Genetic and Morphological Characterization of *Mangifera indica* L. Growing in Egypt

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Additional index words. mango cultivars, EST, SSR, marker, genetic, morphological

Abstract. Mango (*Mangifera indica* L.) is a fruit crops belong to the family Anacardiaceae and is the oldest cultivated tree worldwide. Cultivars maintained in Egypt have not been investigated previously. Mango was first brought to Egypt from South Asia. Morphological and molecular techniques were used to identify the genetic diversity within 28 mango cultivars. SSR and EST-SSR were used for optimizing germplasm management of mango cultivars. Significant variations were observed in morphological characteristics and genetic polymorphism, as they ranged from 0.71% to 100%. High diversity was confirmed as a pattern of morphological and genotypes data. Data from the present study may be used to calculate the mango relationship and diversity currently grown in Egypt.

In Egypt, statistics of The Ministry of Agriculture and Land Reclamation (2015) provided that a total of 243,028 feddans (≈1.038 acres 61 or 0.42 ha) have been planted with mango. Succari and Owais, the most famous types of Egyptian mangoes, are excellent sugary, white sugary, butter-like mango (Zebda), and Hindi Besennara, as well as other famous types of mangoes. Egypt exports mango to several European countries. Previously, DNA markers such as random amplification of polymorphic DNA (RAPD)–polymerase chain reaction (PCR) (Rajwana et al., 2008), restricted fragment length polymorphism,

and amplified fragment length polymorphism (AFLP) (Yamanaka et al., 2006) have been used to assess the genetic diversity among mango genotypes/cultivars.

Recently, mango germplasm has been collected and analyzed using simple sequence repeat (SSR) markers in numerous studies (Dillon et al., 2013; Tsai et al., 2013). Microsatellite markers were developed by Kundapura et al. (2011) to calculate the genetic diversity of mango cultivars with polymorphic information content (PIC %) from 0.185 to 0.920 (Sherman et al., 2015). Wöhrmann and Weising (2011) used an alternative strategy to identify SSR markers using comparative genomics tools called expressed sequence tags (ESTs). It has been hypothesized that the highly repetitive nature of SSRs makes slippage during replication a common event, leading to high levels of polymorphism found between populations.

EST-SSRs are physically linked to expressed genes and therefore represent potentially functional markers. Twenty-four of the 25 EST-SSR markers exhibited polymorphisms, identifying a total of 86 alleles

with an average of 5.38 alleles per locus, and distinguished between all *Mangifera* selections that were analyzed. Private alleles were identified for some *Mangifera* species. The main objectives of the present study were to examine the molecular and horticultural characterizations of several mango cultivars in Egypt from different localities based on different markers.

Materials and Methods

Mango cultivars. Experiments were performed at the Agricultural Botany Department, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt and Botany and Microbiology Department, College of Science, King Saud University, Saudi Arabia. Twenty-eight mango (*Mangifera indica* L.) cultivars were included for horticultural and molecular markers analyses. The cultivars were obtained from the Agricultural Research Center, Horticulture Research Institute, Giza, Egypt, and included four native Egyptian cultivars, i.e., Succari, Hindi Besennara, Zebda, and Princess, as well as other mango cultivars are growing in Egypt, such as Shelly, Kensington Pride, Yasmina, Golek, Alphonso, Piva, R2E2, Sabre, Heidi, Osteen, Langra Benersi, Maya, Nam Doc Mai, Hindi Mloki, Fajri Kalan, Sidik, Joa, Sensation, Tommy Atkins, Kent, Haden, Naomi, Palmer, and Lilly cultivars.

Morphological characterizations. Eight horticultural characters, were measured in 10 replicates (fruit) at maturity and harvest stages including fruit length (cm), width (cm), weight (g), peel (%), pulp (%), fiber length (mm), shelf life (days), and fruit shape according to the recommendations of Bally et al. (1996), and Viocell et al. (2016). DNA was isolated from leaves using the modified cetyltrimethylammonium bromide method described by Dellaporta et al. (1983). Six SSR markers were selected for analysis as described by Begum et al. (2013, 2016) (Table 1). PCR amplification reactions were performed in 17 µL of reaction volume containing 50 ng of DNA, 12.5 µL of Dream Taq master mix (Fermentas Co., Waltham, MA), and 0.5 µmol of each primer.

The primary program was set at six cycles at 94 °C for 1 min, 45 °C for 50 s decreasing 1 °C in every cycle, and 72 °C for 1 min, followed by 28 cycles at 94 °C for 1 min, 40 °C for 1 min, and 72 °C for 1 min. These programs were preceded by a denaturation step at 94 °C for 4 min, followed by an extension step at 72 °C for 7 min. The PCR products were separated on 1.5% agarose gel electrophoresis (Begum et al., 2016). For SSR markers, the annealing temperature ranged from 52 to 59 °C.

Seven EST-SSR markers (Table 1) were selected to achieve the EST analysis for mango cultivars as described by Dillon et al. (2013). The PCR program was performed according to Dillon et al. (2014).

Statistical analysis. Morphological data were subjected to analysis of variance to

Received for publication 20 Mar. 2018. Accepted for publication 19 June 2018.

The authors extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding this Research group No. RG 1435-011. The authors thank the Deanship of Scientific Research and RSSU at King Saud University for their technical support.

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determine variations among the varieties using Statistical Package for the Social Sciences (SPSS 14; SPSS Inc., Chicago, IL). DNA bands of PCR products were visualized on an ultraviolet transilluminator gel documentation system and photographed. The polymorphism percentage (PIC) of each primer pair was calculated. Similarity, co-

efficient matrices were calculated (Jaccard, 1908).

Results and Discussion

Morphological variations of mango cultivars. Table 2 displays the fruit length of the 28 mango cultivars, which shows that

the longest cultivar was Fajri Kalan at 16.5 cm and the shortest was Succari at 9.0 cm. The average fruit length was 12.11 cm, with significant variations observed among the cultivars. Five cultivars from a total of 28 had fruit lengths equal to or less than 10 cm, including Alphonso (9.2 cm), Maya (9.5 cm), Princess (9.6 cm), Sensation (9.5 cm), and Haden (10 cm). These values were lower than the overall average and nearly equaled the minimum values (Table 2).

Data for fruit width (cm) shown in Table 2 ranged from 6.3 to 11.5 cm (average of 10.6 cm). The highest value recorded was for R2E2 (11.5 cm) and the lowest fruit width recorded was for Sabre at 6.3 cm. No significant variations were observed between the maximum and minimum values, whereas between cultivars there were significant difference in relation to fruit width.

Comparing fruit weight, significant variations were observed among all cultivars (Table 2). The heaviest fruit weight was for Piva (650 g), followed by for Hindi Mloki (625 g) and Tommy Atkins (615 g). The lightest value was 225 g recorded for Zebda cultivar. The mean was 416.4 g for the 28 cultivars. Three different categories were observed for fruit weight, the first group was greater than 600 g and included Piva (650 g), Hindi Mloki (626 g), and Tommy Atkins (615 g). The second group was from 300 to 600 g, which included 17 cultivars, and the last group was less than 300 g, which included seven

Table 1. Marker names and sequences for SSR and EST used in the current study.

Marker	Marker name	Forward and reverse sequence
SSR	SSR-16	5'-AGCGATGGTGCTCATGCTTA-3' 3'-TCTCTCACGGAATCACATCTT-5'
	SSR-19	5'-TTTCAGCAAACCTAGAACCAG-3' 3'-GGCATTTCAGTTTTCACCTTGT-5'
	SSR-52	5'-AAAAACCTTACATAAGTGAATC-3' 3'-GAACAGTTGTTTCGTGTCGTA-5'
	SSR-59	5'-GATGTTGTTGGTGTGTTT-3' 3'-CAATTAGGAGCAAAATCAGA-5'
	SSR-65	5'-GGTTTTGAATAGAAATGCAA-3' 3'-AAGATGTGTCATATTGTTT-5'
	SSR-83	5'-GGCTATTGTACGAAACAAAT-3' 3'-GATTCAGACCCGATACATT-5'
EST	QGMi-001	5'-GAAAGGCTTGCAGAGACAGG-3' 3'-GTTTCTTCTGTTTCGGTGATGGAGGAGT-5'
	QGMi-003	5'-CAGGAATCTTCCAAACGAA-3' 3'-GTTTCTTGGCAGTGTCTTACCTTCA-5'
	QGMi-004	5'-TTCACAACGAGAAGACATGGA-3' 3'-GTTTCTTGGGACCTATTCGATCCCACT-5'
	QGMi-005	5'-TGGAGGAATTGAACCGATTG-3' 3'-GTTTCTTCAGTATCGGAGCGTCAGTC-5'
	QGMi-010	5'-GGTTTGAGCTTCCAAATTGC-3' 3'-GTTTCTTCTGGGAAAGTCAACAGCAG-5'
	QGMi-023	5'-TCAATGCAAAGAAGCTCTGAAA-3' 3'-GTTTCTTGCCCTCAGCTCAGTCTCTTG-5'

SSR = simple sequence repeat; EST = expressed sequence tag.

Table 2. Morphological characters of 28 mango cultivars.

No.	Cultivars	Fruit length (cm)	Fruit width (cm)	Fruit wt (g)	Peel (%)	Pulp (%)	Fiber length (mm)	Shelf life (days)	Fruit shape
Egyptian cultivars									
1	Succari	9.0	6.5	275.0	14.0	44.0	12.0	6.0	Cordate
2	Zebda	12.5	9.0	225.0	17.0	33.0	13.0	6.0	Ovate
3	Hindi Besennara	12.0	6.7	300.0	20.0	46.0	13.0	6.0	Cylindrical
4	Princess	9.6	6.9	375.0	24.0	36.0	6.0	5.0	cordate
Growing cultivars in Egypt									
5	Shelly	12.5	11.0	425.0	25.0	33.0	8.0	6.0	Cordate
6	Kensington Pride	12.6	9.8	400.0	18.0	42.3	11.0	7.0	Cordate
7	Yasmina	11.5	6.7	275.0	17.0	35.0	7.0	5.0	Ovate
8	Golek	11.0	7.0	250.0	18.0	29.0	11.0	6.0	Cylindrical
9	Alphonso	9.2	7.8	500.0	30.0	41.0	7.0	7.0	Cordate
10	Piva	13.7	8.8	650.0	28.0	38.0	8.0	5.0	Obliqueovate
11	R2E2	13.5	11.5	500.0	23.0	18.0	12.0	7.0	Cordate
12	Sabre	12.2	6.3	375.0	27.0	39.0	23.0	7.0	Cylindrical
13	Heidi	11.4	9.5	575.0	17.0	22.0	7.0	7.0	Cordate
14	Osteen	12.9	8.0	400.0	22.0	21.0	16.0	7.0	Cylindrical
15	Langra Benersi	10.7	8.1	325.0	26.0	28.0	7.0	6.0	Ovate
16	Maya	9.5	8.0	300.0	18.0	25.0	9.0	6.0	Cordate
17	Nam Doc Mai	13.6	7.2	425.0	28.0	21.0	9.0	5.0	Fusiform
18	Hindi Mloki	12.4	7.0	625.0	33.0	33.0	8.0	6.0	Cylindrical
19	Fajri Kalan	16.5	8.0	500.0	25.0	24.0	8.0	6.0	Cylindrical
20	Sidik	16.4	7.3	425.0	19.0	28.0	16.0	7.0	Cylindrical
21	Joa	12.5	7.5	300.0	22.0	35.0	8.0	7.0	Rectangular
22	Sensation	9.5	7.3	480.0	28.0	41.0	22.0	6.0	Rectangular
23	Tommy Atkins	12.1	8.8	615.0	34.0	16.0	9.0	5.0	Oval roundish
24	Kent	11.9	9.7	310.0	22.0	26.0	10.0	6.0	Cordate
25	Haden	10.0	8.5	505.0	26.0	21.0	11.0	6.0	Cordate
26	Naomi	13.5	9.1	455.0	23.0	28.0	8.0	7.0	Rectangular
27	Palmer	13.0	7.0	490.0	28.0	22.0	15.0	5.0	Cylindrical
28	Lilly	12.5	8.6	380.0	18.0	18.0	12.0	6.0	Ovate oblique
Average		12.1	10.6	416.4	23.2	30.1	10.9	6.1	—
Maximum		16.5	11.5	650.0	34.0	46.0	23.0	7.0	—
Minimum		9.0	6.3	225.0	14.0	16.0	6.0	5.0	—
LSD = 0.05		2.30	3.1	114.30	10.50	8.2	11.5	1.6	—

cultivars, including Yasmina, Succari, Hindi Besennara, Golek, Zebda, Maya, and Joa.

The peel percentage in different mango cultivars demonstrated that Succari had the lowest percentage (14%) and was also the shortest fruit. In contrast, Tommy Atkins showed the highest peel percentage (34%), followed by Hindi Mloki (33%). Piva, Hindi Mloki, and Tommy Atkins also had the largest fruit weights with values of 650, 625, and 615 g, respectively.

For pulp percentage, the data ranged from 16% to 46%, with a mean of 23.2%. The highest pulp percentage was for Hindi Besennara (46%) and the lowest was for Tommy Atkins (16%), although the fruit weight was high (615 g) for the latter cultivar, as shown in Table 2.

For fiber length (mm), data showed that values ranged from 6 to 23 mm, with a mean of 10.9 mm. The shortest cultivars were Princess at 6 mm, whereas the longest was Sabre at 23 mm, with this latter cultivar also having the lowest fruit width.

The shelf life for the studied cultivars ranged from 5 to 7 d, with a mean of 6.1 d. Yasmina, Alphonso, and Nam Doc Mai showed the lowest values (5, 7, and 5 d, respectively) for shelf life compared with the other cultivars (Table 2).

For fruit shape, different morphological variation between the 28 mango cultivars were found (Table 2), such as cordate, ovate, cylindrical, oblique ovate, fusiform, cylindrical oblique, rectangular oblique, and oval roundish (Table 2). The obtained results are in agreement with the results of previous studies (Bally et al., 1996; Rymbai et al., 2014; Singh et al., 2009).

Molecular studies of *M. indica*. During the present study, 13 specific markers were used (SSR and EST-SSR) (Figs. 1 and 2) to calculate the genetic variations between 28 mango cultivars. SSR data in Table 3 show that SSR-16 and 19 markers produced two alleles with the allele size ranging from 169 to 235 and 137 to 173 base pairs (bp), respectively. SSR-52 and SSR-65 detected only one allele at 199–154 bp, respectively. Finally, SSR-59 and SSR-83 recorded two alleles with a molecular weight range of 145–168 bp and 157–183 bp, respectively. The PIC ranged from 0.71% to 1% based on the different markers. The data for SSR-52 and SSR-65 showed PIC of 1% for both, followed by SSR-59 of 0.87%, SSR-19 of 0.83%, SSR-16 of 0.77%, and finally SSR-83 of 0.71%.

Previous studies have reported that molecular analysis is an efficient method of assessing genetic diversity among mango cultivars, and PCR-based genomic polymorphisms have been detected in several mango cultivars (Bally et al., 1996; Rocha et al., 2012). Intracultivar studies of genomes from different locations could confirm whether or not there were any genetic differences among the location-specific cultivars. In the present study with SSR markers, in total 190 amplified fragments, ranging from 137 to 235 bp in length, were detected.

Fig. 1. Amplification patterns of 28 accessions of mango generated by simple sequence repeat (SSR)-16, -19, -52, -59, -65, and -83 primers. M = molecular weight marker (200-bp DNA ladder at left).

Fig. 2. Amplification patterns of 28 accessions of mango generated by expressed sequence tag-01, 03, 04, 05, 10, 20, and 23 primers. M = molecular weight marker (200-bp DNA ladder at left). QGMi001 Irwin red leaf library *Mangifera indica* cDNA clone IRrlf0003B04_g 5- similar to SVP (SHORT VEGETATIVE PHASE), mRNA sequence GenBank: JZ532296.1 <<https://www.ncbi.nlm.nih.gov/nucleotide/JZ532296>>.

Overall, larger intra-cultivars variation and significant differentiation in different cultivar pairs were observed at several loci. The present study is in agreement with Manchekar (2008), who reported the level

of polymorphism in mangoes present in microsatellites was variable and ranged from two to four alleles with an average of 2.48 alleles per SSR. The analysis of 23 SSRs revealed that the PCR product size (bp)

ranged from 100 (SSR-52) to 310 (SSR-20) in 31 mango cultivars (Manchekar, 2008). The results of Manchekar (2008) are in agreement with results from the present study that showed the PIC values varied widely among loci and ranged from 0.77% (SSR-16) to 100.0% (SSR-52 and SSR-65) with an average of 86.33% per locus (Table 4).

Data in Table 4 show that all EST-SSR markers detected one specific allele except for QGMi-001, which recorded three alleles with sizes ranging from 161 to 253 bp. The other primers showed different allele sizes e.g., 172, 227, 315, 240, 110, and 140 for primers QGMi-003, QGMi-004, QGMi-005, QGMi-0010, QGMi-020, and QGMi-023, respectively. Concerning the EST-SSR markers used in the present study (Table 3), different specific genes were selected to identify the genetic diversity between the 28 mango cultivars. The first one was QGMi-001, and the homology traits for this gene were a short vegetative phase (controlling flowering time) or floral development. This marker produced three alleles with a size range from 161 to 253 bp with genetic polymorphism of 0.82%; the next six markers provided just one allele and were related to different homology traits such as disease resistance gene (defense response), cis epoxy carotenoid dioxygenase 5 (abscisic acid biosynthesis); stress response, WRKY40 (transcription factor); and defense response, carotenoid cleavage dioxygenase 1 (carotenoid biosynthesis), IAA-leucine resistant 3 (transcription factor), and phytochrome-associated protein 2 (plant development). The allele size ranged from 110 to 240 bp. Data in Table 4 show the present and absent amplified fragments for both SSR and EST-PCR markers for the 28 mango cultivars.

A dendrogram illustrating the genetic relationships of the 28 mango cultivars using an unweighted pair-group method is shown in Fig. 3. The dendrogram constructed from the matrix of simple matching coefficients revealed two major clusters with genetic similarity of 46%. The first major bifurcation in the dendrogram (Fig. 3) separated the 28 cultivars into two major clusters (56%). Cluster I divided into subclusters (74%) that included Shelly, Golek (100%), Succari (87%), Alphonso (82%), Piva, and Princess (87%). Cluster II (65%) divided into subclusters that included Nam Doc Mai and Sidik in a separate subcluster (87%), Kent (80%), Hindi Besennara, R2E2, Tommy Atkins, Naomi (88%); and the other sub-cluster (80%) included Zebda and Fajri Kalan (87%); and Heidi, Joa, Sensation, and Lilly (87%). The third subcluster (68%) included Sabre, Haden, and Langra Benersi (81%) and Osteen, Maya, Hindi Mloki, and Palmer (87%). Kensington Pride and Yasmina were found to be in a separate cluster (74%). These findings are in agreement with Mukherjee (1972), who worked on genetic diversity of mango cultivars in India during seedling and reported that the selections of the variants due to recombination and segregation of characters in the progenies. Also, it

Table 3. Primers, annealing temperature, alleles size, and polymorphic microsatellite primers used in this study.

No.	Primer	Annealing temperature (°C)	No. of alleles	Allele size range (bp)	PIC (%)
1	SSR-16	54	2	169–235	0.77
2	SSR-19	54	2	137–173	0.83
3	SSR-52	52	1	199	1
4	SSR-59	59	2	145–168	0.87
5	SSR-65	53	1	154	1
6	SSR-83	57	2	157–183	0.71

PIC = polymorphic information content; SSR = simple sequence repeat.

Table 4. Characteristics of seven EST-SSR markers screened across 28 of *Mangifera* cultivars.

Marker	GenBank accession no.	Repeat motif	Homology	No. alleles	Size range	PIC (%)
QGMi-001	JZ532296	(CCTTT)5	(floral development)	3	161–253	1
QGMi-003	JZ532319	(CTT)6	(defense response)	1	172	0.89
QGMi-004	JZ532302	(AAG)5	(abscisic acid biosynthesis; stress response)	1	227	0.88
QGMi-005	JZ532303	(AAC)8	(defense response)	1	315	0.75
QGMi-010	JZ532309	(AGG)4	(carotenoid biosynthesis)	1	240	0.80
QGMi-020	JZ532301	(CT)7	IAA-leucine resistant 3	1	110	0.82
QGMi-0023	JZ532311	(AAC)7	Phytochrome-associated protein 2	1	140	0.77

EST = expressed sequence tag; SSR = simple sequence repeat; PIC = polymorphic information content. QGMi001 Irwin red leaf library *Mangifera indica* cDNA clone IRlf0003B04_g 5- similar to SVP (SHORT VEGETATIVE PHASE), mRNA sequence GenBank: JZ532296.1 <<https://www.ncbi.nlm.nih.gov/nucleotide/JZ532296.1>>.

Fig. 3. Dendrogram of mango cultivars cluster analysis based on simple sequence repeat and expressed sequence tag markers.

has been found that suitable environment can help in wider diversity in the seedling progenies with much improved types as in Florida, where the Haden and other cultivars have bigger-sized fruits with more attractive color than the parent Mulgoa with dull color.

Conclusions

In the present study, detailed information regarding the genetic diversity of mango germplasm in Egypt using 13 specific markers (SSR and EST-SSR) was reported. A high diversity of mango cultivars was confirmed as a pattern of morphology and genotypes. Molecular markers (SSR and EST-SSR) are powerful tools for optimizing germplasm management of mango cultivars in Egypt. All these collected data could be reference material for future re-

searchers when studying mango cultivars in Egypt.

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