International Journal of Botany 1 (2): 188-195, 2005
ISSN 1811-9700

Genetic Diversity among *Mentha* Populations in Egypt as Reflected by Isozyme Polymorphism

[1]Abd El-Zaher M.A. Mustafa, [1]Abdelfattah Badr,
[2]Mohammed A. El-Galaly, [2]Ahmed A. Mobarak and [2]Mervat G. Hassan
[1]Department of Botany, Faculty of Science, Tanta University, 31527 Tanta, Egypt
[2]Department of Botany, Faculty of Science, Benha University, Benha, Egypt

Abstract: Isozyme polymorphism has been used to measure genetic diversity, genetic population structure and for the estimation of the amount of genetic divergence within and between populations and species of *Mentha* in Egypt. Ten isozyme systems revealed 31 isozyme loci and a total of 79 alleles in 32 populations of *M. longifolia*, five populations of *M. spicata* and one population of *M. piperita*. The examined populations of *M. longifolia* and *M. spicata* showed high levels of genetic variation that might be due to out-breeding and wide dispersal of seeds and pollen grains. Three alleles were scored in all populations; however, no diagnostic alleles at the species level were detected in the 31 isozyme loci. This suggests that the three species may be of hybrid origin or have recently been derived from an ancestor harboring high levels of genetic diversity. The tree illustrating genetic distance among the examined accessions of *Mentha* indicated obvious discrimination of the three species and the isolation of their populations from each other. At the infra-specific level, the geographically proximal populations exhibited higher genetic similarity than those that geographically distant, indicating environmental impact on the genetic diversity in the genus *Mentha* in Egypt.

Key words: *Mentha*, genetic diversity, isozymes

INTRODUCTION

The genus *Mentha* L. includes perennial erect herbs with prostrate stem and some erect aerial shoots. It includes 25 species mainly distributed in temperate regions of the world; many of them are inter-specific hybrids[1,2]. The species of *Mentha* have a medicinal and economic importance due to the volatile oils in their vegetative parts[3]. *Mentha* is represented in the Egyptian flora by three species; *M. spicata*, *M. pulegium* and *M. longifolia*[4]. The latter species is widely distributed and includes two subspecies; *M. longifolia* subsp. *typhoides* and *M. longifolia* subsp. *schemprei*[4]. *M. spicata* (spearmint) is cultivated for its volatile oils and is used in food flavoring. *M. piperita* (peppermint) was added to this study due to its wide cultivation in Egypt for its economic uses.

In addition to morphological traits and other cellular biochemical and molecular evidences, isozyme polymorphism has been used effectively to assess genetic variation, taxonomic and phylogenetic relationships within and among populations of the same species and also between closely related species[5-7]. Isozymes are closely tied to the genotype and electrophoretically assayed isozymic variation has been used effectively to estimate the extent of genetic variability[8-10]. Isozyme polymorphism has provided good estimates of genetic variations within and among populations, within species and between closely related species in a number of genera. For example in natural population of wild barely *Hordium spontaneoum*[11], the wild radish *Raphanus sativus*[12] and the wild soybean, *Glycine soja*[13]. Isozyme polymorphism has been also recently used as evidence for natural hybridization in *Phlomis*[14] and reveal the ancestors of white clover *Trifolium repens*[15]. Also, many recent studies used isozyme markers to evaluate the genetic resources and variability of plants for conservation purposes[16,17].

Understanding and evaluation of genetic diversity in plants are important for future crop improvement, which is dependent upon the availability and strategic use of genetic diversity. Estimating the genetic diversity levels both within and among populations of a crop is necessary for the best conservation of its gene pool[18]. 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[18]. However, the variability found within

Corresponding Author: Abd El-Zaher M.A. Mustafa, Department of Botany, Faculty of Science, Tanta University, 31527 Tanta, Egypt Tel: 0020403516041 Fax: 0020403350804

populations of native plant species can also have important implications for their conservation and management[18]. Genetic variation and/or taxonomic relationships in the genus *Mentha* were previously investigated using morphological, chemical, cytological and molecular traits[2,19-25]. However, no previous studies utilizing electrophoretic variation of isozymes in taxonomic, genetic or phylogenetic studies on *Mentha* are known. The objective of this study was to investigate genetic diversity among *Mentha* populations in Egypt inferred from isozyme polymorphism.

MATERIALS AND METHODS

This study was conducted at 2003-2004. Seed materials were collected from a number of localities representing 38 natural populations. These included 32 populations of *M. longifolia* (ML01-ML32); ML01 for the subspecies *schemprei* and ML02-ML32 for the subspecies *typhoides*. They included also five populations for *M. spicata* (MS01-MS05) and one for *M. piperita* (MP).

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[26]. 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 isozyme (Table 3).

Isozymes were separated on 8% PAGE vertical slab gels (16x18x0.2 cm) according to Wendel and Weeden[32]. The gel buffer composed of 45 mM Tris-HCl, 25 mM boric acid and 1mM 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 after electrophoresis by shaking in the dark at 37°C in the appropriate recipe (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.

Data analysis: Interpretation of banding patterns followed standard principles[32,33]. Loci were numbered consecutively from the anodal end and alleles at each locus were labeled alphabetically in the same direction[34]. 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*). A tree illustrating the genetic distance among populations was constructed based on Nei's genetic distance[35,36], using the Unweighed Pair Group Method with Arithmetic average (UPGMA)[37] using the sequential, agglomerative, hierarchical and nested clustering method (SAHN) as defined by Sneath and Sokal[38], using the NTSYS-pc software program[39].

RESULTS

Ten enzyme systems and 31 loci were resolved and scored in the 38 *Mentha* populations (Table 2 and 3). Only IDH showed one locus while the remaining nine enzyme systems exhibited 2-4 loci each. Three of the 31 loci were monomorphic in all the studied accessions (PHOS 2, EST. α-n-val 1 and EST. β-n-acet 3), while the remaining 28 loci were polymorphic (Table 3). The allele frequency of the polymorphic loci is given in Table 2.

In *Mentha longifolia* subspecies *schemprei*, 9 loci were monomorphic (ME 1, PHOS 1, MDH 1, EST. α-n-acet 2, EST. α-n-acet 4, EST. α-n-buter 1, EST. α-n-buter 4, EST. α-n-val 2 and EST. β-n-acet 2). Three loci could not be scored (MDH 3, ME 3 and EST. α-n-buter 3) and the remaining 16 loci were polymorphic. In *Mentha longifolia* subspesies *typhoides* all loci were polymorphic except PHOS 3, while ME 1 was found polymorphic in ML24 only and MDH 3 could not be scored. In *Mentha spicata*, 7 loci were monomorphic (PPO 3, ME 1, PHOS 1, PHOS 3,

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	[27,28]	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	[29]	Oxido-reductase
Malate dehydrogenase (MDH)	E.C. 1.1.1.37	0.1 M Tris-HCl buffer pH 7.5, 0.015% NAD, 0.12 M DL-Malate, 20 mM, MTT and 4 mM PMS	[11]	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	[11]	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	[30]	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	[31]	Oxido-reductase

[1]Nomenclature committee of the international union of biochemistry and molecular biology, [2]Non-specific enzyme

Table 2: Allele frequency of the different isozymes in the studied *Mentha* populations in Egypt

Isozyme	Alleles	ML1	ML2	ML3	ML4	ML5	ML6	ML7	ML8	ML9	ML10	ML11	ML12	ML13	ML14	ML15	ML16	ML17	ML18	ML19
MDH 1	a	0	0	0.11	0.12	0.07	0	0	0	1	0.07	0.15	0.22	0.05	0.12	0.12	0	0	0.13	0.07
	b	0	0.5	0.09	0.09	0.08	0	0.29	0.22	0.15	0.15	0.2	0.28	0.1	0.38	0.26	0.35	0.3	0.14	0.24
	c	1	0.5	0.8	0.79	0.85	1	0.71	0.78	0.75	0.78	0.65	0.5	0.85	0.5	0.62	0.65	0.7	0.73	0.69\
MDH 2	a	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MDH 3	a	0	0.5	0	0.3	0.25	0	0	0	0.5	0.5	0.5	1	0.5	0.5	0.42	0	0	0	0
	b	0	0.5	1	0.7	0.75	1	1	1	0.5	0.5	0.5	0	0.5	0.5	0.58	0	0	0	0
ME 1	a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
ME 2	a	0.41	0.5	0.5	0.36	0.4	0.4	0.4	0.4	0.28	0.25	0.39	0.4	0.18	0.24	0.35	0.5	0	0	0.37
	b	0.59	0.5	0.5	0.64	0.6	0.6	0.6	0.6	0.72	0.75	0.61	0.6	0.82	0.76	0.65	0.5	1	1	0.63
ME 3	a	0	0	0	0	0	0	0	0	0	0.29	0	0.31	0	0	0	1	1	1	1
	b	0	1	1	1	1	1	1	1	1	0.71	1	0.69	1	1	1	0	0	0	0
PHOS 1	a	1	1	0.72	0.7	0.69	1	1	1	1	1	1	1	1	0.65	0.7	1	1	1	1
	b	0	0	0.28	0.3	0.31	0	0	0	0	0	0	0	0	0.35	0.3	0	0	0	0
PHOS 2	a	0.68	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	b	0.32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PPO 1	a	0.23	0	0	0.13	0.34	0.3	0	0	0	1	0	0	0	0	0	0.4	0	0	0
	b	0.77	0	1	0.85	0.66	0.7	1	0	0	0	0	0	0	1	1	0.6	1	0	1
PPO 2	a	0.19	0.19	0.14	0	0.09	0	0.06	0.04	0.02	0.9	0.22	0.15	0.1	0	0.16	0	0	0.1	0
	b	0.51	0.53	0.6	0.71	0.7	0.83	0.83	0.8	0.78	0.69	0.7	0.72	0.8	0.83	0.54	0.73	0.59	0.67	0.71
	c	0.3	0.28	0.26	0.29	0.21	0.17	0.11	0.16	0.2	0.21	0.18	0.13	0.1	0.17	0.3	0.27	0.41	0.23	0.29
PPO 3	a	0.65	0.7	0.58	0	0	0	0	0	0	0	0	0	0	0	0.7	1	1	1	0.66
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	c	0.35	0.3	0.42	0	1	0	1	1	0	0	1	1	1	1	0.3	0	0	0	0.33
ISD 1	a	0.08	0.06	0	0.05	0.09	0.08	0.04	0.05	0	0	0	0	0	0	0	0.19	0.11	0.18	0.35
	b	0.92	0.94	1	0.95	0.91	0.92	0.96	0.95	1	1	1	1	1	1	1	0.81	0.89	0.88	0.65
Est α n.acetate 1	a	0.51	0.6	0	0	0	0	0	0	0.5	0	0.5	0.54	0	1	1	0	1	1	0
	b	0.49	0.4	0	1	1	1	1	0	0.5	0	0.5	0.48	0	0	0	0	0	0	1
Est α n.acetate 2	a	0	0	0	0	0	0	0	0	0.33	0.14	0.3	0.21	0	0.22	0.31	0	0.24	0	0
	b	0	0.38	0.4	0	0	0	0	0	0	0.21	0.19	0.79	0	0.88	0.69	1	0.76	1	1
	c	1	0.62	0.6	1	1	1	1	0	0.77	0.65	0.51	0	1	0	0	0	0	0	0
Est α n.acetate 3	a	0.58	0	0.58	0	0.75	0	0	1	1	0	1	1	0.63	1	1	1	0.5	1	0.67
	b	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33
	c	0.17	0	0.42	0	0.25	.	1	0	0	0	0	0	0.37	0	0	0	0.5	0	0
Est α n.acetate 4	a	0	0	0	0	0	0	0.52	0.5	0	0.49	0	0	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0.48	0.5	0	0.51	0	1	0	0	1	1	1	1	1
	c	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0
Est α n.buterat 1	a	0.67	0.59	0	0.6	0.55	0	0	0	0.52	0.56	1	0	0	1	1	0	0.71	0.68	0.73
	b	0.33	0.41	0	0.4	0.45	0	1	1	0.48	0.44	0	1	1	0	0	0	0.29	0.32	0.27
Est α n.buterat 2	a	1	0.5	1	0.58	0.6	1	0.67	0	0.63	0.61	1	1	1	0.64	0.7	1	1	0.57	0.67
	b	0	0.5	0	0.42	0.4	0	0.33	1	0.37	0.39	0	0	0	0.36	0.3	0	0	0.43	0.33
Est α n.buterat 3	a	0	0.18	0	0	0.1	0	0.11	0	0	0	0	0	0.13	0	0	0	0.7	0.5	0.11
	b	0	0	0	0	0	1	0	0	0	1	0	0	0	0.05	0.12	0	0	0	0
	c	0	0.82	1	1	0.9	0	0.89	1	1	0	1	1	0.87	0.95	0.88	1	0.93	0.95	0.89
Est α n.buterat 4	a	1	0.33	0.36	0.3	0.37	1	0.44	0.3	0.52	0.44	0.64	0.5	0.61	0.58	0.48	0.5	0.53	0.37	0.64
	b	0	0.34	0.36	0.34	0.31	0	0.56	0.36	0.48	0.31	0	0	0	0	0	0	0	0.33	0
	c	0	0.32	0.28	0.36	0.32	0	0	0.34	0	0.25	0.36	0.5	0.39	0.42	0.52	0.5	0.47	0.3	0.36
Est α n.valerat 2	a	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
	b	0	0	0	0	0	0	1	1	1	0	0	1	1	1	1	1	0	0	0
	c	0	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0
Est α n.valerat 3	a	0	0	0	0	0	0	0	0	0	0.23	0.28	0	0.17	0.12	0.21	0.18	0.2	0.15	0.18
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	c	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	d	0	1	1	1	1	1	1	1	1	0.77	0.72	1	0.83	0.88	0.79	0.82	0.8	0.85	0.82
	e	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Est α n.propunat 1	a	0	0	0	0	0	0.08	0	0.19	0	0	0	0	0	0	0	0	0	0	0
	b	0	0.19	0	0	0	0.11	0.08	0	0	0	0.17	0	0.24	0.06	0.13	0.25	0.17	0.17	0.21
	c	0.65	0.6	1	0.71	1	0.56	0.68	0.81	0.79	1	0.63	0.83	0.76	0.56	0.5	0.5	0.5	0.53	0.52
	d	0.35	0.21	0	0.29	0	0.25	0.24	0	0.21	0	0.2	0.17	0	0.38	0.37	0.25	0.33	0.3	0.29
Est α n.propunat 2	a	0.59	0.71	0.74	1	0.82	1	0.86	0	1	0	0.83	1	0.69	0.8	0.85	0.7	0.75	0.78	0
	b	0.21	0	0	0	0	0	0.14	0	0	0.6	0	0	0.2	0	0	0	0	0	0
	c	0.2	0.29	0.26	0	0.18	0	0	0.48	0	0	0	0	0	0	0	0.3	0.25	0.22	0
	d	0	0	0	0	0	0	0	0.52	0	0	0	0	0	0	0	0	0	0	0
	e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	f	0	0	0	0	0	0	0	0	0	0.4	0.17	0	0.13	0.2	0.15	0	0	0	0
	g	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Est α n.propunat 3	a	0.8	0.85	0.79	0.75	0	0.65	0.6	0.87	0.72	0.69	0.75	0.65	0.58	0.71	0.73	0.69	0.74	0.81	0.8
	b	0	0	0	0.06	0.43	0.06	0.12	0.13	0.09	0.11	0	0.13	0.13	0.1	0.07	0.12	0.08	0	0
	c	0.2	0.21	0.21	0.19	0.57	0.29	0.28	0	0.19	0.2	0.25	0.22	0.29	0.19	0.2	0.19	0.18	0.19	0.2
Est β n.acetat 1	a	0.8	0	0	0	0.91	0	0.59	0	0.85	0	0.79	0.83	0	0.77	0.79	0	1	1	1
	b	0.2	0	0		0.09	0	0.41	0	0.15	0	0.21	0.17	0	0.23	0.21	0	0	0	0
Est β n.acetate 2	a	0	0	0	0	0	0	0	0	0	0	0.2	0	0	0	0	0	0.28	0.16	0
	b	0	0.72	0.72	0	0	0	0	0	0	0	0.21	0	0	0	0	0	0	0	0
	c	1	0.28	0.28	1	1	1	1	0.46	0	1	0.59	1	0.41	1	0.35	0	0	0.21	0
	d	0	0	0	0	0	0	0	0.54	1	0	0	0	0.59	0	0.65	1	0.72	0.63	1
Est β n.acetate 4	a	0.31	0.29	0.35	0.29	0.25	0	0.27	0.3	0.32	0.29	0.25	0.31	0.14	0.16	0.12	0.18	0	0	0
	b	0.42	0.51	0.65	0.56	0.52	0	0.73	0.7	0.68	0.71	0.75	0.69	0.65	0.65	0.63	0.65	1	1	1
	c	0.27	0.2	0	0.19	0.23	0	0	0	0	0	0	0	0.21	0.19	0.25	0.17	0	0	0

Table 2: Continue

Isozyme	Alleles	ML20	ML21	ML22	ML23	ML24	ML25	ML26	ML27	ML28	ML29	ML30	ML31	ML32	MS1	MS2	MS3	MS4	MS5	MP
MDH 1	a	0.06	0	0	0.09	0.1	0.18	0	0.09	0.11	0.06	0	0.06	0.06	0	0	0.18	0	0.29	0.5
	b	0.3	0.2	0	0.2	0.2	0	0	0.41	0.4	0.35	0.38	0.36	0.29	0	0	0	0	0	0.5
	c	0.64	0.8	1	0.71	0.7	0.82	1	0.5	0.49	0.59	0.62	0.58	0.65	1	1	0.82	1	0.71	0
MDH 2	a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MDH 3	a	0	0	0.5	1	1	1	0.5	0.5	0.5	0.5	0.5	0	0	0.5	0	0.5	0	0.5	0
	b	0	0	0.5	0	0	0	0.5	0.5	0.5	0.5	0.5	0	0	0.5	0	0.5	0	0.5	0
ME 1	a	0	0	0	0	0.62	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	0	0	0	0	0.38	0	1	1	1	0	1	1	1	1	1	0	0	0	0
ME 2	a	0.35	0.39	0	0.5	0.28	0.37	0.31	0.29	0.34	0.35	0.25	0.26	0.3	0.28	0.28	0.29	1	0.5	0
	b	0.65	0.61	1	0.5	0.72	0.63	0.69	0.71	0.66	0.65	0.75	0.74	0.7	0.72	0.72	0.71	0	0.5	1
ME 3	a	1	1	1	1	1	1	1	1	1	1	1	1	0.35	0.3	0.31	1	0.33	1	1
	b	0	0	0	0	0	0	0	0	0	0	0	0	0.65	0.7	0.69	0	0.67	0	0
PHOS 1	a	0.69	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	b	0.31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PHOS 2	a	1	1	1	1	0	1	0	1	0	0	1	1	0	1	0	0	0	0	1
	b	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PPO 1	a	0	0	0	0	0.27	0	1	0	0	0.21	0	0.2	0.31	0	1	0	0	0.18	0
	b	0	0	0	0	0.73	0	0	1	1	0.79	0	0.8	0.69	0	0	1	1	0.82	0
PPO 2	a	0	0.11	0	0	0	0	0	0	0	0	0	0.4	0	0	0.17	0.2	0.2	0	0
	b	0.74	0.6	0.75	0.76	0.7	0.7	0.76	0.73	0.69	0.65	0.71	0.6	1	1	0.51	0.5	0.49	0.5	1
	c	0.26	0.29	0.25	0.24	0.3	0.3	0.24	0.27	0.31	0.35	0.29	0	0	0	0.32	0.3	0.31	0.5	0
PPO 3	a	0	0	0	0	1	1	1	0	1	1	1	0	1	0	0	1	1	0	0
	b	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ISD 1	a	0.41	0.45	0.29	0.17	0.15	0.52	0.51	0.07	0.11	0.04	0.39	0.37	0.23	0.46	0.48	0.4	0.54	0.59	0.62
	b	0.59	0.55	0.71	0.83	0.85	0.48	0.49	0.93	0.89	0.96	0.61	0.63	0.77	0.54	0.52	0.6	0.46	0.41	0.38
Est α n.acetate 1	a	0.46	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	0.54	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Est α n.acetate 2	a	1	0	0	0	0	0	0	0	0.09	0	0	0	0	0	0	0	0	0	0
	b	0	0	0.86	0	0	0	0	0	0	0	0	0	0	0	1	1	0.25	0.19	1
	c	0	1	0.14	1	1	1	1	1	0.91	1	1	1	1	1	0	0	0.75	0.81	0
Est α n.acetate 3	a	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0
	b	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Estα n.acetate 4	a	0	0	0	1	1	1	0	0	1	0	1	1	0.6	0	0	1	1	1	0.59
	b	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
	c	0	0	0	0	0	0	1	1	0	1	0	0	0.4	0	0	0	0	0	0.41
Est α n.buterat 1	a	0.65	0	0.59	0.6	0.7	0	0	0	0	0.67	0.71	0.57	0.7	0	0	0	0	0	0
	b	0.35	0	0.41	0.4	0.3	0	0	1	0	0.33	0.29	0.43	0.3	0	1	1	1	1	1
Est α n.buterat 2	a	0.63	0	0.51	0.5	0.5	0	0	0	0	0	1	1	0	1	0	0	1	1	0
	b	0.37	0	0.49	0.5	0.5	0	0	0	0	0	0	0	1	0	1	1	0	0	1
Est α n.buterat 3	a	0.07	0	0.06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.11	0
	b	0	0	0	0.14	0	0	0	0.07	0	0.12	1	1	0	1	0.05	1	1	0.89	1
	c	0.93	1	0.94	0.86	1	0	0	0.93	1	0.88	0	0	1	0	0.95	0	0	0	0
Est α n.buterat 4	a	0.51	0.55	0.35	0.64	0.6	1	1	0.59	1	0.65	1	1	1	1	0.69	1	1	1	1
	b	0	0	0.34	0.36	0.4	0	0	0.41	0	0.35	0	0	0	0	0	0	0	0	0
	c	0.49	0.45	0.31	0	0	0	0	0	0	0	0	0	0	0	0.31	0	0	0	0
Est α n.valerat 2	a	0	0	0	0	1	1	1	1	0	0.36	0	0	0.3	0	0.45	0.48	0.5	0.54	0.42
	b	1	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
	c	0	0	0	0	0	0	0	0	0	0.64	0	1	0.7	0	0.55	0.52	0.5	0.56	0.58
Est α n.valerat 3	a	0.19	0.2	0.22	0.16	0.17	0.17	0	0.31	0	0.2	0.22	0.23	0.15	0.2	0.18	0.18	0.25	0.22	0.22
	b	0.1	0	0	0.15	0.11	0.13	0	0	0	0.15	0	0.12	0.15	0.12	0.1	0	0.2	0.13	0.1
	c	0	0.8	0.78	0	0	0	0	0.69	0	0	0	0.65	0.7	0.68	0.72	0.82	0.55	0.65	0.68
	d	0.71	0	0	0.69	0.72	0.7	1	0	1	0.65	0.78	0	0	0	0	0	0	0	0
	e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Est α n.propunat 1	a	0	0	0	0	0	0.13	0	0	0.13	0	0	0.32	0	0	0	0	0	0	0
	b	0.16	0	0	1	0	0.16	0	0	0.12	0	0	0.68	0	0	0	0	0	0	0
	c	0.6	0.79	0.81	0	0	0.51	0	0	0.55	0	0	0	0	0	0	0	0	0	0
	d	0.24	0.21	0.19	0	0	0.2	0	0	0.2	0	0	0	0	1	0	0	0	0	0
Est α n.propunat 2	a	0	0	0	0.85	0.8	0.79	0.83	0	0.86	1	0	0	0.77	0	0	0	0.51	0	1
	b	0	0	0	0.15	0.2	0.21	0.17	1	0.14	0	0	0	0.23	0	0	0	0	0	0
	c	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.19	0.22	0
	d	0	0.19	0.22	0	0	0	0	0	0	0	0	0	0	0	0.5	0.43	0	0	0
	e	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0.57	0	0	0
	f	0.8	0.81	0.78	0	0	0	0	0	0	0	0	0	0	0	0	0	0.17	0.78	0
	g	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.13	0	0
Est α n.propunat 3	a	0.81	0.79	0.85	0.69	0.72	0.68	0.69	0.83	0.82	0.79	0.75	0.81	0.74	0.59	0.5	0.53	0	0	0.5
	b	0	0	0	0.09	0.11	0.13	0.31	0.09	0.08	0.1	0.25	0.19	0.16	0.41	0.5	0.47	1	1	0.5
	c	0.19	0.21	0.15	0.22	0.17	0.19	0	0.08	0.1	0.11	0	0	0.9	0	0	0	0	0	0
Est β n.acetat 1	a	0.83	0.8	0.79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.4	0
	b	0.17	0.2	0.21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.6	0
Est β n.acetate 2	a	0	1	0	0	0	0	0.37	0.3	0.35	0.34	0.35	0	0.25	0	0.4	0.35	0	0.33	0
	b	0	0	0	1	1	1	0.63	0.7	0.65	0.66	0.65	1	0.75	0.54	0	0	0	0.67	1
	c	1	0.21	0	0	0	0	0	0	0	0	0	0	0	0.46	0	0	0.24	0	0
	d	0	0.69	1	0	0	0	0	0	0	0	0	0	0	0	0.6	0.65	0.76	0	0
Est β n.acetate 4	a	0	0	0	0.37	0.3	0.31	0.13	0	0.33	0.19	0.27	0	0	1	0.14	1	0	0.15	0
	b	1	1	1	0.63	0.7	0.69	0.72	0.74	0.67	0.7	0.73	1	0	0	0.86	0	0	0.85	1
	c	0	0	0	0	0	0	0.15	0.26	0	0.11	0	0	0	0	0	0	0	0	0

Table 3: Number of polymorphic and monomorphic loci for each isozyme studied in *Mentha* samples

Isozymes	No. of polymorphic loci	No. of monomorphic loci
MDH.	3	0
ME.	3	0
Phosphorylase	2	1
Isd	1	0
PPO.	3	0
EST α.n.acetate	4	0
EST α.n.buterate	4	0
EST α.n.valerat	2	1
EST α.n.propunat	3	0
EST β.n.acetate	3	1

Table 4: Number of replica (*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 *Mentha*

Populations	N	P	K	Kp	Unique alleles
ML01	5	0.62	1.77	2.25	4
ML02	4	0.68	1.88	2.24	0
ML03	6	0.52	1.74	2.25	0
ML04	5	0.57	1.83	2.31	0
ML05	5	0.62	1.85	2.25	0
ML06	4	0.30	1.45	2.50	0
ML07	4	0.48	1.70	2.23	0
ML08	4	0.50	1.68	2.18	0
ML09	5	0.54	1.75	2.23	0
ML10	6	0.64	2.00	2.36	0
ML11	4	0.58	1.88	2.36	0
ML12	4	0.44	1.60	2.27	0
ML13	5	0.57	1.87	2.38	0
ML14	5	0.58	1.77	2.27	0
ML15	5	0.65	1.85	2.29	0
ML16	4	0.50	1.68	2.27	0
ML17	6	0.52	1.64	2.15	0
ML18	6	0.50	1.79	2.42	0
ML19	5	0.54	1.71	2.15	0
ML20	4	0.58	1.83	2.36	0
ML21	4	0.55	1.70	2.18	0
ML22	4	0.58	1.71	2.07	0
ML23	4	0.54	1.73	2.25	0
ML24	5	0.59	1.86	2.23	1
ML25	5	0.45	1.65	2.45	0
ML26	5	0.42	1.58	2.13	0
ML27	6	0.52	1.67	2.18	0
ML28	6	0.52	1.76	2.36	0
ML29	6	0.70	1.95	2.29	0
ML30	5	0.50	1.50	2.00	0
ML31	5	0.43	1.05	2.23	0
ML32	4	0.60	1.05	2.25	0
MS01	4	0.35	1.40	2.14	0
MS02	4	0.57	1.76	2.17	0
MS03	5	0.45	1.59	2.10	0
MS04	5	0.42	1.68	2.50	0
MS05	6	0.67	1.76	2.07	0
MP	4	0.32	1.37	2.17	0

EST.α-n-acet 3, EST. α-n-buter 1 and EST. α-n-prop 1) and two were absent (MDH 2 and EST. α-n-acet 1). In *Mentha piperita*, 13 loci were monomorphic (ME 2, ME 3, PHOS 1, PHOS 3, PPO 2, EST. α-n-acet 2, EST. α-n-buter 1, EST. α-n-buter 2, EST. α-n-buter 3, EST. α-n-buter 4, EST. α-n-prop 2, EST. β-n-acet 2 and EST.β-n-acet 4) and six loci were polymorphic (MDH1, ISD1, EST. α-n-acet4, EST. α-n-val2, EST. α-n-val3 and

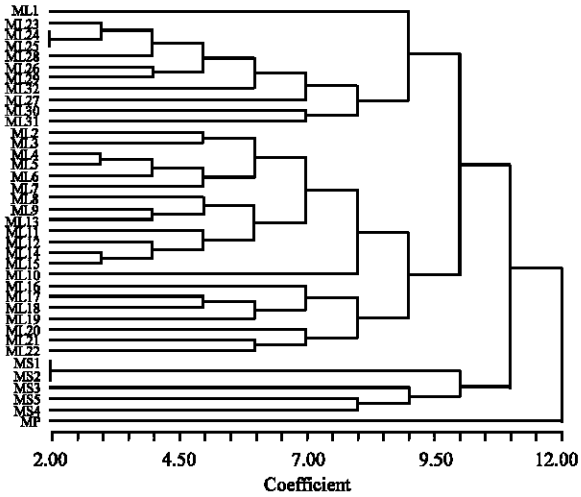

Fig. 1: A UPGMA tree based on electrophoretic data of *Mentha* isozymes

EST. α-n-prop3), while the remaining nine loci were absent in the accession representing *M. piperita*.

The proportion of polymorphic loci (*P*) in the populations of *Mentha longifolia* ranged between 0.3 and 0.7, in *Mentha spicata*, between 0.35 and 0.67 and was 0.32 in the accession representing *M. piperita*. The mean number of alleles per locus (*K*) ranged between 1.05 and 2.0 in *Mentha longifolia* and between 1.4 and 1.76 in *Mentha spicata* and in *Mentha piperita*, K was 1.37. The mean number of alleles per polymorphic locus (*kp*) ranged between 2.0 and 2.5 in *Mentha longifolia*, between 2.07 and 2.5 in *Mentha spicata* and in *Mentha piperita*, *kp* was 2.17 (Table 4).

Some accessions showed unique alleles that were not scored in any other accessions. However, four of the scored five unique alleles were found in *Mentha longifolia* subspecies *schemprei* (ML01) whereas only one unique allele was found in accession ML24 of *Mentha longifolia* subspecies *typhoides* (Table 4).

In the tree illustrating the relationships among the studied *Mentha* populations (Fig. 1), *Mentha piperita* was clearly separated from the populations of both *Mentha longifolia* and *Mentha spicata*. The populations of each of *Mentha longifolia* and *Mentha spicata* were delimited in separate groups (Fig. 1). The group representing *Mentha spicata* was diverged into two clusters; the first included samples having erect shoot (MS01 and MS02) with minimal dissimilarity, while the other cluster included the samples having prostrate shoot (MS03, MS04 and MS05). The group representing *Mentha longifolia* was delimited in two subgroups; one comprising subspecies *schemprei* and accessions ML23-ML32 of subspecies *typhoides*. However, the

accession representing subspecies *schemprei* was clearly distant from accessions of subspecies *typhoides*. The other subgroup of *Mentha longifolia* accessions was differentiated in two clusters; one representing accessions ML02-ML15 and the other representing accessions ML16-ML22.

DISCUSSION

The importance of isozyme polymorphism among the examined populations of *Mentha* is demonstrated by the topology of the UPGMA tree based on isozyme data (Fig. 1) that shows an obvious discrimination of the three species under study (*M. longifolia, M. spicata and M. piperita*). The accession representing *M. piperita*, which reproduce mostly vegetatively was separated from all the accessions representing the other two species, *M. longifolia* and *M. spicata*, which mostly reproduce sexually (by seeds). The validity of electrophoretic assayed isozyme variations to assess the relationships at the species level is further supported by the isolation of the populations representing *M. spciata* from those representing *M. longifolia*.

The grouping of the accessions of *M. spicata* that have an erect shoot in one subgorup and the accessions having prostate shoot in another subgroup reflects genetic basis for the plant form in *Mentha*. It also supports the validity of electrophoretically assayed isozyme polymorphism in assessing the genetic diversity at infraspecific levels and in distinguishing between different genetically-based plant forms. Moreover, it is obvious that the divergence of accessions of both *M. longifolia* and *M. spicata* reflects the impact of environmental variation on the genetic diversity among the populations of the genus *Mentha*. In both species, accessions representing localities within the Nile Delta were delimited from accessions representing localities outside the Nile Delta. However, the accession representing *M. longifolia* subsp. *schemprei* that was collected from Sant Katherine is assigned to the group representing the accessions collected from the Nile Delta, but clearly separated from them. This mode of divergence supports the view of Votava *et al.*[18] that geographically or environmentally proximal populations are more similar genetically than those that are geographically distant.

The amount of genetic polymorphism in a population or a species is dependent on the amount of sexual reproduction, whereas low levels of genetic diversity are often associated with species that are maintained asexually[10]. This view agrees with the results obtained in this study, where genetic diversity, expressed as the mean number of alleles per locus (*K*) or the mean number of alleles per polymorphic locus (*Kp*), was generally higher in the sexually reproducing plants of *Mentha longifolia* than the plants of *Mentha piperita*, which is mostly propagated vegetatively. This higher genetic diversity may be due to intraspecific natural hybridization and the subsequent dispersion of pollen grains and hybrid fruits or seeds in the sexually propagated plants of *Mentha longifolia*. This view agrees with some previous studies on other genera or species such as *Phlomis margaritae*[14], *Raphanus sativus*[40] and *Hemirocallis*[41].

No diagnostic alleles were scored for any of the studied species. However, four unique alleles were scored in the subspecies *schemprei* of *Mentha longifolia* and one unique allele in the accession ML24 of *Mentha longifolia* subspecies *typhoides*. The absence of diagnostic alleles for any of the studied three species suggests that these species may be hybrids or have recently derived from an ancestor harboring high levels of genetic diversity[41]. The hybrid origin of *M. spicata* and *M. piperita* was proposed by Harley and Brighton[1]. Thus, data inferred from isozyme polymorphism support cytogenetic evidence in indicating the hybrid origin of *M. spicata* and *M. piperita*. Natural hybridization is of wide occurrence in the wild *Mentha*[2].

REFERENCES

1. Harley, R.M. and C.A. Brighton, 1977. Chromosome numbers in the genus Mentha L. Bot. J. Linn. Soc., 74: 71-96.
2. Gobert, V., S. Moja, M. Colson and P. Taberlet, 2002. Hybridization in the section *Mentha* (Lamiaceae) inferred from AFLP markers. Am. J. Bot., 89: 2017-2023
3. Simpson, B.B. and M. Conner-Ogorzaly, 1986. Economic Botany, Plants in Our World. McGraw-Hill Book Company, Hamburg, Germany, pp: 640.
4. Mohammed, N.E., 1986. Flora of Egypt; Systematic revision of Labiatae. Ph.D. Thesis, Fac. Sci., Cairo Uni., Cairo, Egypt.
5. Gottlieb, L., 1977. Electrophoretic evidence and plant systematics. Ann. Missouri Bot Gard, 64: 161-180.
6. Crawford, D.J., 1985. Electrophoretic data and plant speciation. Syst. Bot., 10: 405-416.
7. Hamrick, J.L. and M.J.W. Godt, 1997. Allozyme diversity in cultivated crops. Crop Sci., 37: 26-30.
8. Weeden, N.F. and J.F. Wendel, 1989. Genetics of Plant Isozymes. In: Solttis, D.E. and P.S. Soltis, (Eds.). Isozymes in Plant Biology, London Champer and Hall, pp: 46-72.
9. Crawford, D.J., 1983. Phylogenetic and Systematic Inferences from Electrophoretic Studies. In: Tanksley, S.D. and T.J. Orton (Eds.) Isozymes in plant genetics and breeding, Part A: 257-287, Elsevier, Amsterdam.

10. Micales, J.A. and M.R. Bonde, 1995. Isozymes: Methods and Applications. In: Singh, R.P. and U.S. Singh (Eds.) Molecular methods in plant pathology. CRC Press, Inc., Lewis Publishers, London, pp: 115-130.
11. Brown, A.H.D., E. Nevo, D. Zohary and O. Dagan, 1978. Genetic variation in natural population of wild barely (Hordium spontaneum). Genetica, 49: 97-108.
12. Ellstrand, N., 1984. Multiple paternity within the fruits of the wild radish, *Raphanus sativus*. Am. Natl., 123: 819-828.
13. Bult, C.J. and Y.T. Kiang, 1992. Electrophoretic and morohological variation within and among natural populations of the wild soybean, *Glycine soja*. Sieb. Zucc. Bot. Bull. Acad. Sinica, 33: 111-122.
14. Aparicio, A., R.G. Albaladejo, M. Porras and G. Ceballos, 2000. Isozyme evidence for natural hybridgization in *Phlomis* (Lamiaceae): Hybrid origin of the rare *P.X. margaritae*. Ann. Bot., 85: 7-12.
15. Badr, A., H. Sayed-Ahmed, A. El-Shanshouri and L.E. Watson, 2002. Ancestors of white clover (*Trifolium repens* L.), as revealed by isozyme polymorphisms. Theor Applied Genet., 106: 143-148.
16. Gemmill, E.C., T.A. Ranker, D. Ragene, S.P. Perlman and K.R. Wood, 1998. Conservation genetics of the endangered endemic Hawaiian genus *Brighamia* (campanulaceae) Am. J. Bot., 85: 528-539.
17. Godt, M.J.W. and J.L. Hamrick, 1998. Low allozyme diversity in *Schalbea americana* (Scrophulariaceae), an endangered plant species. J. Heredity, 89: 89-93.
18. Votava, E.J., G.P. Nabhan and P.W. Bosland, 2002. Genetic diversity and similarity exhibited via molecular analysis among and within *in situ* population and *ex situ* accessions of chiltepin (*Capsicum annuum* var. glabriusculum). Conservation Geneaics, 3: 123-129.
19. Ikoda, N., S. Ono, T. Tzuchiya and P.K. Gupta, 1991. Cytogenetics of the genus *Mentha*, Chromosome-engineering in plants: Genetics, breeding, evolution. Part B: Development in Plant Genetics and Breedings, 2: 565-580.
20. Srikant S., B.R. Tyagi, S. Mandal, V. Singh, H. Singh and S. Sharma, 1996. Cluster analysis of 38 genotypes of peppermint (*Mentha piperita*) based on essential oil yield and quality traits. J. Med. Arom. Plant Sci., 18: 280-286.
21. Zviniene, N. and F. Pank, 1996. Data processing for numerical taxonomy in genus *Mentha* L. growing in Lithuania. Proceeding of International Symposium. Breeding Research on Medicinal and Aromatic Plants, Quedlinburg, Germany 30 June-4 July, Beitr. Zucht.-Bundesanstalt Fuer Zuchtungs-Forschung-und-Kulturpflanzen, 2: 103-107.
22. Ozguven, M., S. Kirici, N. Zencirci, Z. Kaya, Y. Anihster and T. Admas, 1998. *In situ* conservation of aromatic plants in southeast-Turkey: A wild *Mentha* species. Proceedings of Intl. Symposium on the *in situ* Conservation of Plant Genetic Diversity, Antalya, Turkey, pp: 171-176.
23. Singh, S.P., S. Sharma, R.K. Tewari and S. Sharma, 1998. Genetic improvement of *Mentha arvensis* based on essential oil yield and quality traits. J. Herbs, Spices and Medicinal Plants, 6: 79-86.
24. Khanuja, S.P.S., A.K. Shasany, A. Srivastava and S. Kumar, 2000. Assessment of genetic relationships in *Mentha* Species. Euphytica, 111: 121-125.
25. Shasany, A.K., A. Srivastava, J.R. Bahl, S. Sharma, S.P.S. Khanuja and S. Kumar, 2001. Genetic diversity of *Mentha spicata* L. germplasm through RAPD analysis. Plant Gene. Res. Newslett., 130: 1-5.
26. Marshall, P.R. and A.H.D. Brown, 1975. Optimum Sampling Strategies in Genetic Conservation In: Frankel, O.H. and J.H.Whawkes, (Eds.) Crop Genetic Resources for Today and Tomorrow. Cambridge University Press, Cambridge, UK, pp: 53-80.
27. Tanksley, D. and C. Rick, 1980. Genetics of Esterases in Species of *Lycoporicon*. Theor. Applied Genetic, 56: 209-219.
28. Tanksley, S. and T. Orton, 1983. Isozymes in Plant Genetic and Breeding. Part (B), Elsevier Science Publishers B.V. Amesterdam.
29. Fine, I.H. and L.A. Costello, 1963. The Use of Starch Electrophoresis in Dehydrogenase Studies. In: Colowick, S.P. and N.O. Kaplan, (Eds.). Methods in Enzymology, VI,. Academic Press, New York, pp: 958-972.
30. Siepmann, R. and H. Stegeman, 1967. Enzyme-elektrophorese in einschluß-Polymerisation des acrylamids. Amylase, phosphorylase. Z. Natureforsch., 22: 949-955.
31. Sato, M. and M. Hasegawa, 1976. The patency of spinach chloroplast phenolase. Phytochemistry, 15: 61-65.
32. Wendel, J.F. and N.F. Weeden, 1989. Visualization and Interpretation of Plant Isozymes In: Soltis, D.E. and P.S. Soltis, (Eds.). Isozymes in Plant Biology, London Champer and Hall, pp: 5-45.
33. Murphy, R.W., J.W.J. Sites, D.J. Buth and C.H. Haufler, 1996. Proteins I: Isozyme Electrophoresis. In: Hillis, D.M., C. Moritz and B.K. Mable, (Eds.) Molecular Systematics. Sinauer Associates, Sunderland, Massachusetts, pp: 45-126.
34. Pasteur, N., G. Pasteur, F. Bonhomme, J. Catalan and J. Britton-Davidian, 1988. Practical Isozyme Genetics. Halsted Press, New York.

35. Nei, M., 1972. Genetic distance between populations. Am. Natl., 106: 283-292.
36. Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci., USA, 70: 3321-3323.
37. Sokal, R.R. and C.D. Michener, 1958. A statistical method for evaluating systematic relationships. Univ Kansas Sci, Bull., 28: 1409-1438.
38. Sneath, P.H. and R.R. Sokal, 1973. Numerical Taxonomy. Freeman. San Francisco, pp: 573.
39. Rohlf, F.J., 2000. NTSYSpc, Numerical Taxonomy and Multivariate Analysis System. New York, Exeter Pub., Ltd.
40. Ellstrand, N.C. and K.A. Schierenbeck, 2000. Hybridization as a stimulus for the evolution of invasiveness in plants. Proc. Natl. Acad. Sci. USA, 97: 7043-7050.
41. Kang, S.S. and M.G. Chung, 2000. High levels of allozyme variations and low allozyme divergence within and among species of *Hemerocallis* (Liliaceae). Amer. J. Bot., 87: 1634-1646.