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# Morphological and Molecular Analysis of Genetic Diversity among Some 'Sukkary' Mango (*Mangifera indica* L.) Genotypes

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Abstract: The present study aimed to investigates the morphological characterization and estimate genetic polymorphism and relationships among five mango accessions (collected from different governorates) based on ISSR markers. Seventy-eight morphological characteristics were studied to describe the fruit and stone. The collected mango accessions exhibited a wide range of differences in fruit and stone characteristics. All the studied accessions had a roundish fruit shape smooth and waxy yellow skin, soft pulp texture and obtuse shape of fruit apex. Differences among accessions were observed in fruit ground color, fruit beak type, pulp aroma. Also the quantitative characteristics of fruit and seed showed differences between the collected accessions. Inter-simple sequence repeats (ISSR) markers were used to study the genetic diversity and phylogenetic relationships among the collected accessions. The twelve ISSR primers produced a total number of amplified bands ranged from 6 to 19 fragments. The highest number of fragments was 19 bands for (TC)<sub>8</sub>GT primer. While, (CA)<sub>6</sub>GT primer generated the lowest number of amplicons (6 bands). The average number of fragments/primer was (11.25) and the size of these fragment ranged from 326-3125 bps. The percentage of polymorphism revealed by the different primers ranged from 7.14 to 66.67 % with average of 42.86%.

Key words: Mangifera indica L. • Morphological characterization • Genetic diversity • ISSR markers

## INTRODUCTION

Mango (Mangifera indica L.) is one of the important fruit crops of tropical and sub-tropical regions. India is the major mango producer followed by China, Mexico, Brazil and Australia [1]. Success of crop improvement programs depends on the genetic variability available in crop germplasm. According to Ravishankar et al. [2] the phenomenon of allopolyploid and the high rat of outcrossing produce a high level of genetic diversity in mango. Also there is much confusion and uncertainty concerning the identity of the mango genotypes due to different local names for the same varieties, which making characterization of local accession important for better use of the genetic available resources [3]. In this respect efforts were made for understanding the variability of mango germplasm based on morpho-physiological traits [4-7].

Morphological characterization had been used for many different fruit crops such as mango, banana and citrus [8-11]. The application of morphological markers is the simplest methods of evaluating crop genetic diversity. Also, morphological characterization is the first step that should be done before the molecular studies [12]. Recently the DNA markers generated by PCR methods that are reliable and fast have been used [13]. Markers such as, randomly amplified polymorphic DNA, inter simple sequence repeats-ISSRs, ampli?ed fragment length polymorphism and microsatellites or SSRs have been used in mango characterization [14, 6, 15, 7]. Also, molecular markers have been used to identify mango cultivars by unique patterns of marker alleles. Fingerprinting of some mango cultivars has been used to identify duplicated and misnamed individuals [16, 17]. This has importance for mango improvement programs and management of genetic resource which give insight into genetic markup of related genotypes. The aims of this study are determination of the relationship among five mango accessions of Sukkary mango cv. based on morphological characterization and genetic similarity estimation.

#### MATERIALS AND METHODS

### **Morphological Characterization**

**Plant Materials:** Plant materials used for this study including five mango accessions supposedly belong to the Sukkary cultivar collected from Ismailia, Sharkia, Behera, El-Fayoum and Giza governorates, samples were collected during 2014 and 2015 years (Table1). Each accession represented by three trees, all the collected samples was from mature trees grafted on seedling rootstocks at age of 18-20 years growing in commercial orchards. The standard horticultural management practices were carried out as usual.

Data Collection: Three trees of each accession were used, 20 fruits per tree were collected from all canopy directions. The studied accessions were evaluated for 27 morphological characters. The morphological characteristics based on those previously prescribed for mango by the International Plant Genetic Resources Institute [18]. In this respect, 8 quantitative and 19 qualitative characteristics were used as shown in (Table 2). All observations on the fruit were made at the optimum maturity stage according to IPGRI [18]. Data were recorded for fruit weight, diameter, length and shape, shape of fruit apex and fruit attractiveness, fruit ground color, texture of skin surface and fruit beak type.

Table 1: List of mango plant materials used in this study

Accession number	Accession name	Location
12175	Sukkary-1	Ismailia
12176	Sukkary -2	Sharkia
12177	Sukkary -3	Behera
12178	Sukkary -4	El-Fayoum
12179	Sukkary -5	Giza

Table 2: Codes of morphological traits used in characterization of mango accessions.

Code	Characters	Character states
	Qu	nalitative Traits (19 characters)
Fr01	Fruit shape	(1) Oblong;(2) Elliptic;(3) Roundish;(4) Ovoid;(5) Obovoid.
Fr02	Fruit shape of apex	(1) Acute;(2) Obluse;(3) Round.
Fr03	Fruit attractiveness	(1) Poor;(2) Average;(3) Good;(4) Excellent.
Fr04	Fruit ground colour	(1)Yellow;(2) Orange;(3) Purple;(5) Red.
Fr05	Fruit skin surface texture	(1) Smooth;(2) Rough
Fr06	Depth of fruit stalk cavity	(1)Absent;(2) Shallow;(3) Medium;(4) Deep;(5) Very deep.
Fr07	Fruit stalk attachment	(1) weak;(2)Intermediate;(3)Strong.
Fr08	Fruit neck prominence	(1) Absent;(2) Slightly prominent;(3)Prominent;(4)V. prominent
Fr09	Slope of fruit ventral shoulder	(1) Slopping abruptly;(2) Ending in a long curve;(3) Rising
Fr010	Fruit beak type	(1) Perceptible;(2) Pointed;(3) Prominent;(4) Mammiform
Fr011	Fruit sinus type	(1) Absent;(2) Shallow;(3) Deep.
Fr012	Fruit skin waxiness	(1) waxy;(2) Non-waxy.
Fr013	Fruit skin colour of ripe	(1) Green;(2) Greenish yellow;(3) Yellow;(4) Green with red blush
Fr014	Pulp colour of ripe fruit	(1) light yellow;(2) Golden yellow;(3) Yellow orange;(4) Orange
Fr015	Pulp texture of ripe fruit	(1) Soft; (2) Intermediate; (3) Firm.
Fr016	Adherence of fruit skin to pulp	(1) Absent; (2) Weak; (3) Intermediate; (4) Strong.
Fr017	Pulp juiciness	(1) Slightly juicy;(2) Juicy;(3) Very juicy
Fr018	Pulp aroma	(1) Mild;(2) Intermediate;(3) Strong.
Fr019	Seed shape	(1) Ellipsoid;(2) Oblong;(3) Reniform
	Qι	nantitative Traits (8 characters)
Fr020	Fruit weight (g)	S024 Stone length (cm)
Fr021	Fruit diameter (cm)	S025 Stone width (cm)
Fr022	Fruit length (cm)	S026 Stone thickness (cm)
Fr023	Fruit Pulp thickness(cm)	S027 Stone weight (g)

Table 3: List of ISSR primer names, sequences and annealing temperatures.

Primer Name	Sequence	Annealing Temp. °C
	3'-5' Anchored repea	its
17898-B	(CA) <sub>6</sub> GT	40.0
17898-A	$(CA)_6AC$	40.0
ISSR-1	CAC(TCC) <sub>5</sub>	50.0
ISSR-2	AGA(TCC) <sub>5</sub>	50.0
890	ACG(GT) <sub>7</sub>	50.0
853	(TC) <sub>8</sub> GT	46.0
17	CAGC(AC) <sub>7</sub>	50.0
17899-A	(CA) <sub>6</sub> AG	40.0
844-B	(CT) <sub>8</sub> GC	50.0
HB-9	(GT) <sub>6</sub> GG	40.0
HB-10	(GA)₀CC	40.0
15	GGTC(AC) <sub>7</sub>	56.0

R= purine, Y= pyrimidine (C or T), B=non-A, D=non-C, H=non-G, V=non-T

Measurements also including pulp color of ripe fruit, pulp texture of ripe fruit, pulp juiciness and adherence of fruit skin to pulp. Stones were extracted from 20 fully ripe fruit of each replication and stone shape, type of embryonic, stone length; stone width and stone weight were recorded.

**Statistical Analysis:** The data of fruit and stone characteristics were presented as mean (n=20) and the means were compared using a one-way analysis of variance according to Snedecor and Chochran [19] using MSTAT-C statistical package software according to Freed [20] and means were compared by Least Significant Difference at significance level of 0.05 [21].

#### **Molecular Characterization**

DNA Extraction and ISSR-PCR Amplification Conditions: Total genomic DNA was isolated from young leaves samples using DNeasy Plant Mini Kit (Qiagen<sup>©</sup> Germany) according to the manual procedures. A total of 12 primers (Table 3) were used to amplify DNA fragments. The DNA concentration was quantitatively measured and adjusted to 50 ng/ µl. PCR reaction was performed in 25 µl reaction volume containing 2X read mix (EmeraldAmp Max PCR master mix-320 RR) 20 pM oligonucleotide primer and 50 ng genomic DNA. This reaction was performed on BioRad-Mycyclar thermal cycler, programmed to 35 cycles as follows: an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles of denaturation step at 94°C for 1 minute, annealing temperature (Ta) for 1 minute and an extension step at 72 °C for 1 minutes and final extension step at 72°C for 10 minutes. The amplified bandies scored using Non linear Dynamics software.

The amplified fragments were resolved on 2.5 % agarose (Seakem LE Agarose-Lonza). A set of 12 primers were used in this study, these primers were synthesized by HVD Corporation, Germany.Ladder DNA used was Fermentas 100 pb plus.

**Data Analysis:** The banding patterns generated by ISSR primers were analyzed and compared to determine the genetic relatedness among different mango accessions. The amplified fragments were scored either as present (1) or absent (0). The genetic similarity and similarity matrix were estimated according to Dice coefficient [22]. Dendrograms showing the genetic relationships were constructed using the Un-weighted Pair Group Method with Arithmetic Averages (UPGMA) by Phoretix 1D software (Total Lab, UK).

#### RESULTS AND DISCUSSION

## **Morphological Characterization**

**Qualitative Characteristics:** Table (4) illustrates the fruit qualitative characteristics of the five mango accessions under the study. All the accessions showed roundish fruit shape and obtuse fruit apex. Concerning fruit attractiveness Sukkary-5 had an excellent degree while that of the Sukkary-1, Sukkary-2 and Sukkary-3 accessions has good fruit attractiveness, only the Sukkary-4 showed average fruit attractiveness. The fruit ground color ranged from orange in Sukkary-5 to greenyellow in the other accessions. Regarding fruit beak type, only Sukkary-1 showed pointed type and Sukkary-2 has prominent type, while the other accessions exhibited perceptible type. All the studied accessions showed smooth fruit surface, shallow fruit sinus type, waxy fruit skin and yellow skin color of ripe fruit. The color of fruit pulp was yellow orange in the Sukkary-1 and Sukkary-3, whereas it was golden yellow and light yellow in the Sukkary-2 and Sukkary-4 respectively. Sukkary-5 showed orange color of fruit pulp.

The pulp texture of rip fruit, were soft in all of the studied accessions. Sukkary-4 had weak adherence of fruit skin to pulp, while it was strong in Sukkary-5, the other accessions had intermediate degree. Regarding the pulp juiciness Sukkary-5 showed slightly juicy, the rest of the accessions exhibited juicy pulp juiciness. Sukkary-4 had intermediate pulp aroma, while the other accessions had strong pulp aroma. Sukkary-5 presented an oblong seed shape while seed shape of the rest of accessions was reniform. Sukkary-3 and Sukkary-4 showed medium depth of fruit stalk while the other accessions had shallow depth. The fruit stalk attachment ranged from weak in

Table 4: Fruits morphological qualitative characteristics of five mango accessions.

		Shape of	Fruit	Fruit	Fruit Skin	
Accessions	Fruit shape	fruit apex	attractiveness	ground color	surface texture	Fruit beak type
Sukkary-1	Roundish	Obtuse	Good	Yellow	Smooth	Pointed
Sukkary-2	Roundish	Obtuse	Good	Yellow	Smooth	Prominent
Sukkary-3	Roundish	Obtuse	Good	Yellow	Smooth	Perceptible
Sukkary-4	Roundish	Obtuse	Average	Yellow	Smooth	Perceptible
Sukkary-5	Roundish	Obtuse	Excellent	Orange	Smooth	Perceptible
		Fruit skin	Skin color	Pulp texture	Adherence of fruit	
Accessions	Fruit sinus type	waxiness	of ripe fruit	of rip fruit	skin to pulp	Pulp juiciness
Sukkary-1	Shallow	Waxy	Yellow	Soft	Intermediate	Juicy
Sukkary-2	Shallow	Waxy	Yellow	Soft	Intermediate	Juicy
Sukkary-3	Shallow	Waxy	Yellow	Soft	Intermediate	Juicy
Sukkary-4	Shallow	Waxy	Yellow	Soft	Weak	Juicy
Sukkary-5	Shallow	Waxy	Yellow	Soft	Strong	Slightly Juicy
			Depth of	Fruit stalk	Fruit neck	Slope of fruit
Accessions	Pulp aroma	Seed shape	fruit stalk	attachment	prominence	ventral shoulder
Sukkary-1	Strong	Reniform	Shallow	Strong	Absent	Ending in a long curve
Sukkary-2	Strong	Reniform	Shallow	Intermediate	Absent	Ending in a long curve
Sukkary-3	Strong	Reniform	Medium	Intermediate	Absent	Rising and then rounded
Sukkary-4	Intermediate	Reniform	Medium	Intermediate	Slightly prominent	Rising and then rounded
Sukkary-5	strong	Oblong	Shallow	Weak	Absent	Ending in a long curve

Table 5: Fruit morphological quantitative characteristics of five mango accessions.

Accessions	Fruit weight (g)	Fruit diameter (cm)	Fruit length (cm)	Fruit pulp thickness (mm)
		First seas	on	
Sukkary-1	261.17ab	7.71 a	10.72 a	5.07a b
Sukkary-2	245.00 b	6.78 c	9.67 b	3.96 c
Sukkary-3	265.00ab	6.83 c	10.13 ab	4.20 bc
Sukkary-4	270.00 a	7.03 bc	9.88 b	4.33 bc
Sukkary-5	267.38 a	7.29 b	9.56 b	5.40 a
		Second se	ason	
Sukkary-1	330.57 a	8.11 a	9.91 ab	4.82b
Sukkary-2	349.3 a	8.06 a	10.11 ab	5.27ab
Sukkary-3	349.50 a	7.44 ab	10.58 a	5.23ab
Sukkary-4	351.40 a	7.71 ab	10.12 ab	4.92b
Sukkary-5	267.73 b	7.29 b	9.56 b	6.03a

Values have the same letter(s) in the same column are not significantly different at LSD=0.05 level

Sukkary-5 to strong in Sukkary-1; the other accessions had an intermediate value. Sukkary-4 had slightly prominent fruit neck prominence and the other accessions showed absent fruit neck prominence. Among the studied accessions Sukkary-3 and Sukkary-4 showed a rising and then rounded slope of fruit ventral shoulder while in Sukkary-1, Sukkary-2 and Sukkary-5 it was ending in along curve. In pervious study, Bhuyan and Guha [23] observed a wide range of variability in respect of different characteristics of mango fruits. Jintanawong *et al.*, [24] determined the quality standards for mango genotypes by observing the fruit size, shape color, weight, texture and fiber. Naik, [25] reports variability among trees of the same variety with respect to fruit size, shape, color and quality.

Also, Ozkaya *et al.* [26] reported that the olive accessions collected from different locations showed differ degrees of morphological variations from the standard cultivar.

Quantitative Characteristics: According to the data in Table (5) in both seasons Sukkary-4 had the heights fruit weight compared with the other accession, Sukkary-1 recorded the highest fruit length and diameter. Regarding the pulp thickness, Sukkary-5 had the highest value among the tested accessions. The variation in mango fruit parameters were reported previously. The mango can have a fruit weight range that varies from as little as a few grams up to 1 kg and fruit lengths can vary from 2.5 to 30.0 cm in different varieties [27, 28].

Table 6: Seed morphological quantitative characteristics of five mango accessions.

Accessions	Stone length (cm)	Stone width (cm)	Stone thickness (cm)	Stone weight (g)
		First sea	son	
Sukkary-1	8.62a	3.79a	2.29a	57.30a
Sukkary-2	8.35a	3.85a	2.57a	48.00bc
Sukkary-3	8.34a	3.93a	2.46a	51.93ab
Sukkary-4	8.07a	3.53a	2.34a	53.65ab
Sukkary-5	7.57a	3.52a	2.22a	39.72c
		Second se	eason	
Sukkary-1	8.02a	3.99ab	2.31b	61.08a
Sukkary-2	8.74a	4.58a	2.71a	43.73d
Sukkary-3	8.70a	4.28ab	2.41b	51.67c
Sukkary-4	8.26a	3.88ab	2.42b	57.32b
Sukkary-5	7.57 a	3.52b	2.21b	39.72e

Values have the same letter(s) in the same column are not significantly different at LSD=0.05 level

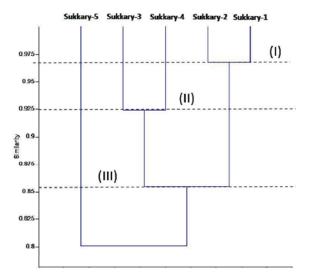


Fig. 1: Dendrogram using the similarity measure by Rho based on morpho-Pomological distances of five mango accessions.

The quantitative characteristics of studied mango stones are showed in Table (6) in the 1<sup>st</sup> season the examined accessions had a similar value of stone length, width and thickness. Sukkary-1 had the highest stone weight while Sukkary-5 had the lowest value during both seasons. In the second season there was a non-significant difference between the studied accessions in fruit length. Sukkary-2 recorded the highest stone width and thickness.

The observed variations between the studied mango accessions reflect the existence of a genetic variation. Morphological analysis of some Indian mango cultivars detected a variation between the standard cultivar and their landraces [6]. According to Haque *et al.* [29] the different mango genotypes maintained distinctive stone

characteristics like length, width and thickness. Also, Pendey [30] observed some variation between different accessions of Alphonso mango cultivar. In study to describe mango genotypes growing in Thailand using IPGRI descriptors, the authors concluded that the studied characters were useful to characterize all cultivars [22].

**Dendrogram of Morphological Relationship:** The cluster analysis produced a dendrogram with three main clusters (Figure 1). The first cluster (I) including Sukkary-1 and Sukkary-2, which was collected from nearly the same geographical zone, the genetic distance was (0.96). The second cluster (II) includes the Sukkary-3 and Sukkary-4 with a genetic distance (0.925). The last group (III) had only Sukkary-5. These results were in the same line with [31]. Fruit characteristics have a strong discriminating power and considered as useful tool for the identifying particular landrace [4, 5, 32, 33]. Morphological diversity within local mango accessions from Giza and Lower Egypt was very high for some qualitative characteristics; qualitative characteristics such as fruit shape, fruit apex and fruit stalk depth are less prone to influences from environmental factors [34]. According to the presented results we recommend to use the mentioned morphological descriptors for identification of mano landraces.

## **Molecular Characterization**

**Polymorphism Detected by ISSR Analysis:** ISSR amplification from all DNA samples of five Mango accessions collected from Ismailia, Sharkia, Behera, El-Fayoum and Giza governorates produced prolific banding profiles for all 12 primers (Figure 2). The total number of amplified amplicons among tested primers ranged from 6 to 19 fragments. 3'anchored (TC)<sub>8</sub> GT primer amplified

Table 7: Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by ISSR markers among five Sukkary accessions.

Primer	Size of fragments (bP)	Total amplicons	No. of monomorphic bands	No. of polymorphic bands	Polymorphism (%)
17898-B	852-2564	6	2	4	66.67
17898-A	710-1928	7	3	4	57.14
ISSR-1	418-3097	10	5	5	50.00
ISSR-2	454-2927	13	7	6	46.15
890	432-1834	14	7	7	50.00
853	326-2075	19	14	5	26.32
17	394-1826	14	13	1	7.14
17899-A	605-1580	9	5	4	44.44
844-B	786-3125	7	4	3	42.86
HB-9	620-1376	11	5	6	54.55
HB-10	445-2585	12	9	3	25.00
15	577-1794	13	12	1	7.69
Total		135	86	49	
Average		11.25	7.16	4.08	42.86

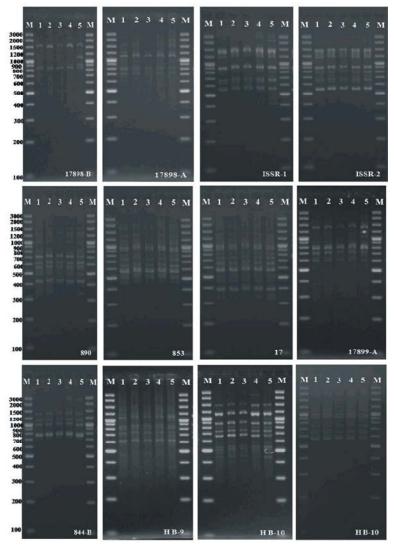


Fig. 2: DNA polymorphism of the five mango Sukkary accessions collected from different location amplified with 12 primers using ISSR-PCR (M) DNA ladder marker(bP) (1) Sukkary from Ismailia,(2) Sukkary from Sharkia, (3) Sukkary from Behera, (4) Sukkary from El-Fayoum and (5) Sukkary from Giza.

Table 8: Genetic similarity matrix detected between five Sukkary accessions collected from different governorates with ISSRs markers based on UPGMA analysis. (1) Ismailia, (2) Sharkia, (3) Behera, (4) El-Fayoum and (5) Giza.

	<i>X Y Y Y Y Y Y Y Y Y Y</i>				
Genotype	Sukkary-1	Sukkary-2	Sukkary-3	Sukkary-4	Sukkary-5
Sukkary-1	1.00				
Sukkary-2	0.95	1.00			
Sukkary-3	0.92	0.91	1.00		
Sukkary-4	0.94	0.92	0.89	1.00	
Sukkary-5	0.84	0.84	0.83	0.86	1.00

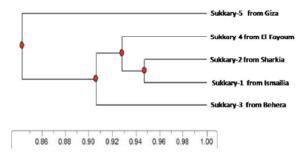


Fig. 3: Dendrogram for five Sukkary accessions constructed from the ISSR generated data using UPGMA method and similarity matrices computed according to Dice's similarity coefficient.

the highest number of fragments (19 bands). However, 17898-B primer generated the lowest number of amplicons (6 bands). The average number of fragments/primer was (11.25) and the approximate size of these fragments ranged from 326-3125 bps. All the tested primers produced polymorphic bands (Table 7) of the total 135 scorable fragments, 49 were polymorphic among the accessions (Figure 2). The number of polymorphic bands ranged from 1 to 7 resulting in an average of polymorphism/primer of (4.08). Primers (TC)<sub>8</sub>GT revealed the maximum number of polymorphic bands (7) conversely; the lowest number of polymorphic amplicons (1) was generated by CAGC(AC)<sub>7</sub> and GGTC(AC)<sub>7</sub>. The percent of polymorphism revealed by different primers ranged from 7.14 to 66.67 % with an average of 42.86 %.

## Genetic Relationships as Revealed by ISSRs Markers:

The 12 tested primers used to compute the similarity matrices according to Dice [20].. The genetic similarity ranged from 0.83 to 0.95 (Table 8). The highest genetic similarity revealed by the ISSRs analysis (0.95) was between Sukkary-1 collected from Ismailia and Sukkary-2 collected from Sharkia this was followed by 0.94 between Sukkary-1 and Sukkary-4 collected from El-Fayoum.

On the other hand, the genetic similarity between Sukkary-5 collected from Giza and any of the other accessions ranged from 0.83 to 0.86. It has been 0.86 between Sukkary-5 from Giza and Sukkary-4 from El-

Fayoum. While, the coefficient was 0.84 between Sukkary-1 from Ismailia and Sukkary-2 from Sharkia. The lowest percentage of similarity observed between Sukkary-3 collected from Behera and Sukkary-5 collected from Giza.

Clustering Analysis: The UPGMA cluster analysis of genetic distance among mango accessions is shown in (Figure 3) although there is a little genetic variation was detected among the tested accessions, ISSR phylogenetic analysis declared enough differences that could differentiate local accessions. Phylogenetic tree separated Sukkary accessions from different governorates into two major clusters at 84 % level of similarity, the first included only one genotype (Sukkary-5). However, the second cluster included Sukkary-1, Sukkary-2, Sukkary-3 and Sukkary-4 accessions. The highest genetic similarity was detected between Sukkary-1 collected from Ismailia and Sukkary-2 collected from Sharkia with 95% level of similarity; nevertheless the highest genetic difference was identified between the Sukkary-4 from El-Fayoum and Sukkary-3 from Behera accessions with 91 % level of similarity.

In pervious studies a genetic dissimilarity of 0.05% was observed among 27 accessions of 'Kensington Pride', using RAPD markers (14). Addition, an intracultivar variability in 'Banganapalli', 'Dashehri' and 'Langra' cultivars of mango detected using ISSRs (6).

## Genotype Identification by Unique ISSR Markers:

The phylogenetic analysis declared high degree of genetic relationships among Sukkary accessions. However the genotype-specific ISSR unique markers were able to differentiate the studied genotype accessions. The ISSR markers generating primers and the positive and/or negative markers with approximate size are shown in (Table 9) out of all tested ISSR primers, 10 primers were able to generate unique markers (positive and/or negative) that could differentiate mango accessions with the percent of 83.3%. However, two primers (HB<sub>9</sub> and 15) were failed to produce any unique marker. The number of generated unique markers ranged from 2 to 19 bands.

Table 9: Sukkary mango accessions identified by unique positive and/or negative ISSR markers.

Accessions	Primer	Unique positive		Unique negative		
		Size in bp	Total	Size in bp	Total	Total
Sukkary-1	-	-	-	-	-	-
Sukkary -2	17898-B	1288	1	-	-	
	853	2075	1	-	-	3
		776	1	-	-	
Sukkary -3	844-B	-	-	2796	1	2
		-	-	1598	1	
Sukkary -4	ISSR-2	2927	1	-	-	2
	844-B	3125	1	-	-	
Sukkary -5	17898-B	926	1	-	-	19
	17898-A	1928	1	-	-	
		1672	1	-	-	
	ISSR-1	2368	1	-	-	
		418	1	-	-	
	ISSR-2	2401	1	846	1	
		718	1	632	1	
	890	1834	1	-	-	
		1138	1	-	-	
		834	1	-	-	
	853	1587	1	531	1	
		1105	1	-	-	
	17	1122	1	-	-	
	17899-A	1580	1	-	-	
		1056	1	-	-	
	HB-10	1005	1	-	-	

The maximum number of unique markers was identified in Sukkary-5 which was 19 markers. However, other accessions (Sukkary-2, Sukkary-3 and Sukkary-4) were characterized by two or three unique bands. On the other hand, Sukkary-3 was only characterized by a unique negative band.

ISSR has been shown to provide a powerful tool in mango molecular characterization and to determine genetic diversity between cultivars [8, 35, 36].

Using ISSR assessment, it was demonstrated that most of the cultivars can be easily distinguished. Moreover, some fragments were uniquely amplified or absent in some of the landraces. These fragments are of great interest in genetic identification of mango accessions. [39, 15]. Ukoskit [37] reported a 39 band containing di-nucleotide repeats in mango, which was utilized for developing microsatellite markers. Results clearly demonstrated that PCR based assays of dominat markers, is a good tool for the genetic analysis of mango genotypes. The critical number of loci and sample size considerations are met with precision [38, 39]. Using ISSR assessment was demonstrated that most of the cultivars

can be easily distinguished. Moreover, some fragments were uniquely amplified or absent in some of the landraces. These fragments are of great interest in genetic identification of mango accessions in the germplasm collection. Over all these data extends the knowledge of ISSR application as a molecular tool in mango as reported previously for molecular characterization of mango [8, 39, 40, 15].

#### REFERENCES

- Kumar, N.V., H.P. Narayanaswamy, P.D. Theertha, G.K. Mukunda and S.N. Sondur, 2001. Estimation of diversity of commercial mango (Mangifera indica L.) cultivars using RAPD markers. Journal of Horticultural Science and Biotechnology, 76(5): 529-533.
- Ravishankar, K.V., A. Lalitha, M.R. Dinesh and L. Anand, 2000. Assessment of genetic relatedness among mango cultivars of India using RAPD markers. J Hortic Sci Biotechnol., 75(2): 198–201.

- Krishna, H. and S. Singh, 2007. Biotechnological advances in mango (*Mangifera indica* L.) and their future implication in crop improvement - A Review. Biotechnology Advances, 25: 223-243.
- 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 pp: 991-995.
- 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(2): 151-158.
- Singh, S., A.B. Gaikwad and J.L. Karihaloo, 2009. Morphological and molecular analysis of intra cultivar variation in Indian mango (*Mangifera indica* L.) cultivars. Acta Horti., 829: 205-212.
- 7. 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 indigeneous landraces with popular cultivars of mango in Andhra Pradesh, India. The Asian and Australian Journal of Plant Biotechnology, 6(1): 24-37.
- 8. González, A., M. Coulson and R. Brettell, 2002. Development of DNA markers (ISSRs) in Mango. Acta Horticulturae, 575: 139-143.
- Subedi, A., J. Bajracharva, B.K. Joshi, S.R. Gupta, H.N. Regmi and B. Sthapit, 2009. Locating and managing the mango (*Mangifera indica* L.) genetic resources in Nepal. PGR Newsletter, 115: 52-61.
- Gibert, O., D. Dufour, A. Giraldo, T. Sánchez, M. Reynes, J.P. Pain, A. González, A. Fernández and A. Diaz, 2009. Differentiation between cooking bananas and dessert bananas. 1. Morphological and compositional characterization of cultivated Colombian Musaceae (*Musa* sp.) in relation to consumer preferences. Journal of Agricultural and Food Chemistry, 57: 7857-7869.
- Domingues, E.T., 1999. Morphological characterization of Mandarin fruits from Citrus germplasm active bank of Centro de Citricultura Sylvio Moreira/IAC. Scientia Agricola, 56: 197-206.
- 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. pp: 34-40.

- 13. Varshney, R.K., A. Graner and M.E. Sorrells, 2005. Genic microsatellite markers in plants: features and applications. Trends in Biotechnology. 23(1): 48-55.
- Bally, I.S.E., G.C. Graham and R.J. Henry, 1996.
  Genetic diversity of Kensington mango in Australia.
  Aust. J. Exp. Agric., 36: 243-247.
- 15. Eiadthong, W., K. Yonemori, S. Kansaki, A. Sugiura, N. Utsunomiya and S. Subhadrabandhu, 2000. Amplified fragments length polymorphism analysis for studying genetic relationships among Mangifera species in Thailand. J. Am. Soc. Hortic. Sci., 125: 160-164.
- Schnell, R.J., J.S. Brown, C.T. Olano, A.W. Meerow, R.J. Campbell and D.N. Kuhn, 2006. Mango genetic diversity analysis and pedigree inferences for Florida cultivars using microsatellite markers. Journal of the American Society for Horticultural Science, 131: 214-224.
- Dillon, N.L., I.S.E. Bally, L.A. Hucks, C.L. Wright, D.J. Innes and R.G. Dietzgen, 2013. Implementation of SSR markers in mango breeding in Australia. Acta Hortic., 992: 259-267.
- 18. IPGRI, 2006. Descriptors of Mango (*Mangifera indica* L.). Rome, Italy. URL: http://www.ipgri.cgiar.org
- Snedecor, G.W. and W.G. Chochran, 1980.
  Statistical Methods, Seventh Edition, Ames: Iowa State University Press. pp: 507.
- Freed, R., S.P. Eisensmith, S. Goetz, D. Reicosky, V.M. Smail and P. Wollberg, 1990. MSTAT-C A Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments. https://www.msu.edu/~freed/disks.htm
- 21. Duncan, D.B., 1955. "Multiple Range and Multiple F-Tests. Biometrics, 11: 1-42.
- 22. Sokal, R.R. and P.N.A. Sneath, 1963. Principles of Numerical Taxonomy. Free-man, San Francisco.
- 23. Bhuyan, M.A.J. and D. Guha, 1995. Performance of some exotic mango germplasm under Bangladesh conditions. Bangladesh Hort., 23(1&2): 17-22.
- Jintanawong, S., H. Hiranpradit, P. Polprasid and P. Duangpikul, 1992. Group characterization of Thai mango, *Mangifera indica* L. Acta Horticulturae, 321: 254-261.
- 25. Naik, K.C., 1971. Mango improvement. Andhra Agri. J., 18(6): 221-222.
- Ozkaya, M.T., E. Cakir, Z. Gokbayrak, H. Ercan and N. Taskin, 2006. Morphological and molecular characterization of Derik Halhali olive (*Olea* europaea L.) accessions grown in Derik–Mardin province of Turkey. Sci Hortic., 108: 205–209.

- 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. pp: 9-15.
- 28. Morton, J.F., 1987. Fruits of warm climates. Miami. Florida Flair Books, pp: 221-239.
- 29. Haque, A.M.M.M., M.R. Ali, M.R. Uddin and A.K.M.A. Hossain, 1993. Evaluation of elite mango cultivars at southern region of Bangladesh. Bangladesh J. Plant Breed. Gent., 6(2): 21-28.
- 30. Pandey, S.N., 1998. Mango cultivars. In: Mango Cultivation, ed. R.P. Srivastav, pp 39-99. International Book Distributing Company, Lucknow, India.
- 31. Barbagollo, M.G., R.M. Di Lorenzo and F.G. Crescimanno, 1997. Characterization of carob germplasm (*Ceratonia silique* L.) in Sicily. In: J. Hort. Sci., 72: 537-543.
- 32. Illoh, H.C. and O. Olorode, 1991. Numerical taxonomic studies of mango (*Mangifera indica* L.) varieties in Nigeria. Euphytica, 51: 197-205.
- Gàlvez-López, D., M. Salvador-Figueroa, M.L. Adriano-Anaya and N. Mayek-Pérez, 2010. Morphological characterization of native mangos from Chiapas, Mexico Subtropical. Plant Sci., J., 62: 18-26.

- 34. Morell M.K., R. Peakll, R. Apels, L.R. Preston and H.L. Lloyd, 1995. DNA profiling techniques for plant variety identification. Aust. J. Exp Agric., 35: 807-819.
- 35. Zhao, Y., X.Y. Chen, X.R. Wang and R.Q. Pian, 2007. ISSR analysis of genetic diversity among Lespedeza bicolor populations. J. Plant Genet. Res., 2: 195-199.
- 36. Nybom, H., 2004. Comparison of different nuclear DNA markers for estimating intraspecyfic genetic diversity in plants. Mol. Ecol., 5: 1143-1150.
- 37. Ukoskit, K., 2007. Development of microsatellite markers in mango (*Mangifera indica* L.) using 5' anchored PCR. Thammasat Int. J. Sci. Technol., 12: 1-7.
- 38. Staub, J.E., Y. Danin-Poleg, G. Fazio, T. Horejsi, N. Reis and N. Katsir, 2000. Comparative analysis of cultivated melon groups (*Cucumis melo* L.) using random amplified polymorphic DNA and simple sequence repeat markers. Euphytica, 115: 225-241.
- 39. Krauss, S.L., 2000. Accurate gene diversity estimates from amplified fragment length polymorphism (AFLP) markers. Molecular Ecol., 9: 1241-1245.
- Adato, A., D. Sharon, U. Lavi, J. Hillel and S. Gazit, 1995. Application of DNA fingerprints for identification and genetic analyses of mango (*Mangifera indica*) genotypes. J. Am. Soc. Hortic. Sci., 120: 259-264.