

GENETIC VARIABILITY AND MOLECULAR CHARACTERIZATION OF SOME LOCAL AND IMPORTED MANGO CULTIVARS IN EGYPT

OLA A. GALAL¹, HODA A. GALAL² AND AZIZA A. ABOULILA¹

1. Genetics Department, Faculty of Agric., 33516, Kafr-El-Sheikh University, Kafr El-Sheikh, Egypt
2. Enviro. Studies and Research Institute (ESRI), University of Sadat City (USC), Menofeia, Egypt

Mango (*Mangifera indica* L., $2n=40$); belonging to the family *Anacardiaceae*, is one of the oldest and most important tropical fruits worldwide. It originated in the South East Asian or Indo-Burma Region and has been cultivated for 4000 years in India (Mukherjee, 1953; Kostermans and Bompard, 1993). In Egypt, mango has been cultivated since the 19th century until now, there are at least 40 cultivars which are propagated and grown in commercial scale (EMALR, 2004). Great variations have been detected among these cultivars in vegetative canopy and reproductive characteristics, production season, yield and fruit characters (EMALR, 2004; Elsheshetawy *et al.*, 2016).

Many efforts were made to understand the variability of mango germplasm and cultivars identification. Earlier, morphological and physico-chemical characteristics were used to establish and identify cultivars (Singh *et al.*, 2009; Mussane *et al.*, 2010; Rajwana *et al.*, 2011; Begum *et al.*, 2012). The application of these methods is the simplest way and is considered the first step for evaluating the genetic diversity (Hoogendijk and Williams, 2001). In spite of that, actual identi-

ty of some cultivars is still unclear because some of these characters differed from year to year as affected by environmental conditions and agricultural practices. Therefore, there is a great need to identify mango cultivars at the molecular level. Unlike the agronomic and morphologic characteristics, the molecular markers are not subject to the environmental effect. Recently, the DNA markers generated by PCR methods have been used in mango characterization (Singh *et al.*, 2009; Bhargava and Khorwa, 2011; Begum *et al.*, 2012). Among the PCR-based DNA markers, Randomly Amplified Polymorphic DNA (RAPD) is a commonly and extensively used tool for assessment of genetic variability in mango and other crops. This technique has been shown to be easy, rapid, reliable and useful in genetic analysis (Ranade *et al.*, 2006; Souza *et al.*, 2011). This analysis is important for mango improvement programs and management of genetic resources which gives insight into genetic markup of related genotypes.

To produce a new mango cultivar, plant breeders are facing some challenges. Mangoes have a long juvenile period, self incompatibility, cross pollination, high

clonal heterozygosity, polyembryonic seed and limited genetic knowledge. In addition, classical breeding technologies such as back crossing and hybrid-seeds are not applicable to fruit trees. These challenges have resulted in inefficient, long and expensive breeding programs (Usman *et al.*, 2001; Lavi *et al.*, 2006; Iyer and Schnell, 2009). In Egypt, production of a new cultivar is based on: 1) personal selection of superior seedling by mango growers; once selected, this phenotype is usually vegetatively propagated quite easily (i.e. Ewais, Sedeeq and Zebda), or, 2) importing a new cultivar from other countries like introducing Keitt and Tommy Atkins from Florida, Fajri Klan from India, Haidi from South Africa and Naomi from Israel (Litz, 2009).

The main objectives of mango breeding aimed to improve both plant and fruit characteristics such as dwarf trees, profuse and regular bearing, good fruit size and edible quality, less fibers, attractive peel and pulp color, diseases resistance and long storage life (Usman *et al.*, 2001). Most of the Egyptian selected cultivars are vigorous in growth (Rashedy *et al.*, 2014), polyembryonic, with a green or greenish-yellow external color, strong-flavored pulp and usually have little disagreeable fiber (Knight, 2004). However Indian cultivars mostly monoembrionic with red blush and yellow or orange ground color and have strong flavour (Litz, 2009). To establish breeding program and to improve mango cultivars in Egypt, the genetic variability among available cultivars should be understood.

Johnson *et al.* (1955) reported that the genetic improvement for any required character depends upon the amount of genetic variability present in the breeding material. High heritability generally enables the breeder to select plants on the basis of the phenotypic expression. Therefore, selection of these characters would be feasible for mango improvement (Majumder *et al.*, 2012).

The present investigation was conducted to assess the genetic variability and heritability for some physico-chemical traits of some major local and imported mango cultivars grown in Egypt. Moreover, study of the genetic divergence and phylogenetic relationships; based on RAPD markers, among the genotypes studied to provide bases for marker-assisted selection of parents for hybridization and improvement of mango cultivars.

MATERIALS AND METHODS

The present study was conducted during two successive seasons; 2014 and 2015, at the Pomology laboratory, Environmental Studies and Research Institute, University of Sadat City, Menofeia, Egypt and the Laboratory of Genetics Department, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh, Egypt.

Plant materials

Plant materials used in this study included three local mango cultivars; Ewais, Zebda and Sedeeq, as well as five imported mango cultivars; Naomi, Keitt, Fajri Klan, Tommy Atkins and Haidi,

which were cultivated in a private orchard at Cairo-Alexandria Desert Road, Egypt. Each cultivar was represented by four trees and all the collected leaf and fruit samples were taken randomly from mature trees through two successive seasons; 2014 and 2015.

Physico-chemical analysis

The studied cultivars were evaluated for six qualitative and eight quantitative characteristics representing physical and chemical properties.

Observations for tree vigor and alternative bearing were recorded on site, while leaf and fruit samples were taken to Pomology Lab. for further evaluation. Ten mature healthy leaves were taken to estimate length and width. For determining different fruit characters, five mature fruits were harvested randomly and kept for ripening at ambient storage conditions ($25\pm 3^{\circ}\text{C}$). Data were recorded for peel color of ripe fruits, fiber in pulp, eating quality and seed embryonic type. Measurements also included fruit length, breadth and weight in addition to the percentage of stone weight.

Longitudinal slices of fruit pulp were used to extract juice to measure total soluble solids (TSS) and acidity. The TSS was determined in each sample by using hand refractometer (model Atago, Tokyo, Japan) and expressed as $^{\circ}\text{Brix}$. Titratable acidity was determined by titrating with 0.1 N NaOH in the presence of phenolphthalein indicator as described by AOAC (1995). The results were expressed as %

citric acid which is the main organic acid in mango fruit (Ueda *et al.*, 2000).

Molecular analysis

Young leaves (the first 2-4 leaves from the tips) from each of the eight mango cultivars were collected and washed thoroughly using distilled water. Total genomic DNA was extracted from the collected leaves using the DNeasy Plant Mini Kit (QIAGEN GmbH, Cat. No. 69104).

RAPD analysis was carried out using fourteen decamer random primers which were purchased from Bio Basic Inc, Canada. The list of primers and their sequences are presented in Table (1). The optimization of PCR conditions for each primer was performed in a final volume of 25 μL reaction mixture including 1 μL of isolated DNA template. Final concentrations were 1 x buffer (Mg^{2+} free), 1.5 mM MgCl_2 , 200 μM dNTPs mix, 0.8 μM primer, 1 U Taq DNA Polymerase (ROVALAB, Germany). Amplifications were carried out in a thermal cycler according to manufacture instructions as follow: the initial amplification program started with denaturation step at 94°C for 5 min, followed by 35 cycles consisting of 30 sec of denaturation at 94°C , 45 sec of annealing at 30°C and 1.5 min of extension at 72°C . The program ended with a final extension step at 72°C for 2 min. PCR amplified products were electrophoresed on 1.5% (w/v) agarose gel against a known DNA Ladder (50 bp DNA Ladder ready-to-use, Cat-no: 300003, GeneON).

Data analyses

The data of quantitative characteristics were subjected to analysis of variance (ANOVA) with Randomized Complete Block Design using the Statistical Analysis Software (SAS). Means were compared by Least Significant Difference (LSD) at significance level of 0.05.

Statistical and genetic parameters; phenotypic (PCV%) and genotypic (GCV%) coefficients of variation, phenotypic and genotypic variances and heritability (%) were calculated according to Falconer (1981) based on the combined analysis of the two studied years.

Data generated from banding patterns of the fourteen RAPD primers were introduced to SPSS package program according to binary values of (1) and (0) for the presence and absence of bands, respectively. The genetic distances among the eight mango genotypes were conducted based on Jaccard's similarity coefficient (Jaccard, 1901) using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) analysis (Nei, 1973).

RESULTS AND DISCUSSION

Physico-chemical characterization

In fruit tree species, quantitative and qualitative fruit characters have been found to be useful in identification and assessment of genetic heterogeneity and selection of superior genotypes. EMALR (2004) and Elsheshetawy *et al.* (2016) reported that mango cultivars display a great genetic diversity in tree vigor and

size, bearing habit, fruit yield, shape, size and quality, embryo number in the seed, ripening season, disease resistance and storage characteristics.

a. Qualitative characteristics

Qualitative characters have been used as a traditional method to identify cultivars and to select superior seedlings in fruit crops. Table (2) illustrated the qualitative characteristics of the eight studied mango cultivars.

Peel color is one of the most important fruit characters, since it is the main factor by which most consumers define mango quality and ripening stage. Table (2) showed that peel color of ripe mango differed among the studied cultivars. Peel color was yellow in Ewais, greenish yellow in Keitt, Fajri Klan and Zebda, whereas it was green with red plush in the Sedeek cultivar. Cultivars of Tommy Atkins and Haidi had Red/yellow peel color. Naomi cultivar showed green, red and yellow colors in the fruit peel. The change in peel color from green and the appearance of yellow or red colors is one of the most obvious signs of fruit ripening; although some cultivars do not change their green color upon ripening. These changes are due to the disappearance of chlorophyll and the appearance of other pigments depending on the cultivar. For green cultivars, Ketsa *et al.* (1999) suggested that green color was due to a lower activity of chlorophyllase and/or peroxidase activity rather than low ethylene production. While for the yellow cultivars, carotenoids and xanthophylls are the pre-

dominant pigments (Litz, 2009). On the other hand, peel color development can be affected by environmental conditions and agricultural practices such as nitrogen fertilization and light intensity which varied according to the fruit position on the tree (Yahia, 1999; Litz, 2009).

The quantity of fibers in the pulp was high in Keitt and Zebda cultivars. Fajri Klan, Tommy Atkins, Sedeek and Haidi had intermediate quantity of fibers; while it was absent in the rest of cultivars. Yahia (1999) recorded that the crude fibers around mango seeds varied from 2 to 19.3% according to the cultivar. Cultivars with absent or less fibers are more preferable to the consumers (Pinto *et al.*, 2002).

Concerning eating qualities as flavour and taste, the three cultivars of Ewais, Naomi and Sedeek were more delicious and favorable taste, so they were considered as excellent cultivars. Whereas, Keitt, Fajri Klan, Tommy Atkins, Zebda and Haidi cultivars had good fruit eating qualities.

Examination of mango seeds revealed that the Egyptian cultivars (Ewais, Zebda and Sedeek) have polyembryonic seed, while the other cultivars exhibited monoembryonic seed. Monoembryonic cultivars are hybrid in origin and must be reproduced by asexual propagation. They produce seedlings that are not true to type of the mother plant. Polyembryonic cultivars are those where many embryos may develop from diploid parental nucellar tissue and one of which initiates by fertilization. The sexual embryo often gives a

weak and stunted seedling and should be discarded, while the other seedlings are clones of the mother tree (Hartmann *et al.*, 2011). Our results agree with those of Knight (2004) and Litz (2009) that most of the Egyptian selected cultivars are polyembryonic, while Indian cultivars mostly monoembryonic.

Results of tree vigor observations were recorded in Table (2). From the obtained data, the studied cultivars can be classified into three groups according to tree growth behavior. 1) The dwarf group; which grows with low vigor, included the cultivars Naomi and Keitt. 2) The medium group; included Tommy Atkins and Haidi cultivars. 3) The large group; which recorded the highest height compared to the other tested cultivars, included the Egyptian cultivars (Ewais, Zebda and Sedeek) as well as Fajri Klan. Campbell (1991) reported that the large size of mango trees is one of the most important problems in mango production. Dwarf cultivar is the preferable in breeding since huge size of mango trees causes problems in agricultural practices and harvesting. Recently, the high density orchard system became a new trend in fruit production that depends on low vigor cultivars or dwarfing rootstocks. In this concern, Rashedy *et al.* (2014) noticed that mango tree vigor was related to stem anatomy since vigor cultivars had high xylem and low phloem percentage, and high number of the large vessels.

Alternative bearing is a major problem in mango production. It refers to trees that have an irregular crop load from

year to year resulting in significant reduction in yield. With regard to our observations on the flowering and yield of the eight mango cultivars, the recorded data revealed that Zebda cultivar suffered strongly from alternative bearing, while Fajri Klan and Sedeek showed moderate effects. Alternative bearing was absent in the other studied cultivars. This problem is due to several factors such as genetic, physiological, environmental and nutritional practices (Monselise and Goldschmidt, 1982; Bangerth, 2006).

b. Quantitative characteristics

The quantitative characteristics of the eight mango cultivar leaves and fruits are shown in Table (3). Based on the measured data of mango leaves, Naomi had the highest leaf length, while Fajri Klan recorded the highest leaf width compared to the other studied cultivars. On the other hand, cultivars of Tommy Atkins and Haidi had the lowest values of leaf length, while Haidi had the lowest value of leaf width.

According to the mango fruit parameters, Sedeek had the highest fruit length compared to the other cultivars, while Keitt recorded the heights fruit breadth and weight. Regarding the stone weight (%), Sedeek had the highest value among the studied cultivars which is considered unfavorable character for consumer. The variations in mango fruit parameters were reported previously by Bhuyan and Guha (1995) who observed a wide range of variability in mango fruit charac-

teristics. Mango can have a fruit weight ranges from a few grams up to 1 kg and fruit lengths can vary from 2.5 to 30.0 cm in different varieties (Morton, 1987; Human, 2008). Also, Jintanawong *et al.* (1992) determined the quality standards for mango genotypes by observing the fruit size, shape, color, weight, texture and fiber. On the other hand, Naik (1971) reported variability among trees of the same cultivar with respect to fruit size, shape, color and quality.

Two essential chemical parameters; TSS and acidity, were recorded in support of quality of mango fruits. It was generally considered that greater than 14 °Brix of TSS indicates the good quality of mangoes. The present findings (Table 3) revealed that the highest TSS content was found as 21.68 °Brix in Ewais followed by 17.90, 16.17, 14.78 and 14.18 °Brix in Keitt, Fajri Klan, Zebda and Naomi, respectively. On the other hand, the lowest TSS content was recorded for Sedeek cultivar (9.88 °Brix). For the acidity content, it ranged from 0.20% for Naomi (which did not differ significantly from Sedeek and Haidi) to 0.63% for Ewais (which did not differ significantly from Tommy Atkins). Ueda *et al.* (2000) reported that TSS and acidity content are related to the maturity of the fruit. During maturation, soluble solids are increased and titratable acidity is decreased.

The observed variations among the studied mango cultivars reflect the existence of a wide range of genetic variations. Similar results were reported by Singh *et*

al. (2009) who studied the morphological characterization of some Indian mango cultivars and detected a variation between the standard cultivar and its landraces.

Mean performance and genetic parameters of leaf and fruit characters

The improvement in any crop depends on better understanding of the nature and magnitude of genetic variability in the material and the extent to which it is heritable (Majumder *et al.*, 2012). Assessment of genetic variability of various plant characters are of interest for plant breeders to know the role of environment in the expression of a trait. Genetic variability for physico-chemical characteristics was determined on the basis of the results of combined analysis of the two seasons; 2014 and 2015, with the use of certain genetic parameters such as coefficients of variation, variance components and heritability as presented in Table (3).

The mean performances of the eight genotypes with respect to different characters showed that the ranges of leaf parameters; length and width, were 5.87 and 1.24 cm with mean values of 19.06 and 5.68 cm, respectively. For the fruit parameters, the results indicated that the ranges of fruit length, breadth, weight and stone weight were 5.28 cm, 2.69 cm, 451.80 g and 8.82% with mean values of 12.38 cm, 8.53 cm, 468.15 g and 9.36%, respectively. The mean values of fruit chemical characters; TSS and acidity, were 14.95 °Brix and 0.39% with ranges varied from 9.88 to 21.68 °Brix and 0.20 to 0.63%, respectively. In general, it can

be emphasized that wide range of phenotypic variation was observed for all characters, except acidity (0.43) followed by leaf width (1.24 cm) and fruit breadth (2.69 cm).

Regarding PCV and GCV parameters, the GCV for the majority of traits was quite close to the estimates of PCV. This indicated that these traits were less affected by environment and amenable to improvement.

The estimates of variance components revealed that the differences between phenotypic and genotypic variances were very narrow and the estimates of phenotypic variance were slightly higher than genotypic variance for all characters, suggesting a little influence of environments in the expression of genetic variability.

The heritability estimates in broad sense for the different characters were presented in Table (3). It was estimated as the ratio of genotypic variance to phenotypic variance for the studied traits. Heritability value was low for leaf length (26.27%); indicating the influence of environmental conditions on the expression of this trait, while it was relatively high for leaf width (76.97%). Concerning fruit parameters, the heritability values were high for fruit length (98.27%), breadth (92.52%) and weight (94.37%), while it was relatively high for stone weight (80.83%). Also, heritability values were high for TSS (98.07%) and acidity (93.15%). These characters which gave the highest values of heritability indicate

that the environmental effect was very low and these traits were primarily under genetic control and selection for them would be effective based on phenotypic performance of these characters (Burton and Devane, 1953). Falconer (1981) stated that heritability is a good index of transmission of a character from parents to their offsprings; in addition, the knowledge of heritability helps the plant breeder in selection for a particular character which would be feasible for yield improvement of mango.

The estimates of genetic parameters reflected the amount of variation presented in the eight mango cultivars for all the studied characters. This variability is expected as a result of natural cross-pollination that is common in mango (Purseglove, 1968). Significant variations in one or more of the characters studied in the present investigation have also been reported previously by Singh (2002), Singh *et al.* (2004), Pradeepkumar *et al.* (2006), Majumder *et al.* (2012) and Dinesh *et al.* (2013).

Molecular characterization

Fourteen oligonucleotide RAPD primers were used to assess the genetic diversity and relationships among the eight studied mango cultivars (Fig. 1). Results of RAPD revealed variations in the number and size of RAPD bands depend on the sequences of primers and DNA templates used (Table 4). Then, numbers of bands varied from three bands (primer OPB-01) to 19 bands (primer OPB-10). Sizes of bands ranged from 180

bp for primer OPB-17 to 2647 bp for primer OPA-09. A total of 154 bands were detected from the fourteen decamer primers. Twenty eight bands were common for all cultivars (monomorphic), however, 126 bands were polymorphic with 81.82% polymorphism. All 154 bands; either monomorphic or polymorphic, were considered to estimate the genetic diversity and elicit the phylogenic relationship among the eight cultivars.

Table (5) showed that the similarity index among the eight studied cultivars ranged from 0.472 to 0.688. The highest similarity index (0.688) was found between the Egyptian cultivar (Zebda) and the Indian cultivar (Fajri Klan), whereas the lowest one (0.472) was found between the Egyptian cultivar (Sedeek) and the imported cultivar (Tommy Atkins). The close relationship between Zebda and Fajri Klan at the molecular level is consistent with some morphological characters; both of them have the same peel color (green/yellow), good eating quality, huge tree vigor, while they were different in quantity of fiber in pulp, embryo type and alternative bearing. This close relation refers to the possibility that Zebda could be a seedling selected from the imported cultivar Fajri Klan and planted as a new local cultivar in Egypt. Although the high similarity between local cultivar (Zebda) and imported one (Fajri Klan) at physical and molecular levels. There was a great variation among other local and imported cultivars. This genetic distance among different cultivars could be contributed in mango improvement and might allow the

implementation of a more efficient breeding program (kumar *et al.*, 2001).

The UPGMA dendrogram; based on the similarity index, separated the eight genotypes into two main groups (Fig. 2). Egyptian cultivar Sedeeq was the most divergent in the first main group and the second main group exhibited less distance, which consisted of all the other cultivars. This was consistent with genetic relationship of pigments in peel color. The Egyptian cultivar Sedeeq which was separated and formed distinct group was the only cultivar that did not have yellow color in ripe fruit peel; as it has green/red color, while all the other cultivars had yellow color in their ripe fruit peel. These results were in agreement with Galal (2008) who found that green mango cultivars were separated from colored cultivars in two groups. On the other hand, Mansour *et al.* (2014) reported that Sedeeq and Succary montaz cultivars; which were closely related and were separated in distinct cluster, had similar bearing habit and the same polyembryonic type. In addition, both cultivars are indigenous to the Delta of Egypt as a superior chance seedling. Thus, RAPD analysis could be effective in separating cultivars according to their morphological characters and/or their geographical location.

On the other hand, the results of phylogenetic relationship was not completely related to their geographical origin and the type of embryo, although earlier studies stated that RAPD markers could segregate mango cultivars depending on

their geographical location (Schnell *et al.*, 1995) or according to the embryo type and bearing habit (Mansour *et al.*, 2014). Thus RAPD analysis needs to be combined with other molecular markers as well as the traditional classification for a comprehensive consideration.

The capability of RAPD to detect genomic variability and validate genetic relationships among mango cultivars was reported previously by Kumar *et al.* (2001), Singh *et al.* (2009), Bhargava and Khorwa (2011) and Mansour *et al.* (2014), indicating the usefulness of RAPD markers for genetic improvement of mango cultivars.

In conclusion, results of this study gave insight into the amount and nature of the genetic variability in eight of the available mango cultivars in Egypt, which was very important for plant breeders to establish breeding program for mango improvement. Considerably high heritability values were found for the majority of traits suggesting a little influence of environments. The knowledge of heritability helps the plant breeder in selection for these characters and improvement of mango cultivars through breeding and selection. Moreover, RAPD marker was found to be effective in study of the genetic divergence and phylogenetic relationships; in addition, separating cultivars according to their physico-chemical characters. Thus it can provide bases for marker-assisted selection of parents for hybridization and improvement of mango cultivars.

SUMMARY

Mango is one of the most important tropical fruits in the world. It has been cultivated in Egypt since 19th century. The usual methodologies to produce a new cultivar in Egypt are based on selection of superior seedling or importing new cultivars. To establish breeding program and improve mango cultivars, genetic variability and relationships among available mango germplasms must be detected. The present study was conducted in two successive seasons (2014 and 2015), on three common local cultivars (Ewais, Zebda and Sedeeq) and five imported cultivars (Nami, Keitt, Fajri Klan, Tommy Atkins and Haidi) which are grown commercially in Egypt. Results revealed a wide range of variability in qualitative and quantitative characteristics of mango cultivars. On the basis of combined analysis of the two years, all fruit parameters; fruit length, breadth and weight, stone weight, TSS and acidity, showed considerably high heritability values which ranged from 80.83 to 98.27%, indicating that genetic improvement for these characters through breeding and selection would be effective. On the other hand, genetic diversity and relatedness among the eight genotypes were assessed based on fourteen decamer RAPD primers. A total of 154 bands were obtained with 81.82% polymorphism. High similarity degree was found between the local cultivar (Zebda) and the Indian cultivar (Fajri Klan) at the molecular level which was consistent with some physical characters. Results of UPGMA dendrogram revealed that the Egyptian

cultivar Sedeeq was the most divergent and separated in a distinct group. This was consistent with genetic relationship of pigments in peel color; Sedeeq was the only cultivar that did not have yellow color in ripe fruit peel. These results indicated that RAPD analysis could be used as an effective tool in separating cultivars according to their physico-chemical properties and could be useful for genetic improvement of mango cultivars.

REFERENCES

- AOAC (1995). Association of Official Analytical Chemists. Official Methods of Analysis, 16th ed. Washington DC, USA.
- Bangerth, F. (2006). Flower induction in perennial fruit trees: still an enigma? *Acta Hort.*, 727: 177-195.
- Begum, H., T. M. Reddy, S. Malthi, B. P. Reddy, S. Archack, J. Nagaraju and E. A. Siddiq (2012). Molecular analysis for genetic distinctiveness and relationships of indigenous landraces with popular cultivars of mango in Andhra Pradesh, India. *The Asian and Australian J. Pl. Biotech.*, 6: 24-37.
- Bhargava, R. and R. Khorwa (2011). Molecular characterization of *Mangifera indica* by using RAPD marker. *Ind. J. Fund. Appl. Life Sci.*, 1: 47-49.
- Bhuyan, M. A. J. and D. Guha (1995). Performance of some exotic mango

- germplasm under Bangladesh conditions. *Bangladesh Hort.*, 23: 17-22.
- Burton, W. G. and E. H. Devane (1953). Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material. *Agron. J.*, 45: 478-481.
- Campbell, C. W. (1991). Progress of mango cultivation. *Proc. Inter. Soc. Tropic. Hortic.*, 32: 8-19.
- Dinesh, M. R., C. Vasugi and R. Venugopal (2013). Heritability studies in mango (*Mangifera indica* L.). *Acta Hortic.*, 992: 321-324.
- Elsheshetawy, H. E., A. Mossad, W. K. Elhelew and V. Farina (2016). Comparative study on the quality characteristics of some Egyptian mango cultivars used for food processing. *Annals of Agric. Sciences*, 61: 49-56.
- EMALR, Egyptian Ministry of Agriculture and Land Reclamation (2004). Mango; cultivation and production. CAAE, ARC, Egypt. Bulletin No. 857.
- Falconer, D. S. (1981). Introduction to quantitative genetics. 2nd ed., Loughman Green, London/New York.
- Galal, H. A. (2008). Storability and genetic evaluation of some mango cultivars. PhD. Thesis, Fac. Agric. Kafr El-Sheikh, Tanta Univ., Egypt.
- Hartmann, H. T., D. E. Kester, F. T. Davies and R. L. Geneve (2011). *Plant Propagation Principles And Practices: Propagation Methods and Rootstocks for Fruit and Nut Species*, 8th ed, chapter 19, PH, Pearson Professional Business. p: 728-766.
- Hoogendijk, M. and D. Williams (2001). Characterizing the genetic diversity of home garden crops: Some examples from Americas. 2nd International Home Gardens Workshop, 17-19 July 2001, Witzenhausen, Federal Republic of Germany, p: 34-40.
- Human, C. F. (2008). Production Areas. In: de Villiers E. A., Joubert P. H. (eds). *The Cultivation of Mango*. ARC-Institute for Tropical and Subtropical Crops, p: 9-15.
- Iyer, C. P. A. and R. J. Schnell (2009). Breeding and genetics, p. 67-96. In: Litz, RE (ed.). *The mango: Botany, production and uses*. CAB International, Wallingford, UK.
- Jaccard, P. (1901). Étude comparative de la distribution florale dans une portion des Alpes et des Jura. *Bull. Soc. Vandoise Sci. Nat.*, 37: 547-579.
- Jintanawong, S., H. Hiranpradit, P. Polprasid and P. Duangpikul

- (1992). Group characterization of Thai mango; *Mangifera indica* L. *Acta Hortic.*, 321: 254-261.
- Johnson, H. W., H. F. Robinson and R. E. Comstock (1955). Estimates of genetic and environmental variability in soybeans. *Agron. J.*, 47: 314-318.
- Ketsa, S., W. Phakawatmongkol and S. Subhadrabhandhu (1999). Peel enzymatic activity and colour changes in ripening mango fruit. *J. Plant Physiol.*, 154: 363-366.
- Knight, R. J. Jr. (2004). *Tropical Visions: Report on the Egyptian Mango Industry*. Tropical Visions, The Rare Fruit Review, 1(4).
- Kostermans, A. J. and J. M. Bompard (1993). *The Mangoes: their Botany, Nomenclature, Horticulture and Utilization*. London, Academic Press. pp. 233.
- Kumar, H., P. Narayanaswamy, T. Prasad, G. K. Mukunda and S. Sondur (2001). Estimation of genetic diversity of commercial mango (*Mangifera indica* L.) cultivars using RAPD markers. *J. Hortic. Sci. Biotechno.*, 76: 529-533.
- Lavi, U., S. Gurevitz, G. Ben Ari, D. Saada, K. Kashkush, T. Paz, T. Twito, Y. Cohen, J. Hillel and G. Simchen (2006). Potential applications of modern biological techniques in breeding fruit trees. *J. Fruit Ornam. Plant Res.*, 14: 13-19.
- Litz, R. E. (2009). *The Mango Botany, Production and Uses*. 2nd ed. Wallingford, UK: CABI Publishing, pp: 669.
- Majumder, D. A. N., L. Hassan, M. A. Rahim and M. M. Kabir (2012). Genotypic and phenotypic variability in mango (*Mangifera indica* L.). *Bangladesh J. Agril. Res.*, 37: 683-690.
- Mansour, H., L. E. Mekki and M. A. Hussein (2014). Assessment of genetic diversity and relationships among Egyptian mango (*Mangifera indica* L.) cultivars grown in Suez Canal and Sinai region using RAPD markers. *Pak. J. Biol. Sci.*, 17: 56-61.
- Monselise, S. and E. Goldschmidt (1982). Alternate bearing in fruit trees. *Hortic. Rev.*, 4: 128-173.
- Morton, J. F. (1987). *Fruits of warm climates*. Miami. Florida Flair Books, p: 221-239.
- Mukherjee, S. K. (1953). The mango. Its botany, cultivation, uses and future improvement, especially as observed in India. *Econ. Bot.*, 7: 130-160.
- Mussane, C. R. B., A. V. Biljon and L. Herselman (2010). Morphological and genetic characterization of

- mango varieties in Mozambique. Second RUFORUM Biennial Meeting 20-24 September, 2010, Entebbe, Uganda. p: 991-995.
- Naik, K. C. (1971). Mango improvement. *Andhra Agri. J.*, 18: 221-222.
- Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA*, 70: 3321-3323.
- Pinto, A. C. Q., J. G. da Costa and C. A. F. Santos (2002). Most importante varieties, in: *Genu PJC, Pinto ACQ* (Eds.), *The Mango Crop*, Embrapa Informaçao Tecnologica, Brasilia, Brazil. *ISHS Acta Hortic.* 645: VII International Mango Symposium.
- Pradeepkumar, T., P. Joseph and I. Johnkutty (2006). Variability in physicochemical characteristics of mango genotypes in Northern Kerala. *J. Trop. Agri.*, 44: 57-60.
- Purseglove, J. W. (1968). *Tropical Crops: Dicotyledons*, Longman Scientific and Technical. John Wiley and Sons Inc., New York.
- Rajwana, I. A., I. A. Khan, A. U. Malik, B. A. Saleem, A. S. Khan, Z. Khurram, A. Raheel and M. Amin (2011). Morphological and biochemical markers for varietal characterization and quality assessment of potential indigenous mango (*Mangifera indica*) germplasm. *Int. J. Agri. Biol.*, 13: 151-158.
- Ranade, S. A., T. S. Rana, A. P. Srivastava and K. N. Nair (2006). Molecular differentiation in *Murraya Koenig* ex L. species in India inferred through ITS, RAPD and DAMD analysis. *Curr. Sci.*, 90: 1253-1258.
- Rashedy, A. A., M. A. El Kheshin and A. M. Abd Allatif (2014). Histological parameters related to dwarfism in some mango cultivars. *World J. of Agric. Science*, 10: 216-222.
- Schnell, R. J., C. M. Ronning and R. J. Jr. Knight (1995). Identification of cultivars and validation of genetic relationships in *Mangifera indica* L. using RAPD markers. *Theor. Applied Genet.*, 90: 269-274.
- Singh, D. B. (2002). Improvement of mango for regular and early fruiting in Andaman. *Indian J. Agric. Sci.*, 72: 631-634.
- Singh, J., R. R. Singh, G. S. Yadav and U. K. Singh (2004). Studies on genetic variability in mango. *J. Applied Biology*, 14: 34-35.
- Singh, S., A. B. Gaikwad and J. L. Karihaloo (2009). Morphological and molecular analysis of intracultivar variation in Indian mango (*Mangifera indica* L.) cultivars. *Acta Hortic.*, 829: 205-212.
- Souza, I. G. B., S. E. S. Valente, F. B. Britto, V. A. B. de Souza and P. S. C. Lima (2011). RAPD analysis of

- the genetic diversity of mango (*Mangifera indica*) germplasm in Brazil. *Genet. Mol. Res.*, 10: 3080-3089.
- Ueda, M., K. Sasaki, N. Utsunomiya, K. Inaba and Y. Shimabayash (2000). Change in physical and chemical properties during maturation of mango fruit (*Mangifera indica* L. rwin) cultured in plastics green houses. *Food Sci. Technol. Res.*, 6: 299-305.
- Usman, M., B. Fatima and M. J. Jaskani (2001). Review breeding in mango. *Int. J. Agri. Biol.*, 3: 522-526.
- Yahia, E. H. M. (1999). Postharvest handling of mango. Technical Report. *Agric. Technology Utilisation and Transfer (ATUT)*. Giza, Egypt, p: 57-61.

Table (1): List of RAPD primers and their nucleotide sequences.

| Serial No. | Primer code | Primer sequence (5'→3') |
|------------|-------------|-------------------------|
| 1 | OPA-08 | GTGACGTAGG |
| 2 | OPA-09 | GGGTAACGCC |
| 3 | OPA-14 | TCTGTGCTGG |
| 4 | OPA-20 | GTTGCGATCC |
| 5 | OPB-01 | GTTTCGCTCC |
| 6 | OPB-05 | TGCGCCCTTC |
| 7 | OPB-06 | TGCTCTGCCC |
| 8 | OPB-07 | GGTGACGCAG |
| 9 | OPB-08 | GTCCACACGG |
| 10 | OPB-10 | CTGCTGGGAC |
| 11 | OPB-11 | GTAGACCCGT |
| 12 | OPB-12 | CCTTGACGCA |
| 13 | OPB-14 | TCCGCTCTGG |
| 14 | OPB-17 | AGGGAACGAG |

Table (2): Qualitative characteristics of the eight mango cultivars.

| Cultivar | Peel color of ripe fruit | Fiber in pulp | Eating quality | Embryo type | Tree vigor | Alternative bearing |
|--------------|--------------------------|---------------|----------------|-------------|------------|---------------------|
| Ewais | Yellow | Absent | Excellent | Poly | Huge | Absent |
| Naomi | Green/Red/Yellow | Absent | Excellent | Mono | Dwarf | Absent |
| Keitt | Green/Yellow | High | Good | Mono | Dwarf | Absent |
| Fajri Klan | Green/Yellow | Moderate | Good | Mono | Huge | Moderate |
| Tommy Atkins | Red/Yellow | Moderate | Good | Mono | Medium | Absent |
| Zebda | Green/ yellow | High | Good | Poly | Huge | High |
| Sedeek | Green/Red | Moderate | Excellent | Poly | Huge | Moderate |
| Haidi | Red/Yellow | Moderate | Good | Mono | Medium | Absent |

Table (3): Mean performance and genetic parameters of quantitative characteristics of the eight mango cultivars.

| Variety | Leaf length (cm) | Leaf width (cm) | Fruit Length (cm) | Fruit breadth (cm) | Fruit weight (g) | Stone weight (%) | TSS (°Brix) | Acidity (%) |
|------------------------------------|---------------------|---------------------|---------------------|--------------------|----------------------|----------------------|---------------------|--------------------|
| Ewais | 18.20 ^{bc} | 5.48 ^{bc} | 10.23 ^f | 7.35 ^d | 262.17 ^d | 7.39 ^{cd} | 21.68 ^a | 0.63 ^a |
| Naomi | 22.38 ^a | 6.13 ^{ab} | 12.73 ^{cd} | 9.25 ^{ab} | 516.55 ^{bc} | 4.26 ^d | 14.18 ^d | 0.20 ^d |
| Keitt | 18.55 ^{bc} | 6.20 ^{ab} | 13.60 ^{bc} | 9.70 ^a | 713.97 ^a | 4.68 ^d | 17.90 ^b | 0.51 ^{ab} |
| Fajri Klan | 18.37 ^{bc} | 6.55 ^a | 14.02 ^b | 8.88 ^{bc} | 564.78 ^b | 11.73 ^{ab} | 16.17 ^{bc} | 0.29 ^{cd} |
| Tommy Atkins | 17.87 ^c | 5.10 ^{cd} | 12.03 ^{de} | 8.78 ^{bc} | 502.42 ^{bc} | 12.45 ^{ab} | 11.88 ^e | 0.55 ^a |
| Zebda | 21.20 ^{ab} | 5.70 ^{bc} | 11.63 ^e | 8.87 ^{bc} | 446.83 ^c | 9.78 ^{abc} | 14.78 ^{cd} | 0.39 ^{bc} |
| Sedeek | 18.40 ^{bc} | 5.77 ^{abc} | 15.03 ^a | 7.02 ^d | 424.75 ^c | 13.08 ^a | 9.88 ^f | 0.27 ^d |
| Haidi | 17.52 ^c | 4.53 ^d | 9.75 ^f | 8.40 ^c | 313.73 ^d | 10.08 ^{abc} | 13.10 ^{de} | 0.24 ^d |
| Statistical and genetic parameters | | | | | | | | |
| Mean | 19.06 | 5.68 | 12.38 | 8.53 | 468.15 | 9.36 | 14.95 | 0.39 |
| Range | 5.87 | 1.24 | 5.28 | 2.69 | 451.80 | 8.82 | 11.80 | 0.43 |
| PCV (%) | 12.96 | 16.10 | 20.95 | 15.20 | 43.01 | 44.31 | 34.79 | 58.79 |
| GCV (%) | 6.64 | 14.13 | 20.76 | 14.63 | 41.78 | 39.83 | 34.45 | 56.74 |
| Phenotypic variance | 6.10 | 0.84 | 6.72 | 1.68 | 40540.63 | 17.21 | 27.04 | 0.06 |
| Genotypic variance | 1.60 | 0.64 | 6.61 | 1.56 | 38258.40 | 13.91 | 26.52 | 0.05 |
| Heritability (%) | 26.27 | 76.97 | 98.27 | 92.52 | 94.37 | 80.83 | 98.07 | 93.15 |

Means within a column followed by different letter (s) are statistically different at 5% level.

PCV (%): Phenotypic coefficient of variability,

GCV (%): Genotypic coefficient of variability.

Table (4): Total number of bands, monomorphic bands, polymorphic bands and percentage of polymorphism as revealed by RAPD markers among the eight mango cultivars.

| Primer | Size of bands (bp) | Total amplified fragments | Monomorphic bands | Polymorphic bands | Polymorphism (%) |
|--------|--------------------|---------------------------|-------------------|-------------------|------------------|
| OPA-08 | 495-935 | 5 | 1 | 4 | 80.00 |
| OPA-09 | 181-2647 | 13 | 3 | 10 | 76.92 |
| OPA-14 | 335-2056 | 8 | 0 | 8 | 100.00 |
| OPA-20 | 548-1544 | 6 | 0 | 6 | 100.00 |
| OPB-01 | 379-1128 | 3 | 3 | 0 | 0.00 |
| OPB-05 | 332-1973 | 12 | 3 | 9 | 75.00 |
| OPB-06 | 214-1969 | 12 | 9 | 3 | 25.00 |
| OPB-07 | 250-1617 | 14 | 0 | 14 | 100.00 |
| OPB-08 | 343-2000 | 9 | 1 | 8 | 88.89 |
| OPB-10 | 336-2320 | 19 | 1 | 18 | 94.74 |
| OPB-11 | 654-2077 | 14 | 0 | 14 | 100.00 |
| OPB-12 | 239-1720 | 15 | 6 | 9 | 60.00 |
| OPB-14 | 227-2491 | 15 | 0 | 15 | 100.00 |
| OPB-17 | 180-2173 | 9 | 1 | 8 | 88.89 |
| Total | | 154 | 28 | 126 | 81.82 |

Table (5): Genetic similarity matrix among the eight mango cultivars based on RAPD markers.

| Genotype | Ewais | Naomi | Keitt | Fajri Klan | Tommy Atkins | Zebda | Sedeek |
|--------------|-------|-------|-------|------------|--------------|-------|--------|
| Naomi | 0.536 | | | | | | |
| Keitt | 0.595 | 0.605 | | | | | |
| Fajri Klan | 0.676 | 0.600 | 0.555 | | | | |
| Tommy Atkins | 0.538 | 0.588 | 0.556 | 0.634 | | | |
| Zebda | 0.614 | 0.570 | 0.551 | 0.688 | 0.602 | | |
| Sedeek | 0.508 | 0.484 | 0.488 | 0.600 | 0.472 | 0.517 | |
| Haidi | 0.571 | 0.556 | 0.500 | 0.685 | 0.586 | 0.637 | 0.595 |

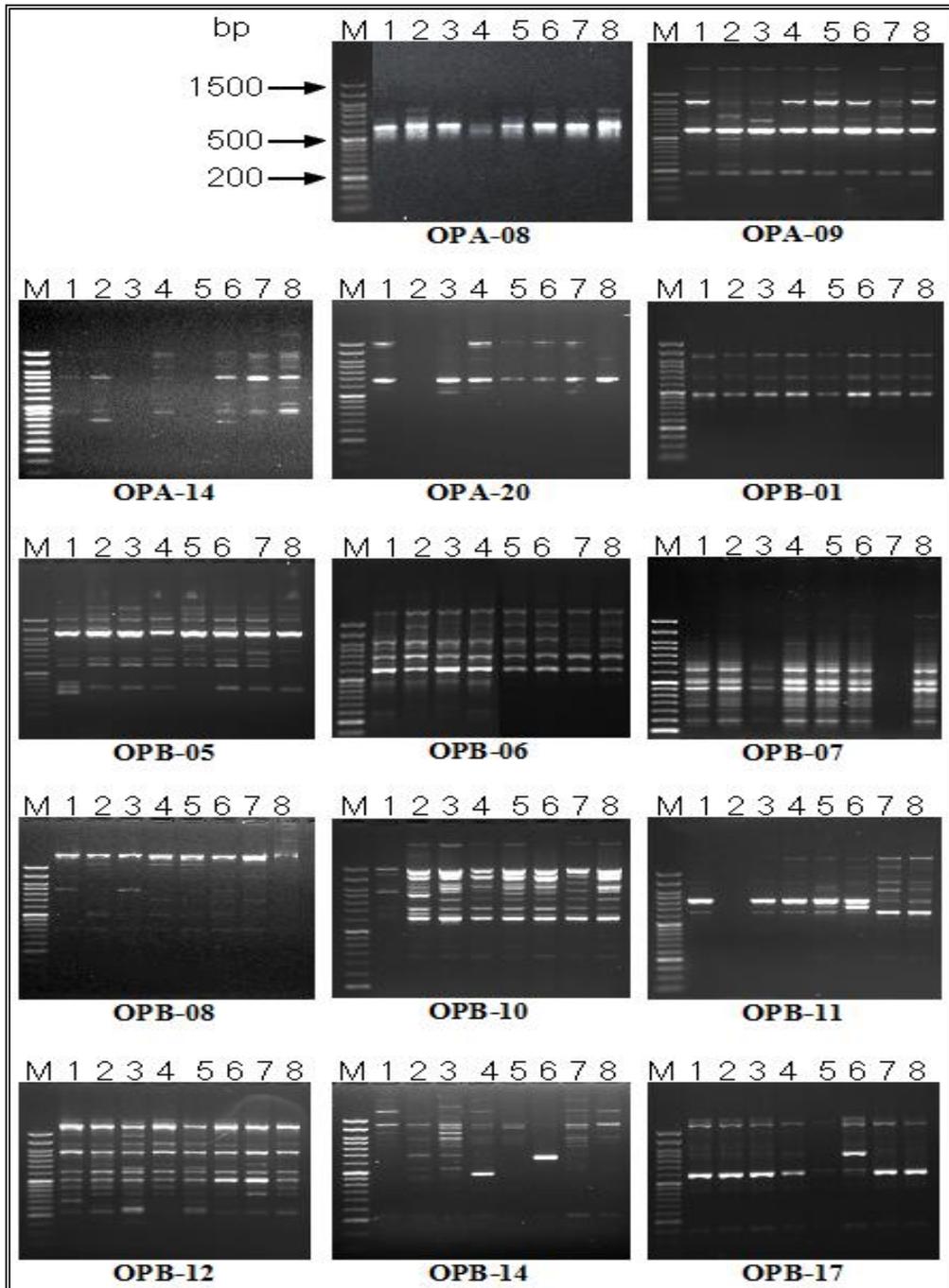


Fig. (1): RAPD profiles of the eight mango cultivars. M: 50 bp DNA leader; 1: Ewais; 2: Naomi; 3: Keitt; 4: Fajri Klan 5: Tommy Atkins; 6: Zebda; 7: Sedeek and 8: Haidi.

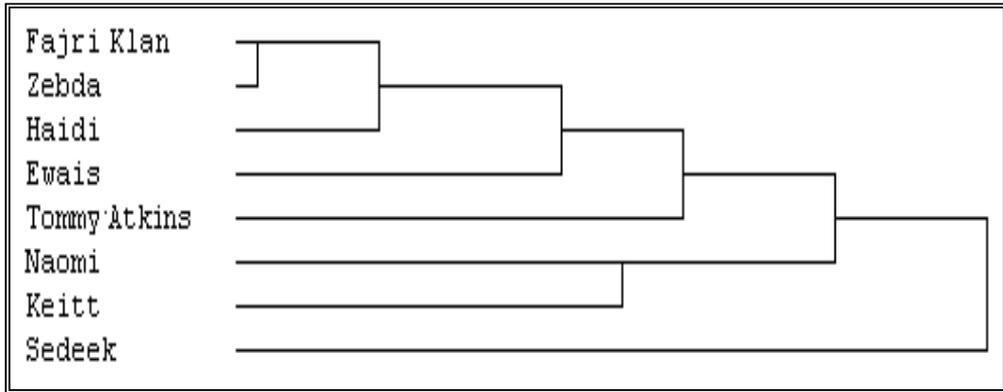


Fig. (2): UPGMA phylogenetic relationship among the eight mango cultivars based on RAPD markers.