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MOLECULAR MARKERS FOR SELF INCOMPATIBILITY PHENOMENA IN SOME MANGO CULTIVARS

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Mango (*Mangifera indica* L.) is considered one of the oldest cultivated trees in the world. The genus *Mangifera* is one of 73 genera belonging to the family Anacardiaceae (Litz, 1997). Mango (2n=40), is an allopolyploid plant, most probably amphidiploids, out breeding species (Mukhejee, 1953). The mango crop, typically tropical fruit, is 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 most of all the production comes from developing countries (Litz, 1997). In Egypt, the total cultivated area with mango reached 209040 Fed. in 2010 (Statistics' of 2011, Ministry of Agriculture, Egypt). The average yield per feddan is only 3.33 Ton.

Low cropping of some mango cultivars is associate with low fruit set and or high fruit drop of immature fruits. The self and cross incompatibility was reported as one of the serious factor affecting low fruit set in many mango cultivars (Singh *et al*., 1962). Self incompatibility is a genetic mechanism used by hermaphroditic plants to prevent self-fertilization and to promote out-breeding gametophytic self in-compatibility (GSI)) and sporophytic self incompatibility (SSI) are among the several types of self-incompatibility systems that exist. Self-incompatibility in mango was confirmed to be of the sporophytic type (Ram *et al*., 1976; c.f. Litz, 1997). It was reported in several commercial Indian mangos (*Mangifera indica*) such as the Langra and Dusheri cultivars (Singh, 1978). However, Floridian cultivars appear to be self-fertile (Dag *et al*., 1998). Cross pollination increased fruit set and retention in Alphonso, Goamankur and Kesar mango cultivars (Desai *et al*., 1985).

Random Amplified Polymorphic DNA (RAPD) 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). The protocol is quick, easy to perform and only nanograms of template DNA are required. The RAPD technique has been

employed to develop sex-linked markers in *Brassica oleracea* (Camargo, *et al*., 1997), hazelnut (*Corylus avellana*) (Pomper *et al*., 1998), *Medicago sativa* (Campbell, 2000), Chinese cabbage (*B. campestris* subsp. *chinensis* var. *communis* [*B. chinensis*]) Shi Gong Jun and Hou XiLin (2004), *A. comosus* Tapia Campos *et al*. (2005), *Mangifera indica* (Damodaran *et al*., 2009).

In this respect, five elite mango cultivars were investigated for their self- and cross-compatibility, and RAPD analysis was tried to assess the genetic variation in cross-compatibility between them.

MATERIALS AND METHODS

Plant materials

This study was conducted at the Horticulture Research Station in El-Kanater El-Kheireia, Kalubeia governorate, and Agricultural Genetic Engineering Research Institute, ARC Giza, Egypt during two successive seasons of 2009 and 2010. Five mango cultivars namely; Alphonse, Ewais, Hindi khassa, Keitt and Zebda were used in this study.

Methods

Pollination experiments

Five as far as possible trees in randomized complete block design from each cultivar were chosen, similar in vigor and size and in the same bearing phase, each tree represented one replicate. Self and cross pollination was performed between trees of the experimental cultivars. Flowers at a similar stage of development were chosen, then each inflorescence was well bagged with pergamin bag before anthesis to prevent insect pollination and the pollen grains of each pollinator were collected to using for hand pollination (cross-pollination) after emasculation, then each inflorescence was bagged after pollinated in pergamin bags.

Microscopic preparations

Fifteen pistils from each replicate were collected just after pollination and daily for the seven successive days after pollination and fixed in FPA (Formalin: Propionic acid: Alcohol, 90:5:5). Samples of pistils were softened in 8N NaOH for 2 hours, washed with distilled water for 24 hours and stained in 0.1% aniline blue (W/S), dissolved in 0.1 N K3PO4 and examined with Leica fluorescence microscope (WILD LEITZ GMBH, Type 020-505-030., LEITZ WETZLAR GERMANY) according to the method of Kho and Baer (1970) and Ebeed (1996).

RAPD - PCR analysis

Genomic DNA was extracted from the leaves of the five mango cultivars (*Mangifera indica* L.) using the Nucleon DNeasy plant mini Kit (Qiagen, CA, USA). The purified genomic DNA was subjected to PCR for RAPD analysis using 10 random primers each of twelve mer from BEX, Japan (Table 1). The PCR reaction mixture consisted of 50 ng genomic DNA, 200 µM each of 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 sterile ultra-pure water. The PCR was performed in a Perkin Elmer 9700 thermal cycler for 40 cycles of denaturation at 94ºC for 1 min, annealing at 42ºC for 1 min and extension at 72ºC for 2 min followed by final extension at 72ºC for 7 min then.

Data analysis

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

RESULTS AND DISCUSSION

The rate of pollen tube growth in the style is affected by many factors such as soil fertility and fertilization regimes (Williams, 1965), flowering dates (Church and Williams, 1983) temperature and relative humidity (Williams, *et al*., 1984), and sexual compatibility (Stott, 1972; Spiegel Roy and Alston, 1982).

Our results showed that when Alphonse cultivar was self pollinated it exhibited zero percent of pistils with pollen tubes reaching the base of the style after 6 days from pollination which suggests that it should be highly self incompatible with microscopic studies (Fig. 1). Rao *et al.* (1984) showed that Himayuddin, Jahangir and Alphonse mango varieties are self incompatible. Figure (2a) showed that pollen tubes grew to about 1/3 length of the style. When Alphonse cultivar was pollinated with Ewais, Hindi khassa, Keitt and Zebda cultivars it gave percentage of pistils with pollen tubes reaching the base of the style after 6 days from pollination 2.56%, 2.5%, 2.5% and 1.78%, respectively (Fig. 1) which explain the partial cross incompatible. Figure (2:b to e) showed that, pollen tubes grew and reached to the base of Alphonse style after 6 days from pollinated with Ewais, Hindi khassa, Keitt and Zebda cultivars, respectively as male parents.

When Ewais cultivar was pollinated with Alphonse, hand selfing pollination and Hindi khassa cultivar it showed zero percent which suggest that it should be considered incompatible combinations. But when Ewais cultivar was pollinated with Keitt and Zebda cultivars, it gave 5.12% (partial cross compatible) and 2.56% (partial cross incompatible), respectively as illustrated in Fig. (1). Additionally, Fig. (2:f to j) showed that, low number of germinated pollens was observed in the stigma surface in these combinations; some plugs at along of the style, this lag in pollen tube growth were grown slightly and delays its arrival to the base of the style end. Stosser & Anvari (1982), Brain *et al*. (1989) and Abou El-Nasr *et al*. (1997) reported that,

incompatible tubes contained frequent large callose plugs which, sometimes continuous depositions along the tubes.

When Hindi khassa mango cultivar were pollinated with Alphonse , Hindi khassa (selfing) and Zebda cultivars, it gave percentage of pistils with pollen tubes reaching the base of the style after 6 days from pollination 2.5%, 1.75% and 2.38%, respectively, which clear the partial cross incompatibility (Fig. 1). In these combinations, style of Hindi khassa contains a short pollen tubes from the Alphonse and Hindi khassa cultivars on the upper part of the style (Fig. 2: k and m), but, Zebda cultivar produced high number of pollens in this combination than in other ones. When Hindi khassa cultivar was pollinated with Ewais, it gave zero percent which suggest that it should be considered cross incompatible. Figure (2l) shows that, most of the pollen tubes grew through the styles revealed abnormal development. When Hindi khassa cultivar was pollinated with Keitt, it gives 5.12 % which suggest the partial cross compatible. Figure (2 n) showed that, pollen tubes reached the base of the style. This combination seemed to be as compatible combination when use Keitt cv. as a pollinizer. The appearance and behaviors of pollen tubes were as detected in the observations made by Modilibowska (1945), Williams (1966), Abou El Nasr and Stosser (1989) and Abou El Nasr & Wanas (1992) who found that, tubes resulting from compatible cross pollination, grew rapidly down the style.

When Keitt cultivar were pollinated with Alphonse, Ewais and Hindi khassa, it gives percentage pistils with pollen tubes reaching the base of the style after 6 days from pollination of 1.72%, zero percent and 2.5%, respectively (Fig. 1). In this respect, Fig. (2) showed that, most of pollen tubes contained frequently callus plugs at a along of the tubes and they reached the base of the style after pollinated with Alphonse (Fig. 2p), Ewais (Fig. 2q) and Hindi khassa (Fig. 2r), respectively. But, when Keitt cultivar was self pollinated, it gave 2.43 % which suggests that it should be of low self compatible. Additionally, Fig. (2s) showed that, tubes started reached the base of the style 6 days after self pollination. However, when Keitt cultivar was pollinated with Zebda, it gives 4.87% which explain the partial cross compatible. Figure (2t) showed that pollen observed short tubes in the stigma surface after pollinated with Zebda pollens.

When Zebda cultivar were pollinated with Alphonse, Ewais, Hindi khassa and Keitt, it gave percentage pistils with pollen tubes reaching the base of the style after 6 days from pollination of 1.69%, 1.53%, 1.81% and 2.5%, respectively (Fig. 1) which suggests the partial cross incompatible. Figure (2) showed that, most of the pollen tubes grew to the end of the style after pollinated with Alphonse pollens (u). Hindi khassa pollens (w) and Keitt pollens (x), respectively. But pollen tubes grew slowly when use Ewais cv. as a pollinizer

this slowly in pollen tube delays its arrival to the base of the style. But when Zebda cultivar was self pollinated, it gives zero percent for pistils contained a pollen tubes growth and reaching the base of the style after 6 days from pollination which suggests the completely self incompatible. However, Fig. (2y) showed that pollen tube delays its arrival to the base of the style end after 7 days from pollination. Similar finding was early in Taimour stigma when pollinated with Zebda pollens were reported by Abou El-Nasr *et al.* (1997), they found that the germination of Zebda pollen on Taimour stigma was poor and the pollen tubes grew very slowly.

RAPD-PCR analysis

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

The five mango cultivars were tested using RAPD-PCR analysis to assess their genetic variation (Fig. 3). RAPD profile of each mango cultivar was generated using BEX primers (*BEX*, Japan) and compared to each other. Out of 21 primers tested, ten primers were selected as they gave clear, reproducible, and polymorphic banding profile. Using 10 arbitrary 12-mer primers, 101 distinct fragments of DNA were identified with an average of 10.1 DNA fragments per primer. Total of 91 DNA fragments was polymorphic with average 9.1 polymorphic bands per primers. Fragment sizes ranged from 322 to 3311 bp.

Results of similarity index based on RAPD-PCR with the 10 primers using UPGMA computer analysis are shown in Table (2). A distance matrix between cultivars showed a similarity distance ranged from 0.254 to 0.533 with a mean value of 0.3905. Thus, the cultivars tested in this study were highly similar at the DNA level. The highest similarity value was recorded between Hindi khassa and Ewais cultivars (0.533), while the lowest similarity value was recorded between Ewais and Zebda cultivars (0.254). Dendrogram as shown in Fig. (4) illustrated the genetic relationships among the studied cultivars, where the two groups of Ewais and Hindi khassa were clustered in one group, while Alphonse, Keitt and Zebda cultivars were clustered in the other group.

It is of interesting to note that, the two cultivars Ewais and Hindi khassa which revealed low percentage of pistils with pollen tubes reaching the base of the style after 6 days from pollination had the highest genetic similarity values (0.533), while the high percentage of pollen tubes reaching the base of the style after 6 days from pollination that showed between Keitt and Zebda revealed the lowest similarity values of (0.324). The results for these dendrogram which showed genetic relationships among the five mango

cultivars which across the ten primers were agreed with Ismail, O.M.M (2003).

SUMMARY

Mango (*Mangifera indica* L.) is considered one of the oldest cultivated trees in the world. Low productivity of some mango cultivars is associate with low fruit setting and/or high fruit drop of immature fruits. The self and cross incompatibility has been reported as one of the serious factor affecting fruit set in many mango cultivars. In this respect, five elite mango cultivars (Alphonse, Ewais, Hindi khassa, Keitt and Zebda) were examined for their self and cross-incompatibility. RAPD analysis was performed to assess the genetic variation in cross-incompatibility between them. The cross-compatibility was estimated by counting the number of setted fruits per panicles at 21 days after cross pollination between every two cultivars. At the same time, the fluorescence microscope was used to determine the growth of pollen tube in the style tissue after pollination. The obtained results showed that, the five tested mango cultivars in this study were highly similar at the DNA level. Ewais, Hindi khassa and Alphonse can be grouped together showing high similarity between them. The highest similarity value was observed between Hindi khassa and Ewais cultivars, while the lowest similarity was recorded between Ewais and Zebda cultivars. Hindi Khassa and Ewais which appear to be the most similar cultivars showed the lowest percentage of pistils with pollen tubes reaching the base of the style after 6 days from pollination. On the other hand, the highest percentage was between Keitt and Zebda which is less similar.

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Table (1) Sequences and names of the selected primers

Primer	Sequence (5' - 3')	Primer	Sequence (5' - 3')
A0	ATC AGC GCA CCA	A11	ACT GAC CTA GTT
A1	AGC AGC GCC TCA	A16	ATT TGG ATA GGG
A2	GCC AGC TGT ACG	A17	GGT TCG GGA ATG
A4	GCC CCG TTA GCA	A21	GTG ACC GAT CCA
A9	AGA ATT GGA CGA	A23	AAG TGG TGG TAT

Table (2): Similarity indices among the five mango cultivars based on RAPD-PCR using 10 primers.

	Alphonse	Ewais	Hindi khassa	Keitt
Alphonse				
Ewais	0.381			
Hindi khassa	0.507	0.533		
Keitt	0.273	0.400	0.413	
Zebda	0.415	0.254	0.405	0.324

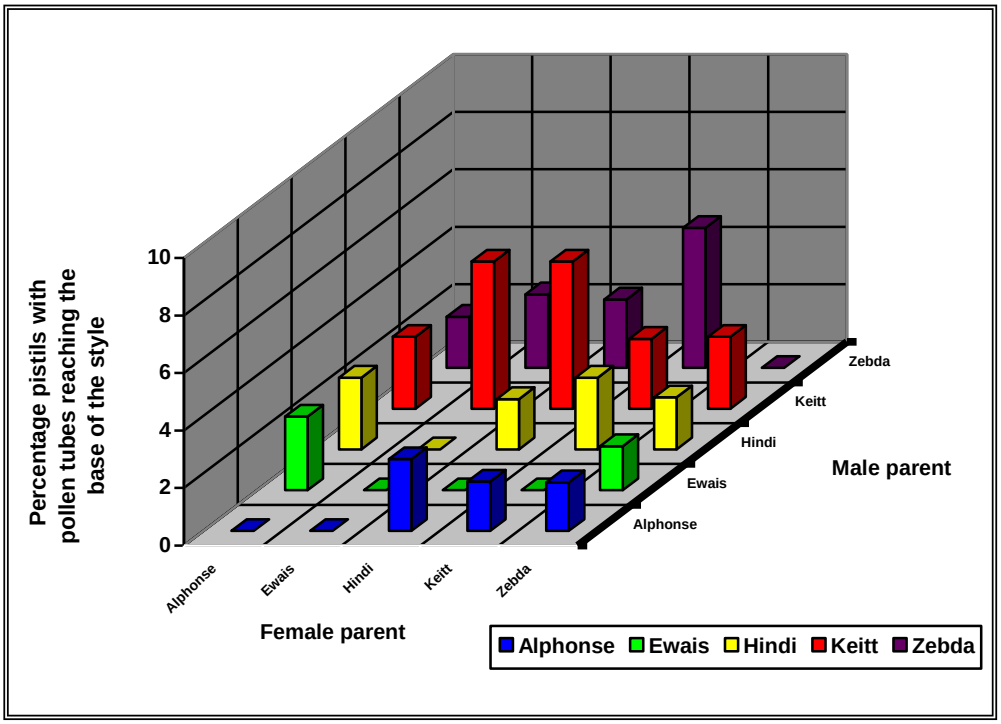

Fig. (1): Growth of Alphonse, Ewais, Hindi khassa, Keitt and Zebda pollen tubes in the style of the studied mango cultivar expressed as percentage of penetration after six days from pollination.

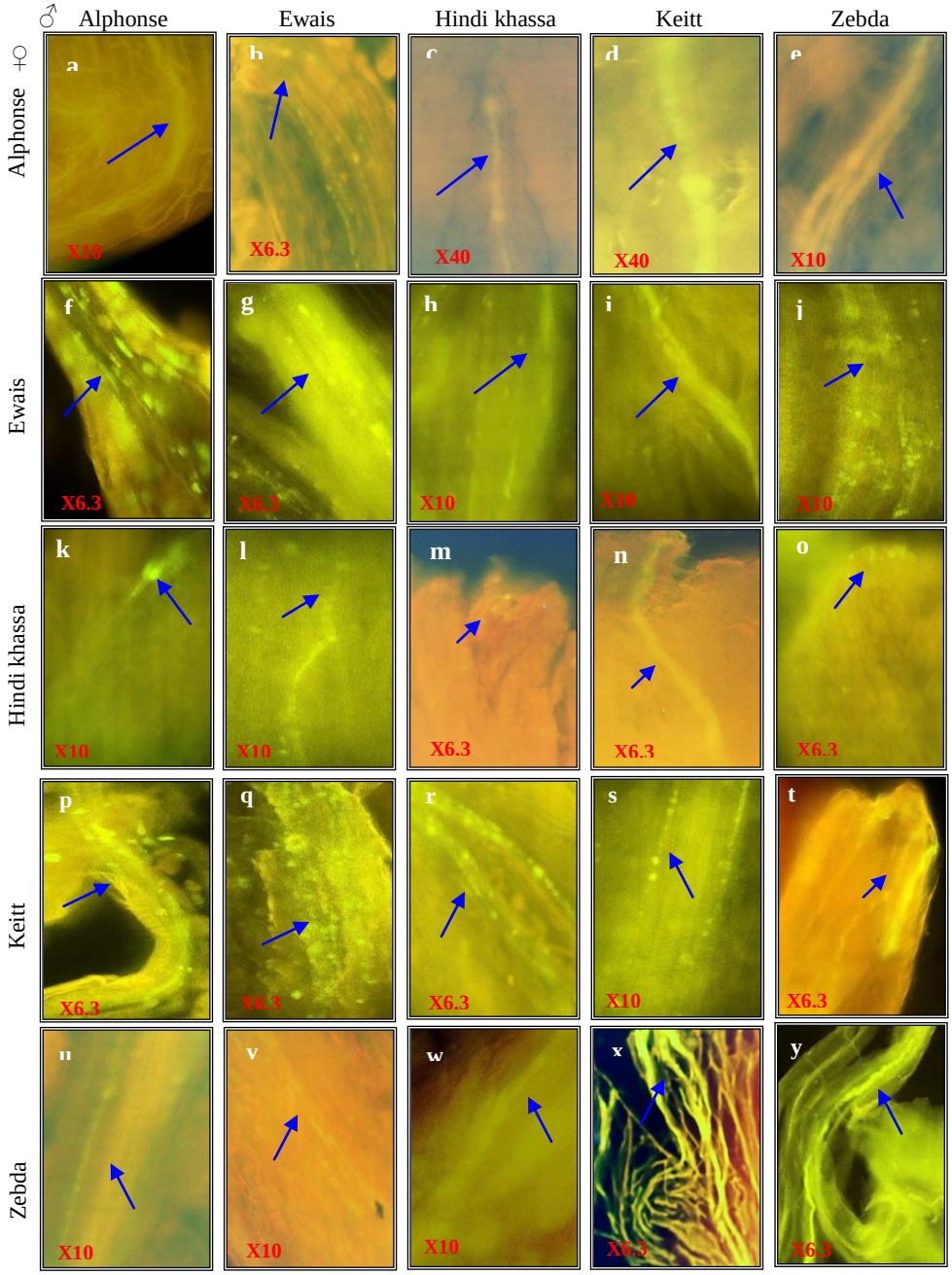

Fig. (2): Pollen tube growth in the studied mango cultivars. The location of the pollen grain the style is denoted by arrows. A to E shows the pollen tube growth of Alphonse, Ewais, Hindi khassa, Keitt and Zebda in the longitudinal styles of Alphonse, respectively. F to J shows the pollen tube growth of Alphonse, Ewais, Hindi khassa, Keitt and Zebda in the longitudinal styles of Ewais, respectively. K to O shows the pollen tube growth of Alphonse, Ewais, Hindi khassa, Keitt and Zebda in the longitudinal styles of Hindi khassa, respectively. P to T shows the pollen tube growth of Alphonse, Ewais, Hindi khassa, Keitt and Zebda in the longitudinal styles of Keitt, respectively. U to Y shows the pollen tube growth of Alphonse, Ewais, Hindi khassa, Keitt and Zebda in the longitudinal styles of Zebda, respectively.

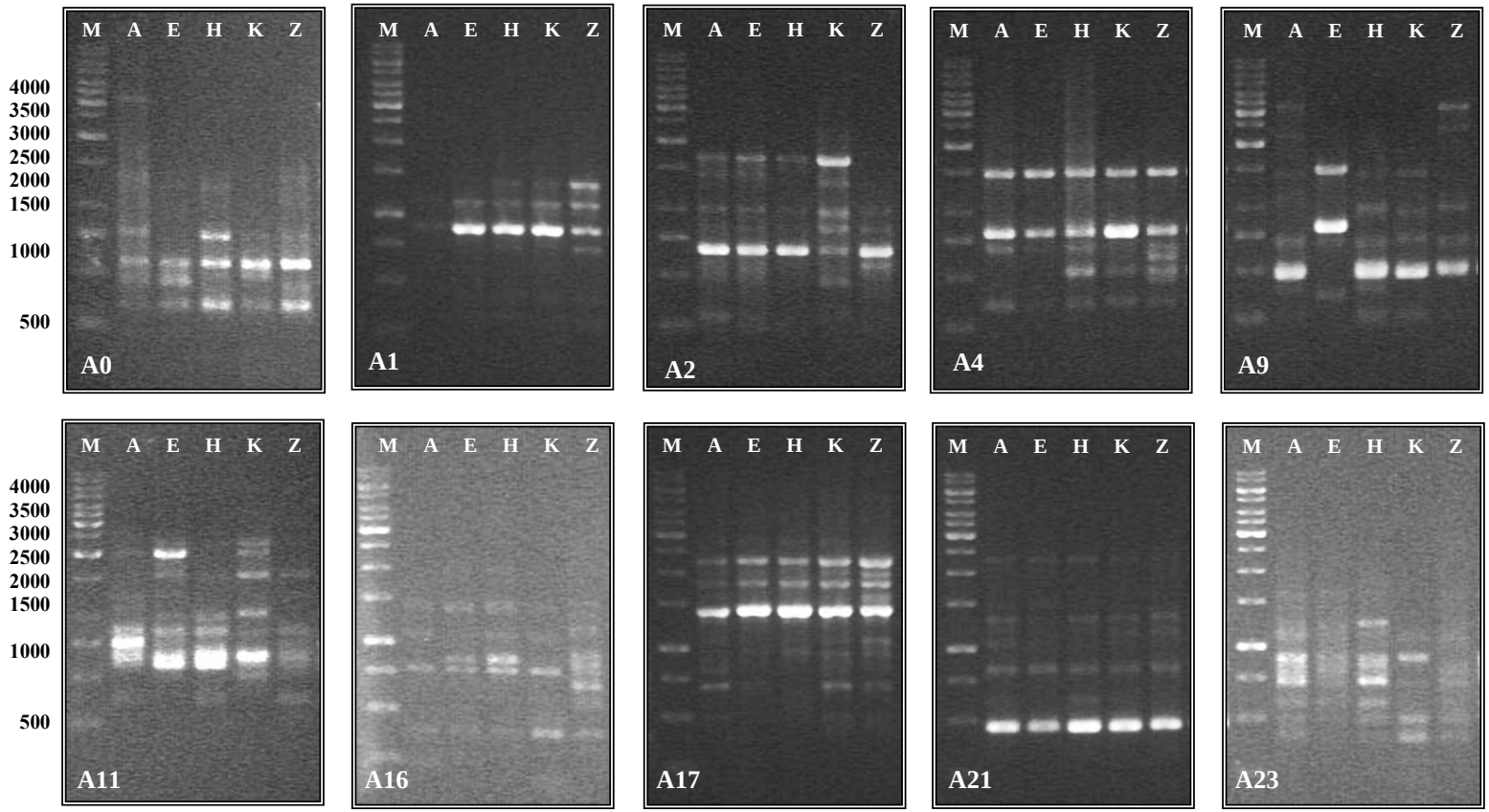

Fig. (3): DNA polymorphism of the five mango cultivars amplified with primers OP- A0, A1, A2, A4, A9, A11, A16, A17, A21 and A23 using RAPD-PCR (M) DNA ladder marker (bp) (A) Alphonse, (E) Ewais, (H) Hindi khassa, (K) Keitt and (Z) Zebda.

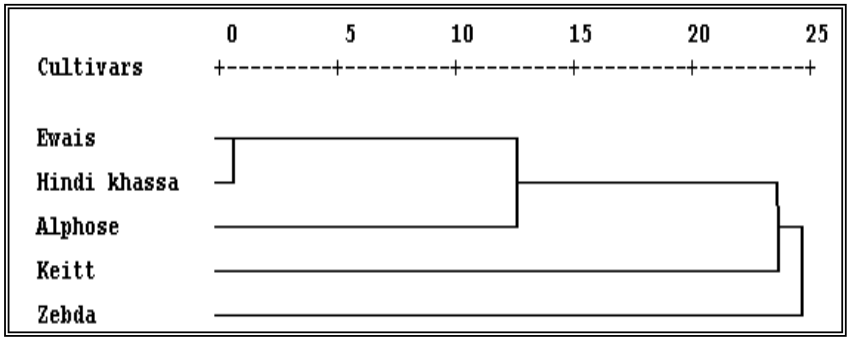

Fig. (4): Dendrogram for the genetic distances relationships among the five mango cultivars based on similarity indices data of RAPD 10 analyses.

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