MOLECULAR PHYLOGENETIC RELATIONSHIPS OF TWO GENERA OF *LABIATAE* FAMILY

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There are hundreds of medicinal plants with useful properties that can be grown in temperate climates. More than two thousand plant species grow wildly in Egypt with no complete inventory of medicinal and aromatic plants in each region and, in general, the list of medicinal plants in Egypt and the Arab countries is inexhaustible (Batanouny, 1999).

It is well-known that most species, especially those belonging to the *Labiatae* (*Lamiaceae*) family, possess a wide range of biological and pharmacological activities. Since ancient times, they have been used to improve the flavor and the organoleptic properties of different types of food. Furthermore, the use of aromatic plants and species in phytotherapy is mostly related to different activities of their essential oils, such as antimicrobial, spasmolytic, carminative, hepatoprotective, antiviral, anticarcinogenic, etc., (Bozin *et al*., 2006). Species of genus *Mentha* (mints) show substantial variation in terms of their natural habitats, growth characteristics, and aromas. Essential oils produced by these plants are of particular economic interest (Shasany *et al.,* 2005). At the same time, the common sweet basil (*Ocimum basilicum* L.) with its several cultivated varieties is an annual aromatic plant, widely grown because of its pleasant odour and taste. It is an economically important herb in several Mediterranean and European countries where it has been a culinary herb for quite long time (Belbahri *et al*., 2005).

Molecular markers have been shown to be useful for diversity assessment in a number of plant species (Waugh and Powell, 1992). These markers, based on the polymerase chain reaction (PCR) technique, are the most commonly used for these purposes. Several different PCR-based techniques have been developed during the last decade, each with specific advantages and disadvantages. The randomly amplified polymorphic DNA (RAPD) markers technique is quick, easy and requires no prior sequence information; it detects nucleotide sequence polymorphisms using a single primer of arbitrary nucleotide sequence (Williams *et al*., 1990). Inter-simple sequence repeat (ISSR) markers permit detection of poly-

morphisms in inter-microsatellite loci, using a primer designed from dinucleotide or trinucleotide simple sequence repeats. ISSR amplifies inter-microsatellite sequences at multiple loci throughout the genome (Salimath *et al.,* 1995; Li and Xia, 2005). An ISSR molecular marker technique permits the detection of polymorphism in microsatellites and inter-microsatellite loci without previous knowledge of DNA sequences (Zietkiewicz *et al.,* 1994). This technique has been widely used to investigate genetic diversity and population genetic structure (Reddy and Nagaraju, 1999; Li and Xia, 2005) because of its advantages in overcoming limitations of allozymes and RAPD techniques (Wolfe *et al.,* 1998; Ratnaparkhe *et al.,* 1998; Esselman *et al.,* 1999). ISSR could be amplified with oligonucleotide primers based on simple sequence repeats anchored at either the 3′ or 5′ end (Lin *et al.*, 2006). RAPD and ISSR markers have been extensively used for DNA fingerprinting (Moreno *et al*., 1998; Gilbert *et al*., 1999; Abdel-Tawab *et al.,* 1998; 2001; 2004; 2006), genetic diversity studies (Hoz *et al.,* 1996; Esselman *et al*., 1999), population genetic studies (Wolfe *et al*., 1998; Nebauer *et al*., 1999) and phylogenetic studies (Hess *et al.,* 2000; Wu *et al*., 2005).

The objectives of this study were to identify some species of two genera of family *labiatae;* (*Mentha* sp. and *Ocimum* sp.) using molecular genetic techniques and to assess the phylogenetic relationships among the different species of the two genera.

MATERIALS AND METHODS

a. Materials

Plant materials

Two genera represented family *Labiatae*; four species of *Mentha* and three of *Ocimum* genera were used in this study. Their codes, scientific names, origins, active constituents and medicinal uses are shown in Table (1). Field grown leaves of the seven genotypes were freshly harvested for DNA isolation.

b. Methods

1. Molecular genetic studies

1.1. Genomic DNA extraction

Isolation of genomic DNA was done according to Pirttilä *et al.* (2001).

1.2. RAPD-PCR analysis

Reaction conditions were optimized as Maniatis *et al.* (1982) and Sambrook *et al.* (1989). The amplification was performed using a thermocycler (HYBAID® PCR EXPRESS) which was programmed as follows; initial denaturation at 94°C for 4 min, one cycle, denaturing at 94°C for 1 min, annealing at 36°C for 1 min, extension at 72°C for 2 min (40 cycle), final extension72°C for 10 min (one cycle) and hold at 4°C. The product fractionated on agarose gel (1.2 %) in TAE buffer, stained with 0.2g/ml ethidium bromide and 100bp ladder was used as a DNA molecular marker. Twenty-one random primers were used for RAPD-PCR amplification; their

names, sequences and GC percentages are summarized in Table (2).

1.3. ISSR-PCR analysis

An alternative method to SSR, called inter-simple sequence repeats (ISSRs)-PCR, has also been used with these species to get molecular markers (Ratnaparkhe *et al.,* 1998). Ten primers composed of a microsatellite sequence anchored at the 3' or 5' end with 2-4 arbitrary, often degenerate, nucleotides used in this study. Table (3) shows names of these primers, their sequences and GC percentages. Amplification was performed using a thermocycler (HYBAID® PCR EXPRESS) which was programmed as follows; initial denaturation at 94°C for 4 min, denaturing at 94°C for 30 sec, annealing at 55°C for 45 sec and extension at 72°C for 1.5 min (40 cycle), final extension 72°C for 10 minutes (one cycle) and hold at 4°C. The product was fractionated on agarose gel (1.2%) in TAE buffer, stained with 0.2 g/ml ethidium bromide and 100 bp ladder was used as a DNA molecular marker.

1.4. Phylogenetic relationships

The banding patterns of 21 RAPD and 10 ISSRs primers were scored and data were inserted in a computer as values of (1) and (0) for the presence and absence of bands, respectively. The data were analyzed using UPGMA cluster analysis program in order to estimate the similarity indices among the seven genotypes and to develop their consensus tree relationships.

RESULTS AND DISCUSSION

1. RAPD-PCR analysis

1.1.Genotype-specific markers based on RAPD analysis

The results of total amplified fragments (TFA), amplified fragments (AF) specific markers (SM) for each genotype of mints and basil using RAPD-PCR analysis with the 21 random primers are shown in Table (4) and Figure (1). A total number of 399 amplified fragments were obtained using the 21 RAPD-primers, all primers showed high polymorphisms which agreed with Welsh and McClelland (1990) who found that simple and reproducible fingerprints of complex genomes can be generated using single 10-mer primers and PCR. The species-specific markers differed among the seven genotypes (100 markers) and appeared in Tables (4 and 6); *M. viridis* and *M. piperita* exhibited 10 and 8 species-specific fragments, respectively. *M. arvensis* showed 3 specific markers, whereas, *M. longifolia* implied 12 specific markers. At the same time, *O. basilicum* showed 10 SM fragments, while *Ocimum canum* exhibited 49 species-specific bands, moreover, *O. basilicum Marseille* had 8 specific fragments.These results confirmed the importance of using RAPD analysis to characterize each genotype with the appearance of specific markers and produce informative bands that distinguished all the tested species, similar findings were obtained in mints by Gaboreanu *et al.* (2003), Hassan (2005) and Momeni *et al.* (2006) and in basil

with Vieira *et al*. (2001) and in other genera as Choi *et al.* (1999), Benedetti *et al.* (2000) and Abdel-Tawab *et al.* (2001 and 2006).

1.2. *Genetic similarity and cluster analysis based on RAPD markers*

The RAPD data were used to estimate the genetic similarities and the phylogenetic relationships among the four *Mentha* and the *three Ocimum* species. The highest similarity index (84.5%) was observed between the two species; *Ocimum basilicum* and *Ocimum basilicum Marseille*, while the lowest similarity index (29.6%) was recorded between *Mentha longifolia and Ocimum canum* species. A dendrogram for the phylogenetic relationships among the two genera (Fig. 2) separated the seven genotypes into two clusters; cluster one included *Ocimum* species, while cluster two combined *Mentha* species. Within the first cluster, two sub-clusters were obtained; the first one contained *Ocimum canum* alone, while the second sub-cluster included *Ocimum basilicum* and *Ocimum basilicum Marseille*. The second cluster was divided into two sub-clusters, *Mentha longifolia* was alone in one and the other included two sub-divisions; *Mentha viridis* alone, while the other subdivision cotained *Mentha piperita* and *Mentha arvensis.*

The aforementioned data asserted that RAPD-PCR analysis is good for phylogenetic relationships studies and could identify a high level of interspecific polymorphism among the seven species. This was consistent with their classification that agreed with Vieira *et al*. (2001) and Luigi *et al*. (2006) in *Ocimum* genotypes and Gaboreanu *et al*. (2003) and Momeni *et al*. (2006) in *Mentha* species. The same results were obtained in different genera (Han *et al*., 1998; Zhuravlev *et al*., 1998; Kim *et al*., 1999; Benedetti *et al*. (2000); Abdel-Tawab *et al.,* 2004 and 2006).

2. ISSR-PCR analysis

2.1. Genotype-specific markers based on ISSR primers

The results of total amplified fragments (TFA), amplified fragments (AF), specific marker (SM) for each genotype of mints and basil using ISSRs-PCR analysis with 10 primers are shown in Table (5) and Fig. (3). Primers produced high polymorphisms with a total of 164 total amplified fragments ranging from 11 (HB09 primer) to 23 fragments (844A primer). A total of 50 species-specific markers were obtained (Tables 5 and 6) as follows; *M. viridis* and *M. piperita* showed 12 and 3 specific markers, respectively. Moreover, *M. arvensis* exhibited 3 specific ones and *M. longifolia* showed 5 specific markers. In addition, *Ocimum basililcum* expressed 5, *O. canum* exhibited 15 and *O. basilicum Marseille* showed 7 specific markers. ISSR primers could produce polymorphic markers which were able to discriminate and identify the different species. ISSR markers are useful in gene tagging and can be used for finding species- specific markers. Moreover, ISSRs are consider-

ing a universal marker, which can be used in different species. This agreed with the results of Hodkinson *et al.* (2002), Nan *et al.* (2003), Hassan (2005) and Gobert *et al.* (2006).

2.2. Genetic similarity and cluster analysis based on ISSR analysis

The genetic similarities among the seven species showed that the highest one was obtained between *Mentha viridis* and *Mentha arvensis* species (69.6%), followed by 60.8% between *O. basilicum* and *O. basilicum Marseille*, while, the lowest similarity index was (27.4%) between *Mentha arvensis and Ocimum basilicum Marseille* species.

A dendrogram for the phylogenetic relationships among the seven genotypes was carried out as shown in Fig. (4). The seven genotypes were separated into two clusters; cluster one included the three *Ocimum* species, while cluster two combined the four *Mentha* species. Within cluster one, two sub-clusters were obtained, the first one contained *Ocimum canum* alone, while the second sub cluster included *Ocimum basilicum* and *Ocimum basilicum Marseille*. The second cluster was divided into two sub-clusters, *Mentha longifolia* was alone in one sub-cluster and the other sub-cluster comprised two sub-divisions; the first one contained *Mentha piperita*, while *Mentha viridis* and *Mentha arvensis* appeared in the other subdivision. The aforementioned results confirmed that ISSRs profiling is a powerful method for identification and molecular classification which agreed with Hassan (2005), Pharmawati *et al.* (2005) and Gobert *et al.* (2006).

3. Combined identification based on RAPD-PCR and ISSR-PCR analyses

In general, the overall results indicated the possible use of these methods to detect species-specific markers and to characterize the seven *Labiatae* genotypes. A total number of 150 markers out of 563 RAPD and ISSR-bands (about 27%) were found to be useful as species-specific ones (Table 6), each marker uniquely appeared in one species.

RAPD and ISSR techniques indicated that they are useful in the estimation of phylogenetic relationships among the four *Mentha* and three *Ocimum* species. Inspite of differences in the number of used RAPD-primers (21) and ISSR primers (10), they showed nearly equal efficiency in the detection of polymorphism. In addition, estimation of the phylogenetic relationships among these genotypes can assist in breeding programs. The molecular genetic studies of the four *Mentha* and the three *Ocimum* species are efficient tools for the characterization of these species, which could be used as marker-assisted selection (MAS) in breeding programs for these *Labiatae* species and in filing data for gene bank.

Cluster analysis based on the combined data of RAPD- and ISSR-PCR was carried out. The highest similarity index recorded was 78.3% between *O. basilicum* and *O. basililcum Marseille*

species, while the lowest similarity index (29.6%) was observed between the *M. viridis* and *O. basilicum Marseille*. A dendrogram for the phylogenetic relationships among the seven species (Fig. 5) separated them into two clusters; cluster one included the three *Ocimum* species, while cluster two included the four *Mentha* species. Within cluster one, two sub-clusters appeared; the first one included *Ocimum canum* alone, while the second comprized *Ocimum basilicum* and *Ocimum basilicum Marseille*. The second cluster was divided into two sub-clusters, *Mentha longifolia* was alone in one, and the other comprised two sub-divisions; the first one contained *Mentha viridis*, while the second included *Mentha piperita* and *Mentha arvensis.*

The results of RAPD, ISSR and combined (RAPD & ISSR) analyses showed different assessment among them. The combined analysis of RAPD & ISSR exhibited high degree of the relationship between *O. basilicum* and *O. basilicum Marseilli*. Otherwise, either primer alone or the combined analysis succeeded in resolving the high associations, which were confined to either *Mentha* species or *Ocimum* species, yet with variable degrees. RAPD and ISSR polymorphisms could be used as efficient tools for the detection of similarities and phylogenetic relationships of the studied genotypes. The same conclusion was obtained by several authors (King and Ferris, 2000; Alexander, 2002; Abdel-Tawab *et al.,* 1998, 2001 and 2006).

SUMMARY

Leaves of seven genotypes of *Labiatae* family represented two genera; four species of *Mentha* genus (*M. Viridis, M. Piperita, M. Arvensis* and *M. longifolia*) and three species of genus *Ocimum* (*O. basilicum, O. canum* and *O. basilicum Marseille*) were collected to identify molecular markers for each and to study the similarity indices and phylogenetic relatinships among these species. Two PCR- based analysis systems were carried out using randomly amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR). RAPD-PCR analysis using 21 random primers successfully exhibited a total of 399 DNA fragments across the seven genotypes, with 100 species-specific markers. Similarity indices ranged from 29.6% to 84.5% among the 7 species using RAPD-data. A dendrogram for phylogenetic relationships separated the seven genotypes into two clusters. ISSR-PCR technique using 10 primers showed a total number of 164 fragments across the seven genotypes. Fifty species-specific markers were detected. Similarity indices ranged between 27.4% and 69.6%. Dendrogram tree based on ISSR analysis showed two clusters, while, combined data of RAPD- and ISSR-PCR showed similarity indices ranged between 29.6% and 78.3 and a dendrogram tree with the same two clusters; one for *Mentha* species and the other for *Ocimum* species.

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Table (1): List of the two species of family *Labiatae*; their scientific names, origin, active constituents and medicinal uses.

Code	Scientific names*	Origin	Active constituents	Medicinal uses
1	*Mentha viridis* (Spear or baladi mint)	Meditranian and north africa	Menthol Menthone- Isomenthone-	for colic, wind , indigestion, hiccups and feverish childhood illnesses
2	*Mentha piperita* (Felfeli mint)	Meditranian and north africa	Menthone- Isomenthone- Menthol	Menthol is an antiseptic, Decongestant and analgesic, nausa, indigestion, gastric ulcer, colic, influenza, colds, respiratory tract infection And rheumatism.
3	*Mentha arvensis* (japanese mint)	Japan east asia	Carvon, Limonene α-pinene, β-pinene	for colic, wind , indigestion, hiccups
4	*Mentha longefolia (Silver mint or horsemint)*	South Africa	Piperitone piperitenone	for colic, wind , indigestion,
5	*Ocimum basilicum* (baladi basil)	Meditranian and north africa	α-pinene, β-pinene ocimene linalool Methyl chavecol	Treatment of abcesses, skin pustules, headach of common cold and antispasmodic for renal and gastric colics.
6	*Ocimum canum* (American basil)	Meditranian and north africa	N/A	
7	*Ocimum basilicum Marseilli* (frence basil)	Meditranian and north africa	ocimene linalool Methyl chavecol	

*Data were kindly obtained from the aromatic plants book by Dr. El- Shahat Nasr Abo Zaid 1988 & some internet resources

Table (2): Names, sequences and G C% for the twenty-one random primers used in RAPD-PCR analysis.

Serial number	Primer code	Sequence	GC %	Serial number	Primer code	Sequence	GC %
1	OP-A01	5′ CAG GCC CTT C 3′	70%	12	OP-B14	5′ TCC GCT CTG G 3′	70%
2	OP-A03	5′ CAG GCC CTT C 3′	70%	13	OP-B15	5′ GGA GGG TGT T 3′	60%
3	OP-A06	5′ GGT CCC TGA C 3′	70%	14	OP-B17	5′ TTT CCC ACG G 3′	60%
4	OP-A07	5′ GAA ACG GGT G 3′	60%	15	OP-B19	5′ ACC CCC GAA G 3′	70%
5	OP-A08	5′ GTG ACG TAG G 3′	60%	16	OP-B20	5′ GGA CCC TTA C 3′	60%
6	OP-A09	5′ GGG TAA CGC C 3′	70%	17	OP-D14	5′ CTT CCC CAA G 3′	60%
7	OP-A17	5′ GAC CGC TTG T 3′	60%	18	OP-D16	5′AGG GCG TAA G 3′	60%
8	OP-B07	5′ GGT GAC GCA G 3′	70%	19	OP-G02	5′ GGC ACT GAG G 3′	70%
9	OP-B09	5′ TGG GGG ACT C 3′	70%	20	OP-G03	5′ GAG CCC TCC A 3′	70%
10	OP-B10	5′ CTG CTG GGA C 3′	70%	21	OP-G07	5′ GAA CCT GCG G 3′	70%
11	OP-B12	5′ CCT TGA CGC A 3′	60%				

Table (3): Names, sequences and G C% for the ten ISSR primers used in ISSR-PCR analysis.

Serial number	Primer code	Sequence	GC %	Serial number	Primer code	Sequence	GC %
1	844A	5′$(CT)_8$ AC 3′	50%	6	HB15	5′$(GTG)_3$ GC 3′	72.7%
2	17899B	5′$(CA)_6$ GG 3′	50%	7	IS05	5′CAT$(CA)_7$ 3′	47.5%
3	HB09	5′$(CT)_6$ GG 3′	57%	8	IS07	5′ATTA$(CA)_7$ 3′	38.8%
4	HB11	5′$(GA)_6$ CC 3′	57%	9	IS09	5′AAC$(TG)_7$ T 3′	44.4%
5	HB13	5′$(GAG)_3$ GC 3′	73 %	10	IS10	5′$(TCC)_5$ AC3′	44.4%

Table (4): Number of amplified fragments and specific markers of the four *Mentha* and the three *Ocimum* species using RAPD analysis with 21 primers.

Primer	TAF	1		2		3		4		5		6		7		TSM
		AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
A01	10	4	0	3	1	3	0	2	0	5	1	4	1	2	0	3
A03	23	9	0	9	0	14	1	14	1	10	0	8	1	9	0	3
A06	18	7	0	8	2	7	0	8	0	9	0	7	1	9	0	3
A07	23	11	1	10	1	8	0	5	0	10	0	12	4	9	0	6
A08	19	7	3	9	0	8	0	8	1	8	0	10	3	8	0	7
A09	16	6	0	5	0	7	0	3	1	6	0	9	4	7	0	5
A17	13	5	0	5	0	5	0	4	0	7	0	6	1	8	0	1
B07	19	4	0	5	0	5	0	5	0	10	2	11	4	8	0	6
B09	19	7	1	10	0	7	0	10	0	10	3	7	1	11	1	6
B10	10	6	1	4	0	5	0	5	0	6	1	2	0	3	0	2
B12	22	7	0	7	0	7	0	7	2	8	1	10	4	11	2	9
B14	22	9	0	8	0	12	0	6	1	12	0	11	1	12	1	3
B15	17	5	0	9	0	11	0	10	0	4	1	9	5	3	0	6
B17	22	9	2	6	2	11	0	6	0	8	0	8	1	8	0	5
B19	22	11	0	14	1	11	0	7	0	10	0	13	1	12	0	2
B20	22	8	0	8	0	11	0	9	1	10	0	15	5	10	1	7
D14	21	2	0	3	0	4	1	2	0	12	0	8	4	13	1	6
D16	21	7	0	4	0	7	0	9	1	5	0	9	4	7	1	6
G02	21	11	1	8	0	9	0	9	3	7	0	8	0	10	1	5
G03	18	11	0	9	0	11	0	7	0	6	1	9	2	5	0	3
G07	21	4	1	6	1	9	1	7	1	8	0	5	2	7	0	6
Total	399	150	10	150	8	172	3	144	12	171	10	179	49	172	8	100

(1) *M. viridis* (2) *M. piperita* (3) *M. arvensis* (4) *M. longifolia* (5) *O. basilicum* (6) *O. canum* (7) *O. basilicum Marseille*.
TAF: total amplified fragment AF: amplified fragment SM: specific marker TSM: total specific markers

Table (5): Number of amplified fragments and specific ISSR markers of the four *Mentha* and the three *Ocimum* species using 10 ISSR primers.

Primer	TAF	1		2		3		4		5		6		7		TSM
		AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
844A	23	6	1	8	2	8	0	7	0	6	0	9	7	4	0	10
17899A	17	7	2	5	0	5	0	4	0	10	3	3	1	6	0	6
HB09	11	5	0	4	1	4	1	3	1	3	0	5	0	3	0	3
HB11	18	6	0	4	0	4	0	5	0	5	1	9	4	6	3	8
HB13	12	8	3	6	0	6	0	5	0	5	0	5	1	6	0	4
HB15	13	5	1	2	0	5	0	7	1	4	0	5	1	5	0	3
IS05	21	10	3	5	0	9	0	12	2	10	0	9	1	7	0	6
IS07	19	12	2	5	0	12	1	7	0	13	0	9	0	5	0	3
IS09	14	4	0	4	0	5	1	6	1	3	0	7	0	8	2	4
IS10	16	7	0	7	0	6	0	3	0	6	0	7	0	10	2	2
	164	70	12	50	3	64	3	59	5	55	4	68	15	60	7	49

(1) *M. viridis* (2) *M. piperita* (3) *M. arvensis* (4) *M. longifolia* (5) *O. basilicum* (6) *O. canum* (7) *O. basilicum Marseille*.
TAF: total amplified fragment AF: amplified fragment SM: specific marker TSM: total specific markers

Table (6): Molecular charachterization of the four *Mentha* and three *Ocimum* species based on the specific-markers of RAPD and ISSR-PCR analyses.

Genotype	RAPD-primer	Molecular size (bp)	ISSR-primer	Molecular size (bp)
M. viridis	OP-A07	1380	844A	252
	OP-A08	2900, 672, 497	17899B	731, 388
	OP-B09	640	HB13	1306, 381, 300
	OP-B10	415	HB15	380
	OP-B17	1100, 942	IS05	738, 675, 435
	OP-G02	934	IS07	1107, 140
	OP-G07	314		
M. piperita	OP-A01	800	844A	598, 339
	OP-A06	860, 220		
	OP-A07	870		
	OP-B17	1291, 650	HB09	190
	OP-B19	1094		
	OP-G07	1680		
M. Arvensis	OP-A03	779	HB09	280
	OP-D14	1180	IS07	274
	OP-G07	222		
M. longifolia	OP-A03	755, 295	HB09	261
	OP-A09	280		
	OP-B12	2667, 1190	HB15	400
	OP-B14	1500		
	OP-B20	2190	IS05	660, 303
	OP-D16	1500		
	OP-G02	1127, 994, 905		
	OP-G07	160		
O. basilicum	OP-A01	869	17899B	511, 272, 244
	OP-B07	1714, 450		
	OP-B09	1456, 527, 489	HB11	228
	OP- B10	636		
	OP- B12	390	IS07	996
	OP-B15	1413		
	OP-G03	250		
O. canum	OP-A01	850	844A	2157, 1554, 1464, 1054, 881, 515, 360
	OP-A03	520, 305		
	OP-A06	800		
	OP-A07	950, 505, 470, 334	17899B	893
	OP-A08	1424, 1341, 614		
	OP-A09	1455, 1290, 607, 180		
	OP-A17	594	HB11	1622, 590, 482, 390
	OP-B07	1330, 621, 390,150		
	OP-B09	1603		
	OP-B12	475, 320, 200, 100	HB13	1473
	OP-B14	450		
	OP-B15	839, 674, 638, 541, 411		
	OP-B17	1857	HB15	610
	OP-B19	2233		
	OP-B20	2300, 1750, 1654, 510, 500		
	OP-D14	2077, 1842, 1041, 323	IS05	320
	OP-D16	1207, 658, 530		
	OP-G03	984, 280		
	OP-G07	939, 592		
O. basilicum Marseilli	OP-B09	220	HB11	917, 550, 404
	OP-B12	550, 441		
	OP-B14	267	IS09	958, 389
	OP-B20	400		
	OP-D14	160	IS10	1277, 950
	OP-D16	2300		
	OP-G02	1238		

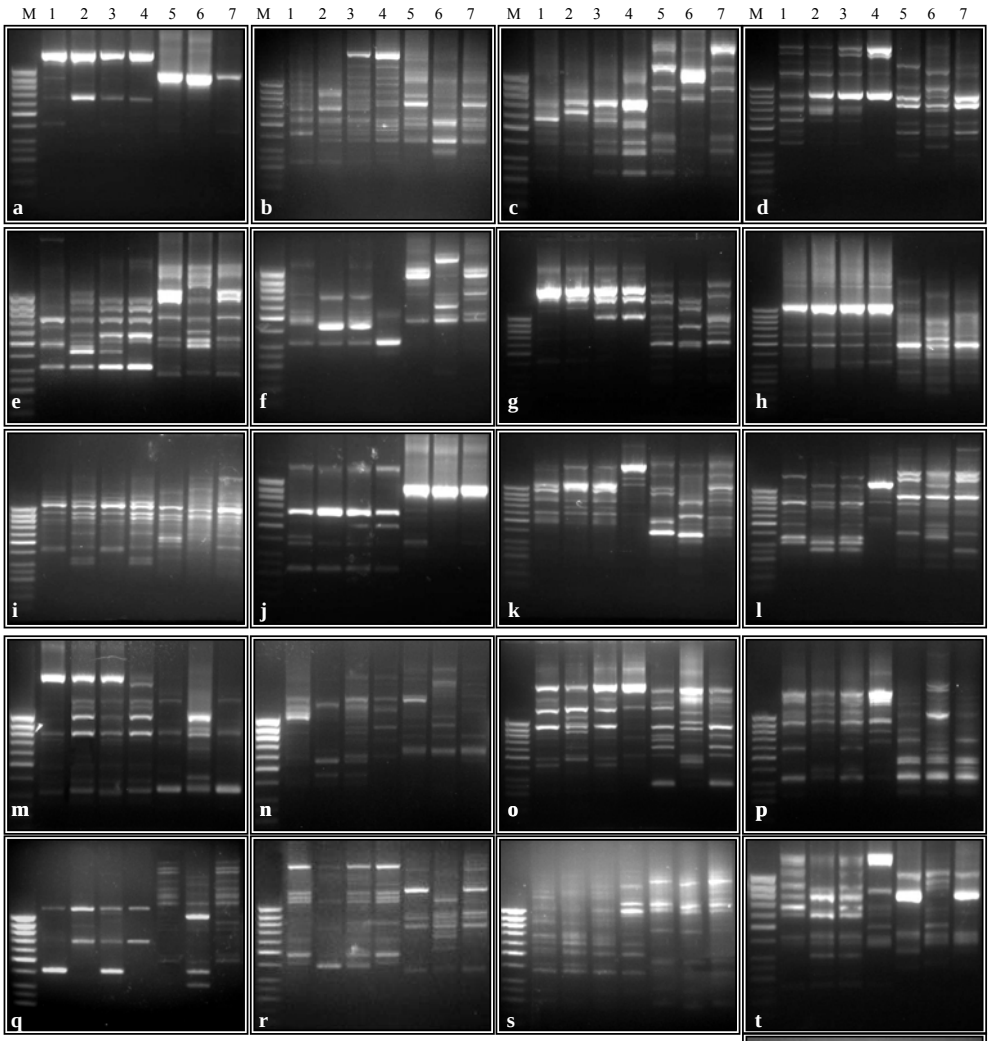

Fig. (1): DNA patterns of the seven genotypes from *Labiatae* family amplified with 21 10-mer random primers; (a) OP-A01, (b) OP-A03, (c) OP-A06, (d) OP-A07, (e) OP-A08, (f) OP-A09, (g) OP-A17, (h) OP-B07, (i) OP-B09, (j) OP-B10, (k) OP-B12, (l) OP-B14, (m) OP-B15, (n) OP-B17, (o) OP-B19 , (p) OP-B20, (q) OP-D14, (r) OP-D16, (s) OP-G02, (t) OP-G03 and (u) OP-G07 using RAPD-PCR analysis. (M) Marker (1) *M. Viridis* (2) *M. Piperita* (3) *M. Arvensis* (4) *M. Longifolia* (5) *O. Basilicum* (6) *O. canum* and (7) *O. basilicum Marseille*.

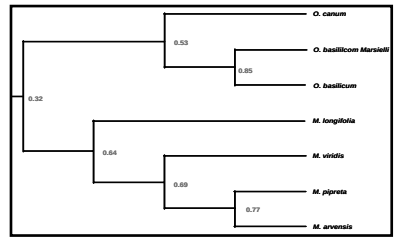

Fig. (2): Dendrogram for the phylogenetic relationships among the four *Mentha* and three *Ocimum* species based on similarity indices data of RAPD analysis.

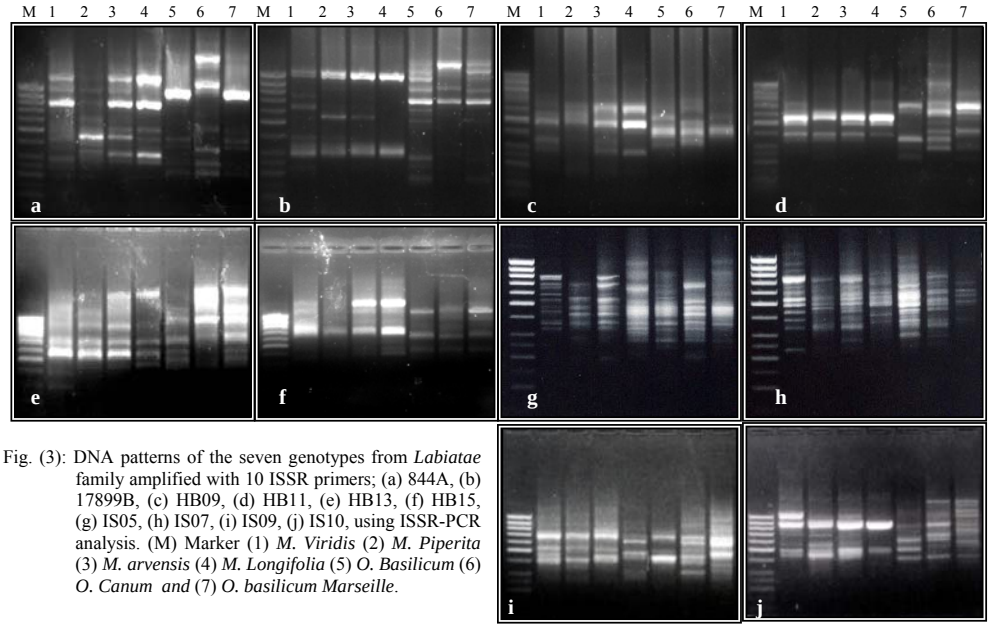

Fig. (3): DNA patterns of the seven genotypes from *Labiatae* family amplified with 10 ISSR primers; (a) 844A, (b) 17899B, (c) HB09, (d) HB11, (e) HB13, (f) HB15, (g) IS05, (h) IS07, (i) IS09, (j) IS10, using ISSR-PCR analysis. (M) Marker (1) *M. Viridis* (2) *M. Piperita* (3) *M. arvensis* (4) *M. Longifolia* (5) *O. Basilicum* (6) *O. Canum and* (7) *O. basilicum Marseille*.

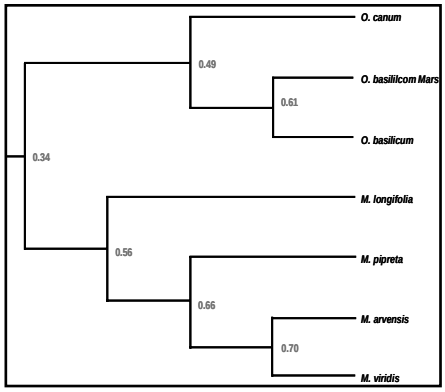

Fig (4): Dendrogram for the phylogenetic relationships among the four *Mentha* and three *Ocimum* species based on similarity indices data of ISSR analysis.

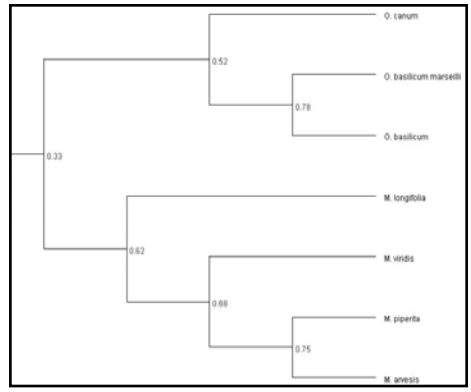

Fig. (5): Dendrogram for the phylogenetic relationships among the four *Mentha* and the three *Ocimum* species based on similarity indices-combined data of RAPD and ISSR analyses.