Molecular Characterization of Seedlings Derived from Two Polyemberyonic Egyptian Mango Cultivars Using SSR Markers

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Abstract: The molecular characterization and genetic variability between two of commercial and polyemberyonic Egyptian mango cultivars, namely: Zebda and Ewais, with thirty seedlings of their offspring were analyzed using 8 Simple Sequences Repeat (SSR) markers. LMMA _15 marker was discarded in data analysis because of producing only one band (monomorphic locus). Other seven markers produced total of 22 alleles with a high level of Polymorphism (~100 percent). The effective number of alleles ranged from 1.7, to 3.4 with average value of 1.47. Heterozygosity per locus varied from 0.00 to 0.75 with an average of 0.36. Polymorphic Information content (PIC) value scored from 0.41 to 0.70 with average of 0.57. The discrimination power (Dp) ranged between 0.11 and 0.72 with an average of 0.50 per locus. Generally, the genetic similarity values varied between 0.12 and 100% over 32 genotypes. A cluster analysis was used to determine genetic similarities. The dendrogram can be grouped into two major clusters (I and II). Cluster I consists of Ewais seedlings exhibiting 94-100% genetic similarity among them. Cluster II consists of all seedlings of Zebda cultivar exhibiting 52-100% genetic similarity and divided to two sub clusters. Seedling (Z_C_S2) was the most divergent in first sub cluster and second sub cluster exhibited less distance and consists of all other Zebda seedlings. This study additionally indicates that SSR markers are useful for distinguishing and characterizing mango genotypes. The genetic relatedness among these genotypes could provide useful information for conservation and selection of cross parents in breeding.

Keywords: Mango, Molecular characterization, Genetic diversity, SSR markers, Genetic relationships.

INTRODUCTION

Mango (*Mangifera indica* L.) is called 'king of fruits' due to its rich taste, flavor, color, production volume and long shelf life. It hails from Anacardiaceae family and it is a diploid plant with 20 pairs of chromosomes and a tiny genome size of 439 Mbp (Viruel *et al*., 2005). Genetic enhancement of mango cultivars is complex by their reproductive system. Some intrinsic characteristics including high level of heterozygosity, long juvenile phase, only one seed per fruit, and heavy fruit drop leading to low maintenance of crossed fruits (Kepiro and Roose, 2010). The cross-pollination nature and a wide range of common agro-climatic conditions have involved in a wide genetic diversity of mango (Abdalla *et al*., 2006). In polyembryonic cultivars, seedlings originate from somatic tissue and from a zygote, but differentiating between the two seedlings types can be confused (Rocha *et al*., 2014). Also, polyembryony makes breeding schemes complicated. Previously, the morphological traits were regarded the base of mango characterization (Farooq and Azam, 2002), but identification depending on morphological features is in competent and inaccurate. Furthermore, morphological characters are complicated due to the continual nature of the crop, vulnerably to environmental conditions and their restricted number (Kundan, 2013). Newly, molecular markers, based on polymorphisms at the DNA level, are increasingly used and proved effective to assess genetic diversity. Data based on molecular markers such as Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLPs) and Random Amplified Polymorphic DNA (RAPD) has been utilized to identify variant genotypes. Microsatellites, also known as Simple-Sequence Repeats (SSRs), constitute an appropriate tool for genotyping because of their co-dominant manner and their high levels of allelic diversity at different loci. The utility of microsatellite markers for assessing the genetic variability in a wide range of plants has been recently reported (Fatimah *et al.,* 2016). On account of their high mutation rates and the ease of analysis, microsatellite markers were useful and effective for phylogenetic studies, genetic fingerprinting, and cultivar identification among different mango genotypes (Adato *et al.,* 1995). In recent years, genetic markers are increasingly used for the study of genetic diversity. Therefore, the polymorphism determined by these markers is one of the valuable parameters for studying cultivars and understanding their genetic difference. The high reproducibility of microsatellite markers may be due to their huge number, distribution throughout the genome, co-dominant inheritance, neutrality with respect to selection, and ease automation of analytical procedures. This study used SSR analysis of thirty two genotypes of mango to estimate the genetic relatedness between two mango cultivars and seedlings resulting from polyemberyonic seeds.

MATERIALS AND METHODS

Plant material and sampling

Two Egyptian polyembryonic mango cultivars (Zebda and Ewais) were used in this study growing at Canal University farm - Ismailia - Egypt in October 2018. 10 mature, healthy mango fruits of each cultivar have been collected and the seeds were implanted at 35°C in sand & soil mixture (50:50) and appropriate wetness. The germination ratio was calculated by dividing the germinated seeds by the total number of seeds. The number of samples obtained from Zebda

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was 17 and from Ewais was (13) with overall 32 samples including every parental samples were labeled. From 32 fresh, young, tender leaves including the parental samples, 5 g of each sample were taken and enveloped in aluminum foil and immediately conserved in liquid nitrogen tank to prevent endo-enzymes activation.

DNA extraction

Total DNA of 32 samples were extracted by the CTAB method according to (Keb-Llanes *et al.,* 2002). Mango leaves were crushed to fine powder by small amount of liquid nitrogen, around (200 mg) of powder was transferred to (1.5ml) Eppendorf tube then 800 µl of preheated extraction buffer (2 g Cetyl trimethyl ammonium bromide (CTAB) (w/v), 100 mM Tris-HCl PH8), 20 mM EDTA pH 8.0 (w/v), 1.4 M NaCl, 4% PVP, 10 mM β-mercaptoethanol) was added immediately vortexed for 30 seconds and incubated at 65°C for 60 min. After incubation, Eppendorf tubes were centrifuged at (4,696.8 xg) for 10 min, the pellets discarded and the aqueous phase transferred to a new Eppendorf tube. Approximately (250 µl) of chloroform/iso-amyl alcohol (24:1) was add to each tube and mixed by inversion several times. The tubes were centrifuged at 13,000 rpm for 1 min. The upper clear layer was transferred to new tube then 50 µl of 7.5 M ammonium acetate followed by 500 µl of ice cold absolute ethanol were added and mixed gently and the mixture was incubated overnight at -20°C. The tubes were centrifuged at (4,696.8 x g) for 1 min and the pellets were washed twice with 70% ethanol, and dried for 15 min. After washing, the pellets were dissolved in 35 µL TE (10 mM Tris-HCl pH 8.0; 1 mM EDTA pH 8.0). To remove RNA, 4 µl RNase A (10 mg/ml) were added and incubated at 65°C for 20 min. The resulting DNA was stored at -20°C. To quantify DNA, the samples were electrophoresised onto 1% agarose (1 g of agarose in 100 ml of 1x TAE buffer) containing 10 µl/ ml ethidium bromide by electrophoresis at 100 V for 45 min. The samples were measured at 260\280 nm by Nano Drop® ND-1000 (Thermo Fisher Scientific™, USA).

DNA amplification

In the current study, five SSR primers pairs were employed for PCR amplification as reported by Viruel *et al*. (2005), and Honsho *et al*. (2005). Three EST-SSR primers reported by Kumar (2015). These SSRs markers were synthesized by Alpha DNA, (Canada) Table (1). These primers were used to analyze the profile band of SSR markers namely (LMMA1, LMMA2, LMMA8, LMMA15, ESTD1, ESTD2, ESTD10 and MIAC-3) Table (1). The amplification mixture contained 12.5 µl of master mix ready to use (dNTPs + Taq DNA polymerase + $MgCL_2$). 2.0 µL of DNA (20 ng/µl,) 2.0 µl of each primers (10 ng/µl) and 10.5 µl demonized water to a final volume 25 µl. PCR reaction was executed in (IBPR laboratory. Institute, PTC-100 thermocycler, Co) as follows: 4 min at 94°C (initial denaturation) followed by 35 cycles for 30s at 94°C (denaturation); 1 min at 60°C (annealing) and 2 min at 72°C for extension and a final extension at 72°C for 5 min. The amplification products were run on an agarose gel and stained with ethidium bromide (10 µl/ 100 ml) and visualized under by ultraviolet light. Gels were done by use Gene Sys Gbox System.

Data analysis

The reproducible bands from selected primers were scored visually and were recognized as polymorphic based on the presence (1) or absence (0) in the different samples regardless the differences in band intensity.

Table (1): List of 8 SSR primers and their sequence

No	Locus name	Sequence (5´-3´)	Ta (C°)	Accession No	Reference
1	**LMMA1**	F:ATGGAGACTAGAATGTACAGAG R:ATTAAATCTCGTCCACAAGT	**53**	**AY628373**	
2	**LMMA 2**	F:AAATAAGATGAAGCAACTAAAG R:TTAGTGATTTTGTATGTTCTTG	**47**	**AY628374**	**Viruel *et al*. (2005)**
3	**LMMA8**	F:CATGGAGTTGTGATACCTAC R:CAGAGTTAGCCATATAGAGTG	**53**	**AY628380**	**Viruel *et al*. (2005)** **Viruel *et al*. (2005)** **Viruel *et al*. (2005)**
4	**LMMA15**	F:AACTACTGTGGCTGACATAT R:CTGATTAACATAATGACCATCT	**62**	**AY628387**	
5	**MIAC_3**	F: TAAGCTAAAAAGGTTATAG R: CCATAGGTGAATGTAGAGAG	**53**	**AB190346**	**Honsho *et al*. (2005)**
6	**ESTD1**	F:TGCTAATTTAGGCACTACCG R:ATCATTATCCACCTCCTCCT	**53**	**-**	
7	**ESTD2**	F:TACCACTCGTAGCCTCAACT R:CCATTGTCGTTGTTGTTATG	**53**	**-**	**Kamlesh (2015).** **Kamlesh (2015).** **Kamlesh (2015).**
8	**ESTD10**	F:GATCTGACCCAACAAAGAAC R:ACGTAGATCTGCTTAACCCA	**53**	**-**	

The percentage of polymorphism, the number of specific alleles and the observed heterozygosity (Ho) were calculated. The effective number of alleles (NE) was calculated for each locus using the formula: NE = 1/∑ (E/F) 2 according to Hart and Clark (1997). The polymorphic information content (PIC) or heterozygosity index was calculated from the formula: PIC= 1-∑ pi2 where pi is the frequency of each allele the discriminating power per locus (PD) was scored as reviewed by Nei (1973) with replacing the allele frequency by the fragment frequency (kloosterman *et al.,* 1993). The matching fingerprints were estimated according to Jones (1972). All previous calculations and genetic parameters were executed with the programs Microsoft Excel, Quantity one, and GENEPOP version 1.31 (Raymond and Rouset, 1995). The similarity degree was calculated according to Dice coefficient (Sneath and Sokal, 1973), using the SPSS software ver. 16.0. The dendrogram were created depending on the average Linkage (Between Groups) using all recorded fragments over all the loci used to elucidate the genetic relationships and similarity between all genotypes.

RESULTS AND DISCUSSION

Experimental field:

In this study, the polyembryony percentage was 71% with average of 3.4 seedlings/seed in Zebda and 83% with average of 2.6 in Ewais the germination ratio was recorded as70%, 60% in Zebda, and Ewais respectively, (Table 2).

Table (2): Germination and polyembryony percentage in both "Zebda" and "Ewais" CV

Cultivars	Number of embryo/ seed										Number of seedling	Germination rate	Polyembryony (%)	Average of polyembryony
	A	B	C	D	E	F*	G*	H*	I*	J*				
Zebda	3	3	5	3	3	1	1	0	0	0	19	70	71	3.4
Ewais	3	4	2	2	2	1	0	0	0	0	14	60	83	2.6

From A – J name of stones of every cultivar, (*) unused seeds in the molecular analysis

Molecular characterization and the discrimination power

The results of using SSR markers developed for mango cultivars (Viruel *et al*., 2005; Honsho *et al*., 2005; Kumar, 2015) gave successful amplifications across the 1SSR Markers Informative and Performance 32 samples. One marker (LMMA_15) did not show any polymorphism among the tested seedlings and gave only one monomorphic band at 220 bp. The LMMA_15 locus might be conserved or homozygous in the samples or homozygosity in the genome.

For the thirty two samples, other seven SSR markers produced total of 22 alleles with a high level of Polymorphism (~100 percent). The high ratio of polymorphism might be a consequence of replication slippage (Powell *et al.,* 1996) or it might be due to low genetic stability and high variability of the DNA sequences in the amplified non-coding regions of the mango genome (Fatima *et al*., 2018).

The overall size of amplified PCR products ranged from 60 bp in LMMA_8 to 578 bp in ESTD_2. This difference in size might correlate with the number of repeats within each particular locus (Cole, 2005). In view of such widely divergent sizes, the actual number of nucleotides in these alleles would need to be established by sequencing. This suggests a wide genetic diversity in the tested seedlings that may be used in mango breeding programs. The identification by SSR markers of allele size can be subjected to pair-wise comparison to detect genotypic differences (Galbacs *et al.,* 2009).

The number of alleles per locus varied from 2 to 5 alleles with average number of 3 alleles per locus. ESTD_1marker has the largest number of alleles (5) Table (3). Whereas, the low number of alleles (2) in each of LMMA_2 marker and MIAC_3 marker might due to the quality of agarose used to resolve the amplified products or the exclusion of the monomorphic and spurious bands from analysis, reducing the number of alleles (Shah *et al*., 2013). Other explanations might be due to the 'short allele dominance', where, in heterozygote's including a short and a long allele, only the short allele is sufficiently amplified in the PCR reaction (Wattier *et al*., 1998). The variability in the number of alleles per locus could results from diverse locus-specific mutation rates and reproduces strong variations in allelic diversity between SSRs loci (Piyusha and Singh, 2018). Higher allelic numbers were detected in six cultivated mangoes and two wild species by Chunwongse *et al*. (2015). The comparisons with the allelic diversities reported by other studies should be regarded with caution, taking into consideration the different sample sizes used. Moreover, the same mean number of alleles may not indicate the same amount of variability (Paiva *et al.,* 2014). Two markers, ESTD_2 and ESTD_10 did not produce any alleles that distinguish Ewais seedlings in this study.

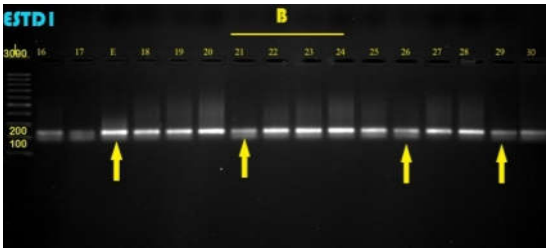

Fig. (1): Profile band of ESTD_1 primer of Zebda . L = 100 bp ladder, Z= mother sample, 1-15 = seedlings samples, C = stone code that contain [7,8,9,10,11], The left arrow = mother sample bands, the middle arrow = different band (tri band), the last arrow = different band (single band)

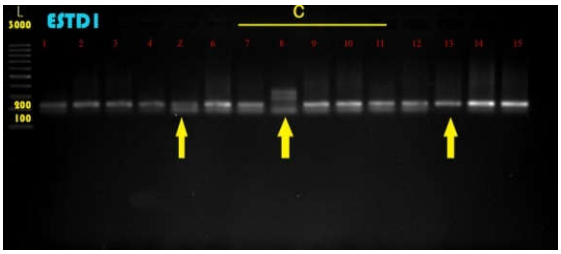

Fig. (2): Profile band of ESTD_1 primer of Ewais. L = 100 bp ladder, E= mother sample, 18-30 = seedlings samples, B = stone code that contain [21,22,23,24], The left yellow arrow = mother sample bands, the other yellow arrows = clear different bands

However, from the 7 SSR markers analyses of 32 mango seedlings, either one or two PCR products were observed for each sample, representing homogeneity and heterogeneity, respectively. One or more of the SSR loci detected multiple bands, which can be attributed to the allopolyploid nature of mango as described by Mukherjee (1972).

Meanwhile two loci (ESTD_1 and ESTD_2) produced more bands than expected based on the diploid construction of this species, this may be due to the duplication of these loci or the genomic rearrangements accumulated and thus it is enable to describe as multiple loci (Callen *et al.,* 1993; Viruel *et al*., 2005).

Effective number of alleles (Ne) is the measure of allelic evenness. In this study, the results showed that the effective number of alleles (Ne) for the polymorphic markers ranged from 1.7, for LMMA _2 and MIAC_3 to 3.4 for ESTD_1 with average value of 1.47. The total number of effective alleles produced by the 7 SSR loci was 10.3. Table (3) showed that the average of effective number of alleles was lower (1.47) than observed number of alleles (3). Because of low frequencies alleles had little contribution to the effective number of alleles. According to the selective standard of the microsatellite loci, it ought to have at least four alleles to be considered supportive for the evaluation of genetic diversity. Bases on this criterion, the 7 SSR loci used in this study were useful for the evaluation of genetic diversity in 32 Mango genotypes. These results imply that abundant genetic polymorphism exist in mango cultivars.

Heterozygosity (He) refers to the presence of different alleles at one or more loci on homologous chromosomes. Heterozygosity per locus varied from 0.00 (MIAC-3 and ESTD_10) to 0.75 ((LMMA1)) with an average of 0.36 (Table 3). The heterozygosity observed at some of the loci could also be due to high mutational rate and mutational bias at SSR loci. The loci with a large number of repeat units (SSR units) tend to show a high mutational rate. As a result, any mutations in any one of the alleles may create a heterozygous condition (Bharathi, 2011). The measure of the level of heterozygosity across loci can be used as an indicator of the amount of genetic variability (Zulkifli *et al.*, 2012). However, Allelic diversity and heterozygosity are important features for the establishment of microsatellite markers for linkage studies (Chiaramonte *et al.*, 2002).

Polymorphic Information content (PIC) value varied from 0.41 (LMMA _2) to 0.70 (ESTD_1) with average of 0.57. The broad range of PIC values in present study was indicative of the presence of unique alleles in some seedlings which facilitates their differentiation from another. According to Botstein *et al.* (1980), the mean value of PIC recodes more than 0.5 that is considered informative markers and reflected the high level of polymorphisms of the used set of microsatellites and heterogeneity in 32 mango seedlings. This is higher than that reported by Schnell *et al*. (2005) in their work with 15 microsatellite loci ranging from 0.21 to 0.63 for the polymorphic among59 Florida cultivars and four related species from the USDA germplasm collection for mango. This may probably be due to the different diverse genotypes analyzed and to the different number of analyzed samples. Nevertheless, the PIC depended on the number of alleles detected and on their distribution frequency. Also, PIC was influenced by location of primers in the genome used for study and genotype sensitivity to the method used (Pachauri *et al*., 2013). Hence, PIC values increased proportionally in ESTD_1, ESTD_2 and LMMA_1 with increasing heterozygosity at each locus. Whereas, the lower PIC value (0.41) for LMMA _2 might be attributed to the concentration of gene frequencies, which leads to deviation from the condition of maximum information content of a locus. This occurs when all alleles have similar frequencies (Paiva, *et al.,* 2014). The discrimination power (Dp) was found to be high in the majority of the 7 SSR markers (Table3), ranged between 0.11 and 0.72 with an average of 0.50 per locus. However, the discrimination power is an extension of the polymorphism information content (PIC), which actually describes the efficiency of a given marker to discriminate between genotypes, i.e., the probability that two randomly selected individuals have different arrays (Anderson *et al.*, 1993). Thus, high PIC parallel with Dp values exhibited that these markers have the potential to disclose allelic variation and each of these markers had a greater affinity towards discriminating between two genotypes (Ashraf *et al.*, 2016).

Table (3): Various parameters related to7markers for SSR analysis in 32 Mango seedlings

SSR loci code	Size	Total alleles (na)	NE [a]	Polymorphic band	HO[b]	PIC[c]	DP[d]
LMMA_1	150-322	3	2.7	3	0.75	0.63	0.60
LMMA _2	95-200	2	1.7	3	0.47	0.41	0.44
LMMA _8	60-378	3	2.3	3	0.06	0.56	0.57
MIAC_3	186-205	2	1.7	2	0.00	0.49	0.50
ESTD_1	188-330	5	3.4	5	0.66	0.70	0.72
ESTD_2	188-578	4	3.1	4	0.56	0.68	0.55
ESTD_10	80-214	3	2.1	3	0.00	0.53	0.11
Total		22	10.3		2.5	4	3.49
Average		3	1.47		0.36	0.57	0.50

Genetic similarity and cluster analysis

Genetic Similarity (GS) matrices constructed on shared allele bases over 32 tested seedlings varied from 0.12 to 1.00 Table (4). The highest percentage of genetic similarity (100%) was in each cultivar and its seedlings. This indicates that the seedlings of both cultivars are highly similar with their mothers. In general, the lowest percentage (12%) was recorded between seedling (Z_B_S1) and each one of 4 seedlings of Ewais, namely (E_E_S1, E_E_S2, E_B_S1 and E_C_S2). The huge variation between the two mango cultivars might be due to a long period of cultivation, polyembryonic nature and germplasm exchange followed by much possibility of hybridization and high clonal heterozygosity. However, Ewais cultivar and its seedlings showed a great similarity among them. This deserves further exploration (Table 4 and Figure 3). Regarding to Zebda cultivar and their seedlings, some distinctions in genetic similarity were noticed and ranged from 52% to 100%. The lowest percentage of genetic similarity (52%) was between Z_B_S1and Z_D_S2. This result might be due to mango pollinators (mango is anallogamus, cross pollinated species) and, especially, human intervention by transferring specimens fromone population to another (Kiambi *et al*., 2005). Up to this point, it is needed to expand the scope of gene flow detection in further studies. The dendrogram can be grouped into two major clusters (I and II). Cluster I consists of Ewais's seedlings exhibiting 94-100% genetic similarity among them (Figure 3). Cluster II consists of all seedlings of Zebda cultivar exhibiting 52-100% genetic similarity and divided to two sub-clusters. Seedling (Z_C_S2) was the most divergent in first sub cluster and second sub cluster exhibited less distance and consists of all other Zebda seedlings.

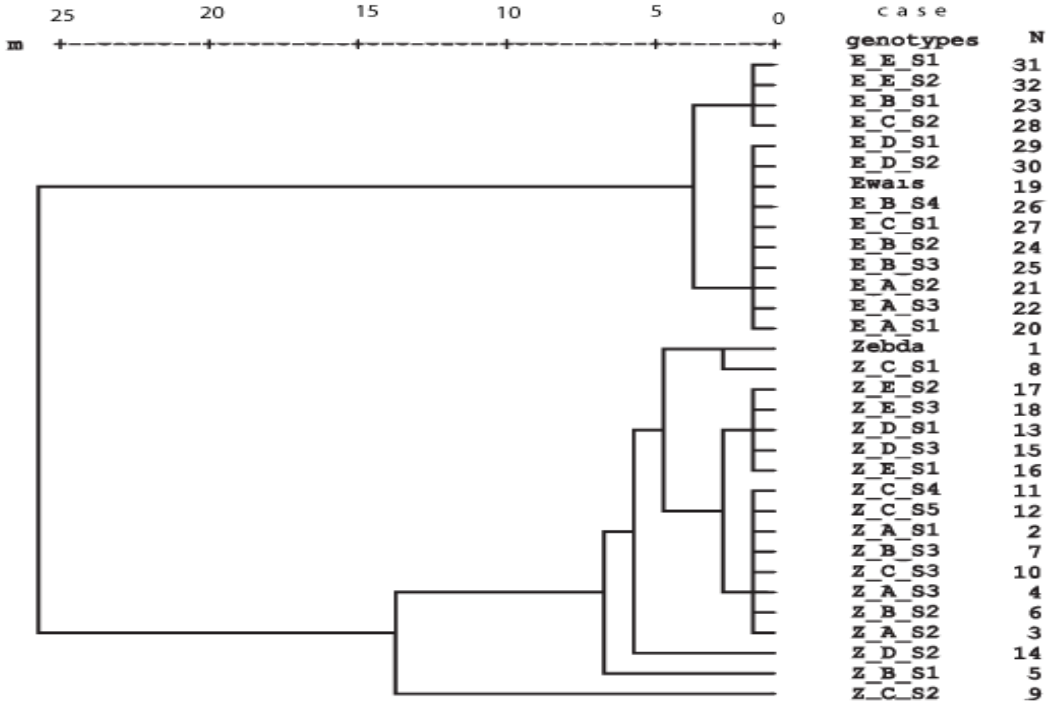

Fig. (4): Dendrogram using Average Linkage (Between Groups) Rescaled Distance Cluster Combine

Table (4): Pair-wise similarities matrix of 32 mango accessions according to the index of (Sneath and Sokal, 1973) based on SSRs data analysis using SPSS program

Genotyp	Zebda	Z_A_S	Z_A_S	Z_A_S	Z_B_S	Z_B_S	Z_B_S	Z_C_S	Z_C_S	Z_C_S	Z_C_S	Z_C_S	Z_D_S	Z_D_S	Z_D_S	Z_E_S	Z_E_S	Z_E_S	Ewais	E_A_S	E_A_S	E_A_S	E_B_S	E_B_S	E_B_S	E_B_S	E_C_S	E_C_S	E_D_S	E_D_S	E_E_S
Zebda	1.0																														
Z_A_S	0.9	1.0																													
Z_A_S	0.9	1.0	1.0																												
Z_A_S	0.9	1.0	1.0	1.0																											
Z_B_S	0.7	0.8	0.8	0.8	1.0																										
Z_B_S	0.9	1.0	1.0	1.0	0.8	1.0																									
Z_B_S	0.9	1.0	1.0	1.0	0.8	1.0	1.0																								
Z_C_S	0.9	0.9	0.9	0.9	0.8	0.9	0.9	1.0																							
Z_C_S	0.6	0.6	0.6	0.6	0.5	0.6	0.6	0.7	1.0																						
Z_C_S	0.9	1.0	1.0	1.0	0.8	1.0	1.0	0.9	0.6	1.0																					
Z_C_S	0.9	1.0	1.0	1.0	0.8	1.0	1.0	0.9	0.6	1.0	1.0																				
Z_C_S	0.9	1.0	1.0	1.0	0.8	1.0	1.0	0.9	0.6	1.0	1.0	1.0																			
Z_D_S	0.8	0.9	0.9	0.9	0.8	0.9	0.9	0.9	0.6	0.9	0.9	0.9	1.0																		
Z_D_S	0.7	0.8	0.8	0.8	0.7	0.8	0.8	0.8	0.5	0.8	0.8	0.8	0.9	1.0																	
Z_D_S	0.8	0.9	0.9	0.9	0.8	0.9	0.9	0.9	0.6	0.9	0.9	0.9	1.0	0.9	1.0																
Z_E_S	0.8	0.9	0.9	0.9	0.8	0.9	0.9	0.9	0.6	0.9	0.9	0.9	1.0	0.9	1.0	1.0															
Z_E_S	0.8	0.9	0.9	0.9	0.8	0.9	0.9	0.9	0.6	0.9	0.9	0.9	1.0	0.9	1.0	1.0	1.0														
Z_E_S	0.8	0.9	0.9	0.9	0.8	0.9	0.9	0.9	0.6	0.9	0.9	0.9	1.0	0.9	1.0	1.0	1.0	1.0													
Ewais	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	1.0												
E_A_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	1.0	1.0											
E_A_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	1.0	1.0	1.0										
E_A_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	1.0	1.0	1.0	1.0									
E_B_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.2	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.9	0.9	0.9	0.9	1.0								
E_B_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	1.0	1.0	1.0	1.0	0.9	1.0							
E_B_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	1.0	1.0	1.0	1.0	0.9	1.0	1.0						
E_B_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0					
E_C_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0				
E_C_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.2	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.9	0.9	0.9	0.9	1.0	0.9	0.9	0.9	0.9	1.0			
E_D_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	0.9	1.0		
E_D_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	1.0	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	0.9	1.0	1.0	
E_E_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.2	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.9	0.9	0.9	0.9	1.0	0.9	0.9	0.9	0.9	1.0	0.9	0.9	1.0
E_E_S	0.3	0.3	0.3	0.3	0.1	0.3	0.3	0.2	0.1	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.9	0.9	0.9	0.9	1.0	0.9	0.9	0.9	0.9	1.0	0.9	0.9	1.0

Whereas Z and E refers to Zebda and Ewais mothers, (A-E) refers to stone key and (S) refers to seedling key

CONCLUSION

The use of SSR analysis in the present study revealed an extensive amount of divergence leading to cultivar identification in mango. The level of polymorphism observed was high (100%), indicating a wide and diverse genetic based for 32 samples used. According to PIC, Ho and DP values, SSR markers seem to be the suitable technique for characterization mango genotypes. The genetic similarity values varied between 0.12 and 1.00 over 32 samples. This might shed more light on the genetic relatedness of mango cultivar sand assist breeders to set up the appropriate guidelines for successful breeding of mango cultivars based on the established relationships. Finally, this study could provide useful information to address breeding programs and germplasm resource management.

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تقدير الخصائص الجزيئية لبادرات ناشئة من صنفين من المانجو المصرية عديدة الأجنة باستخدام معلمات التتابعات المكررة البسيطة

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في هذه الدراسة تم تقدير الخصائص الجزيئية والتنوع الوراثي بين صنفين من أصناف المانجو المصرية التجارية عديدة الأجنة وهما صنفي العويس والزبدة بالإضافة لثلاثون بادرة نشئت من بذور كلاهما. حيث تم تضخيم ثمانية مواقع وراثية بمساعدة معلمات التتابعات المكررة البسيطة المتخصصة والتي تم اختيارها بناء على ثبات نتائجها وقدرتها على التمييز بين التراكيب الوراثية تحت الدراسة. وقد اظهر تحليل النتائج عدم فائدة الموقع الوراثي المعروف ب (LMM_15) في التمييز بين التراكيب الوراثية الاثنين والثلاثون حيث أنتج هذا الموقع حزمة واحدة في حين أن السبعة مواقع وراثية الأخرى أنتجت 22 حزمة أو أليل مع مستوى عالي من التباين وصل لـ (100%). تراوح عدد الأليلات الفعالة من 1,7 إلى 3,4 بمتوسط 1,47 لكل موقع وراثي في حين تراوحت نسبة الخلط داخل المواقع الوراثية المضخمة على مستوى جميع التراكيب الوراثية تراوحت من صفر إلى 0,75 بمتوسط 0,36 بينما كان مقدار ما يوفره كل موقع وراثي من معلومات عن الاختلافات الوراثية بين التراكيب الوراثية تحت الدراسة والمعروفة بـ (PIC value) يتراوح من 0,41 إلى 0,70 بمتوسط 0,57. هذا وقد كانت القدرة التمييزية للمواقع الوراثية (DP) ما بين 0,11 إلى 0,72 بمتوسط 0,50 لكل موقع حيث أظهر موقع ESTD_1 أكبر طاقة تمييزية وكفاءة في تحقيق أهدف الدراسة بقيمة 0,72. وفيما يتعلق بتحليل العلاقات الوراثية ودرجة التشابه، قسمت النتائج التراكيب الوراثية إلى مجموعتين رئيستين وفقا لدرجة التشابه والتي ظهرت ما بين المنخفضة بنسبة 0,12 إلى المرتفعة جدا بنسبة 100% بمتوسط 0,50 فيما بين الـ 32 تركيب وراثي. حيث قد ضمت المجموعة الأولى البادرات الناشئة من صنف العويس وتراوحت درجة التشابه بينهم من 0,94 إلى 100% في حين المجموعة الثانية قد ضمت البادرات الناشئة من الصنف زبدة بنسبة تشابه تراوحت من 0,52 إلى 100% وهذه المجموعة انقسمت بدورها لمجموعتين فرعيتين الأولى انفردت باحتوائها فقط علي البادرة المعروفة بكود (Z_C_S2) والتي ظهرت بأنها الأكثر اختلافا وتباعد عن باقي التراكيب الوراثية في حين المجموعة الفرعية الثانية تضمنت باقي البادرات الناشئة من صنف الزبدة والتي كانت أكثر تشابها فيما بينهم.تشير هذه الدراسة وتؤكد على أن معلمات التتابعات المكررة البسيطة مفيدة جدا وكفؤ في تمييز وتوصيف التراكيب الوراثية لفاكهة المانجو, بالإضافة إلى أن تقدير مستوي الاختلافات الوراثية وفهم طبيعة العلاقات الوراثية من شأنه المساعدة توفير معلومات مفيدة ليس فقط لصيانة وحماية هذه التراكيب الوراثية بل لاختيار المناسب منها كآباء في برامج التربية والتحسين.

الكلمات الدالة: المانجو، الخصائص الجزيئية، الاختلافات الوراثية، العلاقات الوراثية، معلمات التتابعات المكررة البسيطة.