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Genetic Diversity among *Ocimum* Populations in Egypt as Reflected by Morphological, Seed Proteins and Isozyme Polymorphism

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Abstract: Seven populations of two species of *Ocimum* were analyzed using data from morphological and biochemical (seed proteins and isozymes) characters to measure genetic variation within and between populations and species of *Ocimum* in Egypt. Ten isozyme systems revealed 23 loci and a total of 49 alleles in the studied *Ocimum* populations. The constructed trees based on variation in morphological, seed proteins or isozyme data clearly demonstrated the existence of genetic diversity among and within populations of *Ocimum* that might be related to natural hybridization and fluctuations in environmental conditions. Seed proteins and isozyme polymorphism exhibited validity for studying genetic diversity and taxonomic relationships in *Ocimum* at both species and infra-specific levels. Moreover, *Ocimum kilimandscharicum* exhibited higher levels of genetic variation and also higher number of unique alleles than *Ocimum basilicum*.

Key words: *Ocimum*, genetic diversity, proteins, isozymes, morphology

INTRODUCTION

The genus *Ocimum* (basil) comprises same of the most popular herbs in the world. It belongs to the family Lamiaceae, subfamily Ocimoideae and includes over 150 different species and varieties distributed in tropical regions of Asia, Africa, Central and South America (Darrah, 1980). Because of its popularity, basil is often referred to as the "king of the herbs, being widely utilized due to its economic, nutritional, industrial and medicinal importance (Simpson and Conner, 1986; Simon *et al*., 1990; Khosla, 1995). Volatile compounds produced by sweet basil were shown to have allelopathic effects and influence the composition, distribution and spore germination of some fungal populations (Reuveni and Putiersky, 1984).

Inter-specific hybridization and polyploidy are of common occurrence within the genus *Ocimum* and have created taxonomic confusion and challenges (Anonymous, 1980). The morphological diversity within basil has been accenturated by centuries of cultivation with great variation in pigmentation, leaf shape and size (Anonymous, 1980). Using the morphological data in studying genetic diversity and taxonomic relationships of plants was previously scored for many plants (Bult and Kiang, 1992; Zviniene and Pank, 1996).

In addition to morphological traits, phytochemical analysis and chromosomal criteria, seed protein electrophoretic patterns have provided valid evidence for addressing genetic, taxonomic and evolutionary relationships in plants. Evidences provided by the separation of seed protein components in polyacrylamide gels are mostly informative at the species and infra-specific levels (Nei *et al*., 1978; Ladizinsky and Hymowitz, 1979; Cooke, 1984; Badr, 1995). Seed proteins data have therefore been applied to study species relationships in many plant genera (Boulter *et al*., 1970; Ladizinsky, 1979a, b; Sammour, 1989, 1994; Sammour *et al*., 1991; Paino *et al*., 1990; Saraswati *et al*., 1993; Badr, 1995) and genetic diversity at infraspecific levels (Badr *et al*., 1998; Al-Nowaihi *et al*., 2002; Badr *et al*., 2003).

Many previous applications have exploited the ability of electrophoresis to provide efficient data about the variation, heterozygosity and divergence of alleles at many gene loci coding enzymes in large population samples. Most isozyme data have been applied to species or infraspecific taxa rather than at higher taxonomic ranks (Kang and Chung, 2000; Badr *et al*., 2002; Mustafa *et al*., 2005).

Genetic variation and taxonomic relationships in the genus *Ocimum* were previously investigated using morphological, chemical, cytological and molecular traits

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(Khosla, 1995; Grayer *et al*., 1996; Paton and Putievsky, 1996). Estimating the genetic diversity levels both within and among populations of a crop is necessary for the best conservation of its gene pool (Votava *et al*., 2002). The genetic variation found within wild relatives of domesticated species offer novel gene complexes for strategic improvement of crop tolerance to biotic and abiotic stresses (Votava *et al*., 2002). Such variation can also have important implications for their conservation and management (Votava *et al*., 2002). However, no previous studies utilizing electrophoretic variation of seed proteins or isozymes in taxonomic, genetic or phylogenetic studies on *Ocimum* are known. The objectives of this study is to investigate genetic diversity in *Ocimum* populations in Egypt as inferred by variations in their morphology and electrophoretic profiles of both seed proteins and isozymes as a necessary step for best conservation of *Ocimum* gene pool in Egypt and for future improvement of crop characters.

MATERIALS AND METHODS

Samples collection: This study was conducted at Botany Department, Faculty of Science, Tanta University at 2003-2004. Complete mature plants were collected from a number of localities representing natural populations of two species of the genus *Ocimum* in Egypt. *Ocimum basilicum* was represented by 3 varieties, var. *grand vant* (OB1-OB3), var. *parpurascens* or dark opel basil (OP) and var. *finoverde* (OF1 and OF2), while *O. kilimandscharicum* was represented by only one accession (OK). Mature plants were collected during the flowering season for measuring morphological traits and mature seeds were collected for electrophoretic analysis of both seed proteins and isozymes.

Morphological traits: For each accession, the following morphological traits were scored; stem height, width, diameter and habit, internode number and length, leaf width, inflorescence length and width, number of flowers, root width, diameter and length and germination period. Each trait was scored as a mean value of ten individuals for each accession.

Seed proteins: From each population, a composite sample of mature dormant seeds was mixed with an equal weight of sterile fine sand, ground well in a mortar, then defated several times with acetone in the refrigerator until oils could not be observed. The meal was air-dried and samples of 400 mg were extracted overnight at 4°C in 1 mL 0.125 M Tris-borate buffer (pH 8.9) containing 2% β-mercaptoethanol. Extracts were centrifuged at 12000 × g for 20 min, 10% sucrose was added to the supernatant that was used directly for electrophoresis or kept at -20°C until use.

Dissociating polyacryamide gel electrophoresis (SDS-PAGE) was adopted after Laemmli (1970) with some modifications. Samples of 100 μL were applied onto main gels of 17% acrylamide concentration using bromophenol blue as a front dye. Electrophoresis was carried out using 25 mM tris/glycine buffer pH 8.3. The gel was stained overnight by slow shaking in 100 mL staining mixture (0.05% Coammassie Brillient Blue-R250 in 50% methanol, 7% glacial acetic acid and 43% distilled water) and destained by slow shaking in the stain solvent. The subunit molecular weight of the protein bands was determined according to Weber and Osborne (1969). Each band was considered as a character for which the presence or absence was coded 1 or 0 respectively in a data matrix for numerical analysis. Density of the protein bands were not considered.

Isozymes: Seeds were germinated and a leaf specimen (0.25 g) of one seedling was used to prepare samples for isozyme electrophoresis according to Marshall and Brown (1975). Twenty enzyme systems were assayed for activity; nevertheless, valid results were obtained for only ten enzyme systems including five esterases. The staining recipes of these enzymes are given in Table 1. About 4-6 individuals were used as replica from every accession for each enzyme (Table 2).

Isozymes were separated on 8% PAGE vertical slab gels (16×18×0.2 cm) according to Wendel and Weeden (1989). The gel buffer composed of 45 mM Tris-HCl, 25 mM boric acid and 1 mM EDTA-Na_2 pH 8.6 and the electrode buffer was composed of 0.18 M Tris-HCl, 0.1 M Boric acid and 4 mM EDTA-Na_2. The gels were stained by shaking in the dark at 37°C in the appropriate staining solution (Table 1). After staining, the reaction was stopped by washing the gel 2-3 times with tap water. The gel was then kept in the fixing solution (glycerol and water 1:1 v/v) for 24 h and rinsed two times in tap water, then photographed. Interpretation of banding patterns followed standard principles (Wendel and Weeden, 1989; Murphy *et al*., 1996). Loci were numbered consecutively from the anodal end and alleles at each locus were labeled alphabetically at the same direction (Pasteur *et al*., 1988). Alleles were directly scored for each isozyme locus and allele frequencies were calculated. Based on allele frequencies, the following estimates of genetic variation were calculated for each population: (1) Proportion of polymorphic loci (P), (2) mean number of alleles per polymorphic locus (Kp) and (3) the mean number of alleles per locus (K).

Table 1: The enzymes assayed and their staining recipes

Enzyme	IUBMB [1]	Staining recipe	Reference	Section
Esterases (EST)	E.C. 3.1.1.- [2]	0.1 M Na-phosphate buffer pH 6.2, 0.1% Fast blue RR salt, 0.05% α-naphthyl acetate, α-naphthyl valerate, α-naphthyl butyrate, α-naphthyl propionate or β-naphthyl acetate	Tanksley and Rick (1980) Tanksley and Orten (1983)	Hydrolases
Isocitric dehydrogenase (IDH)	E.C. 1.1.1.42	0.1 M Tris-HCl buffer pH 7.5, 10 mM $MnCl_2$, 0.% DL-Isocitrate (Na_3), 0.015% NADP and 0.2% MTT	Fine and Costello (1963)	Oxido-reductase
Malate dehydrogenase (MDH)	E.C. 1.1.1.37	0.1 M Tris-HCl buffer pH 7.5, 0.12 M DL-Malate, 0.015% NAD, 20 mM MTT and 4 mM PMS	Brown *et al.* (1978)	Oxido-reductase
Malic Enzyme (ME)	E.C. 1.1.1.40	0.1 M Tris-HCl buffer pH 7.5, 20 mM $MgCl_2$, 0.12 M DL-Malate, 0.015% NAD, 20 mM MTT and 4 mM PMS	Brown *et al.* (1978)	Oxido-reductase
Phosphorylase (PHOS)	E.C. 2.4.1.1	0.1 M Na-phosphate buffer pH 6.8, 25 mM G1-P, 10 mM I2 and 14 mM KI	Siepmann and Stegemann (1967)	Hydrolases
Polyphenol oxidase (PPO)	E.C. 1.14.18.1	0.1 M Na-phosphate buffer pH 6.8, 15 mg catechol and 50 mg sulfonic acid	Sato and Hasegawa (1976)	Oxido-reductase

[1] Nomenclature Committee of the International Union of Biochemistry and Molecular Biology, [2] Non-specific enzyme

Table 2: Number of studied individuals (N), proportion of polymorphic loci (P), mean number of alleles per locus (K), mean number of alleles per polymorphic locus (Kp) and number of unique allels in accessions of *Ocimum* sp.

Accessions	N	P	K	Kp	Unique alleles
OB01	6	0.36	1.36	2.00	2
OB02	6	0.38	1.43	2.13	0
OB03	5	0.39	1.38	2.00	0
OF01	6	0.39	1.38	2.00	1
OF02	5	0.39	1.39	2.00	0
OP	5	0.45	1.45	2.00	2
OK	6	0.48	1.52	2.10	7

Data analysis: Cluster analyses of the data obtained from morphology, seed proteins or isozyme polymorphism were made using the software SPSS for windows package (Version 10). A tree illustrating the genetic distance among populations was constructed based on Euclidean squared distance, Kulczynski Measure 2 and Dice similarity measure for morphological, seed proteins or isozyme data, respectively.

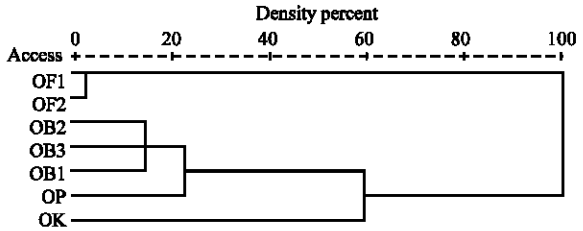

Fig. 1: Dendrogram based on *Ocimum* morphological data

RESULTS

Morphological traits: Figure 1 and Table 3 illustrate the genetic distance among the studied accessions of *Ocimum* based on morphological traits, it can be noticed that farthest genetic distance was exhibited between *O. kilimandscharicum* and *O. basilicum* var. *fino verde*, while nearest distance was exhibited among the accession of the same variety or between sweet basil (OB) and dark opel basil (OP). *O. basilicum* var. *grand vant* and var. *parpurascens* exhibited similarity to *O. kilimandscharicum* more than to var. *fino verde*.

Seed proteins: The results obtained in this study showed a large variation in the number of protein bands among the studied accessions (Fig. 2 and Table 4), the least number of bands (12) was found in *O. basilicum* var. *purpurascence* (OP) and the highest number (36) for sweet basil (*O. basilicum* var. *grand vant* OB2) with molecular weight between 60 and 20 KDa. The accessions of *O. basilicum* exhibited four bands that could not be scored in *O. kilimandscharicum*, which showed four bands that could not be scored by *O. basilicum*. The cluster analysis using Kulczynski Measure 2 (Table 5) showed highest similarity within each variety and farthest genetic distance between OK and both OB01 and OP. These data were best represented in the dendrogram based on Kulczynski measure 2 similarity index (Fig. 3), which exhibited best discrimination between the two *Ocimum* species and also among the three varieties of *O. basilicum*.

Isozymes: Ten enzyme systems and 23 loci were resolved and scored in the studied seven accessions

Table 3: The genetic distance among *Ocimum* accessions based on morphological data

Accession	OB01	OB02	OF01	OK	OP	OB03	OF02
OB01	0						
OB02	**0.016**	0					
OF01	0.453	0.266	0				
OK	0.061	0.154	**0.826**	0			
OP	0.117	**0.031**	0.103	0.346	0		
OB03	0.056	**0.015**	0.236	0.240	**0.021**	0	
OF02	0.582	0.368	**0**	**1**	0.170	0.326	0

Table 4: The protein bands in the electrophoregram of the studied *Ocimum* accessions

	Accessions						
Bands	OB01	OB02	OB03	OF01	OF02	OP	OK
1	0	1	1	1	1	1	1
2	0	1	1	1	1	1	1
3	1	1	1	1	1	1	1
4	0	1	1	0	0	0	0
5	0	1	1	0	0	0	0
6	1	0	0	1	1	0	0
7	0	1	1	1	1	0	0
8	0	1	1	0	0	0	0
9	0	1	0	0	0	0	0
10	0	1	1	0	1	0	1
11	0	1	1	0	0	0	0
12	0	0	0	0	0	0	1
13	0	0	0	**1**	**1**	0	0
14	**1**	**1**	**1**	0	0	0	0
15	***1***	***1***	***1***	1	1	1	0
16	**1**	**1**	**1**	0	0	0	0
17	0	0	0	1	1	1	0
18	1	1	1	0	0	0	1
19	0	1	1	1	1	0	1
20	1	1	1	0	0	0	1
21	0	0	0	1	1	1	1
22	**1**	**1**	**1**	0	0	0	0
23	1	1	1	1	1	0	0
24	0	1	1	0	0	0	0
25	0	0	0	1	1	0	1
26	0	1	1	1	1	0	1
27	0	0	0	0	0	0	**1**
28	1	1	1	0	0	1	0
29	***1***	***1***	***1***	***1***	***1***	***1***	0
30	0	0	0	0	0	0	**1**
31	1	0	0	1	1	0	0
32	**1**	**1**	**1**	0	0	0	0
33	0	0	0	0	0	0	1
34	**1**	**1**	**1**	0	0	0	0
35	**1**	**1**	**1**	0	0	0	0
36	**1**	**1**	**1**	0	0	0	0
37	***1***	***1***	***1***	***1***	***1***	***1***	0
38	***1***	***1***	***1***	***1***	***1***	***1***	0
39	0	1	1	1	1	0	1
40	1	1	1	1	1	1	1
41	1	1	1	1	1	0	0
42	1	1	1	1	1	1	1
43	1	1	1	1	1	0	1
44	1	1	0	1	1	0	1
45	1	0	0	0	0	0	0
46	0	0	0	1	1	0	1
47	1	1	1	1	1	0	1
48	1	1	1	1	1	0	0
Total	26	36	34	26	27	12	21

of *Ocimum* One of these ten enzymes (IDH) was represented by only one locus while the remaining nine enzymes were represented by 2-3 loci each. Seven of the 23 loci were monomorphic in all the studied accessions of *Ocimum* (ME 2, PHOS 2, PPO1, EST α-naphthyl valerate 3, EST α-naphthyl propunate 1 and EST β-naphthyl acetate 2 and 3), while the remaining 16 loci were polymorphic (Table 6 and 7). Twelve unique alleles were scored in the studied accessions of *Ocimum* sp. (Table 2 and 6), seven of which in

Table 5: The genetic similarity index among the accessions of *Ocimum* sp. based on seed protein data. (Kulczynski Measure 2)

Accession	OB01	OB02	OB03	OP	OF01	OF02	OK
OB01	1						
OB02	0.655	1					
OB03	0.631	0.985	1				
OP	0.224	0.332	0.344	1			
OF01	0.365	0.447	0.418	0.511	1		
OF02	0.348	0.477	0.450	0.499	1	1	
OK	0	0.288	0.245	0.076	0.405	0.456	1

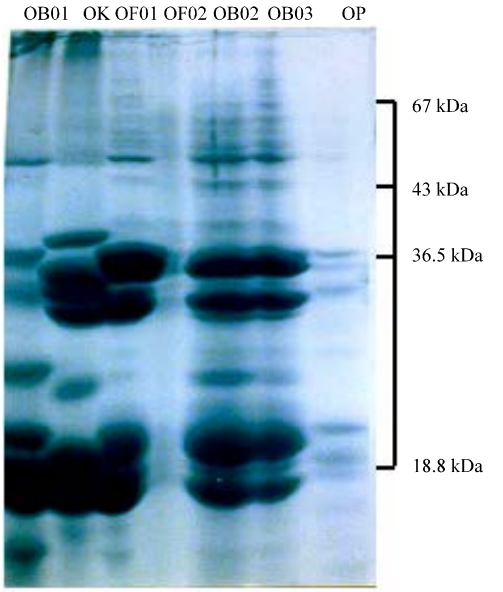

Fig. 2: Seed protein electrophoretic profile of *Ocimum* accessions

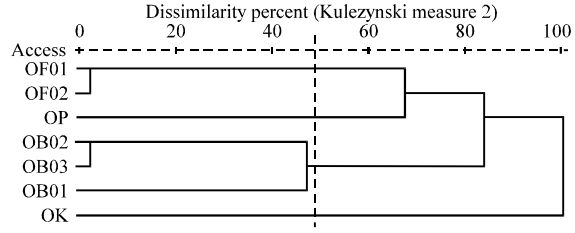

Fig. 3: Dendrogram based on electrophoretic data of *Ocimum* seed proteins

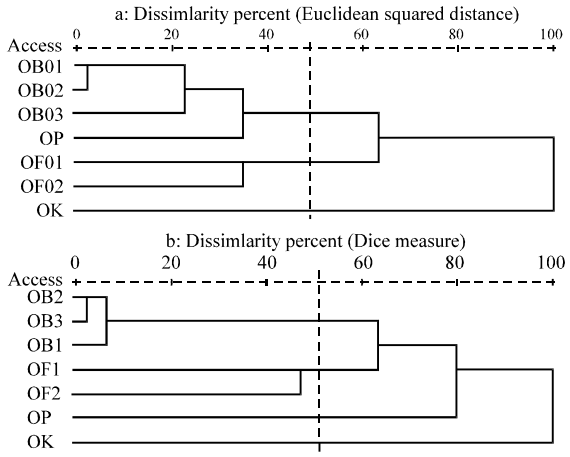

Fig. 4: Dendrogram Based on electrophoretic data of *Ocimum* isozymes

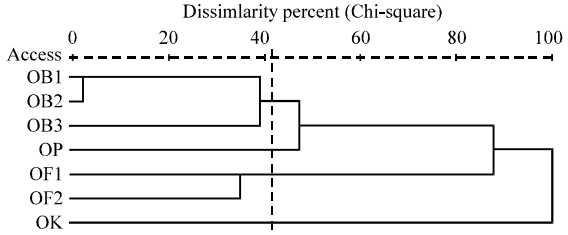

Fig. 5: Dendrogram based on data of morphology and electrophoretic polymorphism of seed proteins and isozymes

O. kilimandscharicum, two in each of the accessions OB1 and OP and only one in OF1. The accession representing *O. kilimandscharicum* exhibited higher values of genetic variation parameters (P, K, Kp) than the accessions of *O. basilicum* (Table 2).

Cluster analysis of the allele frequency data using Euclidean squared distance and Dice similarity measure (Table 8) showed farthest genetic distance between *O. kilimandscharcum* and both of the accessions of *O. basilicum* except the accession OF02. Minimal Euclideane distances were noticed within each variety or between var. *purpurascence* (OP) and var. *grandvant* (OB01, OB02 and OB03) of *O. basilicum*. The dendrograms based on either Euclidean squared distance or Dice similarity measure (Fig. 4) showed best discrimination of the accessions under study at both species and infra-specific levels. At 50% similarity, the accessions were classified according to Dice similarity measure into four categories representing the three cultivars of *O. basilicum* and the accession of *O. kilimandscharcum* while they were grouped into three categories according to squared Euclidean distance aggregating the two varieties *grandvant* and *purpurascence* in one group.

Cluster analysis of the combined data (morphology, seed proteins and Isozymes) depending on Chi-square between sets of frequencies (Table 9 and Fig. 5) showed

Table 6: Allele frequency of polymorphic loci of the studied *Ocimum* samples; unique alleles are shaded and *italic*

Isozymes	Alelles	OB01	OB02	OB03	OF01	OF02	OP	OK
MDH1	A	0	0	0	0	0	***0.11***	0
	B	1	1	1	1	1	0.89	1
MDH2	C	1	1	1	0	0	0	0
	D	0	0	0	0	0	***1***	0
	E	0	0	0	0	0	0	***0.54***
	F	0	0	0	0	0	0	***0.46***
ME1	A	0.53	0.56	0.51	0.33	0.4	0.36	0.5
	B	0.47	0.44	0.49	0.66	0.6	0.64	0.5
PHOS1	A	1	1	1	0.5	0.3	0.51	0.25
	B	0	0	0	0.5	0.7	0.49	0.75
IDH1	A	0.78	0.75	0.73	0.65	1	0.6	1
	B	0.22	0.25	0.27	0.35	0	0.4	0
PPO2	B	0	0	0	0	0.61	0	0.5
	C	1	1	1	1	0	1	0
	D	0	0	0	0	0.39	0	0.5
EST α n. acetat 1	A	0.27	0.24	0.26	0	0.17	0.15	0.56
	B	0	0	0	0	0	0	***0.44***
	C	**73**	**0.76**	**0.74**	**1**	**0.83**	**0.85**	0
EST α n. acetat 2	D	0	0	0	0	0	0	***1***
	E	**1**	**1**	**1**	**0.46**	**1**	**1**	0
	F	0	0	0	***0.54***	0	0	0
EST α n. buterat1	A	1	0.61	0	0	1	1	0.61
	B	0	0.39	0	0	0	0	0.22
	C	0	0	0	0	0	0	***0.17***
EST α n. buterat2	D	0	0	0	0	0	0	***0.48***
	E	0	0	0	1	1	0	0.52
	F	***1***	0	0	0	0	0	0
EST α n. buterat3	G	***0.41***	0	0	0	0	0	0
	H	0.59	1	0	0	0	0	1
EST α n. valerat1	A	0	0	0	0	0	0.13	0.19
	B	1	1	0	1	1	0.87	0.81
EST α n. valerat2	C	0.8	0.78	0.81	0	0	1	1
	D	0.2	0.22	0.19	0	0	0	0
EST α n. pro 2	B	0	0.24	0.49	0.29	0.29	0.31	0.38
	C	0.5	0.45	0.51	0.71	0.71	0.69	0.62
	D	0.5	0.31	0	0	0	0	0
EST α n. pro 3	E	0	0	0	0	0.69	0	0.57
	F	0.68	0.72	0.7	0.71	0	0.69	0
	G	**0.32**	**0.28**	**0.3**	**0.29**	**0.31**	**0.31**	0
	H	0	0	0	0	0	0	***0.43***
EST β n. acetat1	**A**	**0.35**	**0.3**	**0.31**	**0.51**	**0.25**	**0.54**	0
	B	0.65	0.7	0.69	0.49	0.75	0.46	1

Table 7: Number of polymorphic and monomorphic loci for each isozyme studied

Isozymes	No. of polymorphic loci	No. of monomorphic loci
MDH.	2	0
ME.	1	1
PHOS	1	1
IDH	1	0
PPO.	1	1
EST α.n.acetate	2	0
EST α.n.buterate	3	0
EST α.n.valerat	2	1
EST α.n.propunat	2	1
EST β.n.acetate	1	2

Table 8: The genetic relationships among *Ocimum* accessions based on isozyme polymorphism

Accession	OB1	OB2	OB3	OF1	OF2	OP	OK
(a): Euclidean squared distance							
OB1	0						
OB2	**0.001**	0					
OB3	0.242	0.101	0				
OF1	0.556	0.427	0.362	0			
OF2	0.687	0.647	0.713	0.305	0		
OP	0.319	0.278	0.307	0.314	0.466	0	
OK	**0.985**	**0.804**	**1.000**	**0.886**	0.537	**0.882**	0

Table 8: Continued

Accession	OB1	OB2	OB3	OF1	OF2	OP	OK
(b): Dice similarity measure							
OB1	1.000						
OB2	**0.800**	1.000					
OB3	**0.769**	**0.833**	1.000				
OF1	0.462	0.500	0.400	1.000			
OF2	0.571	0.462	0.364	0.545	1.000		
OP	0.462	0.333	0.400	0.200	0.364	1.000	
OK	0.143	0.308	0.182	0.182	0.333	0.182	1.000

Table 9: The genetic distance (Chi-square) among *Ocimum* accessions (total data)

Accession	OB1	OB2	OB3	OF1	OF2	OP	OK
OB1	0						
OB2	0	0					
OB3	0.3507	0.3491	0				
OF1	0.6948	0.6207	0.7623	0			
OF2	0.955	0.8699	0.9893	0.3124	0		
OP	0.4083	0.3723	0.4385	0.6147	0.7543	0	
OK	0.7947	0.7974	0.9881	0.8563	1	0.9098	0

farthest genetic distance between *O. kilimandscharcum* and each of the accessions of *O. basilicum* and also between the two varieties of *O. basilicum*; *grandvant* and *finoverde*. Nearest genetic distances were revealed among the accessions of sweet basil. At 45% dissimilarity, the accessions were grouped into four categories representing the three varieties of *O. basilicum* and the accession of *O. kilimandscharcum*

DISCUSSION

The importance of morphological variation among the examined populations of *Ocimum* is demonstrated by the topology of the dendrogram based on Euclidean distance that showed discrimination of the two species under study. The separation of the two accessions of *O. basilicum* var. *fino verde* from all other accessions in one cluster is correlated with clear differences in most of the scored morphological characters. Grouping the accessions of each cultivar in one cluster in spite of the different environmental conditions of each accession reflects a genetic basis of the plant form in *Ocimum*.

The large variation in the number of protein bands among the studied *Ocimum* accessions reflects the high level of genetic variation among and within *Ocimum* populations in Egypt. The presence of certain protein bands characteristic to certain varieties or species, in addition to the topology of the tree illustrating the genetic distances among the studied *Ocimum* accessions based on the data obtained from the electrophorertic analysis of the seed proteins, supports the validity of the used technique (seed protein electrophoresis) as a tool for cultivar identification as well as studying genetic diversity and taxonomic relationships in *Ocimum* at both specific and infra-specific levels. Electrophoresis of seed proteins was previously used for cultivar identification for many other plants; as *Vicia faba* (Stegemann *et al*., 1980) and *Linum usitatissimum* (Sammour, 1988). It was also utilized for studying genetic diversity and addressing taxonomic relationships in *Glycine soja* (Bult and Kiang, 1992), *Lens* (Ladizinsky, 1979a, b; Sammour, 1994), *Vicia faba* (Sammour, 1989), *Lotus* (Sammour *et al*., 1991), *Sesbania* (Saraswati *et al*., 1993) and *Mentha* (Badr *et al*., 2003).

The dendrograms based on data obtained from isozyme polymorphism and allele frequency according to Euclidean distance or Dice measure reflect the intra-and inter-populational genetic variation in the genus *Ocimum* in Egypt. The higher genetic polymorphism in a population or a species is dependent on the amount of sexual reproduction, whereas low levels of genetic variation are often associated with asexual propagation (Micales and Bonde, 1995; Mustafa *et al*., 2005). According to this view, the results obtained in this study that showed lower genetic variation parameters for *O. basilicum* than those of *O. kilimandscharicum* might reflect the relative equilibrium state between sexual and vegetative reproduction that tends towards higher percentage of sexual propagation in *O. kilimandscharicum*. The topology of the tree based on the isozyme data in addition to such large number of unique alleles reflect the validity of the isozyme data to study genetic diversity and taxonomic relationships at both species and infra-specific levels in *Ocimum*. Such discrimination at the species and infra-specific levels using electrophoretically assayed isozyme variation was previously scored by many authors for different plant genera (Schmit *et al*., 1996; Kang and Chung, 2000; Batista *et al*., 2001; Badr *et al*., 2002; Mustafa *et al*., 2005). The very few number of cultivar diagnostic alleles within *O. basilicum* suggests that the cultivars under study might be hybrids or have recently derived from an ancestor harboring high levels of genetic diversity (Kang and Chung, 2000; Mustafa *et al*., 2005). This derivation might be due to intra-specific natural hybridization and the subsequent dispersion of pollen grains and hybrid fruits or seeds. Inter-specific

hybridization within *Ocimum* in Egypt may be also indicated by the farther genetic distance within *O. basilicum* than between some of its accessions and *O. kilimandscharicum*. The inter- and intra-specific natural hybridization was previously reported for *Ocimum* (Anonymous, 1980; Khosla and Sobti, 1984) and other plant genera (Aparicio *et al*., 2000; Ellstrand and Schierenbeck, 2000; Gobert *et al*., 2002; Mustafa *et al*., 2005).

The grouping of the accessions of each cultivar in one cluster in each of the trees based on data inferred from electrophoretic seed protein or isozyme polymorphism or both with morphological data may indicate the impact of environmental conditions on genetic variation rather than genetic divergence in *Ocimum* populations.

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