Middle East Journal of Agriculture Research
ISSN 2077-4605
Volume : 07 | Issue : 02 | April-June | 2018
Pages:473-480

Evaluation of Local Mango Cultivars Grown under Different Locations in Egypt using Fruit Quality and RAPD analysis

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Received: 12 Mar 2018 / Accepted: 30 April 2018 / Publication date: 20 May 2018

ABSTRACT

Five cultivars of mango (Succari, Taimour, Hindi, Zebda and Ewais) grown under different locations in Egypt (Ismailia and Idko) were evaluated using flower quality and RAPD marker analysis during 2015 and 2016 seasons. In most cases, the estimated values of flower quality in the cultivars grown in Ismailia increased significantly than that from Idko.

RAPD analysis using ten primers amplified a total of 93 bands, 64 of them were polymorphic. Polymorphism percentage ranged from 33.3% to 100 %. Different profile among genotypes was found. The phylogenetic tree grouped the genotypes into three clusters, Ewiss cultivar from the two locations was scored in cluster A with genetic distance of 9.4 %. Sukkary genotypes from Ismailia grouped in cluster C, while that from Edko grouped in cluster B with genetic distance of 22%. Timour genotypes classified in the two different clusters. Hindi and Zebda genotypes, collected from the two locations grouped in cluster B.

Key words: Mango Cultivars, flower quality, RAPD marker analysis

Introduction

Mango (*Mangifera indica* L.) is one of the important of the tropical and subtropical region of the world. The genetic variability is source of crop improvement programs. Mango has two ecotypes based upon embryonic (Mukherjee and Litz 2009). Mono-embryonic type , evolved in dry sub-tropical and very hot summers .However , hot , humid tropics are suitable to polyembryonic type but the dry season is short (Mukherjee 1972) .

Temperature plays a very important role in fruit bud differentiation and fruit set of tropical fruit. The optimum growth of most tropical fruits is about 24 – 30^{o} C. However, mango trees can tolerant temperatures up to 48 ^{o}C for short periods and are sensitive to temperature below 10 C^{o} (Whiley *et al,* 1989 and Tindal 1994). There is sufficient moisture, the TSS of tropical fruit crops increases with the temperature (Sthapit *et al.*, 2012). If panicle in flower development coincides with an unusual cold spell, mango production will face several problems. Most of the fruit crops are highly heterozgous, genotype X environment (G X E) interaction is very high. Hence, the genotypes have to be stable enough to perform under different climatic conditions. Markers such as, randomly amplified polymorphic DNA (RAPD) have been used to identify mango cultivars by unique patterns of marker alleles (Eiadthong *et al.,* 2000).

The target of the present study is to determine the relationship between two mango locations of five mango cultivars based on fruit quality and genetic similarity estimation.

Materials and Methods

The present study was carried out in a commercial orchard located in wadi El-Molak – Ismailia governorate and in Eduko region – El Behera governorate during the two seasons of 2015 and 2016.

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Plant materials used for this study including five mango cultivars namely, Taimour, Zebda, Ewais, Exclent Succari and Hindi Besennara. The same five cultivars were found in two governorates. Fruits of the five mango cultivars were collected at maturity stage as recommended by Khalifa (2006).

A sample of five fruits replicated three times was obtained from each cultivar under two governorates conditions. Average fruit, peel and seed weight were recorded. Also, fruit dimensions (length – width cm) were recorded using a vernier caliper. Soluble solids content (SSC %) by refractometer , fruit acidity were determined as described by A.O.A.C (1995) Soil analysis present in the table (1) .

Table 1: Chemical analysis of Eduko and Wadi El-Molak soils.

Parameters	Eduko	Wadi ElMolak
EC ds/m	1.01	0.2
pH	7.9	7.6
Ca meg/L	2.5	3.2
Mg meg/L	3.0	1.8
K meg/L	3.9	0.09
Na meg/L	0.6	1.5
CO_3 meg/L	-	-
HCO_3 meg/L	3.5	0.05
Cl meg/L	6.0	8.0
So_4 meg/L	0.6	2.34

Genomic DNA of five cultivars Succari, Taimour, Hindi, Zebda and Ewais of *Mangifera indica* L. collected from Ismailia and Edkou, Egypt was extracted from 1 g of young leaf tissues using DNeasy Plant Mini Kit – according to manufactures instructions (QIAGEN). RAPD analysis DNA was performed using ten primers (Tables 2). The amplification was done in volume 25 L consists of 10x Taq polymerase buffer , 50 µL $Mgcl_2$, 0.2mM each of dATP, dTTP, dCTP, dGTP, 25.0 p moles of RAPD primers, 10 ng of genomic DNA and 0.5 Taq DNA polymerase (promega). The amplification was performed by including the reaction mixture for 45 cycles in a thermocycler Gene Amp 9700. Each cycle consisted of denaturation at 96 °c for 1 min followed by annealing at 30 °c for 1 min and extension at 72 °c for 1 min with initial delay for 5 min at 95 °c at the beginning of the cycle and post extension step for 10 min at 72 °c after the end of the last cycle (Ting and Manos, 1990 and Tawfik *et al.,* (2011)

Table 2: Primers sequences employed in the RAPD - PCR

Primer No.	Primer name	Sequence
1	OPK-08	GAACACTGGG
2	OPK-10	GTGCAACGTG
3	OPK-19	CACAGGCGGA
4	OPD-08	GTGTGCCCCA
5	OPO-07	CAGCACTGAC
6	OPO-08	CCTCCAGTGT
7	OPD-07	TTGGCACGGG
8	OPD-12	CACCGTATCC
9	OPE-14	TGCGGCTGAG
10	OPH-08	GAAACACCCC

PCR products were separated on agarose gel electrophoresis using 1.5 % and stained with red safe and then visualized on gel documentation system. The amplified fragments were scored as 1 for presence and 0 for absence of band and the data was analyzed for clustering using the formula of Nei and Li (1979). A similarity coefficient was used for cluster analysis following the UPGMA (unweighted pair grouping method of averages method).

The obtained data was statically analyzed by RCBD according to Sendecor and Corchan.(1980). Means represented as average of two seasons.

Results and Discussion

Fruit weight: Tables (3-7) show that Fruit weight in all cultivars was significantly affected by different regions, except Hindi and Taimour. Generally, fruit weights in Ismailia governorate were higher than El behera governorate. The above mentioned results of our study are in harmony with those attained by El Kheshine *et al.* (2016). Montieth *et al* 1977 and Lakso 1994 stated that Sukkary cultivar collected from five governorates in Egypt and exhibited a wide range of differences in fruit and stone characteristics. Due to, the biological yield of a fruit orchard is a function of the amount of light intercepted by orchard canopy times the photosynthetic effciency of the cultivar minus the respiration cost.

Fruit length and width: Data in all Tables (3-7) indicated that Different in environmental conditions caused a significant difference in Zebda, Hindi, Taimour cultivars. Fruit length in Ismailia was greater than El Behera .In respect to fruit width, it was not significantly affected by different governorate, except for Hindi and Taimour cultivars. Fruit width in Ismailia was higher than El behera. The results are in conformity with those reported by Naik, 1971 on mango .Results showed variability among trees of the same variety with respect to fruit size, shape, color and quality .Besides, Morton, 1987 found that fruit lengths can vary from 2.5 to 30 cm in different varieties. This data can be also correlated with Sthapit *et al.* (2012) who found that higher temperature during fruit development hasten maturity and improve fruit size and quality under sufficient moisture level .

Peel and pulp weight: There were significant differences between governorates, except Ewais. The main value of other cultivars found to be in El behera greater than Ismailia region. Succarri and Ewais were significantly affected by environmental conditions. Pulp weight in Ismailia was greater than El behera region .Our data were in harmony with that found by Human, 2008 and El-Khesshin *et al.,* (2016) where the mango can have a fruit weight range from a few grams up to l Kg.

Seed weight: Seed weight was significantly affected by different environmental effect except Taimour .Seed weight in Ismailia was higher than El Behera, except for Hindi cultivar. The results obtained in the present study coincide with the results of El-Kheshine *et al* (2016) and Naik (1971).

Soluble solids content (SSC): Different governorate caused significant difference in SSC of all cultivars, except Succarri and Zebda cultivars. SSC of Hindi in Ismailia was higher than El Behera. However, SSC of Taimour and Ewais in El Behera were greater than Ismailia governorate. In agreement of our finding, Sthapit *et al* (2012) indicated that there is sufficient moisture, the TSS of tropical fruit crops increases with the temperature. Besides, Naik(1971) observed that trees of the same cultivar varied in fruit quality .

Acidity: There were significant differences between two regions, except Succarri . Acidity of Zebda and Hindi in El Behera were higher than Ismailia wheras acidity of Taimour and Ewais in Ismailia was greater than El Behera . The variation in mango fruit were reported by Naik (1971) and Bhyan and Guha (1995). They observed a wide range of variability respect of different characteristics.

Table 3: Effect of environmental conditions on fruit quality of excellent Succari

	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Peel Weight (g)	Pulp weight (g)	Seed Weight (g)	SSC (%)	Acidity (%)
Elbehera	172.78	8.63	6.68	21.58	117.30	32.55	18.50	1.05
Ismailia	263.06	9.57	7.25	21.25	216.06	40.30	19.75	.85
L.S.D.05	24.00	N.S	N.S	N.S	8.80	4.92	N.S	N.S

Table 4: Effect of environmental conditions on fruit quality of Zebda

	Fruit weight (g)	Fruit length (cm)	Fruit Width (cm)	Peel Weight (g)	Pulp weight (g)	Seed Weight (g)	SSC (%)	Acidity (%)
Elbehera	314	11.16	8.26	38.41	235.30	41.76	15.41	1.73
Ismailia	333.80	14.42	8.40	33.94	247.76	52.03	15.40	1.51
L.S.D.05	10.32	1.42	N.S	N.S	N.S	4.24	N.S	0.22

Table 5: Effect of environmental conditions on fruit quality of Hindi Besennara

	Fruit weight (g)	Fruit length (cm)	Fruit Width (cm)	Peel Weight (g)	Pulp weight (g)	Seed Weight (g)	SSC (%)	Acidity (%)
Elbehera	306.60	11.88	7.76	35.58	195.83	56.86	18.33	4.01
Ismailia	321.55	15.01	6.27	35.37	209.00	47.20	21.30	1.51
L.S.D.05	N.S	1.08	.90	N.S	N.S	5.71	1.12	0.48

Table 6: Effect of environmental conditions on fruit quality of Taimour

	Fruit weight (g)	Fruit length (cm)	Fruit Width (cm)	Peel Weight (g)	Pulp weight (g)	Seed Weight (g)	SSC (%)	Acidity (%)
Elbehera	257.13	10.60	7.10	34.35	178.90	30.13	24.46	1.53
Ismailia	276.67	12.36	8.20	30.43	174.30	33.20	19.20	3.27
L.S.D.05	N.S	0.41	0.75	N.S	N.S	N.S	1.55	0.36

Table 7: Effect of environmental conditions on fruit quality of Ewais

	Fruit weight (g)	Fruit length (cm)	Fruit Width (cm)	Peel Weight (g)	Pulp weight (g)	Seed Weight (g)	SSC (%)	Acidity (%)
Elbehera	229.15	10.75	7.05	24.31	166	32.05	25.75	0.85
Ismailia	262.89	11.35	7.23	18.40	194.54	36.76	21.15	1.13
L.S.D.05	22.64	N.S	N.S	3.00	27.84	4.38	0.79	0.03

The genetic relationship among five cultivars of mango collected from Ismailia and Idkou, Egypt were evaluated using RAPD marker technique. Ten primers amplified a total of 93 bands, 64 of them were polymorphic. The percentage of polymorphism of the all amplified products was 68.8 (Table 8).

Table 8: RAPD marker produced by ten primers among five cultivars Succari, Taimour, Hindi, Zebda and Ewais of *Mangifera indica* L. collected from Ismailia and Edko. Total number of bands, number of polymorphic bands and polymorphism (%)

Primer	Number of amplified band	Number of polymorphic bands	Primer polymorphism (%)
Primer 1	11	7	63.6
Primer 2	9	6	66.7
Primer 3	11	8	72.7
Primer 4	7	7	100
Primer 5	7	4	57.1
Primer 6	7	4	57.1
Primer 7	12	4	33.3
Primer 8	6	6	100
Primer 9	15	10	66.7
Primer 10	8	8	100
Total	93	64	68.8

The largest number of polymorphic bands was ten bands produced by primer nine, while primers 5, 6, and 7 produced low number of polymorphic bands (four bands). All bands produced by primers 4, 8 and 10 were polymorphic. Polymorphism percentages ranged from 33.3% to 100% with an average of 68.8%.

The primer used for RAPD analysis showed size ranged from 150-3000 bp as shown in figures (1, 2). Different profile among genotypes was found. The main changes in the RAPD profile of the present investigation were the appearance or absent of different band with variation in their intensity. The polymorphic bands could be very valuable for DNA fingerprinting and identification between mango genotypes. These results agree with the finding of El Kheshin, 2016.

A dendrogram was constructed using data from unweighted pair group method of arithmetic means (UPGMA) based on the genetic distance (Table 9) and coefficient matrices of the genotypes. The phylogenetic tree grouped the genotypes into three main clusters (A, B and C), Ewiss cultivar from the two locations was scored in cluster A with genetic distance of 9.4 %. Sukkary genotypes from Ismailia grouped in cluster C, while that from Edkou grouped in cluster B with genetic distance of 22%. In the same time Timour genotypes classified in the two different clusters, thus cluster C for Edkou genotypes and cluster B for Ismailia genotype. These results referred to that the observed variations between studied mango genotypes in the morphological parameters due to not only environmental effect but also existence of different degree of genetic variations according to cultivar. The observed variations between studied mango accession reflect the existence of genetic variation (Haque *et al.,* 1993; Pandey *et al.,* 1998; Singh *et al.,* 2006 and Kheshin *et al.,* 2016)

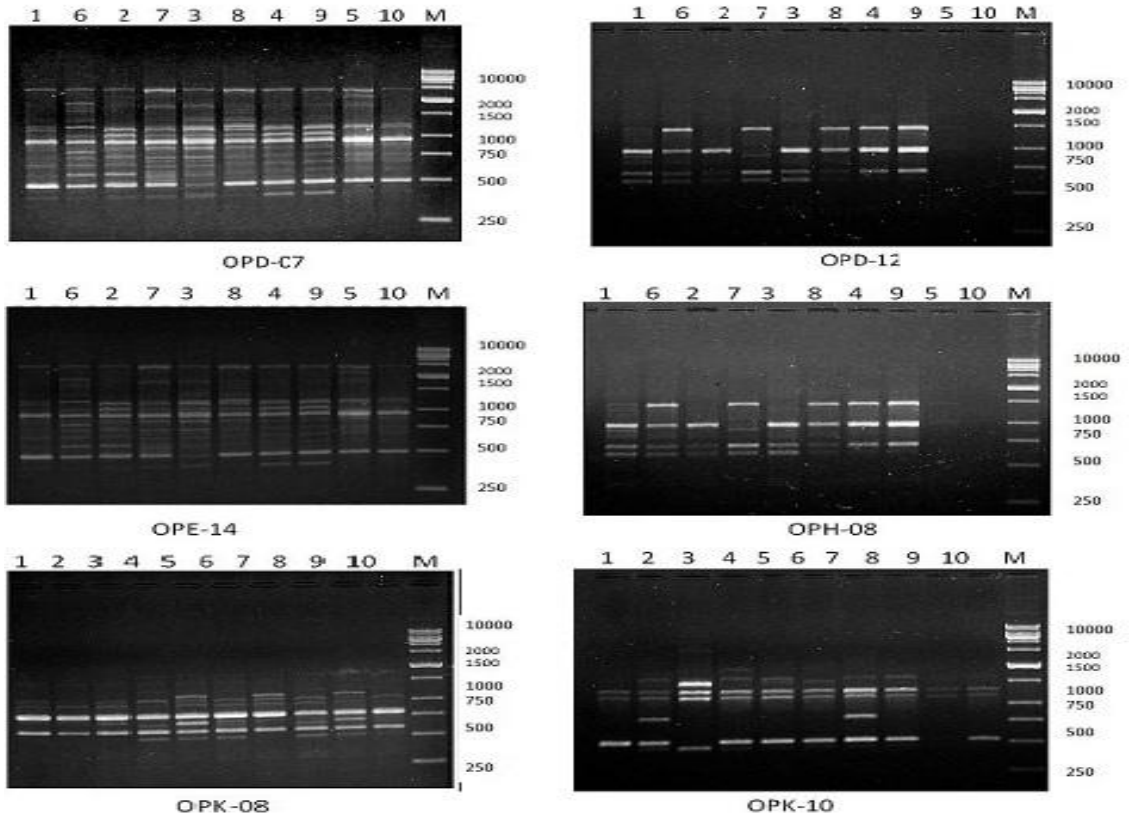

Fig.1: RAPD profile of five cultivars Succari, Taimour, Hindi, Zebda and Ewais (1G, 3G, 5G, 7G, 9G collected from Ismailia, respectively and 2G, 4G, 6G, 8G, 10G collected from Edko, respectively) of *Mangifera indica* L. using primers 1-6

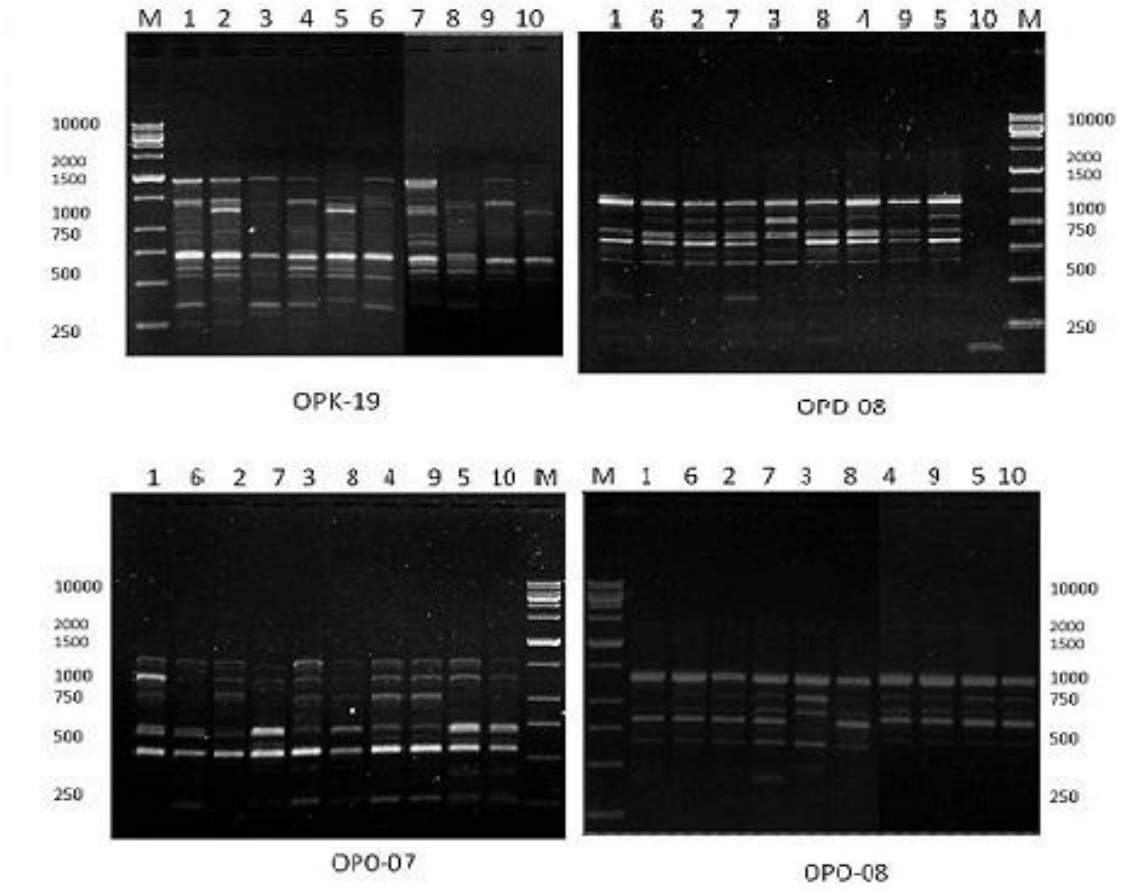

Fig. 2: RAPD profile of five cultivars Succari, Taimour, Hindi, Zebda and Ewais (1G, 3G, 5G, 7G, 9G collected from Ismailia, respectively and 2G, 4G, 6G, 8G, 10G collected from Edko, respectively) of *Mangifera indica* L. using primers 7-10.

The dendrogram grouped Hindi and Zebda genotypes, collected from the two locations in cluster B. Its refer to the role of environmental effect in the observed differences in morphological characteristics between accessions of mango. Also, there is a little genetic variation was detected as shown in Table 9. RAPD phylogenetic analysis declared enough differences that could differentiat mango accessions (Kheshin *et al.,* 2016).

Table 9: Genetic distance of DNA among five cultivars Succari, Taimour, Hindi, Zebda and Ewais of *Mangifera indica* L. collected from Ismailia and Edko.

	1G	2 G	3 G	4 G	5 G	6 G	7 G	8 G	9 G	10 G
1	0.0									
2	22	0.0								
3	26.4	21.3	0.0							
4	17.3	21.2	17.4	0.0						
5	23.4	20.9	20.1	28.7	0.0					
6	18.9	10.3	16.5	20.6	19.4	0.0				
7	16.1	17.8	10.5	14.6	19.9	10.2	0.0			
8	21.2	13.3	11.9	19.0	20.2	9.7	9.2	0.0		
9	32.2	28.4	32.0	23.9	39.1	23.8	28.3	23.4	0.0	
10	26.6	22.6	20.4	45.2	30.6	17.4	23.7	18.8	9.4	0.0

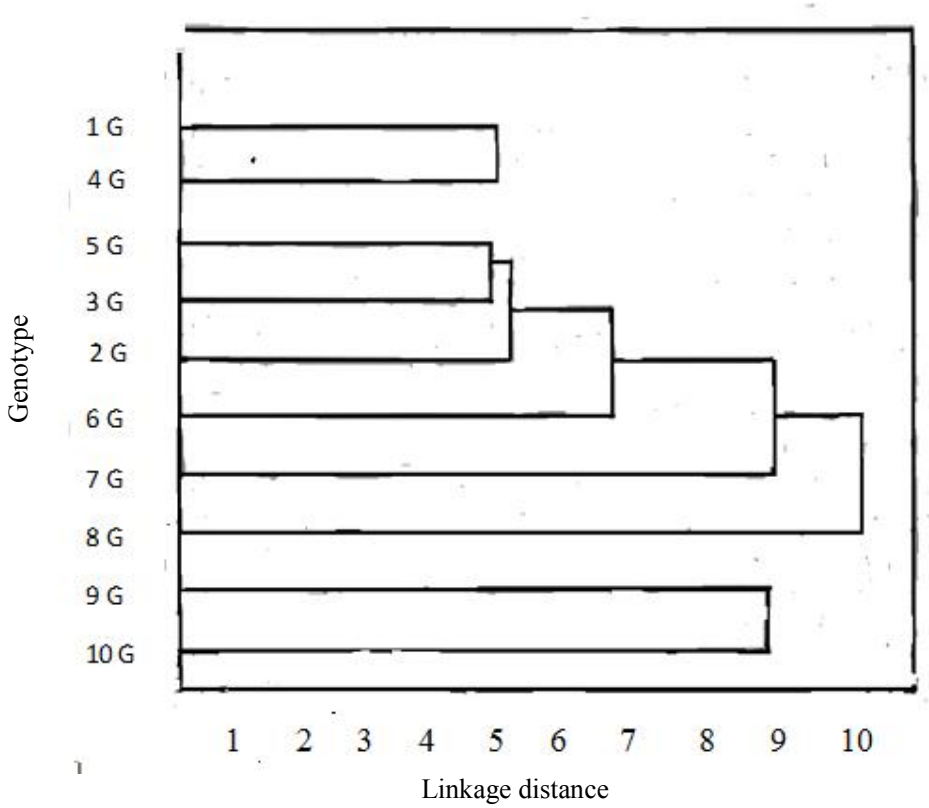

Fig. 3: Dendrograme of five cultivars Succari, Taimour, Hindi, Zebda and Ewais (1G, 3G, 5G, 7G, 9G collected from Ismailia, respectively and 2G, 4G, 6G, 8G, 10G collected from Edko, respectively) of *Mangifera indica* L.

The conservation of plant populations is mostly concerned with the number of genetics individuals present in population in order to asses factors, the breeders need to be able to estimate the degree of relatedness between the existing materials. The genetics relationship can be useful for designing strategies for breeding programs (Nada *et al.,* 2004 and Elansary *et al.,* 2011).

Finally, it can be concluded that RAPD marker is a useful technique to distinguish the genetic differences between genotypes of mango under different environmental effect conditions.

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