Genetic variations among two Egyptian mint species (*Mentha spicata* and *Mentha piperita*) using random amplified Polymorphic DNA markers

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ABSTRACT

In the present study, RAPD-PCR was used for identification of two mint species (*Mentha spicata* and *Mentha piperita*) collected from Egypt and studying the genetic variations among them. RAPD-PCR was conducted using ten oligonucleotide primers. The ten primers succeeded in amplifying of DNA fragments for the two *Mentha* species. These primers produced multiple fragments profiles ranging from 3 to 19 fragments. The polymorphism levels differed from primer to another. The number of polymorphic fragments ranged from 0 to 66.29 per primer. The total number of the obtained fragments from the ten primers was 83 fragments with 39 polymorphic fragments (46.98 %) across the two mint species. Forty percent of primers did not exhibit any polymorphism. This study provides evidence that RAPD Markers can be used as an efficient tool for the detection of the genetic variations and identifications of different mint species.

Keywords: *Mentha* species, RAPD-PCR, genetic differences, molecular markers

INTRODUCTION

Mint plants are perennial herbs. They are cultivated for their aromatic oils (produced as secondary metabolites) that are important in a great variety of products and for medicinal and aromatic purposes. (Vining *et al*., 2017 and Ahmad, 2018).Mint belong to the Genus *Mentha,* family *Labiatae*. The genus which includes about 25 to 30 species is widely distributed in the temperate areas (Douhan and Johnson, 2001, Rabia *et al*., 2015, Vining *et al*., 2017 and Ahmad, 2018). In Egypt, there are three species of *MenthaM. spicata*, *M. longifolia* and *M. pulegium*. *M. spicata* (spearmint) is used in food flavoring and cultivated for the volatile oils. Also, *M. piperita* (peppermint) is widely cultivated in Egypt for its economic importance (Mohammed, 1986 and Rabia *et al*., 2015).

To examine the diversity of *Mentha,* different methods have been used in the past using morphological, chemical, cytological, and chromosomal markers. The genus polymorphism needs accurate taxonomic methods with modern technologies (Ahmad, 2018).

The using of DNA markers techniques can generate a profile or "fingerprint" unique to a particular species (Fenwick and Ward, 2001 and Ahmad, 2018). Randomly amplified polymorphic DNA (RAPD) analysis is one of these markers. In RAPD, short DNA primers are used to generate genome-specific patterns by polymerase chain reaction (Williams *et al*., 1990). RAPD-PCR has been used for cultivari dandification and genetic analysis in a different plants (Nazar and Mahmood, 2011; Mahmood *et at*., 2011, Abd El-Azeem *et al*., 2011 and Ibrahim *et al*., 2014)

In the present study, RAPD-PCR was used for identification of two mint species (*Mentha spicata* and *Mentha piperita*) collected from Egypt and studying the genetic variations among them.

MATERIALS AND METHODS

Experimental plants

Mint plants were obtained from Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat city, Sadat city, Egypt.

DNA extraction

DNA extraction kit from Fermentas (Gene JETTM, plant genomic DNA purification mini kit) was used for genomic DNA extraction. Polyvinyl pyrrolidone (PVP) was added to lysis Buffer before used at 2% (W/V) final concentration. DNA was quantified by using Gene quant spectrophotometer. The quality was further detected through 1% agarose gel electrophoresis.

RAPD analysis

RAPD-PCR was conducted using ten oligonucleotide primers (Table, 1) that were chosen from the Operon Kit (Operon Technologies Inc., Alabameda, CA). PCR amplification was carried out in 20µl volume contains 30 ng of DNA, 0.4 µM of primer, 200 µM of each dNTP, 2 mM MgCl2, 1x PCR buffer and 0.8U of Tag DNA polymerase. PCR amplification was performed by using Thermal cycler of Biometra T1 gradient with the following steps: 94°C for 5 min, then 94°C for 1 min, 35°C for 40 sec, and 72°C for 1 min for 35 cycles. A final extension cycle was performed at 72°C for 7 min (Gebhart, et al., 1991 and Ibrahim et al., 2014). PCR products were tested on 1.5 % ethidium bromide stained agarose gel electrophoresis. The products were separated at 90 volts for 1.0 hr in I x TAE buffer.

Data analysis

The obtained fragments were scored as present/absent using Phoretix 1D advanced Version 4.00 (Phoretix International, Newcastle upon Tyne, UK). Similarity and coefficients clustering methods were tested using version 2 of the program NTSYSpc (Applied Biostatistics, USA). Clustering methods WPGMA, UPGMA, Complete-link, and Single-link were used in all possible combinations with the similarity coefficients Jaccard, Dice, and simple matching. (Gebhart et al., 1991).

Table1. The nucleotide sequences of primers that were used for RAPD analysis.

Primer code	Sequence(5`-3`)
OPA-15	TTCCGAACCC
OPA-19	CAAACGTCGG
OPA-06	GGTCCCTGAC
OPA-17	GACCGCTTGT
OPA-04	AATCGGGCTG
OPA-11	CAATCGCCGT
OPB-06	TGCTCTGCCC
OPC-01	TTCGAGCCAG
OPC-09	CTCACCGTCC
OPC-20	ACTTCGCCAC

RESULTS

In this study genetic, variations of the two mint species (Spearmint and Peppermint) was studied by using RAPD markers. Ten primers were used; OPA-11, OPA-04, OPA-06, OPA-15, OPA-17, OPA-19, OPB-06 OPC-01, OPC-09 and OPC-20 (Table 2 and Figure 2).

The ten primers succeeded in amplifying of DNA fragments for the two *Mentha* species. These primers produced multiple fragments profiles ranging from 3 to 19 fragments. The polymorphism levels differed from primer to another. The number of polymorphic fragments ranged from 0 to 66.29 per primer. The total number of the obtained fragments from the ten primers was 83 fragments with 39 polymorphic fragments (46.98 %) across the two mint species. The highest number of amplified fragments (19) was obtained from primer OPC-20, while the primer OPA-17 exhibited the lowest number (3). The primer OPC-09 gave the highest % of polymorphism (69.23) whereas the primers, OPA-11, OPA-04, OPA-15 and OPA-17 did not exhibit any polymorphism.

Table2. The obtained amplified fragments from the DNAs of the two mint species.

Band	OPB-06			OPA-11			OPA-04			OPA-06			OPC-20		
	Size in bp	S1	S2	Size in bp	S1	S2	Size in bp	S1	S2	Size in bp	S1	S2	Size in bp	S1	S2
1	1454	0	1	2984	1	1	1454	1	1	1664	1	1	1454	1	0
2	1271	1	1	2384	1	1	1271	1	1	1271	1	1	1329	1	0
3	970	1	1	1904	1	1	970	1	1	1110	1	1	1110	1	1
4	811	1	0	1740	1	1	811	1	1	970	1	1	928	0	1
5	775	0	1	1591	1	1	-	-	-	848	1	1	741	1	1
6	708	1	0	1454	1	1	-	-	--	741	1	1	677	1	0
7	677	1	0	1215	1	1	-	-	-	541	1	1	648	1	1
8	648	0	1	1110	1	1	-	-	-	473	1	1	592	1	0
9	541	1	1	848	1	1	-	-	-	395	1	1	566	0	1
10	473	1	1	775	1	1	-	-	-	345	1	1	541	1	0
11	432	0	1	708	1	1	-	-	-	288	1	1	494	1	0

Band	OPB-06			OPA-11			OPA-04			OPA-06			OPC-20		
	Size in bp	S1	S2	Size in bp	S1	S2	Size in bp	S1	S2	Size in bp	S1	S2	Size in bp	S1	S2
12	395	1	1	566	1	1	-	-	-	230	1	1	473	0	1
13	345	1	1	494	1	1	-	-	-	192	1	1	413	1	1
14	316	0	1	452	1	1	-	-	-	168	0	1	395	0	1
15	302	1	1	395	1	1	-	-	-	147	1	1	361	1	0
16	264	1	1	302	1	1	-	-	-	-	-	-	316	1	1
17	201	1	1	211	1	1	-	-	-	-	-	-	230	1	1
18	-	-	-	176	1	1	-	-	-	-	-	-	192	1	0
19	-	-	-	-	-	-	-	-	-	-	-	-	161	1	1
D.F		14	12		18	18		4	4		14	15		15	11
P.F(%)		47.05			Zero			Zero			6.66			63.15	

Band	OPC-01			OPA-19			OPA-15			OPA-09			OPA-17		
	Size in bp	S1	S2	Size in bp	S1	S2	Size in bp	S1	S2	Size in bp	S1	S2	Size in bp	S1	S2
1	670	1	1	854	1	1	1390	1	1	1532	1	0	500	1	1
2	579	1	1	775	0	1	1261	1	1	1261	1	1	323	1	1
3	476	0	1	607	1	0	941	1	1	1201	1	0	148	1	1
4	454	1	1	525	1	1	854	1	1	1038	0	1	-	-	-
5	392	1	0	476	1	1	775	1	1	941	0	1	-	-	-
6	356	1	1	432	1	1	638	1	1	813	1	1	-	-	-
7	307	0	1	356	0	1	579	1	1	579	1	1	-	-	-
8	265	1	1	339	1	0	476	1	1	500	1	1	-	-	-
9	198	1	1	279	1	1	392	1	1	454	0	1	-	-	-
10	-	-	-	198	0	1	356	1	1	392	1	0	-	-	-
11	-	-	-	122	0	1	279	1	1	373	0	1	-	-	-
12	-	-	-	87	1	1	-	-	-	323	0	1	-	-	-
13	-	-	-	-	-	-	-	-	-	265	0	1	-	-	-
D.F		7	8		8	10		11	11		7	10		3	3
P.F(%)		33.33			50			Zero			69.23			Zero	

S1: spearmint specie, S2: peppermint specie, D. F: detectable fragments, P. F (%): polymorphic fragments percent.

Total P.F (%) =46.98.

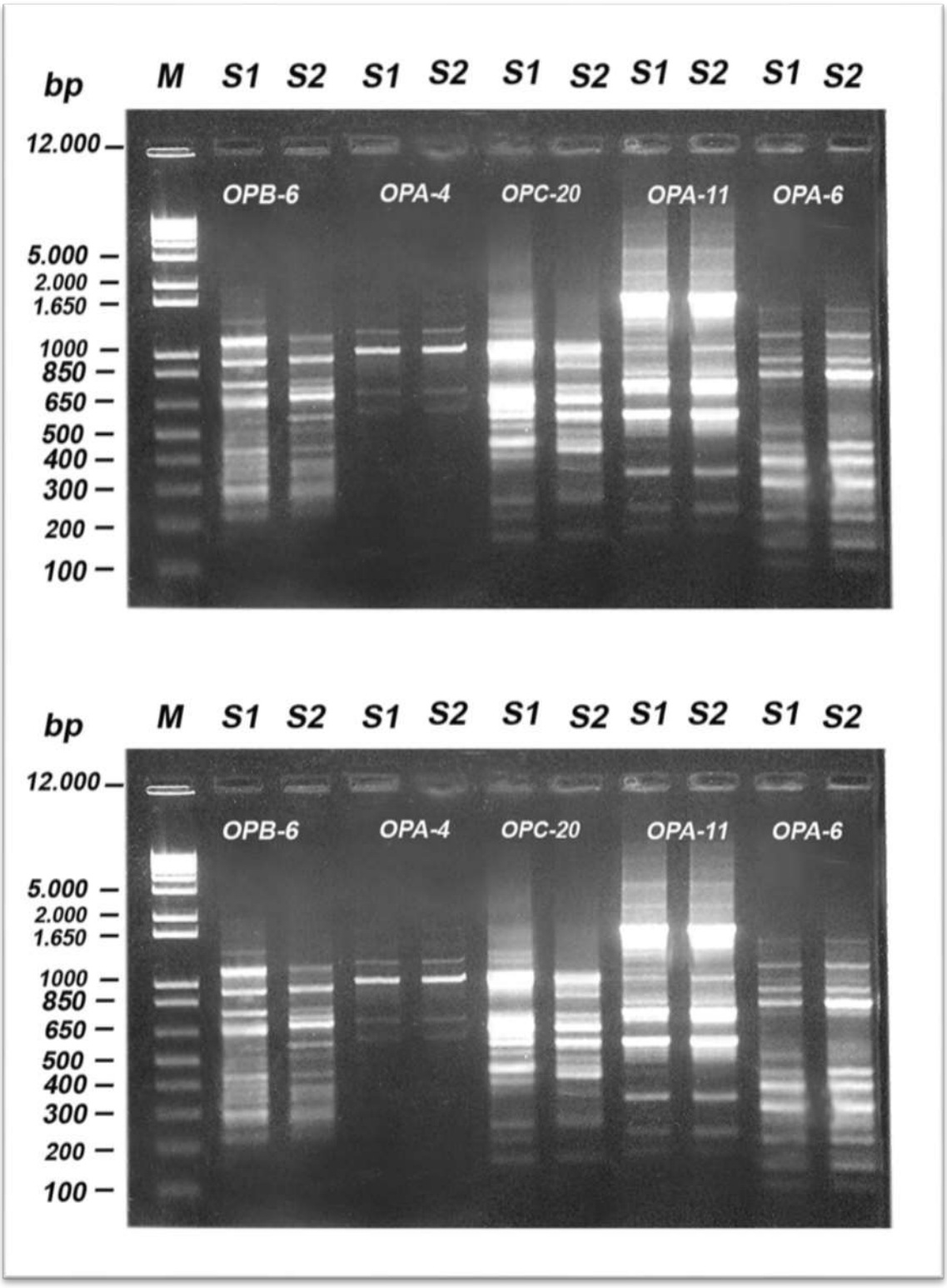

Figure 2. RAPD-PCR analysis of the two mint species by using ten random primers.
M: DNA marker, S1:Spearmint and S2:Peppermint

DISCUSSION

The study of genetic diversity of plants is the process by which the difference among the individuals or populations is detected by a specific genetically technique or a combination of such techniques. The most important data is that obtained by DNA based marker and can be used to identify different genomes (**Abd El-Azeem *et al*., 2011)**.

Worldwide, many DNA based marker have been used to identify the different genotypes of plants such as RAPD that is the simplest test of all recently applied DNA-based markers for plant identification (**Trifi *et al*., 2000and Ibrahim *et al*., 2014**).

In the present work, ten RAPD primers were used to study the genetic variation between two mint species (*Mentha spicata* and *Mentha piperita*) from Egypt. The obtained data indicated that some primers exhibit high percentage of polymorphism (OPC-20 and OPA-9). As well as, primers such as OPA-11, OPA-04, OPA-15 and OPA-17 did not exhibit any polymorphism. These results support the finding of **Rabia *et al*. (2015)**. The high percentage of polymorphism indicated that the used primer can detect more genetic differences between the studied mint species than others. **Fenwick and Ward, (2001)**used RAPD markers for cultivar identification in Mint. They reported that some RAPD markers primers that gave percentage of polymorphism can be used in mint to differentiate among species and among genotypes within a species. The results of the present study indicated that 40% of the used primers (4 from 10 primers) did not give any polymorphism. This lack of variation between the studied mint species suggests a very high degree of genetic similarity. On other hand 60% of primers exhibit polymorphic fragments between the two species such as OPC-09 primer that gave the highest percent of polymorphism (69.23).**Rabia *et al*. (2015)**found that the polymorphism produced from the used primers ranged from low to high level (50 to 92%, respectively). **Gobert *et al*. (2006) and Rabia *et al*. (2015)**reported that DNA based studies indicated that mint species can be genetically distinguished**.**

In conclusion**,** this study provides evidence that RAPD Markers can be used as an efficient tool for the detection of the genetic variations and identifications of different mint species. The same conclusion was reported by **Heikal *et al*. (2007)** and **Rabia *et al*. (2015 and Ahmad *et al*. 2018).**

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