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Molecular evaluation of selected mango genotypes grown in Egypt using SCoT and ISSR markers

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Abstract: The mango (*Mangifera indica* L.) is a common tropical and subtropical fruit and the second main fruit crop in Egypt. In this work, start codon targeted (SCoT) and inter simple sequence repeat (ISSR) markers were employed to study the genetic relationship among twenty-three mango genotypes grown under the El-Giza district conditions. These genotypes consist of four newly introduced cultivars, six new progenies and thirteen closely related mango cultivars which were included as cultivar references for the best definition. Based on the overall SCoT and ISSR data, a total of 52 bands were generated (18 for SCoT and 34 for ISSR) with a total polymorphic percentage equal to 53.84% (44.44% for SCoT and 58.82% for ISSR). Based on the whole set of SCoT and ISSR marker data, the genetic similarity coefficients ranged from 80 to 97% with a mean equal to 88.5% and the first two principal axes explained about 31% of the total genetic variation among the mango accessions. The SCoT and ISSR data revealed that the four newly introduced mango genotypes, namely 'Aya', 'Kasturi', 'Maya' and 'Omer', are novel genetic resources based on their genetic profiles, which can be used for future breeding programmes in Egypt.

Keywords: *Mangifera indica* L.; molecular marker; identification; germplasm

The mango (*Mangifera indica* L.) is the most important fruit in Egypt belonging to the family Anacardiaceae. In 2018, the mango production was 880 875 tonnes produced from 117 192.04 hectares cultivated by about 50 local and imported cultivars with a mean production of 7.5 tonnes/hectares according to the Ministry of Agriculture and Land Reclamation, Egypt. New imported mango cultivars are necessary for mango breeding to suit local and export market requirements. Also, the new genetic resources of mangoes are important for crop improvement programmes in different climate conditions.

Molecular markers are commonly used in plant genetic relationships and diversity as they are not affected by the environment. The advent of various molecular techniques has led breeders, based on the data produced by the markers, to estimate the genetic diversity among species, genotypes, varieties, cultivars, etc. Currently, many molecular markers have been employed along different loci of genomes that produce a high level of polymorphism, such as random amplification of polymorphic DNA (Mgendi et al. 2010; Abubakar et al. 2011), inter simple sequence repeat (Crespel et al. 2009; Verm, Rana 2011), simple sequence repeat (Zhang et al. 2006; Crespel et al. 2009; Ganesan et al. 2014), amplified fragment length polymorphism (Muluvi et al. 1999; Yang et al. 2013), restriction fragment length polymorphism (Eiadthong et al. 2000), sequence-related amplified polymorphism (Li, Quiros 2001), target region amplification polymorphism (Hu, Vick 2003), conserved region amplification polymorphism (Wang et al. 2009) and start codon targeted polymorphism (Collard, Mackill 2009). Several

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studies have been reported on the application of different molecular markers with different mango genotypes for genetic diversity analyses such as RAPD (Schnell et al. 1995; López-Valenzuela et al. 1997; Kumar et al. 2001; Karihaloo et al. 2003; Rahman et al. 2007; Mansour et al 2008; Díaz-Matallana et al. 2009; Mansour et al. 2014; Ahmedand, Mohamed 2015; Rashed, Maklad 2016; Galal et al. 2017; El-Sayed et al. 2018; He et al. 2021), AFLP (Kashkush et al. 2001; Yamanaka et al. 2006; Zhang et al. 2014; Wang et al. 2015; Dang, Chen 2017) and SSR (Duval et al. 2005; Honsho et al. 2005; Singh et al. 2012; Tsai et al. 2013; Azmat et al. 2016; Lal et al. 2017; Ajayi et al. 2019; Razak et al. 2019; Yamanaka et al. 2019). The start codon targeted (SCoT) polymorphism is a simple and effective gene targeted marker technique based on the conserved region surrounding the translation codon ATG (Collard, Mackill 2009). The technique uses single primers designed to anneal the flanking regions of the ATG start codon on both DNA strands. Like RAPDs, SCoT molecular markers are dominant and could be used for genetic analyses, quantitative trait loci (QTL) mapping and bulk segregation analyses (Collard, Mackill 2009). The SCoT molecular marker has been successfully used in a diverse set of plant species including mangoes and other related horticultural species for genetic diversity analyses (Gorji et al. 2011; Luo et al. 2011; Xiong et al. 2011; Amirmoradi et al. 2012; Bhattacharyya et al. 2013; Shahlaei et al. 2014). In the last few years, a few researchers started using SCoT primers with mangoes and this proved to be a reliable marker system that was able to preferentially detect the polymorphisms in the coding sequences (Luo et al. 2010, 2011, 2012; Gajera et al. 2014; Huang et al. 2014; Zhou et al. 2020). The SCoT technique has been applied in the identification of mango varieties in India (Gajera et al. 2014) and China (Luo et al. 2010, 2011, 2012; Huang et al. 2014; Zhou et al. 2020).

In several horticultural species, including the mango, the ISSR technique has the benefit of being low cost and has been widely used to detect DNA polymorphisms or to identify closely related cultivars (González et al. 2002; Luo et al. 2011; Ariffin et al. 2015; Dang, Chen 2017). The ISSR marker system detects polymorphisms in inter-microsatellite DNA regions without any prior sequence knowledge (Zietkiewicz et al. 1994). Primers are based on a repeat sequence and amplify the sequence between two micro-satellites resulting in many amplification products per primer being produced, offering good reproducibility at a low cost as well. ISSR has been applied in the identification of mango varieties in different regions including Australia (González et al. 2002), China (He et al. 2005, 2007; Luo et al. 2011; Dang, Chen 2017), Egypt (Mansour et al. 2008) and India (Pandit et al. 2007; Gajera et al. 2011; Tomar et al. 2011; Damodaran et al. 2012; Samal et al. 2012; Srivastava et al. 2012; Uddin et al. 2015). In Egypt, a limited number of researchers have used different molecular markers to study the biodiversity and relationships of different mango cultivars. For example, Mansour et al. (2008) investigated the mango genomic variation within twelve cultivars using the RAPD and ISSR molecular marker techniques. Also, ten mango genotypes were selected to assess the genetic diversity and relatedness using the RAPD marker system (Mansour et al. 2014; El-Sayed et al. 2018). Moreover, the genetic divergence and phylogenetic relationships among eight mango genotypes were assayed with RAPD markers to provide bases for the marker-assisted selection of parents for hybridisation and improvement of mango genotypes (Galal et al. 2017).

Molecular characterisation is an important step in tracking the success of any studied cultivars which would help to introduce, select and improve the existing mango varieties. Moreover, the genetic evaluation of mango cultivars in various regions is crucial for mango production, mainly due to the great diversity of cultivars and hybrids available for planting. Hence, the main objective of the present study was to assess the genetic variability and relationships of 23 mango genotypes grown in Egypt, including some newly introduced mango cultivars and newly selected seedy progenies, using two molecular marker systems.

MATERIAL AND METHODS

Plant materials. Fresh leaf samples of 17 mango cultivars along with six newly selected open-pollinated progenies or hybrids of unknown parentage (Table 1) were collected in April 2019 from a private orchard located at the side of an desert road in Alexandria (Cairo-Alexandria Road, 62 km long from Cairo), Giza governorate. The collected leaf samples were immediately stored in liquid nitrogen until DNA extraction could be performed.

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Table 1. Mango genotypes included in this study for molecular evaluation

Code	Cultivar	Code	Cultivar	Code	Cultivar
1	Zebda	9	Omer	17	Lily
2	Keitt	10	Maya	18	Kent
3	Naomi	11	Shenawy–02*	19	Shelly
4	Ewais	12	Shenawy–03*	20	Shenawy–06*
5	Haden	13	Seddek	21	Tommy–Atkins
6	Shenawy–01*	14	Carrie	22	Heidi
7	Aya	15	Shenawy–04*	23	Valencia–Pride
8	Kasturi	16	Shenawy–05*		

Genotypes in bold are new genetic resources to the Egyptian mango germplasm (Figure 1)
*indicates the new open-pollinated progenies included in this study

DNA extraction. The total DNA was isolated from 100 mg of the leaf tissue using the modified Cetyltrimethylammonium bromide (CTAB) method (Doyle, Doyle 1987) at the Biotechnology Lab, Cairo University Research Park, Faculty of Agriculture, Cairo University. The DNA quality was checked by means of the absorbance A_{260}/A_{280} ratios through a UV-spectrophotometer where the DNA is pure with an A_{260}/A_{280} ratio from 1.8–2.0. Moreover, a NA qualitative check was undertaken using electrophoresis in a 1% agarose gel with ethidium bromide.

SCoT and ISSR primers and polymerase chain reaction (PCR) condition. A total of ten highly polymorphic SCoT and ISSR were selected, five primers of each, which were used to perform the mango molecular analysis. The nomenclature and sequences of the SCoT and ISSR primers were obtained from (Collard, Mackill 2009; Luo et al. 2010) and (Mansour et al. 2008; Alansi et al. 2016), respectively, as indicated in Table 2. The DNA amplifications were performed in an automated thermal cycle (Bibby Scientific™ Techne™ TC-512 Gradient Thermal Cycler, UK) programmed for one cycle at 94 °C for 4 minutes followed by 45 cycles of 1 min at 94 °C, 1 min at 57 °C, and 2 minutes at 72 °C. The reaction was finally stored at 72 °C for 10 min. Polymerase chain reaction (PCR) was performed in 30-µL volume tubes according to Williams et al. (1990) that contained the following: Deoxyribonucleotide triphosphates (dNTPs) (2.5 mM; 3.00 µL), $MgCl_2$ (25 mM; 3.00 µL), Buffer (10 x; 3.00 µL), Primer (10 pmol; 2.00 µL), Taq DNA polymerase (0.20 µL), Template DNA (25 ng; 2.00 µL) and H_2O (d.w.; 16.80 µL). The DNA amplified product (15 µL) was loaded in pre-prepared Agarose (1.50 g) with (100 ml) l × TBE (Tris-Borate-Ethylenediaminetetraacetic acid) buffer. Ethidium bromide (5 µL) was added to the melted gel after the temperature was increased to 55 °C. A DNA ladder (1Kbp) mix, with molecular weights of 3 000, 1 500, 1 000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp was used as the standard DNA to control the fragment sizes and to control the run of each primer on two separate gels as well. The run was performed for about 30 min at 80 V in the mini submarine gel BioRad (Hercules, CA, USA).

Table 2. List of the primer names and their nucleotide sequences used in the study for the SCoT and ISSR procedures

Number	Name	Sequence (from 5' to 3')
1	SCoT–21	ACGACATGGCGACCCACA
2	SCoT–54	ACAATGCTACCACCAAGC
3	SCoT–55	ACAATGGCTACCACTACC
4	SCoT–65	ACCATGGCTACCACGGCA
5	SCoT–70	ACCATGGCTACCAGCGCG
6	ISSR–17899A	CACACACACACAAG
7	ISSR–HB10	GAGAGAGAGAGACC
8	ISSR–HB–19B1	GTGTGTGTGTGTCC
9	ISSR–HB–12	CACCACCACGC
10	ISSR–HB–13	GAGGAGGAGC

Data Analyses. Based on the electrophoresis results of the product amplified by SCoT and ISSR, "1" was recorded for the presence or "0" was recorded for the absence and "9" was recorded as missing data. The similarity matrices were calculated based on the UVP gel documentation system (Gel Works ID advanced software, UVP, England). The relationships among the genotypes, as revealed by the dendrograms, were performed using the SPSS windows (Version 10) program. The DICE computer package was used to calculate the pairwise difference matrix and plot the phenogram among the cultivars (Yang,

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Quiros 1993). A Principal Coordinate Analysis (PCA) was conducted using NTSYS-pc2.02 (Rohlf 1998).

RESULTS AND DISCUSSION

Genetic and molecular analysis using SCoT and ISSR markers for polymorphism identification. DNA molecular markers are a new type of genetic marker developed by investigating the polymorphisms present between the nucleotide sequences of different individuals. Nineteen mango genotypes were selected based on their phenotypic characteristics to be run along with some newly introduced mango cultivars for the best cultivar definition. SCoT and ISSR based-PCR approaches were selected. The genetic diversity of 17 mango cultivars along with six new progenies was assessed using SCoT and ISSR markers, and a cluster analysis was performed based on the genetic similarity coefficient. The overall data (Figure 1 and Table 3) can be described as a total of 52 bands were produced, out of which 28 (53.84%) were polymorphic bands. Generally, the percent of polymorphism reported here was similar to that reported earlier in mangoes by Gitahi et al. (2016) and Hidayat et al. (2021), but lower than that reported by Surapaneni et al. (2013), Zhou et al. (2020), Anggraheni and Mulyaningsih (2021), He et al. (2021) and Srivastav et al. (2021). This polymorphism could be the result of replication slippage (Powell et al. 1996) or it could be the result of the low genetic stability and high variability of the mango genome in the amplified non-coding regions (Fatima et al. 2016).

From the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis (Figure 2), 17 mango cultivars and the six newly selected progenies were classified into two major clusters divided into seven groups with a similarity coefficient of 0.84. The first group primarily comprised three mango cultivars ('Keitt', 'Omer' and 'Zebda'). The second group consisted of four mango cultivars

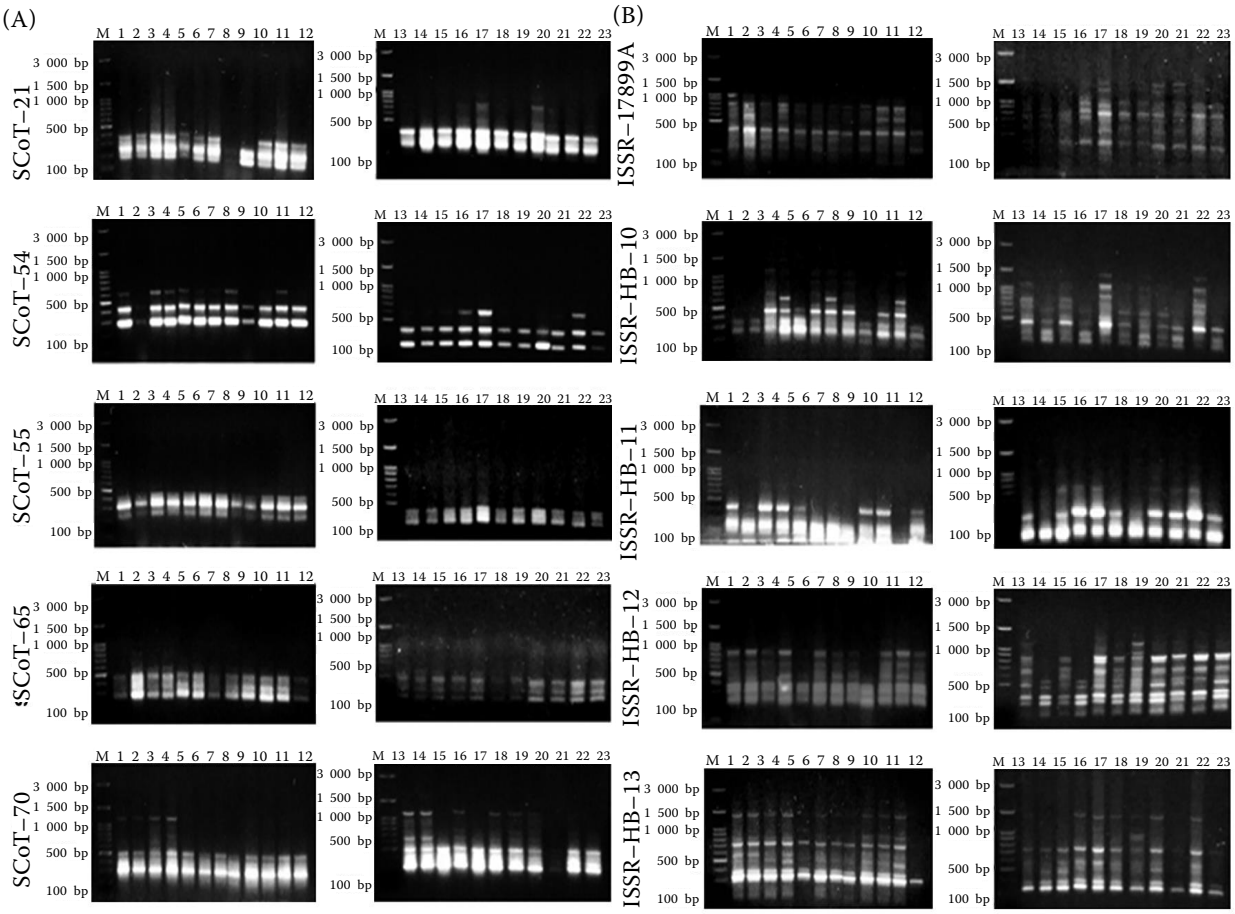

Figure 1. (A) – SCoT–PCR profile of 23 mango genotypes generated by the five selected SCoT primers; (B) – ISSR–PCR profile of 23 mango genotypes generated by the five selected ISSR primers; lane M represents the 1 Kbp ladder and lanes 1 : 23 represent mango samples as shown in order in Table 1.

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Table 3. Descriptive data of the SCoT and ISSR primers amplified results

Primer Name	Total band	Monomorphic band	Polymorphic band	Unique band	Polymorphic (%)
SCoT–21	4	2	2	–	50.00
SCoT–54	3	2	1	–	33.33
SCoT–55	3	2	1	–	33.33
SCoT–65	3	2	1	–	33.33
SCoT–70	5	2	3	1	60.00
Total	18	10	8	1	44.44
ISSR–17899A	9	3	6	2	66.66
ISSR-HB–10	7	2	5	–	71.42
ISSR-HB–11	3	1	2	–	66.66
ISSR-HB–12	8	4	4	–	50.00
ISSR-HB–13	7	4	3	–	42.85
Total	34	14	20	2	58.82
SCoT + ISSR	52	24	28	3	53.84

including one of the new progenies ('Aya', 'Kasturi', 'Shenawy–01' and 'Naomi').

Interestingly, six new closely related mango progenies or hybrids of the 'Shenawy' group, selected from open-pollinated hybrids of unknown parentage, were included in this analysis. The third group comprised seven mango genotypes ('Ewais', 'Heidi', 'Kent', 'Maya', 'Shelly', 'Shenawy–02' and 'Shenawy–04'). The fourth group included two mango cultivars ('Seddek' and 'Valencia–Pride'). The 'Seddek' mango cultivar, one of the main local cultivars in Egypt, was separated and formed a distinct group with the 'Valencia-Pride' cultivar which shares a fruit peel colour with a nephrotic fruit shape. These results agreed with Mansour et al. (2008); Mansour et al. (2014); and Galal et al. (2017). The fifth group comprised only one mango progeny ('Shenawy–03'). 'Shenawy–03' was the most divergent and separated in a distinct group. The sixth group consists of three mango genotypes ('Carrie', 'Haden' and 'Shenawy–05') and the last group also comprised three mango genotypes ('Lily', 'Shenawy–06' and 'Tommy-Atkins'). It should be mentioned that the four newly introduced mango cultivars, which were evaluated earlier from the agronomical and horticultural sides by Haseeb et al. (2020), were found within three different groups.

Interestingly, the phenotypic traits within the genotypes of each group confirmed the obtained results of the genetic affinity. For example, the flowering, fruiting and harvest time of the genotypes of the first group ('Keitt', 'Omer' and 'Zebda') are the same which mostly occurs in March till September or October of each year (late cultivars). Also, 'Aya', 'Kasturi' and 'Shenawy–01' (group II) are early maturing genotypes. Furthermore, the genotypes 'Ewais', 'Heidi', 'Shenawy–02', and 'Maya' are all grouped together, and they all have high fruit quality in terms of their flavour and sugar content. Moreover, the two mangoes 'Seddek' and 'Valencia-Pride' cultivars are genetically close with both having a red cheek with a nephrotic fruit shape. The 'Kent' and 'Shelly' cultivars are genetically similar, and their fruits have a red peel. There are distinctions in the genetic traits amongst different mango selections, such as high or low yield, very early or later maturity and fruit quality. It is very essential to use the mango genetic variety or diversity and ultimately preserve the vital germplasm of promising and threatened mango varieties/cultivars to broaden the genetic resource database (Litz 2004). The genetic diversity analysis in Table 3 illustrated that the selected five SCoT primers generated a total of 18 bands ranging from 3 to 5 with an average of 3.6 bands per primer. Eight bands of which were polymorphic with a percentage of polymorphic bands of 44.44%. The SCoT–70 primer generated a unique band and gave the highest percentage of polymorphism equal to 60%. Concerning the five selected ISSR primers, a total of 34 bands were produced with an average of 6.8 bands per primer, out of which 20 (58.82%) were polymorphic bands. The primer ISSR-49A generated two unique bands and gave 66.66% polymorphism, while the ISSR-HB-10 primer recorded the highest percentage of polymorphism equal to 71.42%. This study suggested that the ISSR technique is

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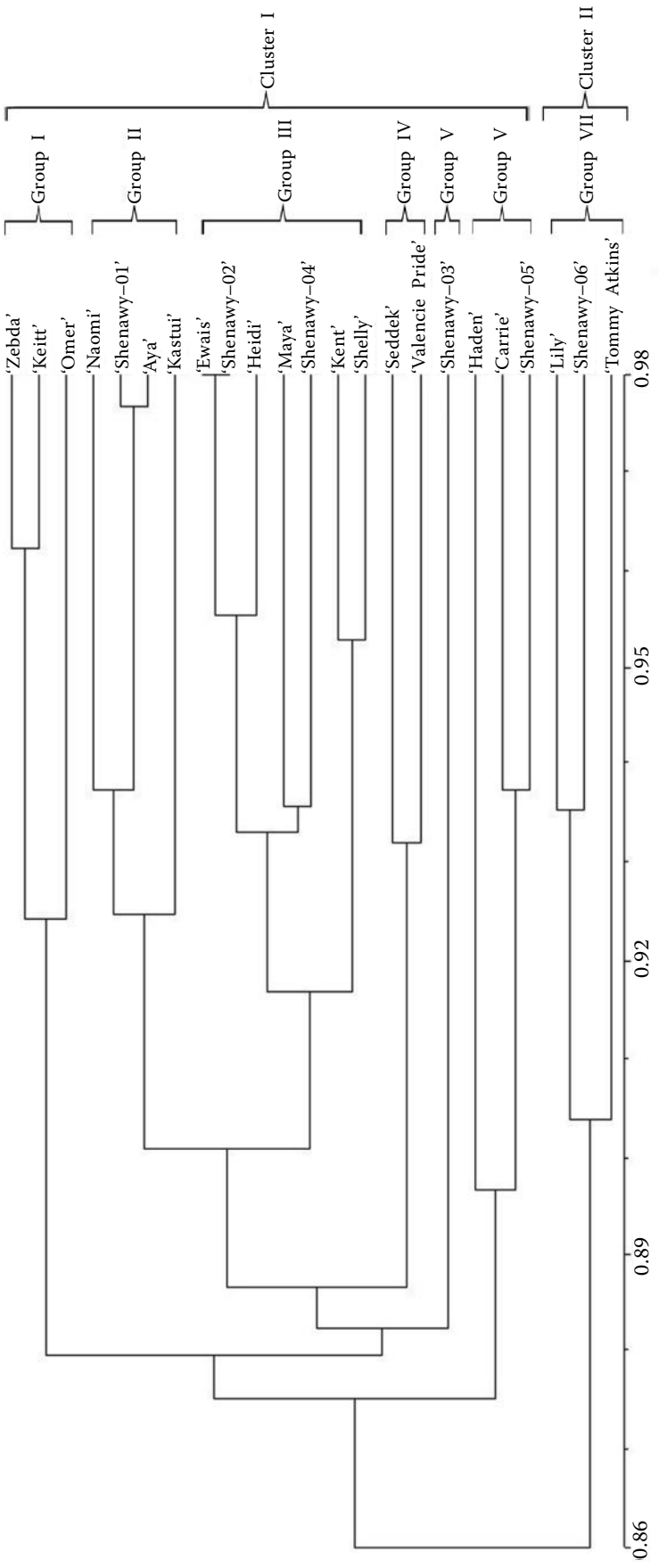

Figure 2. Dendrogram of the SCoT and ISSR analysis for 23 mango genotypes
The dendrogram was constructed based on the combined SCoT and ISSR data

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more powerful and efficient than the SCoT technique for the purpose of genetic identification and discrimination of the mango genotypes. To obtain more accurate genetic estimates, 52 bands based on the combined SCoT and ISSR data set were integrated into one matrix to assess the genetic relationship among the 17 mango cultivars along with the six new progenies. The genetic similarity coefficients ranged from 80 to 97% with a mean equal to 88.5% (Table 4). The polymorphism percentage obtained in this study is consistent with the results from previous studies conducted on different mango cultivars using SCoT and ISSR molecular markers in Egypt (Mansour et al. 2008), Australia (Gonzalez et al. 2000), China (He et al. 2005; 2007; Luo et al. 2010, 2011, 2012; Huang et al. 2014; Zhou et al. 2020) and India (Pandit et al. 2007; Gajera et al. 2011; Tomar et al. 2011; Damodaran et al. 2012; Samal et al. 2012; Srivastava et al. 2012; Gajera et al. 2014; Uddin et al. 2015). In this study, the use of five SCoT and five ISSR primers allowed for the screening of 52 (18 SCoT and 34 ISSR) fragments. Of these, 28 (53.84%) were polymorphic. As identified, the unique SCoT/ISSR markers and unique banding patterns created by different primer combinations, the high discrimination ability of these markers, especially the ISSR markers, will be useful for the management of a germplasm bank of mangoes and provide the nursery industry with a useful tool for certification of their plant material as well. In general, this study verified that both SCoT and ISSR markers are useful tools for mango varietal identification, allowing all the examined cultivars to be accurately characterised. This knowledge can assist greatly in the selection of optimal varieties and help to facilitate further progress in the strategy of mango breeding. To further understand the genetic relationship and diversity among the studied 17 mango cultivars and the six new progenies, a principal coordinate analysis (PCA) based on the genetic similarity matrix generated by the combined SCoT and ISSR data was performed (Figure 3). The PCA generally produced similar results obtained by the UPGMA. Based on the whole set of SCoT and ISSR marker data, the first two principal axes explained about 31% of the total genetic variation among the mango accessions, of which 16.8% attributed to the first coordinate and 14.2% to the second one.

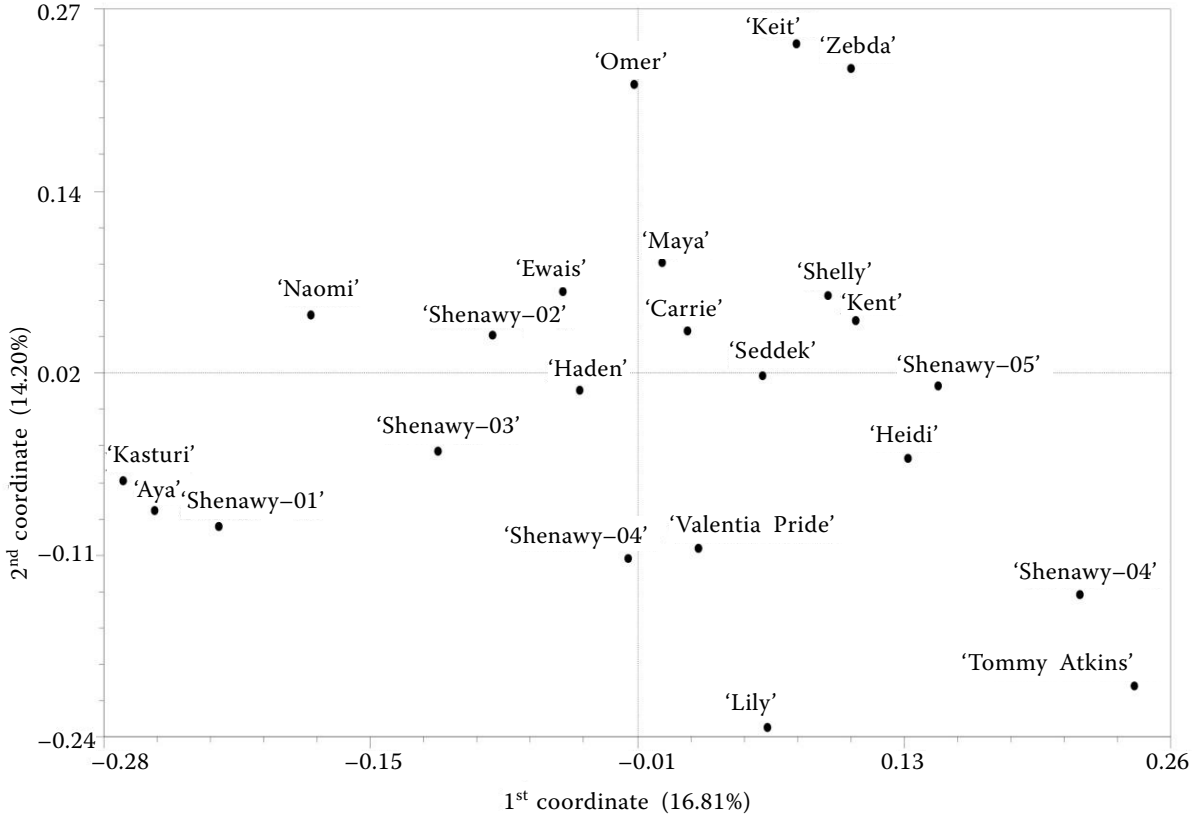

Figure 3. Principal coordinate analysis (PCA) of 23 mango genotypes based on the combined SCoT and ISSR data

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Table 4. Similarity Index based on the combined SCoT and ISSR data of 23 mango genotypes

	'Zebda'	'Keitt'	'Naomi'	'Ewais'	'Haden'	'Shenawy–01'	'Aya'	'Kasturi'	'Omer'	'Maya'	'Shenawy–02'	'Valencia Pride'
'Zebda'	1.000											
'Keitt'	0.960	1.000										
'Naomi'	0.911	0.897	1.000									
'Ewais'	0.902	0.914	0.941	1.000								
'Haden'	0.861	0.873	0.880	0.872	1.000							
'Shenawy–01'	0.842	0.880	0.937	0.902	0.889	1.000						
'Aya'	0.842	0.853	0.937	0.902	0.861	0.974	1.000					
'Kasturi'	0.838	0.849	0.909	0.875	0.886	0.946	0.919	1.000				
'Omer'	0.904	0.944	0.868	0.886	0.870	0.877	0.849	0.873	1.000			
'Maya'	0.895	0.933	0.911	0.927	0.917	0.921	0.895	0.865	0.932	1.000		
'Shenawy–02'	0.900	0.911	0.940	0.977	0.895	0.925	0.925	0.897	0.909	0.950	1.000	
'Shenawy–03'	0.845	0.857	0.892	0.857	0.896	0.901	0.901	0.870	0.853	0.901	0.880	
'Seddek'	0.868	0.907	0.886	0.902	0.889	0.895	0.868	0.865	0.849	0.895	0.900	
'Carrie'	0.827	0.865	0.846	0.889	0.901	0.880	0.880	0.849	0.861	0.907	0.886	
'Shenawy–04'	0.857	0.895	0.900	0.916	0.904	0.935	0.909	0.907	0.892	0.935	0.938	
'Shenawy–05'	0.875	0.861	0.868	0.907	0.895	0.850	0.850	0.821	0.857	0.900	0.905	
'Lily'	0.847	0.833	0.886	0.901	0.840	0.894	0.871	0.868	0.829	0.871	0.899	
'Kent'	0.923	0.909	0.914	0.905	0.865	0.897	0.897	0.842	0.880	0.923	0.902	
'Shelly'	0.900	0.911	0.916	0.930	0.842	0.900	0.875	0.846	0.883	0.925	0.929	
'Shenawy–06'	0.868	0.878	0.861	0.876	0.835	0.868	0.843	0.815	0.850	0.892	0.874	
'Tommy Atkins'	0.842	0.853	0.810	0.829	0.861	0.842	0.816	0.811	0.822	0.868	0.850	
'Heidi'	0.889	0.875	0.905	0.943	0.883	0.914	0.914	0.886	0.872	0.914	0.965	
'Valencia Pride'	0.877	0.889	0.842	0.861	0.899	0.877	0.849	0.845‘	0.857	0.904	0.883	1.000

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Table 4 to be continued

	'Shenawy–03'	'Seddek'	'Carrie'	'Shenawy–04'	'Shenawy–05'	'Lily'	'Kent'	'Shelly'	'Shenawy–06'	'Tommy Atkins'	'Heidi'
'Zebda'											
'Keitt'											
'Naomi'											
'Ewais'											
'Haden'											
'Shenawy–01'											
'Aya'											
'Kasturi'											
'Omer'											
'Maya'											
'Shenawy–02'											
'Shenawy–03'	1.000										
'Seddek'	0.873	1.000									
'Carrie'	0.857	0.907	1.000								
'Shenawy–04'	0.917	0.935	0.895	1.000							
'Shenawy–05'	0.827	0.875	0.937	0.889	1.000						
'Lily'	0.825	0.847	0.810	0.907	0.876	1.000					
'Kent'	0.904	0.923	0.909	0.911	0.927	0.874	1.000				
'Shelly'	0.853	0.925	0.886	0.914	0.905	0.876	0.951	1.000			
'Shenawy–06'	0.846	0.868	0.854	0.905	0.897	0.935	0.918	0.897	1.000		
'Tommy Atkins'	0.845	0.868	0.827	0.909	0.850	0.894	0.872	0.875	0.916	1.000	
'Heidi'	0.868	0.889	0.875	0.951	0.941	0.933	0.916	0.918	0.909	0.889	1.000
'Valencia Pride'	0.882	0.932	0.861	0.919	0.857	0.854	0.907	0.883	0.875	0.904	0.897

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CONCLUSION

The use of SCoT and ISSR molecular markers was valuable in studying the genetic diversity and genetic relationships among all the mango genotypes. Based on the results of the current study, it can be concluded that the four newly introduced mango cultivars ('Aya', 'Kasturi', 'Maya' and 'Omer') are partially different based on their genetic profiles. The use of five SCoT and five ISSR primers allowed for the screening of 52 (18 SCoT and 34 ISSR) markers specific to the different mango cultivars as identified by the unique SCoT/ISSR markers and unique banding patterns created by the different primer combinations. The high discrimination ability of the SCoT and ISSR markers will be useful for the management of a germplasm bank and provide the nursery industry with a useful tool for certification of their plant material as well. This study verified that both SCoT and ISSR markers are useful tools for mango varietal identification, allowing all the examined cultivars to be accurately characterised. This knowledge can greatly assist in the selection of optimal varieties and help to facilitate further progress in the strategy of mango breeding. The newly introduced genotypes of mangoes in Egypt could be used as breeding materials to improve the Egyptian mango germplasm.

REFERENCES

Abubakar B.Y., Wusirika R., MuA'zu S., Khan A.U., Adamu A.K. (2011): Detection of genetic variability using random amplified polymorphic DNA markers in some accessions of *Moringa oleifera* Lam. from northern Nigeria. International Journal of Botany, 7: 237–242.

Ahmedand T.H.M., Mohamed Z.M.A. (2015): Genetic diversity of mango (*Mangifera indica* L.) cultivars in Shendi Area. Extensive Journal of Applied Sciences, 3: 219–224.

Ajayi I.I., Olawuyi O.J., Ayodele A.E., Faneye A.O. (2019): Molecular relationship among *Mangifera indica* L. (Mango) varieties using simple sequence repeat (SSR) marker. Journal of Advances in Biology & Biotechnology, 22: 1–16.

Alansi S., Tarroum M., Al-Qurainy F., Khan S., Nadeem M. (2016): Use of ISSR markers to assess the genetic diversity in wild medicinal *Ziziphus spina-christi* (L.) Willd. collected from different regions of Saudi Arabia. Biotechnology & Biotechnological Equipment, 30: 942–947.

Amirmoradi B., Talebi R., Karami E. (2012): Comparison of genetic variation and differentiation among annual *Cicer* species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers. Plant Systematics and Evolution, 298: 1679–1688.

Anggraheni Y.G.D., Mulyaningsih E.S. (2021): Diversity assessment of mango (Mangifera spp) plant collection of cibinong germplasm garden based on leaves morphology and RAPD markers. In IOP Conference Series. Earth and Environmental Science, 715: 1–12.

Ariffin Z., Sah M.S.M., Idris S., Hashim N. (2015): Genetic diversity of selected Mangifera species revealed by inter simple sequence repeats markers. International Journal of Biodiversity: 1–8.

Azmat M.A., Khan A.A., Khan I.A., Rajwana I.A., Cheema H.M.N., Khan A.S. (2016): Morphological characterization and SSR based DNA fingerprinting of elite commercial mango cultivars. Pakistan Journal of Agricultural Sciences, 53: 321–330.

Bhattacharyya P., Kumaria S., Kumar S., Tandon P. (2013): Start Codon Targeted (SCoT) marker reveals genetic diversity of Dendrobium nobile Lindl., an endangered medicinal orchid species. Gene, 529: 21–26.

Collard B.C., Mackill D.J. (2009): Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Molecular Biology Reporter, 27: 86.

Crespel L., Pernet A., Le Bris M., Gudin S., Oyant L.H.S. (2009): Application of ISSRs for cultivar identification and assessment of genetic relationships in rose. Plant breeding, 128: 501–506.

Damodaran T., Kannan R., Ahmed I., Srivastava R.C., Rai R.B., Umamaheshwari S. (2012): Assessing genetic relationships among mango (*Mangifera indica* L.) accessions of Andaman Islands using inter simple sequence epeat markers. New Zealand Journal of Crop and Horticultural Science, 40: 229–240.

Dang, Z., Chen Y. (2017): Construction of a genetic linkage map of mango based on SRAP, AFLP and ISSR markers. Agricultural Biotechnology, 6: 9–16.

Díaz-Matallana M., Schuler-García I., Ruiz-García M., Hodson de Jaramillo E. (2009): Analysis of diversity among six populations of Colombian mango (*Mangifera indica* L. cvar. Hilacha) using RAPDs markers. Electronic Journal of Biotechnology, 12: 1–8.

Doyle J.J., Doyle J.L. (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19: 11–15.

Duval M.F., Bunel J., Sitbon C., Risterucci A.M. (2005): Development of microsatellite markers for mango (*Mangifera indica*). Molecular Ecology Notes, 5: 824–826.

Eiadthong W., Yonemori K., Kanzaki S., Sugiura A., Utsunomiya N., Subhadrabandhu S. (2000): Amplified fragment length polymorphism analysis for studying genetic relationships among Mangifera species in Thailand. Journal of the American Society for Horticultural Science, 125: 160–164.

https://doi.org/10.17221/112/2021-HORTSCI

El-Sayed M.E.A., El-nawam S.M.A., Mostafa G. (2018): Evaluation of Local Mango Cultivars Grown under Different Locations in Egypt using Fruit Quality and RAPD analysis. Middle East Journal of Agriculture Research, 7: 473–480.

Fatimah F., Husni A., Kosmiatin M., Karsinah K., Baroya M. (2016): Characterization of zygotic and nucellar embryo of six indonesian mango cultivars using molecular markers. Annales Bogorienses, 20: 69–75.

Galal O., Galal H., Aboulila A. (2017): Genetic variability and molecular characterization of some local and imported mango cultivars in Egypt. Egyptian Journal of Genetics and Cytology, 46: 121–138.

Gajera H.P., Bambharolia R.P., Domadiya R.K., Patel S.V., Golakiya B.A. (2014): Molecular characterization and genetic variability studies associated with fruit quality of indigenous mango (*Mangifera indica* L.) cultivars. Plant Systematics and Evolution, 300: 1011–1020.

Gajera H.P., Tomar R.S., Patel S.V., Viradia R.R., Golakiya B.A. (2011): Comparison of RAPD and ISSR markers for genetic diversity analysis among different endangered *Mangifera indica* genotypes of Indian Gir forest region. Journal of Plant Biochemistry and Biotechnology, 20: 217–223.

Ganesan K.S., Singh R., Choudhury D.R., Bharadwaj J., Gupta V., Singode A. (2014): Genetic diversity and population structure study of drumstick (*Moringa oleifera* Lam.) using morphological and SSR markers. Industrial Crops and Products, 60: 316–325.

Gitahi R., Kasili R., Kyallo M., Kehlenbeck K. (2016): Diversity of threatened local mango landraces on smallholder farms in Eastern Kenya. Forests, Trees and Livelihoods, 25: 239–254.

González A., Coulson M., Brettell R. (2002): Development of DNA markers (ISSRs) in mango. Acta Horticulturae (ISHS), 575: 139–143.

Gorji A.M., Poczai P., Polgar Z., Taller J. (2011): Efficiency of arbitrarily amplified dominant markers (SCoT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. American Journal of Potato Research, 88: 226–237.

Haseeb G.M., Ghounim I.E.S., Hmmam I., Mustafa M.R. (2020): Evaluation of four newly introduced mango (*Mangifera indica* L.) cultivars grown under El-Giza conditions. Plant Archives, 20: 9405–9410.

He X.H., Li Y.R., Guo Y.Z., Ou S.J., Li R.B. (2007): Identification of closely related mango cultivars by ISSR. Guihaia, 27: 44–47.

He X.H., Li Y.R., Guo Y.Z., Tang Z.P., Li R.B. (2005): Genetic analysis of 23 mango cultivar collection in Guangxi province revealed by ISSR. Molecular Plant Breeding, 3: 829–834.

He X.H., Razak S.A., Luo C. (2021): Genetic Diversity Analysis of Mango. The Mango Genome, 75–94.

Hidayat A., Rahayu E.S., Abdullah M., Retnoningsih A. (2021): Microsatellites to reveal genetic diversity and to distinguish four mangoes of Tegal District, Central Java, Indonesia. Biodiversitas Journal of Biological Diversity, 22: 3467–3473.

Honsho C., Nishiyama K., Eiadthong W., Yonemori K. (2005): Isolation and characterization of new microsatellite markers in mango (*Mangifera indica*). Molecular Ecology Notes, 5: 152–154.

Hu J., Vick B.A. (2003): Target region amplification polymorphism: a novel marker technique for plant genotyping. Plant Molecular Biology Reporter, 21: 289–294.

Huang X., Zhang X., Huang L., Ma Y., Yin G., Lee S., Zeng J., Liu H. (2014): Genetic diversity of Hemarthria altissima and its related species by EST-SSR and SCoT markers. Biochemical Systematics and Ecology, 57: 338–344.

Karihaloo J.L., Dwivedi Y.K., Archak S., Gaikwad A.B. (2003): Analysis of genetic diversity of Indian mango cultivars using RAPD markers. The Journal of Horticultural Science and Biotechnology, 78: 285–289.

Kashkush K., Jinggui F., Tomer E., Hillel J., Lavi U. (2001): Cultivar identification and genetic map of mango (*Mangifera indica*). Euphytica, 122: 129–136.

Kumar H., Narayanaswamy P., Prasad T., Mukunda G.K., Sondur S. (2001). Estimation of genetic diversity of commercial mango (*Mangifera indica* L.) cultivars using RAPD markers. The Journal of Horticultural Science and Biotechnology, 76: 529–533.

Lal S., Singh A.K., Singh S.K., Srivastav M., Singh B.P., Sharma N., Singh N.K. (2017): Association analysis for pomological traits in mango (*Mangifera indica* L.) by genic-SSR markers. Trees, 31: 1391–1409.

Li G., Quiros C.F. (2001): Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. Theoretical and Applied Genetics, 103: 455–461.

Litz R.E. (2004): Biotechnology and mango improvement. Acta Horticulturae (ISHS), 645: 85–92.

López-Valenzuela J.A., Martinez O., Paredes-Lopez O. (1997): Geographic differentiation and embryo type identification in *Mangifera indica* L. cultivars using RAPD markers. HortScience, 32: 1105–1108.

Luo C., He X.H., Chen H., Hu Y., Ou S.J. (2012): Genetic relationship and diversity of *Mangifera indica* L.: revealed through SCoT analysis. Genetic Resources and Crop Evolution, 59: 1505–1515.

Luo C., He X.H., Chen H., Ou S.J., Gao M.P. (2010): Analysis of diversity and relationships among mango cultivars using Start Codon Targeted (SCoT) markers. Biochemical Systematics and Ecology, 38: 1176–1184.

Luo C., He X.H., Chen H., Ou S.J., Gao M.P., Brown J.S., Tondo C.T., Schnell R.J. (2011): Genetic diversity of mango cultivars estimated using SCoT and ISSR markers. Biochemical Systematics and Ecology, 39: 676–684.

https://doi.org/10.17221/112/2021-HORTSCI

Mansour A., Ismail O.M., El-Din S.M. (2008): Diversity assessment among mango (*Mangifera indica* L.) cultivars in Egypt using ISSR and three-primer based RAPD fingerprints. The African Journal of Plant Science and Biotechnology, 2: 87–92.

Mansour H., Mekki L.E., Hussein, M.A. (2014): Assessment of genetic diversity and relationships among Egyptian mango (*Mangifera indica* L.) cultivers grown in Suez Canal and Sinai region using RAPD markers. Pakistan Journal of Biological Sciences, 17: 56–61.

Mgendi M.G., Manoko M.L., Nyomora A. (2010): Genetic diversity between cultivated and non-cultivated *Moringa oleifera* Lam. provenances assessed by RAPD markers. Journal of Cell and Molecular Biology, 8: 95–102.

Muluvi G.M., Sprent J.I., Soranzo N., Provan J., Odee D., Folkard G., McNicol J.W., Powell W. (1999): Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. Molecular Ecology, 8: 463–470.

Pandit S.S., Mitra S., Giri A.P., Pujari K.H., Patil B.P., Jambhale N.D., Gupta V.S. (2007): Genetic diversity analysis of mango cultivars using inter simple sequence repeat markers. Current Science, 93: 1135–1141.

Powell W., Morgante M., Andre C., Hanafey M., Vogel J., Tingey S., Rafalski A. (1996): The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding, 2: 225–238.

Rahman M.L., Rabbani M.G., Siddique M.N.A., Rahman M.A., Garvey E.J., Rahaman E.H.M.S. (2007): Molecular characterization of 28 mango germplasm using RAPD. Plant Tissue Culture and Biotechnology, 17: 71–77.

Rashed M., Maklad M. (2016): Genetic relationships among six mango (*Mangifera indica* L.) cultivars using rapd markers. Egyptian Journal of Genetics and Cytology, 45: 105–112.

Razak S.A., Azman N.H.E.N., Ismail S.N., Yusof M.F.M., Ariffin M.A.T., Sabdin Z.H.M., Hassan M.H.M., Nasir K.H., Sani M.A., Abdullah N. (2019): Assessment of diversity and population structure of mango (*Mangifera indica* L.) germplasm based on microsatellite (SSR) markers. Australian Journal of Crop Science, 13(2): 315–320.

Rohlf F.J. (1998): NTSYS-pc Version 2.02 Numerical Taxonomy and Multivariate Analysis System. Applied Biostatistics Inc., Exeter Software," Setauket, New York, 43.

Samal K.C., Jena R.C., Swain S.S., Das B.K., Chand P.K. (2012): Evaluation of genetic diversity among commercial cultivars, hybrids and local mango (*Mangifera indica* L.) genotypes of India using cumulative RAPD and ISSR markers. Euphytica, 185: 195–213.

Shahlaei A., Torabi S., Khosroshahli M. (2014): Efficacy of SCoT and ISSR markers in assessment of tomato (*Lycopersicum esculentum* Mill.) genetic diversity. International Journal of Biosciences, 5: 14–22.

Schnell R.J., Ronning C.M., Knight R.J. (1995): Identification of cultivars and validation of genetic relationships in *Mangifera indica* L. using RAPD markers. Theoretical and Applied Genetics, 90: 269–274.

Singh A., Singh A.K., Singh S.K. (2012): SSR markers reveal genetic diversity in closely related mango hybrids. Indian Journal of Horticulture, 69: 299–305.

Srivastav M., Singh S.K., Prakash J., Singh R., Sharma N., Ramchandra S., Devi R., Gupta A., Mahto A.K., Jayaswal P.K., Singh S. (2021): New hyper-variable ssrs for diversity analysis in mango (*Mangifera Indica* L.). Indian Journal of Genetics and Plant Breeding, 81: 119–126.

Srivastava N., Bajpai A., Chandra R., Rajan S., Muthukumar M., Srivastava M.K. (2012): Comparison of PCR based marker systems for genetic analysis in different cultivars of mango. Journal of Environmental Biology, 33: 159–166.

Surapaneni M., Vemireddy L.R., Begum H., Purushotham R.B., Neetasri C., Nagaraju J., Anwar S.Y., Siddiq E.A. (2013): Population structure and genetic analysis of different utility types of mango (*Mangifera indica* L.) germplasm of Andhra Pradesh state of India using microsatellite markers. Plant Systematics and Evolution, 299: 1215–1229.

Tomar R.S., Gajera H.P., Viradiya R.R., Patel S.V., Golakiya B.A. (2011): Phylogenetic relationship among Mango (*Mangifera indica* L.) Landraces of Saurashtra based on DNA fingerprinting. Journal of Horticulture and Forestry, 3: 379–385.

Tsai C.C., Chen Y.K.H., Chen C.H., Weng I.S., Tsai C.M., Lee S.R., Lin Y.S., Chiang, Y.C. (2013): Cultivar identification and genetic relationship of mango (*Mangifera indica*) in Taiwan using 37 SSR markers. Scientia Horticulturae, 164: 196–201.

Uddin M.S., Cheng Q. (2015): Recent application of biotechniques for the improvement of mango research. 12-Applied Plant Genomics and Biotechnology, 195–212.

Verma S., Rana T.S. (2011): Genetic diversity within and among the wild populations of *Murraya koenigii* (L.) resources and their potential parents with start codon targeted (SCoT) markers. Genetic Resources and Crop Evolution, 67: 41–58.

Zietkiewicz E., Rafalski A., Labuda D. (1994): Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20: 176–183.

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