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Appraisal of biochemical and genetic diversity of mango cultivars using molecular markers

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Mango (*Mangifera indica* L.) is one of the oldest fruit crops and is broadly cultivated worldwide. To determine the level of genetic diversity, a total of 13 mango genotypes have been collected from different farms of Fayoum oasis in Egypt and were analyzed using molecular (DNA) and biochemical (SDS-PAGE) markers along with the quantification of soluble carbohydrates, chlorophyll and carotenoids. These profiles were evaluated as characters to identify the taxonomic relationships of these genotypes. A total of 433 protein bands (ranged from 8 to 180 KDa) from all genotypes, were detected in SDS-PAGE. A total of 306 RAPD fragments were produced by 19 primers and among them 123 (40.2%) were polymorphic. The similarities between different taxa were estimated by Jaccard's similarity index and clustered in neighbour joining clustering tree. Among the 13 tested mango samples, the total carbohydrate contents ranged between 31.9 and 40.8 µg/100 mg fresh weights, which represents Taymour cultivar and accession No. 7, respectively. Of the 13 mango cultivars and accessions studied, the highest chlorophyll content (386.9 µg/g) was found in accession No. 10; whereas, the lowest value was observed with accession No. 12 (202.5 µg/g). The amounts of carotenoids were wide-ranging and reached a maximum value of 106.2 µg/g with accession No. 9, however, accession No. 8 recorded the lowest concentration (19.9 µg/g). In conclusion, RAPD-PCR and SDS-PAGE were proved to be an efficient tool in assessing the genetic diversity of mango genotypes. It will also provide an important input to breeders for mango improvement program.

Key words: Mango, genetic diversity, chlorophyll, SDS-PAGE, RAPD.

INTRODUCTION

Mango (*Mangifera indica* L.) is commonly known as the 'king of fruits'. A native of Southeast Asia is one of the important fruit crops in the tropical and subtropical regions thought to have been introduced to Africa in the

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Abbreviations: SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; **RAPD,** random amplified polymorphic DNA.

14th century (Singh, 1960; Duval et al., 2005). Most cultivated mangoes belong to the species *Mangifera indica*; one of 600 species in the family *Anacardiaceae*. The genus *Mangifera* contains around 70 species, which can be divided into two subgenera, Limus and Mangifera (Kostermans and Bompard, 1993) with at least 26 species producing edible fruits (Mukherjee, 1997; Tanaka, 1976). Mango plays a major role in the global trade as it constitutes approximately 50% of all tropical fruits produced worldwide, equivalent to 5.5% of all fruit produced globally (Jedele et al., 2003; Vasanthaiah et al., 2007). In Egypt, mango is an important fruit crop and according to the latest statistics provided by the Ministry of Agriculture and Land Reclamation of Egypt (2007), a total of 184204 Feddan are cultivated with mango.

Mango has economic as well as therapeutic value due to its high vitamin, mineral and fiber content (Lakshminarayana, 1980). Ripe fruits are freshly eaten or prepared as juice, jams, jellies, and squash or canned, while unripe fruits are used in pickles, chutneys and other culinary arrangements. The various parts of mango are used as antihelmintic, diaphoretic, and refrigerant agents and in bleeding piles, manorrhagia, scabies and cutaneous infections, leucorrhoea, diarrhea and dysentery (Lakshminarayana, 1980; Mukherjee, 1997).

Genetic diversity is one of the key factors for the improvement of many crop plants including mango. Plant breeders rely on the availability of genetic diversity during selection in cultivar development.

It has been proposed that traditional agro-ecosystems maintain a high diversity of cultivated plants, both in terms of crop species and genotypes within each species (Alcorn, 1981; Altieri et al., 1987; Bellon, 1996; Brush, 1989, 1992, 2000). Human action, as well as ecological and evolutionary processes, promotes the maintenance of genetic variation of crops within traditional agro ecosystems (Lambert, 1996).

Varieties developed with wider genetic base may be helpful in enhancing the yield under various agro-climatic conditions (Asif et al., 2005). Diverse genetic base may also resist the spread of diseases (Zhu et al., 2000) in approved varieties. The study of genetic diversity is also important for varietal identification, proper purity maintenance, for the implementation of plant variety protection rights and export under WTO regulations. Mango has been reported to have extensive diversity due to continuous grafting and phenotypic differences arising from varied agro climatic conditions in different areas (Young and Ledin, 1954; Ravishankar et al., 2000).

Genetic diversity can be accessed from pedigree analysis, morphological traits or using molecular markers (Pejic et al., 1998).

A number of reports are available on the use for DNA markers to assess genetic diversity among species of several horticultural crops, as well as validation of genetic relatedness among them (Bhat et al., 2010).

Molecular markers have diverse applications in crop improvement, particularly in the areas of genetic diversity and varietal identification studies, gene tagging, disease diagnostics, pedigree analysis, hybrid detection, sex differentiation and marker assisted selection. DNA markers can be used to diagnose the presence of the gene without having to wait for gene effect to be seen (Bhat et al., 2010; Botez et al., 2009; Sisko et al., 2009; Thimmappaiah et al., 2009).

Molecular markers are useful tools for estimating genetic diversity as these are not influenced by environment, are abundant and do not require previous pedigree information (Bohn et al., 1999). Among the biochemical markers, SDS-PAGE has been widely used due to its simplicity and effectiveness for estimating genetic diversity.

Among the different DNA marker types, random amplified polymorphic DNA (RAPD) markers have frequently been used for genetic analyses (Langridge et al., 2001) due to simplicity, efficiency and non requirement of sequence information. RAPDs assay detects nucleotides sequence of polymorphisms in DNA using only a single primer pair of arbitrary nucleotide sequence (Welsh and McClelland, 1990; Williams et al., 1990). RAPDs have been widely used for identification of genotypes in crop plants, for investigating the genetic variability within species and to show relationships among populations (Freitas et al., 2000).

Like all living organisms, plants require energy in chemical form so they can grow and carry out basic life functions. Plants produce, store and burn carbohydrates in the form of sugar to provide them energy (Bieleski, 1962; Bieleski et al., 1992). Carbohydrates are the most abundant single class of organic substances found in nature and initially synthesized in plants from a complex series of reactions involving photosynthesis (Giaquinta, 1979; Gayler and Glasziou, 1972).

There are many pigments in the higher plants, such as chlorophyll, carotenoids, phtochrome, flavonoid, anthocyanin, tannin, and many others (Yang et al., 1998). Chlorophyll is an extremely important bio-molecule that absorbs sunlight and uses its energy to synthesize carbohydrates from CO_2 and water. This process is known as photosynthesis and is the basis for sustaining the life processes of all plants (Blachburn, 1998). Plants contain both chlorophyll a and chlorophyll b, which have slightly different structures (Carter and Spiering, 2002). Carotenoids are composed of two small six-carbon rings connected by a "chain" of carbon atoms. Carotenoids act as accessory pigments, harvesting light for photosynthesis and as photo protective agents limiting the damaging effects of high irradiance (Johnson et al., 1993; Feruse and Arkosiova, 2001). Carotenoids protect cells and tissues from free radicals and also function as light collectors (Blachburn, 1998). During recent years there has been remarkable progress in chlorophyll and carotenoids quantification as an intriguing tool that can reveal information on plant performance and cultivars relationship (Kulshreshtha et al., 1987).

Table 1. List of mango cultivars and accessions used in the current study.

Code	Variety and status	Location
1	Alphons (commercial cultivar)	Fayoum oasis
2	Taymour (commercial cultivar)	Fayoum oasis
3	Ewais (commercial cultivar)	Fayoum oasis
4	Zebda (commercial cultivar)	Fayoum oasis
5	Mabrouka (commercial cultivar)	Fayoum oasis
6	Local cultivar (accession)	Fayoum oasis
7	Local cultivar (accession)	Fayoum oasis
8	Local cultivar (accession)	Fayoum oasis
9	Local cultivar (accession)	Fayoum oasis
10	Local cultivar (accession)	Fayoum oasis
11	Local cultivar (accession)	Fayoum oasis
12	Local cultivar (accession)	Fayoum oasis
13	Local cultivar (accession)	Fayoum oasis

Table 2. Primer sequences examined for categorization of mango cultivars and accessions.

Primer code	Primer Sequence (5' → 3')	Primer code	Primer Sequence (5' → 3')
OP-A01	CAGGCCCTTC	OP-B16	TTTGCCCGGA
OP-A02	TGCCGAGCTG	OP-B18	CCACAGCAGT
OP-A04	AATCGGGCTG	OP-B20	GGACCCTTAC
OP-A05	AGGGGTCTTG	OP-C01	TTCGAGCCAG
OP-A09	GGGTAACGCC	RMn-P1	CAGAAGCGGA
OP-A11	CAATCGCCGT	RMn-P2	GGGTAACGCC
OP-A16	AGCCAGCGAA	RMn-P3	TGTCATCCCC
OP-A18	AGGTGACCGT	RMn-P4	AAGTGCGACC
OP-A20	GACCAATGCC	RMn-P5	ACTGAACGCC
OP-B15	GGAGGGTGTT		

The objective of the present study was to assess the level of genetic diversity in the gene pool of mango using RAPD and SDS-PAGE with the quantification of soluble carbohydrates, leaf chlorophyll and carotenoids.

MATERIALS AND METHODS

Plant materials

The experimental materials for the present study comprised of 13 local and commercial Egyptian mango cultivars and accessions collected from different farms of Fayoum oasis in Egypt. The taxonomy and classification of the used samples were identified according to Hussein (2009) as demonstrated in Table 1.

SDS-PAGE analysis

To study the protein banding pattern of 13 mango genotypes, we used SDS-PAGE, which was performed according to the method of Laemmli (1970), as modified by Studier (1973). Total proteins were extracted from mango trees leaves. Protein fractionations were performed exclusively on vertical slab (19.8 × 26.8 × 0.2 cm) gel using the electrophoresis apparatus manufactured by Laboconco. The Blueye Prestained Protein Ladder (GeneDirex) was used as a standard marker. The bands were detected and analyzed using Total Lab software.

DNA extraction, primers and DNA amplification

Genomic DNA was extracted from small amount of young and fresh leaves (0.1 g) from the 13 cultivars and accessions of mango trees by the Biospain Plant Genomic DNA Extraction Kit (BioFlux).

Nineteen primers (Table 2), obtained from Pharmacia Biotech. (Amersham Pharmacia Biotech UK Limited, Ebgland HP79 NA), were used for identification of different mango genotypes.

PCR reaction test was performed in a 25 µL volume reaction mixture containing: 5 µL of 5X green Taq DNA polymerase buffer, 100 µM of primer (1 µL), 20 ng of total cellular DNA, 10 mM of each dNTP (0.5 µL), 25 mM $MgCl_2$ (4 µL), 5 U/µL of GoTaq DNA polymerase (0.25 µL) (Promega), up to 25 µL by nuclease-free water. PCR was performed in a DNA thermo cycler (Biometra, Germany). Samples were first heated at 94°C for 3 min and subjected to 35 cycles of the following cycle: 45 s at 94°C, 45 s at 37°C, 1.5 min at 72°C. A

Table 3. Total soluble carbohydrates µg/100mg), total chlorophyll (µg/g), and carotenoids content (µg/g) in different genotypes of mango trees (mean ± SE).

Code	Total soluble carbohydrates µg/100mg)	Total chlorophyll (µg/g)	Carotenoids (µg/g)
1	35.5 ±3.79	210.9 ± 14.5	28.0 ± 2.43
2	31.9 ±4.04	205.2 ±11.78	29.4 ±2.56
3	38.5 ±2.45	273.2 ±21.2	40.9 ±3.5
4	37.2 ±3.39	272.9 ±18.7	30.9 ±1.51
5	34.6 ±2. 9	323.3 ±24.8	99.9 ±4.81
6	32.6 ±3.97	366.9 ±22.91	54.8 ±2.47
7	40.8 ±3.54	378 ± 21.1	66.8 ±4.69
8	32.4 ±4.13	276.3 ±15.9	19.9 ±1.23
9	39.8 ±3.93	331.9 ±20.5	106.2 ±7.17
10	35.4 ±3.28	386.9 ±23.8	37.3 ± 2.61
11	38.4 ± 4.1	318.4 ±27.58	48.8 ±2.48
12	36.3 ±3.97	202.5 ±13.52	28.3 ±1.92
13	32.2 ±3.58	322 ±20.31	47.0 ±2.57

A final step of 5 min at 72°C was always run. PCR reaction was tested on 1.6% agarose (Genetics) gels and 100 bp DNA ladder H3 RTU (Genetics) was used as the standard marker.

Total soluble carbohydrates estimation

Total soluble sugars content were assayed at 625 nm using anthrone reagent (Cao et al., 2007).

Total chlorophyll and carotenoids analysis

Total chlorophyll (The sum of chlorophyll a and b) and total carotenoids (Car) concentrations were determined following the methods of Porra et al. (1989) and Lichtenthaler (1987), respectively on the basis of µg /g fresh weight according to the following formula:

Total chlorophyll (TC) = $17.76A_{646.6} + 7.34A_{663.6}$ (µg/ml)
Total carotenoids = $4.69A_{440.5} – 0.267$ x TC (µg/ml)

Data handling and cluster analysis

The size of DNA fragments and protein band molecular weights were calculated using Total Lab software. Data were scored for computer analysis on the basis of the presence (1) or absence (0) of the amplified products for each primer. Pair wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to determine similarity index, according to Jaccard (1980). The similarity coefficients were, then, used to construct dendrogram, using Neighbour joining cluster algorithm employing the Paleontological Statistics (PAST) software Version 2.17b (Hammer et al., 2001).

RESULTS AND DISCUSSION

Total carbohydrate

Data in Table 3 shows the total carbohydrates did not differ much within the tested 13 mango genotypes. Taymor cultivar showed the lowest level of total carbohydrates (31.9 µg/100 mg) while, the highest (40.8 µg/100 mg) was found in accessions No. 7.

Reid et al. (1989) have reported that decapitation and girdling of *Protea* plant flowers slow down the leaf blackening and he proposed that reduced carbohydrate content was the cause of that phenomena. Bieleski et al. (1992) reported that the changes in carbohydrate content of *P. eximia* leaves are consistent with our hypothesis that the leaf-blackening disorder arises out of a depletion of leaf carbohydrate. Leaf blackening was visible shortly after the starch and sugar content of the leaves had fallen to their minimum level. Moreover, Boldingh et al. (2000) reported that glucose peak in early development of *A. deliciosa* coincides with a peaks of water content. Transient accumulation of sugars at the early stage of growth may lead to an increase in osmotic pressure followed by an increase in cell turgor at the expansion phase.

Total chlorophyll and carotenoids determination

Data presented in Table 3 illustrate the total chlorophyll content (Chl a+b), in leaves of 13 different mango genotypes which ranged between 202.5 to 386.9 µg/g fresh weight. It is clear that, the highest chlorophyll content (386.9 µg/g) was found in accession No. 10, whereas the lowest value was observed with accession No. 12 (202.5 µg/g). Carotenoids had significantly varied results, the concentrations were in a wide scale and ranged from 19.9 to 106.2 µg/g, the accessions No. 9 shows the highest concentration (106.2 µg/g), however, accessions No. 8 recorded the lowest concentration (19.9 µg/g). These results are in agreement with Kershaw and Webber (1986) who examined seasonal changes in chlorophyll concentrations and photosynthetic rates in *Brachythecium*

rutabulum from an apple orchard in Canada. Samples collected from a low-light environment, exhibited higher chlorophyll concentrations and higher rates of photosynthesis at low light, relative to samples collected when understory irradiance was greater. So, it was clear that the variation in pigments concentrations among different genotypes maybe a reason of genetical or environmental variance.

Molecular and biochemical identification of some genotypes of mango

Genome profiling is a strategy that identifies genomic DNA fragments common to closely related species without performing DNA sequencing. Random RAPD-PCR is one of the key technologies of genome profiling (Naimuddin et al., 2002). PCR based methods including RAPD can be effectively used for cultivars identification and the study of phylogeny and genetic diversity (Saengprajak and Saensouk, 2012).

Initial evaluating of 19 RAPD primers against 13 cultivars and accessions of mango trees gave 123 polymorphic bands and 183 fragments were monomorphic between the plant genotypes (Figure 1 and Table 4). The total 306 bands were amplified using 19 RAPD primers; produced an average of 7 (OP-B16) to 24 (Mnp3) bands per primer. The number of RAPD fragments that were amplified ranged from 1 (OP-A01) to 19 (Mnp5) with an average of 16.1 bands per primer and the sizes ranged from about 65 to 2100 bp. However, the highest number of DNA amplified fragments, using the 19 primers, was present in cultivar Taymour (Table 5) (255 fragments), while the accession No. 13 revealed the lowest number (227 fragments).

For the polymorphism percentage presented by the 19 primers which used to identify the genotypes as shown in Table 4, the primer OP-B18 gave the highest number of polymorphic fragments in all genotypes (18 fragments) with 79.3% polymorphism percentage while the primers OP-B15 and OP-B20 gave the lowest number of polymorphic fragments (1 fragment) with 7.1 and 9.1%, respectively of polymorphism percentage.

As showed in Table 6, the band data were utilized to calculate the similarity matrix. Jaccard's similarity coefficient ranged from 0.755 (between the accessions 6 and 13) to 0.893 (accessions 6 and 7). The RAPD results revealed a large set of markers, which can be used for the evaluation of both between- and within-species genetic variation (Guadagnuolo et al., 2001).

Figure 2 illustrate the neighbour joining clustering tree which was constructed on the basis of Jaccard's coefficient based on RAPD-PCR. The tree clustered the cultivars and accessions into two clusters (I and II). The clusters I divided to two groups (A and B). The group A contained most of the accessions (7, 6, 8, 9, 13, 11 and 10) while the group B included the cultivars Taymour and alphons and only one accession No. 12. However, the cluster II consisted of three cultivars which are Mabrouka, Ewais and Zebda.

On the other hand, to identify the 13 genotypes of mango, we used another method which is SDS-PAGE technique. The banding patterns were analyzed on 12% SDS-PAGE. Two bands corresponding to 18.5, 17 and 16 KDa were observed in 12% SDSPAGE (Figure 3).

The 18.5 KDa band was reported in all cultivar genotypes Alphons, Taymour, Ewais, Zebda and Mabrouka but it disappeared in all the accession genotypes. 17 KDa band was detected in cultivars alphons, Taymour, Ewais and Zebda and in the accessions 6, 10, 11, 12 and 13. Moreover, the 16 KDa band appeared in Mabrouka and in the accessions 7 and 8. That is in agreement with the results of Zaied et al. (2007) who found variation between different genotypes of mango at SDS-PAGE level. The similarity between the cultivars and the accession was high which ranged from 91.4 to 100% (Table 7).

Mabrouka cultivar and the accessions 6, 10, 11, 12 and 13 showed 91.4% of similarity. Also, the similarity between the accessions 7 and 8 and four cultivars Alphons, Taymour, Ewais and Zebda was 91.4%. However, the similarity rose up to 100% between 13, 6, 10, 11 and 12 and among 7 and 8. Moreover, the cultivars Alphons, Taymour, Ewais and Zebda revealed the highest similarity (100%). Neighbour joining clustering based on SDS-PAGE results showed that the cultivars and the accessions under study were set in two clusters (I and II). The cluster I contained two groups (A and B). Group A include accessions 8, 7, Mabrouka and accession 9, while group B contain Zebda, Taymour, Ewais and Alphons cultivars (Figure 2). Furthermore, the cluster II consisted of the accessions 6, 13, 12, 11 and 10. Some of the accessions grouped together either from RAPD-PCR results or from SDS-PAGE, and that could refer to their common ancestor which may be one of the cultivars under study or another cultivar was not used in our study. Our results show that RAPD-PCR and SDS-PAGE are useful for taxonomy and evaluation study between different cultivars and accessions of mango. That is in agreement with the study of Ghafoor and Arshad, (2008) that reported that the electrophoretic patterns of total proteins (SDS-PAGE) have been successfully employed to resolve the taxonomic and evolutionary problems of some plant species. The use of RAPD technique for the study of genetic variation has been demonstrated as suitable in many species (Abbas et al. 2009). Moreover, we can report that the RAPD-PCR and SDS-PAGE results indicated existence variations between these genotypes. The molecular and/or biochemical methods are more powerful than morphological traits to study the genetic diversity. Ungerer et al. (2003) and Alan (2007) showed that the estimation of genetic diversity based on the morphological traits alone showed the true level of genetic diversity between genotypes because morphological traits are determined by the interaction between genetic

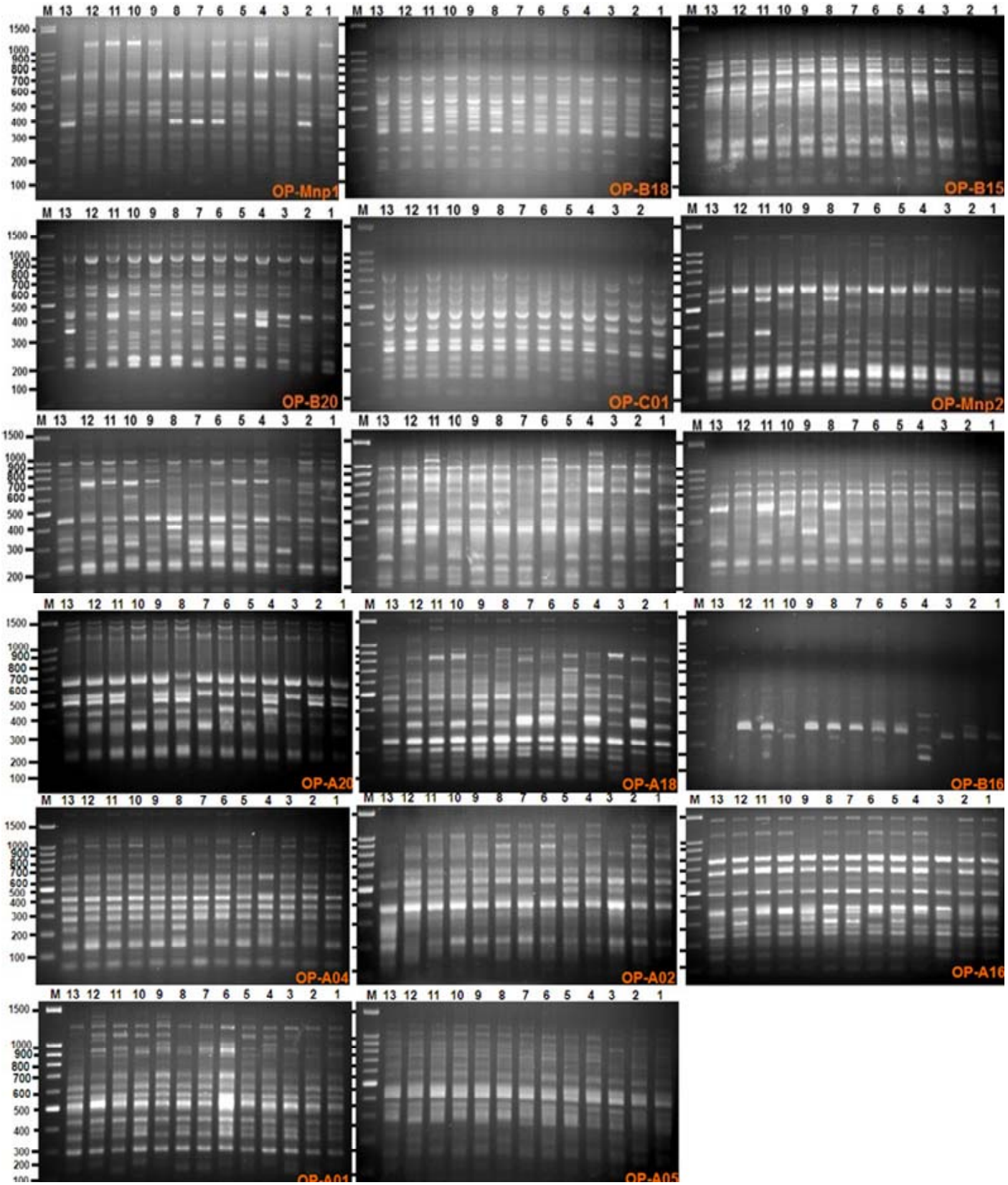

Figure 1. DNA amplified fragments using random primers (OP- Mnp1, B18, B15, B20, Co1, Mnp2, Mnp3, Mnp4, Mnp5, A20, A18, B16, A04, A02, A16, A01 and A05). Commercial cultivars: (1) Alphons, (2) Taymour, (3) Ewais, (4) Zebda and (5) Mabrouka. Accessions (6, 7, 8, 9, 10, 11, 12 and 13) uses as a stock. M: 1Kb markers.

Table 4. Genetic polymorphism between five cultivars (1) Alphons, (2) Taymour, (3) Ewais, (4) Zebda and (5) Mabrouka and eight accessions 6, 7, 8, 9, 10, 11, 12 and 13 of mango detected by RAPD-PCR.

Primer	Size of fragments (bp)	Total bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism percentage (%)
OP-A01	240-1390	19	8	11	57.9
OP-A02	144-1240	15	8	7	46.7
OP-A04	65-2100	14	12	2	14.3
OP-A05	125-1120	18	16	2	11.1
OP-A09	130-1240	12	8	4	33.3
OP-A11	120-1370	16	9	7	43.8
OP-A16	110-1490	20	13	7	35.0
OP-A18	110-1520	23	5	18	78.3
OP-A20	240-1520	11	10	1	9.1
OP-B15	150-1065	14	13	1	7.1
OP-B16	440-1170	7	1	6	85.7
OP-B18	180-1120	16	14	2	12.5
OP-B20	210-1450	24	11	13	54.2
OP-C01	125-725	13	10	3	23.1
Mnp1	150-1240	12	9	3	25.0
Mnp2	110-1240	20	11	9	45,0
Mnp3	140-1270	24	7	17	70.8
Mnp4	190-1250	15	7	8	53.3
Mnp5	125-975	13	11	2	15.4
Total	-	306	183	123	40.2

Table 5. Total bands produced from each primer for the five cultivars (1) Alphons, (2) Taymour, (3) Ewais, (4) Zebda and (5) Mabrouka (1) Alphons, (2) Taymour, (3) Ewais, (4) Zebda and (5) Mabrouka and eight accessions 6, 7, 8, 9, 10, 11, 12 and 13 of mango.

Primers	13	12	11	10	9	8	7	6	5	4	3	2	1
OP-A01	11	17	12	13	14	13	12	14	14	15	14	14	14
OP-A02	7	11	10	10	11	12	12	12	11	10	9	12	11
OP-A04	13	14	14	13	14	12	12	12	13	14	13	13	13
OP-A05	17	17	17	17	17	17	17	17	17	17	17	17	17
OP-A09	10	9	10	9	8	9	10	10	9	9	9	10	9
OP-A11	11	12	12	12	14	14	12	12	14	12	12	12	12
OP-A16	15	16	15	14	13	15	16	15	15	16	16	17	15
OP-A18	14	14	18	20	17	19	15	19	18	14	13	17	13
OP-A20	11	11	11	10	11	11	10	10	10	11	10	11	11
OP-B15	14	14	14	14	14	14	14	14	14	14	14	14	13
OP-B16	1	3	3	2	2	3	2	4	3	5	3	3	3
OP-B18	14	16	16	16	15	16	16	15	14	14	15	15	15
OP-B20	16	19	18	20	19	18	19	21	18	18	19	19	19
OP-C01	11	11	11	12	12	13	12	12	12	12	12	11	11
Mnp1	9	11	10	10	10	9	10	10	10	11	10	9	10
Mnp2	14	14	15	14	15	17	16	17	14	16	14	15	15
Mnp3	15	18	17	16	15	15	15	16	17	17	13	20	18
Mnp4	11	11	11	11	10	10	10	13	9	12	11	13	12
Mnp5	13	13	13	13	12	13	11	11	13	12	13	13	13
Total	227	251	247	246	243	250	241	254	245	249	237	255	244

Table 6. Genetic similarity matrix detected between five cultivars (1) Alphons, (2) Taymour, (3) Ewais, (4) Zebda and (5) Mabrouka and eight accessions 6, 7, 8, 9, 10, 11, 12 and 13 of mango with RAPD markers based on Jaccard's coefficients.

Genotype	13	12	11	10	9	8	7	6	5	4	3	2	1
13	1												
12	0.781	1.000											
11	0.823	0.855	1.000										
10	0.775	0.862	0.871	1.000									
9	0.787	0.854	0.856	0.849	1.000								
8	0.800	0.832	0.875	0.848	0.867	1.000							
7	0.789	0.816	0.817	0.831	0.851	0.877	1.000						
6	0.755	0.820	0.822	0.856	0.827	0.860	0.893	1.000					
5	0.785	0.865	0.860	0.867	0.866	0.865	0.848	0.845	1.000				
4	0.770	0.828	0.797	0.830	0.822	0.808	0.804	0.822	0.833	1.000			
3	0.761	0.842	0.837	0.851	0.802	0.821	0.817	0.809	0.883	0.824	1.000		
2	0.779	0.850	0.846	0.839	0.798	0.870	0.820	0.831	0.815	0.806	0.826	1.000	
1	0.772	0.879	0.860	0.896	0.818	0.844	0.813	0.832	0.864	0.840	0.876	0.876	1.000

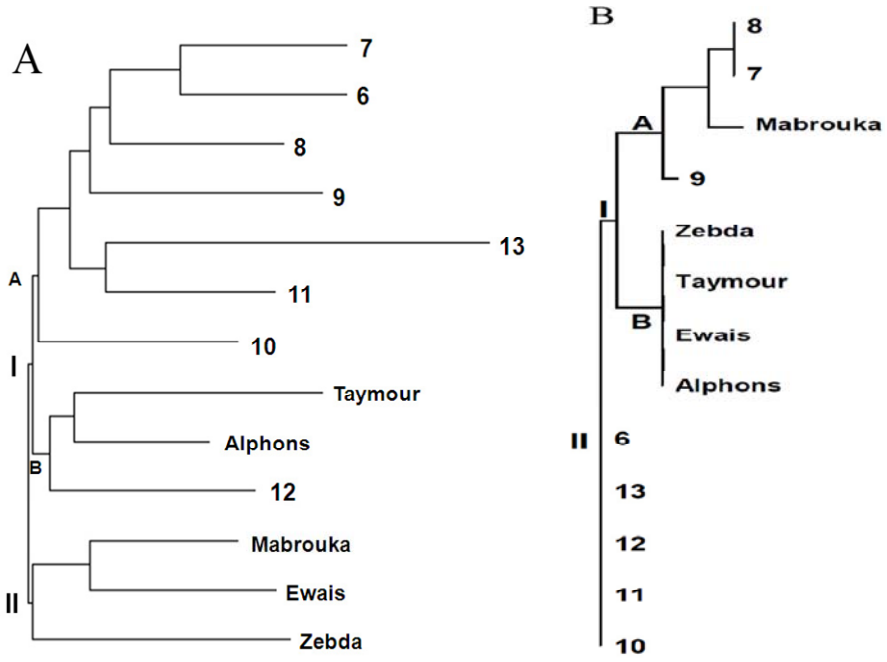

Figure 2. (A) RAPD based dendogram of the thirteen cultivars and accessions constructed using Neighbour joining method based on Jaccard's coefficient. **(B)** Dendogram showing the similarity among the electrophoretic protein patterns (SDS-PAGE) of 13 of cultivars and accessions based on Jaccard's similarity coefficient values which were grouped by the Neighbour joining method.

and environmental factors. Gene expression is influenced by the environment therefore; the selection based merely on morphological traits has been often misleading (Kumar et al., 1998; Astarini et al., 2004 and Asif et al., 2005). In fact RAPD-PCR is a useful technique for providing information on the degree of polymorphism and genetic diversity of our cultivars and accessions. So this analysis could be profitable for breeders for rapid and early identification of most diverse genotypes to improve crop productivity. The knowledge of the genetic diversity of the genotypes is important for parental selection that to maximize the genetic improvement. But we found that RAPD-PCR technique was much higher and an efficient method than that of the SDS-PAGE for genotypes identification because the RAPD-PCR markers are stable but the markers appearing in SDS-PAGE could be affected by

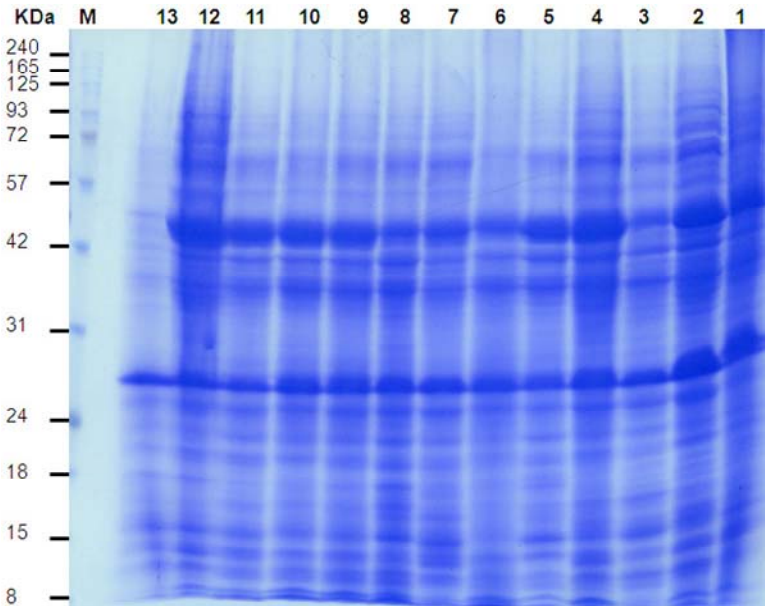

Figure 3. SDS-PAGE banding patterns of 13 cultivars and accessions of mango. Cultivars: (1) Alphons, (2) Taymour, (3) Ewais, (4) Zebda and (5) Mabrouka. Accessions: 6, 7, 8, 9, 10, 11, 12 and 13. Wild type varieties used as a stock M: Protein marker.

Table 7. Genetic similarity matrix appeared between five cultivars (1) Alphons, (2) Taymour, (3) Ewais, (4) Zebda and (5) Mabrouka and eight accessions 6, 7, 8, 9, 10, 11, 12 and 13 of mango with SDS-PAGE based on Jaccard's coefficients.

Genotype	13	12	11	10	9	8	7	6	5	4	3	2	1
13	1.000												
12	1.000	1.000											
11	1.000	1.000	1.000										
10	1.000	1.000	1.000	1.000									
9	0.970	0.970	0.970	0.970	1.000								
8	0.941	0.941	0.941	0.941	0.970	1.000							
7	0.941	0.941	0.941	0.941	0.970	1.000	1.000						
6	1.000	1.000	1.000	1.000	0.970	0.941	0.941	1.000					
5	0.914	0.914	0.914	0.914	0.941	0.971	0.971	0.914	1.000				
4	0.971	0.971	0.971	0.971	0.941	0.914	0.914	0.971	0.943	1.000			
3	0.971	0.971	0.971	0.971	0.941	0.914	0.914	0.971	0.943	1.000	1.000		
2	0.971	0.971	0.971	0.971	0.941	0.914	0.914	0.971	0.943	1.000	1.000	1.000	
1	0.971	0.971	0.971	0.971	0.941	0.914	0.914	0.971	0.943	1.000	1.000	1.000	1.000

the environment.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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