Preliminary Horticultural Studies To Describe And Identify Of Two New Egyptian Mango Strains Using DNA Fingerprint.

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Abstract: Selection of some strains is considered the first step in improving mango production. So, this study was done for a three successive seasons (2003 – 2005) on two Egyptian mango strains "Hania" and "Aml" to describe them, horticulturally and identification those genetically utilizing DNA fingerprint. The vegetative characters (leaf shape, length, width, etc) and histological characteristics (number of stomata per mm (stomatal density), stomata length and width) showed great variation between the two studied strains. The fruit weight was 581 gm for Hania strain and 1020 gm for Aml strain. The two strains fruits had good characters as shape, net weight ratio, firmness, SSC, TA, Vit. C and total sugars. In generally, physical and chemical properties of Aml strain fruits were better than Hania strain. These differences of horticultural aspects due to genetic variances, which were determined by using SSR markers, of the 42 primers screened, 36 primers gave reproducible polymorphic DNA amplification patterns. 60.7 % of the scored fragments are considered putative genotypes-specific markers in both strains. The polymorphic information content (PIC values) ranged from 0.25 to 0.75, with a mean value 0.51 for all loci. The heterozygosity level was 0.68 and 0.53 for Hania and Aml strains, respectively. By banding patterns obtained from these 36 primers, each strain in this study could be distinguished from the other, indicating that, PCR by using SSR primers was an efficient method for genotype identification.
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1. Introduction

Mango (*Mangifera indica* L.) is a diploid fruit tree (2n = 40). The mango is considered as one of the oldest cultivated trees in the world.

Mangoes are an important fruit crop in Egypt. According to the latest statistics provided by the Ministry of Agriculture and Land Reclamation of Egypt (2007), indicated that, a total of 184204 Feddan are planted by mangoes. While, only in Ismailia Governorate the total area was about 92557 Feddan (50.24 % from total area in Egypt), about third of this area was cultivated by local seedling trees. Each seedling tree can be considered as a separate type exhibiting wide variation in different characters. Many of them are still unknown and the information on their ecology, morphology and productivity is limited. Mango cultivars are commonly divided into two groups based on their mode of reproduction from seeds and their origin; monoembryonic (Indian types) and polyembryonic (Indo-Chinese types). Monoembryonic mango seeds contain a single zygotic embryo, mostly are subtropical and the fruit skin is highly colored (mixes of red, purple and yellow). While, polyembryonic seeds (Southeast Asian types) contain several nucellar embryos, mostly tropical with skin is not highly colored (green to light green to yellow) (Iyer and Degani, 1997). There is considerable confusion in mango cultivars nomenclature because many clonally propagated mango cultivars have unique local and regional names and the spelling and name variants have been translated to the Roman alphabet (Viruel *et al.*, 2005), as presently several mango cultivars have many synonyms in different regions, which makes identification difficult.

However, selection and correct identification of genotypes is essential for any breeding and improvement effort, is difficult, inefficient and inaccurate when based on morphological traits only. Even though a high number of descriptors are used (Thomas *et al.,* 1994), this due to some phenotypic traits are difficult to describe, and phenotypic data may be influenced by environmental factors and growing conditions, in addition to quantitative inheritance, or partial and complete dominance often confound the expression of genetic traits.

Recently, as in other fruit tree species molecular identification of mango cultivars has been carried out with different molecular systems as isozymes,

minisatellites (Adato *et al.*, 1995; Eiadthong *et al.*, 1999), AFLPs (Eiadthong *et al.*, 2000) While DNA profiles based on polymorphic band patterns from Random Amplified polymorphic DNA (RAPD) analysis have been described for several fruit species including mango (Schnell *et al.*, 1995; Lopez-Valenzuela *et al.*, 1997 and Hemanth Kumar *et al.*, 2001). RAPD is a dominant marker, does not require target sequence information for design of amplification of primers. RAPDs are easy, cheap and fast and detect genetic differences between organisms, but their reproducibility is low. Different thermocyclers, Taq-polymerases, DNA primer concentrations and even the skill of the experimenter can influence the results, which makes comparisons of results between laboratories unreliable (Sefc *et al.*, 2001). However, DNA-fingerprinting based on simple sequence repeats (SSRs) or microsatellites, in addition to their usefulness in mapping and breeding (McCouch *et al.*, 1997), has become the marker of choice, because of their widespread occurrence throughout the genomes of all eukaryotic species, their co-dominant inheritance and the high level of polymorphism observed due to variations in repeat lengths. The high discriminatory power of SSRs is also important for analyzing variation in gene pool of crops (Powell *et al.*, 1996).

DNA fingerprinting using SSRs has been applied to accession identification programmes and for genetic analyses of a broad range of agricultural and horticultural crops (Pedersen, 2006).

The mango industry in most countries based on a few commercial cultivars, due to numerous problems with most of these cultivars like poor fruit quality, a narrow maturity window and physiological disorders, so the need is for new cultivars to replace them.

Thus, the present work was carried out to describe morphological, horticultural, histological characteristics of two new mango genotypes (Hania and Aml), and identification of them utilizing DNA fingerprint.

2. Material and Methods

The present work was carried out during three successive seasons (2003 – 2005) on two local mango strains namely "Hania" and "Aml". The two strains are grown at private orchard of Ismailia Governorate, Egypt. The trees are about 25 years old.

2.1. Horticultural aspects:-

2.1.1. Physical and chemical properties of fruits:-

Samples of ripe fruits were taken randomly from each tree (strain) at harvest time in each sample, average fruit, peel, seed weight and volume, fruit and seed dimensions were recorded. Moreover, the skin firmness (kg/cm^2) by using effegi pentrometer, fruit length (cm), width (cm), fruit shape index (length/width), fruit thickness (cm), pulp/fruit ratio (net ratio), soluble solids content (SSC %) by hand refractometer, fruit acidity, vitamin C and total sugars were determined as described by A.O. A. C. (1995).

2.1.2. Leaf characters:-

Samples of maturity leaves were outlined on paper, then length and width of blades were measured in cm and the area was estimated by planimeter (cm^2).

2.1.3. Histological study (Number, length and width of stomata):-

The stomata length, width and density of two strains were measured using eye-epiece micrometer (10x eye-piece and 40x objective piece lenses). A fully development leaves from two strains. The stomata were measured from an imprint of the lower leaf surface obtained by painting clear finger-nail polish on the leaf, allowing it to dry and peeling it of (Hamill *et al.*, 1992). The imprint was laid on a microscope slide with a drop of water to allow the analysis of the guard cells. The stomata were measured at 400x magnification. The length, width and density of stomata were measured for each strain using eyepiece graticule in a microscope at 400x magnification.

Stomatal densities were calculated using the following formula:

Stomatal density (SD) =NS / A

Where: NS = number of stomata in the microscope field, and A = area of the microscope field.

The obtained data were statistically analysed using T test according to Snedecor and Cochran (1967).

2.2. DNA fingerprints.

2.2.1. Plant material

Young leaf samples of the two mango strains studied in this research were collected in early spring, lyophilized at -20°C for 48 h and ground to powder.

2.2.2. Total genomic DNA extraction

Total genomic DNA was extracted according to the basic DNA extraction protocol **(**Dellaporta *et al*., 1983) with slight modifications by Porebski *et al*. (1997**)** and adapted to mango, for obtaining good quality total DNA, 500 mg. of ground, lyophilized leaves tissue were extracted by the addition of 10 ml preheated (65ºC) from cetylhexadecyl-trimethyl ammonium bromide (CTAB) extraction buffer [3% CTAB (w/v), 100 mM Tris- HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% (w/v) PVP (Polyvinyl pyrrolidone)], and then 1% (v/v) of β-mercaptoethanol (15 mM) with further grinding.. The mixture was incubated at 65ºC for 60 min, followed by two extractions with chloroform/isoamyl alcohol (24:1). After which the nucleic acids precipitated with Cold isopropanol, and the pellet was dissolved in 1 mL TE 0.1X (Tris-EDTA)

buffer. RNA was removed with RNaseA 4 µl (10 mg/mL). The DNA was purified further by 300 µl phenol: chloroform: isoamyl alcohol (25:24:1), then overnight at (-20 ºC) using 1/10 vol. from 2 M Na acetate (pH 8.0) and one volume of cold isopropanol alcohol. The precipitated was washed twice, and the pellet was dissolved in 0.1X TE buffer. The purified total DNA was quantified by gel electrophoresis, and its quality verified by spectrophotometry.

2.2.3. PCR (SSR) amplification and product electrophoresis

The thirty eight SSR primer pairs used for PCR amplification, previously described by Viruel *et al.* (2005), Duval *et al.* (2005), Honsho *et al.* (2005) and Schnell *et al.* (2005). These primers were synthesized by VBC-Biotech, Vienna, Austria (www.vbc-bioch.com). PCR reactions were performed according to published procedures by Viruel *et al.* (2005) with some modifications using fluorescent fragment detection on a LI-COR 4200 DNA dual-dye sequencing system. For this method either one of SSR primer had a M13 tail as a third primer of a fluorochrome labeled M13-30 oligo (5` CCC AGT CAC GAC GTT G 3`) was add to the PCR reaction. Microsatellites amplification was performed in 10 µl volume contained: 0.02 µM forward primer (M13-30 sequence at the 5` end), 0.18 µM M13-30 oligo infrared fluorescence dye (IRD 700 or IRD 800 labeled), 0.2 µM reverse primer, 0.2 mM of each dNTP, 1.8 mM MgCl2, 0.05 U Taq polymerase, 1X PCR buffer, and 10 ng of template DNA.

The amplification was performed on a `Primus` 384 well thermocycler (MWG Biotech, Germany) using the following temperature: after a first denaturation step at 94 ºC for 2 min, the reaction went through 30 cycles with (94 ºC for 1 min, 0.5 ºC/sec to 51 ºC, 51 ºC for 30 sec, 0.5 ºC/sec to 72 ºC, 72 ºC for 1 min) followed by a final extension step of 5 min at 72 ºC. The analyses were repeated at least twice to assure the reproducibility of the results.

PCR products were detected by electrophoresis on 7 % Polyacrylamide non-denaturing gels to exact allele sizing of the SSR loci, then the products were visualized using fluorescent fragment detection on a LI-COR 4200 DNA dual-dye sequencing system. Quantity-one software was used to estimate the sizes of the products.

2.2.4. Data analysis

Ninety two reproducible bands from selected primers were scored as 1 (presence) or 0 (absence) for the two accessions tested. Allelic composition of each accession and the number of total alleles was determined for each SSR locus. Putative alleles were indicated by the estimated size in bp. The genetic information was assessed for single locus SSRs using the following parameters: number of alleles per locus (A), observed heterozygosity (Ho, direct count), and polymorphic information content values for each locus (PIC) were calculated as follows:

He or PIC = 1- $\sum$ pi2 where pi is the frequency of the ith allele, and summation extends over n alleles (Nei, 1973), Wright's fixation index (F) = (1-Ho/He) (Wright, 1951), and heterozygosity level of the two genotypes assayed.

The computations were performed with the programs, GENEPOP version 1.31 Raymond and Rouset (1995), Quantity one, Irfanview and Microsoft Excel.

3-Results

3.1. Leaf characteristics:-

3.1.1. Morphological characteristics:-

Data in Table (1) revealed that there were significant differences between the two studied strains in both length, width, length/width ratio and leaf area, "Aml" strain showed greater figures than "Hania" one.

3.1.2. Histological characteristics:-

Results pertaining to stomata characteristics in the same table and figure (1) illustrated that, the average number of stomata per mm^2 for the lower epidermis of "Aml" mango strain was (677.5) compared to (384) with "Hania" strain. In the other hand, the average stomata length and width of "Hania" strain were 0.89 and 0.72 µm whereas that of "Aml" strain were 0.68 and 0.55 µm, respectively. These increased were found to be statistically highly significant with t_test.

3.2. Fruit properties:-

Regarding physical characteristics data in Table (2) showed that, significant differences in fruit weight, volume in all seasons. It can notice that "Aml" strain fruit weight and volume were higher than "Hania".

Concerning fruit dimensions, from results of same table it is clear that significant increments were obtained from "Aml strain for fruit length, width, thickness and length/width ratio (shape fruit) compared to "Hania" strain in all seasons. It can concluded that "Hania" fruit shaped is oval to rounded, full while "Aml" fruit shaped is oval, oblong, plump and thick.

In comparing peel and seed weight of "Aml" and "Hania" strains, it noticed that "Aml" fruit was significantly greater in fruit peel and seed weight than "Hania" one and the opposite trend was observed in pulp/fruit ratio (net ratio).

From results of the same table it clear that, significant differences were found between two strains in seed length, width and thickness. In generally, the values of seed length and width of "Aml" strain were

greater than "Hania" one, while the opposite trend was obtained in seed thickness.

Fruit firmness is one of the most important parameters, the greater the firmness, the greater, the quality of the fruit. It was significantly differed in the two evaluated strains and ranged from 1.45 to1.27 kg/cm2 as average of three years for "Hania" and "Aml" strains, respectively.

As regard titratable acidity the highest values (0.68 and 0.74) were obtained with "Aml" compared to (0.47 and 0.66) "Hania" in both seasons, respectively.

Fruits of "Aml" strain contain high percentage of SSC (18 and 15.20 %) comparatively with (14.4 and 15.25 %) for "Hania" strain in both seasons, respectively. Thereat, it can suppose that the values of SSC/acid ratio took against trend of acidity for two studied strains in all seasons. With respect to vitamin C in all seasons, "Aml" fruits had ascorbic acid higher than "Hania" one. At last, as fruit coloration it could be concluded that "Hania" strain had attractive fruit yellow colour, while "Aml" strain had greenish yellow (fig. 2).

3.3. SSRs polymorphism and molecular fingerprinting:-

Of the 42 SSR primer pairs screened, 38 loci generated fragments for the two genotypes. Two loci were monomorphic, each locus showed one fixed band in both genotypes.36 loci (Table 4) were selected in our analysis for their reproducible and polymorphic DNA amplification patterns among genotypes (an example of the amplification pattern obtained is shown in Fig.3 ,with 12 primer pairs only). Analysis of the variability parameters for the 36 SSRs in the two mango strains are shown in (Table 4), detected a total of 92 scorable bands with an average of 2.55 Band/SSR, ranging from 2 to 4 bands/SSR. This is lower than those reported by Viruel *et al.* (2005) in their work with 16 primer pairs among 28 mango genotypes, probably due to the lower number of analyzed samples, as well as due to the less diverse genotypes analyzed. According to the banding patterns obtained with 36 selected primer pairs, one or two bands were present in each genotype; the amplification pattern seems to indicate the detection of a single locus. Mango has been described as allopolyploid (Mukherjee, 1997) and these results suggest a complete depolarization in this species. The strains studied were considered homozygous and heterozygous when one or two fragments were present per locus, respectively (Callen *et al.*, 1993). Consequently, the heterozygosities of the two strains under study were 0.72 for strain "Hania" and 0.52 for strain "Aml", with a mean value of 0.62, and the two strains showed heterozygosities higher than 0.50 (Table 4). The great heterozygosity for the two mango strains can be attributed to the mating system of this species that is normally out cross pollination with some self pollination. The higher level of heterozygosity observed in the present study has been also reported by Shiran *et al*. (2007). From a total of 92 scorable alleles, 66 were polymorphic bands and 26 monomorphic. These results indicate that any 66 out of the 92 (60.7 %) fragments are considered putative genotypes-specific markers in both strains. Strain "Hania" showed the presence of 36 genotype-specific fragments and strain "Aml" was present 30 genotype-specific fragments.

Observed heterozygosity, calculated from direct counts, for the loci identified by each primer pair ranged from 0.1 at most loci, indicating high diversity of the two mango strains to zero with a mean for all the loci of 0.62 (Table 4). The polymorphic information content (PIC values) ranged from 0.25 to 0.75, with a mean value of 0.51 for all loci. Based upon discriminating power (DP) (Table 4). The PIC value provides an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at a locus but also the relative frequencies of these alleles. Expected and observed heterozygosity values were compared using the fixation index (F), the F values indicated the global behavior of these strains, similar to a random mating collection. This could be due to the fact that mango cultivars are the result of selection from open-pollinated seedlings, most of them from chance seedlings from natural cross-pollinations.

SSR profiles, combined over the thirty sex loci, were compared to determine if both strains were genetically identical and through the high average of observed and expected heterozygosity in this work, as well as low number of fixed alleles or monomorphic fragments (26 in 92) compared to the high number of genotype-specific alleles (66 in 92) indicate that a high variability is detected in the strains tested and could be distinguished from each other. Microsatellites are becoming the marker of choice for fingerprinting and genetic diversity studies in a wide range of living organisms (Shiran *et al.*, 2007). Consequently, the approach described in this work shows that microsatellite analysis is a powerful tool also for the characterization and identification of mango strains by comparing the 92 alleles, which were detected using the 36 selected primers (Table 4).

Table (1): Leaf and stomata characteristics of two (mono embryonic) seedling mango strains (Hania and Aml).

Strain	Leaf				Stomata		
	Area (cm^2)	Length (cm)	Width (cm)	Length/Width	Density (mm^2)	Length (µm)	Width (µm)
Hania	104.39	23.95	6.53	3.67	384.00	0.89	0.72
Aml	126.63	25.89	7.14	3.63	677.50	0.68	0.55
T	*	*	*	ns	*	*	*

Table (2): Some fruit characteristics of two (mono embryonic) seedling mango strains (Hania and Aml) from 2003-2005.

Strain	Fruit							Firmness (kg/cm^2)	Peel weight (g)	Seed weight (g)	Net ratio
	Weight (g)	Volume (cm^3)	Specific gravity	Length (cm)	Width (cm)	Shape	Thickness (cm)				
	First season										
Hania	620.00	624	0.99	13.90	9.60	1.45	8.75	1.43	64.70	45.20	82.27
Aml	1012.5	988	1.05	19.05	9.80	1.95	8.95	1.19	145.70	139.55	71.84
T	*	*	ns	*	*	*	ns	*	*	*	*
	Second season										
Hania	658.85	700	0.94	13.25	9.90	1.34	9.30	1.44	36.55	46.80	87.30
Aml	1077.8	1110	0.97	21.25	10.50	2.02	9.65	1.50	82.95	89.30	84.01
T	*	*	ns	*	*	*	*	*	*	*	ns
	Third season										
Hania	465.40	460	1.01	12.71	8.65	1.47	7.60	1.49	49.40	59.25	76.63
Aml	969.75	1010	0.96	19.00	10.30	1.85	9.10	1.13	107.75	71.55	81.33
T	*	*	ns	*	*	*	*	*	*	ns	*
	Average seasons										
Hania	581.42	594.67	0.98	13.29	9.38	1.42	8.55	1.45	50.22	50.42	82.07
Aml	1020.02	1036.00	0.99	19.77	10.20	1.94	9.32	1.27	112.13	100.13	79.06
T	*	*	ns	*	*	*	*	*	*	*	ns

Table (3): Some fruit characteristics of two (mono embryonic) seedling mango strains (Hania and Aml) from 2003-2005.

Strain	Seed			SSC (%)	TA (%)	SSC/TA	Total sugars (%)	VC (mg/100g)
	Length (cm)	Width (cm)	Thickness (cm)					
	First season							
Hania	11.20	3.90	2.25	14.40	0.47	30.98	11.71	25.60
Aml	16.50	4.50	2.20	18.00	0.68	26.68	12.98	41.60
T	*	*	ns	*	*	*	*	*
	Second season							
Hania	10.18	4.25	2.60	15.25	0.66	23.29	15.34	28.40
Aml	16.64	5.10	2.40	15.20	0.74	20.55	15.60	33.60
T	*	*	ns	ns	*	*	*	*
	Third season							
Hania	10.70	4.10	2.65	-	-	-	-	-
Aml	15.10	4.45	2.35	-	-	-	-	-
T	*	*	ns	-	-	-	-	-
	Average seasons							
Hania	10.69	4.08	2.50	14.83	0.57	27.14	13.52	27.00
Aml	16.08	4.68	2.32	16.60	0.71	23.62	14.29	37.60
T	*	*	ns	*	ns	*	*	*

Table (4): Locus name, size range, number of alleles (n), observed (Ho) and polymorphic information content (PIC) of microsatellite data for two mango genotypes.

Locus name	Size range (bp)	n	Ho	F	PIC
MIAC_3	185-193	2	0.5	-0.33	0.375
mMiCiR_5	171-182	2	0.5	-0.33	0.375
mMiCIR_16	240-242	2	0.5	-0.33	0.375
LMMA_7	200-212	2	1.0	1.0	0.05
LMMA_1	199-208	3	1.0	-0.6	0.625
LMMA_15	199-221	3	1.0	-0.6	0.625
LMMA_8	255-267	3	1.0	-0.6	0.625
LMMA_9	171-179	2	0.5	-0.33	0.375
LMMA_11	232-239	2	0.5	-0.33	0.375
LMMA_13	179-198	3	1.0	1.0	0.05
mMiCIR_8	150-166	3	1.0	-0.6	0.625
MiSHRS_4	118-124	3	1.0	-0.6	0.625
MiSHRS_1	190-205	2	0.0	1.0	0.05
MIAC_4	93-112	4	1.0	-0.33	0.75
MIAC_5	117-124	3	0.5	0.2	0.625
MiSHRS_32	205-231	3	0.5	0.2	0.625
mMiCiR_9	158-163	3	1.0	-0.6	0.625
mMiCiR_14	149-152	2	0.5	-0.33	0.375
mMiCiR_10	282-298	3	0.5	0.2	0.625
mMiCiR_22	141-170	4	0.5	1.0	0.25
MIAC_2	139-163	2	0.0	1.0	0.5
MiSHRS_29	175-179	2	0.5	-0.33	0.375
MiSHRS_39	350-369	3	0.5	0.2	0.625
LMMA_4	231-241	2	1.0	1.0	0.5
LMMA_14	161-169	2	1.0	1.0	0.5
LMMA_10	143-170	3	0.5	0.2	0.625
LMMA_5	280-282	2	0.5	-0.33	0.375
LMMA_12	201-205	2	0.5	-0.33	0.375
mMiCiR_3	314-320	2	0.0	1.0	0.5
mMiCiR_18	202-232	3	0.5	0.2	0.625
mMiCiR_25	212-214	2	0.5	-0.33	0.375
MiSHRS_48	206-218	2	0.0	1.0	0.5
MIAC_6	270-307	3	1.0	-0.6	0.625
mMiCIR_36	249-263	3	1.0	-0.6	0.625
mMiCIR_29	175-195	3	0.5	0.2	0.625
mMiCIR_30	186-196	2	0.5	-0.33	0.375
Mean All loci		2.55	0.62	1.64	0.51

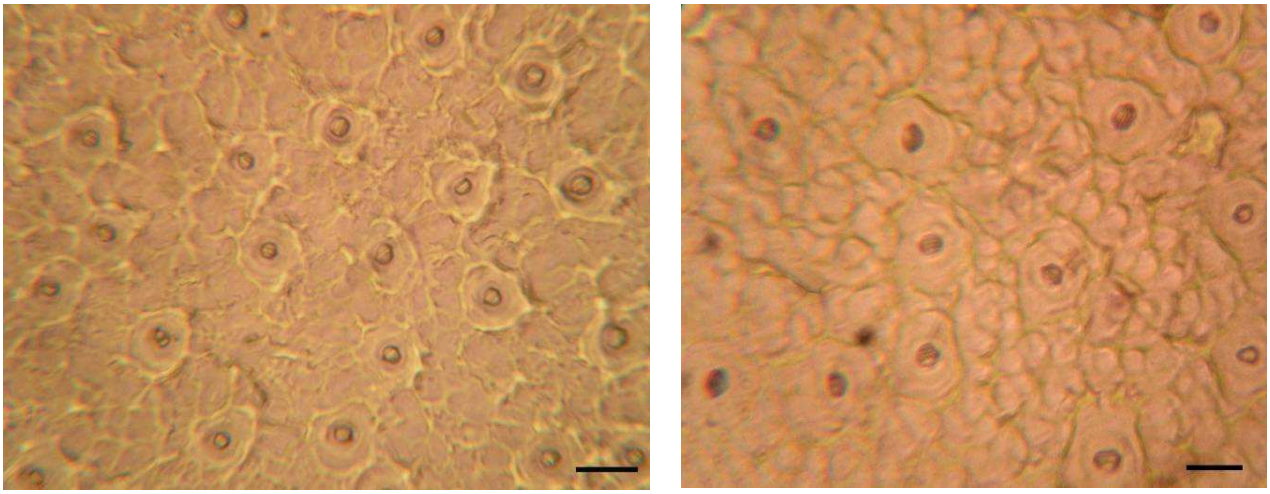

Fig (1): Stomata characteristics in leaf of two strains of mango (left) Hania and (right) Aml (bar 2 µm)

Fig (2): Fruit of Hania strain (left) and Aml strain (right).

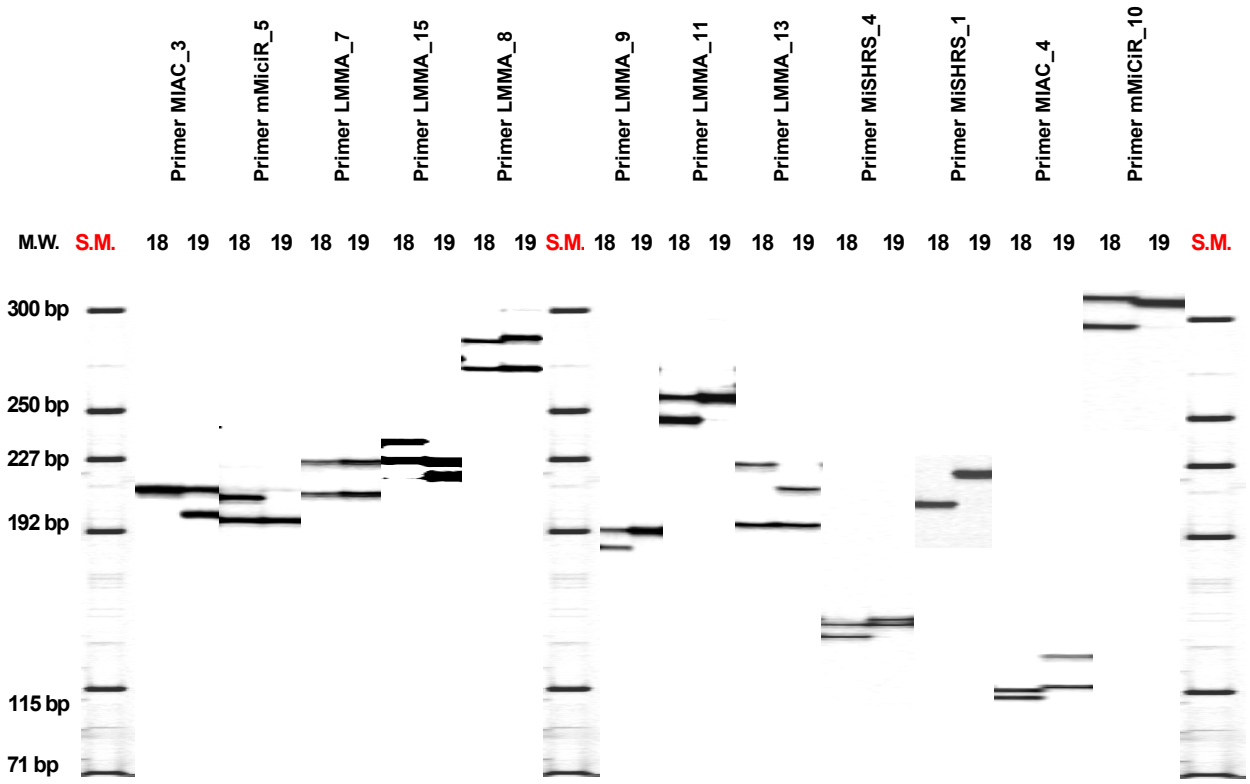

Fig. (3): Heterozygosity level of the two mango strains {(18) (Hania) = 0.68 and (19) (Aml) = 0.53}.

4. Discussion

The two mango strains "Aml" and "Hania" differed in leaf characteristics (length, width and area) and stomata characteristics (length, width and stomatal density (ND)). It concluded that, there are relationship between the number of stomata and it size, consequently decrease the number of stomata when stomata dimensions increased. Also, it can noticed that, clear relationship between increment of leaf and stomata dimensions and fruit quality (weight, size…etc). These results, refereed to evident Genetic variances which found between two strains under study. Where, each seedling tree (strain) can be considered as a referate type exhibiting wide variation in different characters (Singh, 1990).

It can conclude that, the highest pulp (Net ratio) percentage was up to 70% for two strains during three seasons. In general the pulp percentage more than 70% was desirable for fresh consumption and processing. (Naglaa, 2010).

SSC percentages reflect the richness of the studied strains in this regard and consequently excellent taste.

These results, referred to evident genetic variances which found between two strains under study. Where, each seedling tree (strain) can be considered as a separate type exhibiting wide variation in different characters (Singh 1990). Thirty six loci from forty two SSR primer pairs screened, generated fragments for the two strains. Any 66 out of the 92 (60.7 %) fragments are considered putative genotypes-specific markers in both strains. Hania strain sowed the presence of 36 genotype-specific fragments and Aml strain was present 30 genotype-specific fragments. The heterozygosities of the two strains under study were 0.72 for Hania strain and 0.52 for Aml strain with a mean value of 0.62, and the two strains showed heterozygosities than 0.50. The greatest heterozygosities for the two mango strains under study can be attributed to the mating system of this species that is normally out cross pollination with some self pollination (Shiran *et al.*, 2007).

5. Conclusion

Two mango strains "Hania" and "Aml" differed in vegetative characters (leaf shape, length, width, etc)

and histological characteristics (number of stomata per mm (stomatal density), stomata length and width). It can be concluded that these two new strains had a good fruit properties. The fruit weight was 581 gm for Hania strain and 1020 gm for Aml strain. The two strains fruits had good characters as shape, net weight ratio, firmness, SSC, TA, Vit. C and total sugars. In generally, physical and chemical properties of Aml strain fruits were better than Hania strain. These differences of horticultural aspects due to genetic variances, which were determined by using SSR markers, of the 42 primers screened, 36 primers gave reproducible polymorphic DNA amplification patterns. 60.7 % of the scored fragments are considered putative genotypes-specific markers in both strains.

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