using UPGMA revealed 2 clusters (Fig. 2). Cluster 1 (starting from right to left) consisted of 2 species (*O. gratissimum* and *O. tenuiflorum*); while, cluster 2 comprised of three species (*O. basilicum*, *O. canum* and *O. kilimandscharicum*). Result indicated distinct relationship among the number of Basilicum and that of Sanctum groups, thereby corroborating Sobti and Pushpangadan (2) who classified *Ocimum* species into Basilicum and Sanctum groups based on cytomorphological features. Electrophoretic banding pattern in *Ocimum* species led to the detection of species specific band which may be exploited for efficient breeding.

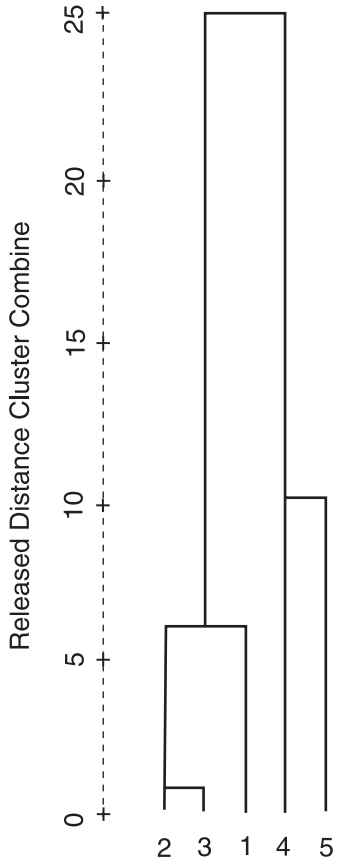

Fig. 2: Dendrogram using Average Linkage (Between Groups) : 1*. Ocimum basilicum*, 2. *Ocimum canum*, 3. *Ocimum kilamandscharicum,* 4. *Ocimum gratissimum*, 5. *Ocimum tenuiflorum*

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