GENETIC RELATIONSHIPS AMONG SIX MANGO (*Mangifera indica* L.) CULTIVARS USING RAPD MARKERS

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Mango (*Mangifera indica* L.) is considered as one of the oldest cultivated tree in the world and important tropical fruits. It has been cultivated for nearly 6000 years (Albert, 1986). It is now cultivated commercially throughout the tropics and in many subtropical areas where it has been considered to be the "king of fruits". The genus *Mangifera* is one of 73 genera belonging to the family *Anacardiaceae* (Litz, 1997). Mango is one at least of 15 Mangifera species that produce edible fruit (Bailey, 1958).

Mango tree is the best adapted to a warm tropical monsoon climate, with a pronounced dry season followed by rains. It is cultivated in many countries of the world, although it is significant that almost all the production comes from developing countries (Litz, 1997).

The total cultivated area with mango reached 241101 Fed. in 2013 (Statistics of 2014, Ministry of Agriculture, Egypt). The average yield per feddan is only 3.547 ton.

Differentiation of cultivars through morphological features is inefficient and inaccurate. This problem is further compounded by the perennial nature of the crop. The use of biochemical and genetic markers for identification of varieties offer a viable alternative method (Williams *et al*., 1990). Fingerprinting for a vast number of mango cultivars is a significant contribution to mango cultivation, as presently several mango cultivars have many synonyms in different regions, which makes identification difficult.

Random Amplified Polymorphic DNA (RAPD) assay detects nucleotides sequence of polymorphisms in DNA using only a single primer pair of arbitrary nucleotide sequence and polymerase chain reaction (PCR) technology has led to the development of several novel genetic assays based on selective DNA amplification. A genetic assay was independently developed by two laboratories. RAPD (Randomly Amplified Polymorphic DNA) assay detects nucleotides sequence of polymorphisms in DNA using only a single primer of arbitrary nucleotide sequence. The protocol is quick, easy to perform and only nanograms of template DNA are required. (Welsh and McClelland, 1990; Williams *et al*., 1990). The RAPD technique has been employed to determine linked markers in some fruits as Olive (*Olea europaea* L.) (Fabbri *et al*., 1995), hazelnut (*Corylus avellana*) (Pomper *et al.*, 1998), Date palm (*Phoenix dactylifera*

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L.) (Soliman *et al.*, 2003), *A. comosus* (Tapia *et al.*, 2005), Citrus cultivars (Baig *et al.*, 2009), *Musa spp.* (Das *et al*., 2009) and *Mangifera indica* (Ismail, 2003; Damodaran *et al*., 2009; Maklad, 2012).

The aim of this study is using the biotechnological markers such as RAPD-PCR to detect some specific markers and the genetic relationships among six mango cultivars.

MATERIALS AND METHODS

Materials

This study was conducted at the Agricultural Genetic Engineering Research Institute, ARC Giza, Egypt. Six mango cultivars namely; Alphonse, Ewais, Hindi khassa, Keitt, Langra and Zebda were used in this study.

Leaf samples of these mango cultivars, were collected from the orchard of the Horticulture Research Station in El-Kanater El-Kheireia, Kalubia governorate. The collected leaf samples were immediately stored in liquid nitrogen until DNA extraction.

Methods RAPD analysis

DNA was extracted from leaf tissues of the six mango cultivars (*Mangifera indica* L.) according to the protocol of Dellaporta *et al.* (1983) with slight modifications as reported by Porebski *et al.* (1997) which adapted to mango. The purified genomic DNA was subjected to PCR for RAPD analysis as described by Williams *et al.* (1990) using (twelve mer) 10 random primers (Table 1). The PCR reaction mixture was consisted of 50 ng genomic DNA, 200 µM of each dNTPs, 20-picomole primer, 1x Taq DNA polymerase buffer and 0.5 units of Taq DNA polymerase (Promega, WS, USA) in a final volume of 25 µl in asterile ultra-pure water. The PCR was performed in a Perkin Elmer 9700 thermolcycler for 40 cycles of denaturation at 94°C for 1 min, annealing at 42°C for 1 min and extension at 72°C for 2 min followed by a final extension at 72°C for 7 min.

RAPD profile of each mango cultivar was generated using 10 primers and compared to each other. Ten primers were selected as they gave clear, reproducible, and polymorphic banding profile.

Data analysis

Each variable RAPD fragment was considered as a locus so that every locus has two alleles and scored as present (1) or absent (0). For data analysis, only polymorphic, reproducible, and clear-cut fragments were kept. The similarity values were calculated by SPSS program. Dendrogram was constructed by the unweighted pair-group method using arithmetic averages (UPGMA) algorithm as described by Sneath and Sokal (1973).

RESULTS AND DISCUSSION

RAPD markers have greater utility than protein markers, because of their abundance in the genome, stability and their high level of DNA polymorphism (Lavi *et al*., 1994; Arumuganathan and

Earle, 1991; Litz, 1997). Therefore, RAPD markers were used in order to identify the genetic relationships among mango cultivars (Schnell *et al*., 1995; Litz, 1997).

The resulted amplified fragments are shown in Fig. (1) and their densitometric analyses are illustrated in Table (2). Amplified fragment were scored as present (1) or absent (0). All the 10 primers were successfully amplified DNA fragments for all cultivars. A total number of fragments were visualized among the six investigated cultivars. Using 10 arbitrary primers, 130 distinct fragments of DNA were identified with an average of 13 DNA fragments per primer. A total of 125 DNA fragments were polymorphic with an average of 12.5 polymorphic fragments per primers. Fragment sizes were ranged from 279 to 3249 bp.

The results of the amplified fragments using these 10 arbitrary primers for the six mango cultivars revealed different levels of polymorphism. The number of total amplified fragments (TAF), polymorphic fragments (PF), amplified fragments (AF) and specific markers (SM) for each cultivar are shown in Table (2). The ten used primers produced number of fragments ranging from six (A1 primer) to 18 (A9 primer) among the six cultivars. All of the ten used primers exhibited 100% of polymorphism, except A4 (77.77%), A21 (81.81%) and A1 (83.33%) primers.

There were 83 specific fragments which can be used to discriminate each cultivar from the others, since these fragments were absent in all cultivar except one cultivar as shown in Table (2).

The ten used primers produced a total number of specific markers which ranged from three (A1 primer) to fourteen (A9 primer) among the six cultivars. The highest number of specific markers (28 markers) was detected by Langra cultivar, while the lowest one (five markers) was detected by Hindi khassa cultivar. Each cultivar detected from one to four specific markers among each primer, except Zebda cultivar (seven markers) with A16 primer and Langra cultivar (six markers) with A11 primer. Only Langra cultivar detected specific markers with all of the ten used primers, followed by Zebda cultivar which detected specific markers with all of the ten used primer, except A21 primer.

Similarity indices based on RAPD-PCR with the 10 primers using UPGMA computer analysis is shown in Table (3). The highest similarity value (0.60) was observed between Hindi khassa and Ewais cultivars, while the lowest one (0.22) was recorded between Ewais and Langra cultivars, The same conclusion was reached by Maklad, (2012) who found that the highest similarity value (0.533) was scored between Hindi khassa and Ewais cultivars

A dendrogram for the genetic relationships among the six mango cultivars using the ten tested primers is shown in Fig. (2). The six mango cultivars were separated into two clusters; cluster 1 was included Ewais, Hindi khassa, Keitt, Alphonse and Zebda cultivars, while cluster

2 was included Langra cultivar. However, Ewais cultivar was closer to Hindi khassa cultivar, while it was distant to Langra cultivar. Because of their abundance in the genome, stability and their high level of polymorphism, DNA markers such as RAPDs have greater utility than protein markers (Lavi *et al*., 1994; Arumuganathan and Earle, 1991; Litz, 1997). However, several reports came out in which RAPD genetic markers were used in order to identify mango cultivars and to determine genetic relationships among them (Schnell *et al*., 1995; Litz, 1997).

SUMMARY

Mango (*Mangifera indica* L.) is considered as one of the oldest cultivated trees in the world. Six elite mango cultivars namely, Alphonse, Ewais, Hindi khassa, Keitt, Langra and Zebda were screened using Randomly Amplified Polymorphic DNA (RAPD) genetic markers with ten random primers (12 - mer) of arbitrary sequence.

The obtained results showed that, the six tested mango cultivars in this study were varied at the DNA level. A total number of 130 fragments were visualized among the investigated cultivars, including 125 polymorphic fragments. Number of produced fragments were ranged from six (A1 primer) to 18 (A9 primer) among the six cultivars. Polymorphism levels were differed from one primer to the other. The ten primers showed 83 specific molecular markers which discriminated each cultivar from the others. The highest similarity index (0.600) was recorded between Ewais and Hindi khassa cultivars, while the lowest similarity index (0.220) was observed between Ewais and Langra cultivars.

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Table (1): Sequence and G+C percentage content of the (12-mer) 10 arbitrary primers.

Primer	Primer sequence (5' - 3')	G+C % content
A 0	ATC AGC GCA CCA	58.33 %
A 1	AGC AGC GCC TCA	58.33 %
A 2	GCC AGC TGT ACG	66.66 %
A 4	GCC CCG TTA GCA	66.66 %
A 9	AGA ATT GGA CGA	41.66 %
A 11	ACT GAC CTA GTT	41.66 %
A 16	ATT TGG ATA GGG	41.66 %
A 17	GGT TCG GGA ATG	58.33 %
A 21	GTG ACC GAT CCA	58.33 %
A 23	AAG TGG TGG TAT	41.66 %

Table (2): Number of amplified fragment and specific markers of the six mango cultivars based on RAPD-PCR analysis using 10 primers.

Mango cultivars															
Primers	TAF	PF	Alphonse		Ewais		Hindi khassa		Keitt		Zebda		Langra		TSM
			AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
A0	12	12	4	3	3	1	3	-	3	1	3	1	4	3	9
A1	6	5	1	-	2	-	3	-	3	-	4	2	3	1	3
A2	13	13	4	-	5	1	2	-	6	1	3	1	6	2	5
A4	9	7	4	1	2	-	6	1	4	-	6	1	6	1	4
A9	18	18	3	2	3	3	3	1	4	3	5	2	4	3	14
A11	17	17	2	1	4	1	3	1	6	2	4	1	7	6	12
A16	16	16	2	1	3	-	4	1	2	-	8	7	4	3	12
A17	14	14	5	1	4	-	5	-	8	2	8	3	3	2	8
A21	11	9	5	1	4	1	5	1	3	-	4	-	7	4	7
A23	14	14	4	1	1	-	5	-	3	1	5	4	7	3	9
TSM				11		7		5		10		22		28	83

TAF = Total amplified fragments PF = Polymorphic fragments
AF = Amplified fragments SM = Specific markers
TSM = Total number of specific markers

Table (3): Similarity indices among the six mango cultivars based on RAPD-PCR using 10 primers.

	Alphonse	Ewais	Hindi khassa	Keitt	Zebda
Ewais	0.492				
Hindi khassa	0.493	0.600			
Keitt	0.368	0.466	0.568		
Zebda	0.405	0.346	0.472	0.478	
Langra	0.282	0.220	0.356	0.344	0.277

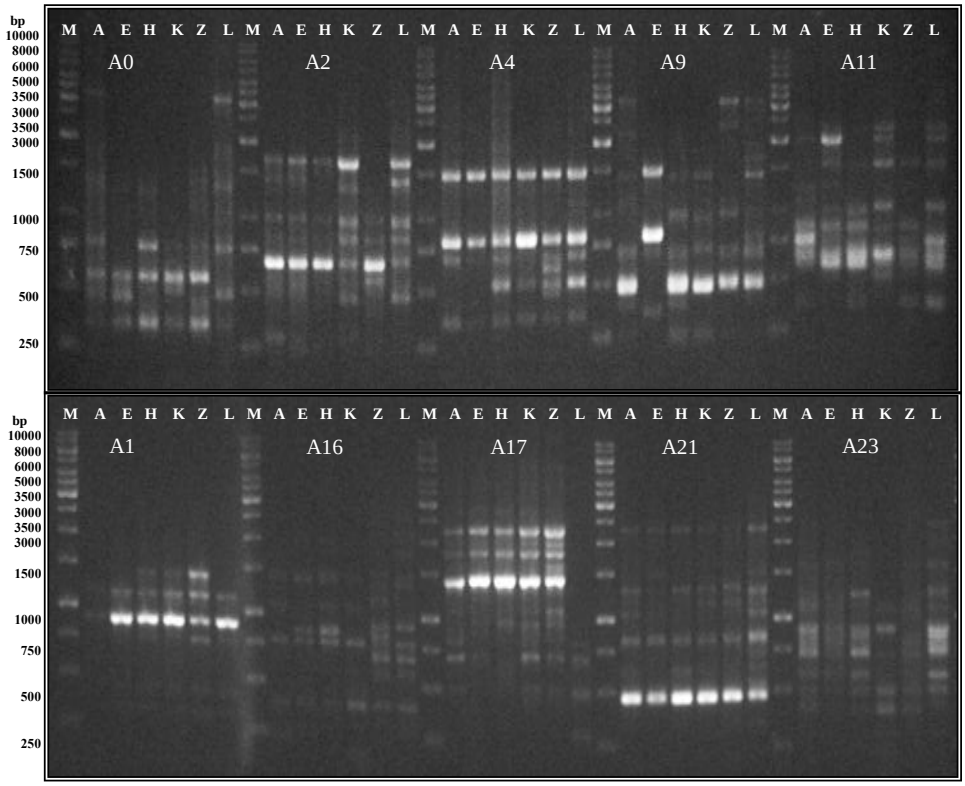

Fig (1): RAPD-PCR fragments of the six mango cultivars which amplified using A0, A2, A4, A9, A11, A1, A16, A17, A21 and A23 Primers (M) DNA ladder marker (bp) (A) Alphonse, (E) Ewais, (H) Hindi khassa, (K) Keitt, (Z) Zebda and (L) Langra.

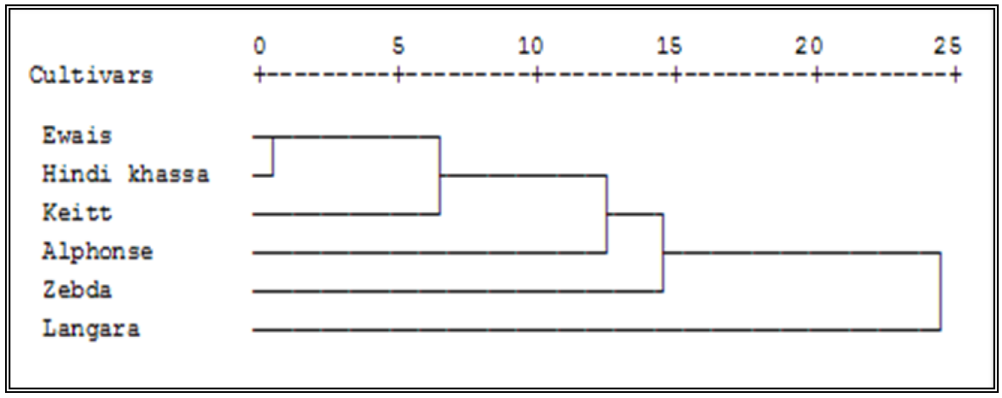

Fig (2): Dendrogram for the genetic relationships among the six mango cultivars based on similarity indices of RAPD analysis