# Efficiency Parameters of SSR Markers for Characterization of Some Mango Cultivars and Their Suitability in Molecular Bar-coding

*Manal Eid and M. A. Hussein

Department of Botany, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt
eid_manl@hotmail.com

**Abstract:** In the present study, **25** Egyptian cultivars of mango were characterized by means of - simple sequence repeats (SSRs) to distinguish the extent of genetic variation and to develop a fingerprinting key. Thirty five SSR markers selected based on their repeatability, scorability and their ability to discrete between cultivars. **35** SSR loci produced **219** alleles with high level of Polymorphism **(~100 per cent**). Primary allelic variability and the genetic bases of the cultivated germplasm were computed through parameters of percentage of polymorphic loci, observed number of alleles, effective number of alleles, observed heterozygosity, expected heterozygosity, fixation index and gene flow. The number of total alleles per locus varied from **3 to 10** alleles with an average of **6.25** across the genotypes. The effective number of alleles ranged between **2** to **6.25** with average value of **4.02.** The observed heterozygosity ranged from 0.**28 to 0.92** with average value of **0.62**. The expected heterozygosity ranged from **0.53** to **0.84** average value **of 0.72.** The results showed the mean of Fixation index was **0.13** whereas the mean gene flow was **1.71**. The mean polymorphic information content value of 0.**70**. The Marker index values for SSR ranged from **1.2** to **8.2** with an average of **4.46** per marker. The Resolving power values ranged from **2.4** to **3.76** with a mean of **3.17** Also, SSRs of diagnostic and curatorial importance were discerned as 'stand alone' molecular descriptors for bar coding the application of DNA sequences of standardized genetic markers for the identification of mango cultivars. The present study could be of much use for the introgression of new characters from cultivar to other, isolation of stable segregating markers, and selection of improved varieties and conservation of germplasm resources.

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## 1. Introduction

Mango (*Mangifera indica* L.) is known as the 'king of fruits' for its rich taste, flavor, color, production volume and diverse end usage. It belongs to plant family Anacardiaceae and it is a diploid fruit tree with 20 pairs of chromosomes and a small genome size of 439 Mbp (Singh *et al*., 2016). Genetic improvement of Mango cultivars is complicated by their reproductive biology. Some inherent characteristics including long juvenile phase, high level of heterozygosity, only one seed per fruit, and heavy fruit drop leading to low retention of crossed fruits (Iyer and Schnell, 2009). The cross-pollination nature and a wide range of prevailing agro-climatic conditions have contributed to its wide genetic diversity in mango (Mukherjee, 1972). In addition, polyembryony in mango complicates breeding schemes. In polyembryonic cultivars, seedlings arise from nucellar tissue or from a zygote, but distinguishing between the two can be complicated (Schnell *et al*., 1994). Hence, it is difficult to differentiate true novel forms from cultivated ones.

In Egypt, Mango economically ranked third after citrus and grapes. According to the statistics provided by the Ministry of Agriculture indicated that, a total of 184204 Feddan are planted by mangoes (2007). Several varieties grow in Egypt from different origins; from India and Sri Lanka; Hindi Bicenara, Long, Ewis and Mabroka and from Florida and South Africa; Carrie, Glenn, Keitt and Kent. Moreover, local varieties exist such as Zebda, Taimor, Mesk and Dabsha. These cultivars vary in the shape of the fruit, size, skin color, and flavor. However, crosses between these cultivars may result in homonymy and synonymy and may cause cultivar confusion (Parfitt *et al.,* 1991).

Traditionally, morphological characteristics have been used to discriminate different cultivars but this approach had limited success. Many economically important traits are controlled by multiple loci and it will be necessary to develop a comprehensive genetic linkage map to investigate them. More effective markers are needed to overcome these problems of identification. Notwithstanding the increasing use of DNA sequence-based approaches, fingerprinting techniques continue to be used for genomic profiling for characterization of germplasm and establishment of the identity of varieties /hybrids/ parental sources of Mango (Begum *et al.,* 2013).

Molecular markers, particularly including microsatellites or Simple Sequence Repeats (SSRs), are the most suitable tools for fingerprinting plant genotypes due to their high polymorphism, co-dominance and reproducibility (Ravishankar *et al*., 2011). Microsatellites are tandem repeats of 1–6 bp nucleotide motifs that are evenly distributed throughout eukaryotic genomes. They are abundant sources of variation in many organisms and have been widely used as genetic markers since their first description (Azmat *et al*., 2016). There were several reports on applications of mango microsatellite markers, including genetic diversity (Duval *et al*., 2005; Suprapaneni *et al.* 2013), cultivar identification (Eiadthong *et al*., 1999), and pedigree analysis (Olano *et al*., 2005). Viruel *et a*l. (2005) developed the first reported set of 16 SSR markers for mango, of which 14 produced the expected one or two amplification products per genotype. These 14 SSRs were used to evaluate 28 mango genotypes that included 14 Florida cultivars. Discrimination of all 28 genotypes was possible, and the average number of alleles per locus was 5.3**.** Schnell *et al*. (2005) developed a second set of 15 SSR markers and analyzed 59 Florida cultivars and four related species. Two of the SSRs were monomorphic among the Florida cultivars; the other 13 had an average number of alleles per locus of 4.2**.** Prevost and Wilkinson (1999) point out that such variation complicate the comparisons between studies. They urged to evolve some suitable function that should be strongly correlated to the proportion of the genotypes identification independent of number of genotypes studies. Powell *et* al. (1996**)** used parameters, namely, expected heterozygosity, multiplex ratio and marker index and resolving power as functions for comparison of the diagnostic capacity of ISSR primers in potato.

Therefore, in the present study, we utilized SSR-PCR assay to enhance genetic informativeness of parameters of SSR marker and to define a fingerprinting identification system. This may provide an additional tool for genetic studies in mangoes in future to investigate genetic relationships among wild species and cultivars of mango, construct a genetic map, carry out functional mapping, and perform marker-assisted selection.

## 2. Material and Methods

**Ethics statement—Field sampling**

The studied 25 mango cultivars are located on private orchards under Ismailia governorate conditions in Egypt, which are not designated as protected areas. The field sampling was done in consultation with the representatives of the owners that manage private orchards. Therefore, no specific permission was required for field sampling from the studied locations. Our study did not involve an endangered species. The cultivars arranged alphabetically are, Alphonse, Bullock's Heart, Company, Dabsha, Ewais, Fajri Kalan, Hindi-Besannara, Hindi -Khassa, Joolik, Keitt, Kent, Langra Benares, Mabrouka, Mesk, Mestkawy, Nabiel, Naomi, Pairi, Sedeka, Sennary, Sukari Momtaz, Taimour, Tommy Atkins, Tota Pari, and Zebda. These cultivars showed high production with high quality fruits.

**Samples collection**

From each cultivar three trees were used for collecting the leaves and from each tree three leaves were taken randomly for investigations.

**DNA Extraction**

The Genomic DNA from leaf samples was extracted by a modified Cetryl Trimethyl Ammonium Bromide (CTAB) method (Porebski *et.al.,* 1997).

**SSR Amplification**

Thirty five of the microsatellite markers used in this study were previously reported by Schnell *et al*. (2005), Viruel *et al*. (2005), (Duval *et al*. 2005) and (Honsho *et al.* 2005). ) These markers were synthesized by Oligo Macrogen, Seoul, korea. Forward primers were labeled with a fluorescent dye on the 5 end and all 35 primer pairs were used on all individuals for the analysis. Microsatellite loci names are listed in Table**1**. PCR amplification reactions were carried out as described by Schnell *et al*. (2005) in a thermos cycler (Eppendorf Master Cycler Gradient Eppendorf, Hamburg, Germany).

The analyses were repeated at least twice to assure the reproducibility of the results. PCR products were separated on 2 % agarose gel and stained with ethidium bromide to check the PCR amplification and determine approximately the size of the amplified fragments. After that, The PCR products of the Microsatellite were detected by electrophoresis on Polyacrylamide non-denaturing gels, because Microsatellite alleles may vary in length by only few base pairs. Therefore, 7 % Polyacrylamide gels were used to exact allele sizing of the SSR loci, and then stained with ethidium bromide solution and documented by gel documentation model. Quantity-one software was used to estimate the sizes of the products by comparison to size marker.

**Molecular data analysis:**

The simple sequence repeat (SSR) bands were scored visually on the basis of their presence (1) or absence (0), separately for each cultivar of mango and each SSR primer. The scores obtained using all polymorphic primers in the SSR analysis were then calculated for effective number of alleles **(*Ne*),** observed heterozygosity **(*Ho*),** expected heterozygosity (***He***), fixation index **(*F*),** estimate of gene flow (***Nm***), using Pop gene 1.31 (Yeh, *et al.*1999). Power Marker version 3.25 was used to

determine the polymorphism information content **(PIC)** (Liu & Muse 2005). Efficiency of polymorphism detection as the Marker index (MI), defined by Powell *et* al. (1996). The resolving capacity of primers (**Rp**) was determined according to Prevost & Wilkinson (1999) as Rp =ΣIb, where Ib (band informativeness) = $1-(2\times|0.5-p|)$, and p is the proportion of genotypes in samples containing the band.

**Table1.** List of polymorphic microsatellite primers used in this study

| No. | Locus | SSR primers sequence 5---»3 | No. | Locus | SSR primers sequence 5---»3 |
|---|---|---|---|---|---|
| 1 | LMMA_1 | F: ATGGAGACTAGAATGTACAGAG | 19 | MiSHRS_18 | F: AAACGAGGAAACAGAGCAC |
| | | R: ATTAAATCTCGTCCACAAGT | | | R: CAAGTACCTGCTGCAACTAG |
| 2 | LMMA_6 | F: ATATCTCAGGCTTCGAATGA | 20 | MiSHRS_33 | F: CGAGGAAGAGGAAGATTATGAC |
| | | R: TATTAATTTTCACAGACTATGTTC | | | R: CGAATACCATCCAGCAAAATAC |
| 3 | LMMA_7 | F: ATTTAACTCTTCAACTTTCAAC | 21 | MiSHRS_36 | F: GTTTTCATTCTCAAAATGTGTG |
| | | R: AGATTTAGTTTTGATTATGGAG | | | R: CTTTCATGTTCATAGATGCAA |
| 4 | LMMA_8 | F: CATGGAGTTGTGATACCTAC | 22 | MiSHRS_37 | F: CTCGCATTTCTCGCAGTC |
| | | R: CAGAGTTAGCCATATAGAGTG | | | R: TCCCTCCATTTAACCCTCC |
| 5 | LMMA_9 | F: TTGCAACTGATAACAAATATAG | 23 | MiSHRS_48 | F: TTTACCAAGCTAGGGTCA |
| | | R: TTCACATGACAGATATACACTT | | | R: CACTCTTAAACTATTCAACCA |
| 6 | LMMA_10 | F: TTCTTTAGACTAAGAGCACATT | 24 | mMiCIR_5 | F: GCCCTTGCATAAGTTG |
| | | R: AGTTACAGATCTTCTCCAATT | | | R: TAAGTGATGCTGCTGGT |
| 7 | LMMA_11 | F: ATTATTTACCCTACAGAGTGC | 25 | mMiCIR_8 | F: GACCCAACAAATCCAA |
| | | R: GTATTATCGGTAATGTCTTCAT | | | R: ACTGTGCAAACCAAAAG |
| 8 | LMMA_13 | F: CACAGCTCAATAAACTCTATG | 26 | mMiCIR_9 | F: AAAGATAAGATTGGGAAGAG |
| | | R: CATTATCCCTAATCTAATCATC | | | R: CGTAAGAAGAGCAAAGGT |
| 9 | LMMA_14 | F: ATTATCCCTATAATGCCCTAT | 27 | mMiCIR_13 | F: GCGTAAAGCTGTTGACTA |
| | | R: CTCGGTTAACCTTTGACTAC | | | R: TCATCTCCCTCAGAACA |
| 10 | LMMA_15 | F: AACTACTGTGGCTGACATAT | 28 | mMiCIR_14 | F: GAGGAACATAAAGATGGTG |
| | | R: CTGATTAACATAATGACCATCT | | | R: GACAAGATAAACAACTGGAA |
| 11 | LMMA_16 | F: ATAGATTCATATCTTCTTGCAT | 29 | mMiCIR_18 | F: CCTCAATCTCACTCAACA |
| | | R: TATAAATTATCATCTTCACTGC | | | R: ACCCCACAATCAAACTAC |
| 12 | MIAC_2 | F: GCTTTATCCACATCAATATCC | 30 | mMiCIR_21 | F: CCATTCTCCATCCAAA |
| | | R: TCCTACAATAACTTGCC | | | R: TGCATAGCAGAAAGAAGA |
| 13 | MIAC_3 | F: TAAGCTAAAAAGGTTATAG | 31 | mMiCIR_22 | F: TGTCTACCATCAAGTTCG |
| | | R: CCATAGGTGAATGTAGAGAG | | | R: GCTGTTGTTGCTTTACTG |
| 14 | MIAC_4 | F: CGTCATCCTTTACAGCGAACT | 32 | mMiCIR_25 | F: ATCCCCAGTAGCTTTGT |
| | | R: CATCTTTGATCATCCGAAAC | | | R: TGAGAGTTGGCAGTGTT |
| 15 | MIAC_5 | F: AATTATCCTATCCCTCGTATC | 33 | mMiCIR0_27 | F: ACGGTTTGAAGGTTTTAC |
| | | R: AGAAACATGATGTGAACC | | | R: ATCCAAGTTTCCTACTCCT |
| 16 | MIAC_6 | F: CGCTCTGTGAGAATCAAATGGT | 34 | mMiCIR_29 | F: GCGTGTCAATCTAGTGG |
| | | R: GGACTCTTATTAGCCAATGGGATG | | | R: GCTTTGGTAAAAGGATAAG |
| 17 | MiSHRS_1 | F: TAACAGCTTTGCTTGCCTCC | 35 | mMiCIR_30 | F: GCTCTTTCCTTGACCTT |
| | | R: TCCGCCGATAAACATCAGAC | | | R: TCAAAATCGTGTCATTTC |
| 18 | MiSHRS_4 | F: CCACGAATATCAACTGCTGCC | | | |
| | | R: TCTGACACTGCTCTTCCACC | | | |

## 3. Results and Discussion

### 3-1. SSR Marker informative

At present, SSRs are the most preferred marker types because they are highly polymorphic even between closely related lines, require low amounts of DNA, can be easily automated and allow high throughput screening, can be exchanged between laboratories and are highly transferable between populations. SSR markers are efficient, time consuming and cost-effective approaches for diversity analysis. Molecular marker analysis is an efficient method of assessing genetic heterogeneity within the cultivars of mango and PCR-based genomic polymorphism has been detected in several cultivars of mango (de Souza and Lima, 2004; Diaz *et al*., 2009; Rocha *et al*., 2012). However, from the 35 SSR loci analyses of 25 mango cultivars, either one or two PCR products were observed for each sample, representing homogeneity and heterogeneity, respectively. The results indicate that the mango cultivars studied are diploid plants and SSR loci detected multiple loci, which can be attributed to the allopolyploid nature of mango as described by Mukherjee (1972). All of 35 SSR loci produced 219 alleles with high level of Polymorphism (~100 per cent). The number of total alleles per locus varied

from 3 (LMMA- and mMiCIR_13) to 10 (MIAC_5) alleles with an average of 6.25 across the genotypes (Table**2**). According to the banding patterns obtained from 35 SSR loci, all 25 Mango cultivars tested in this study could be distinguished from each other. This is because of highly divergent cultivars were included in the study. Consequently, SSR analysis described in this work represents an effective method for cultivar identification. 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 between 2, for mMiCIR_13, and 6.25 for LMMA_1 and MIAC_5, with average value of 4.02. The total number of effective alleles produced by the 35 SSR loci was 140.75.

According to the selective standard of the microsatellite loci, it ought to have at least four alleles to be considered useful for the evaluation of genetic diversity. Bases on this criterion, the 35 microsatellite loci used in this study were useful for the evaluation of genetic diversity in 25Mango genotypes. These results imply that abundant genetic polymorphism exist in Mango cultivars.

**3-2. SSR Marker Performance:**

**Heterozygosity**

Heterozygosity refers to the presence of different alleles at one or more loci on homologous chromosomes. The observed heterozygosity (Ho) ranged from 0.28 in MIAC_2 and MiSHRS_48 to 0.92 in LMMA_8 with average value of 0.62. Some of markers showed a higher level of observed heterozygosity (Ho) than expected heterozygosity (He). It is likely that these results reflect the effects of out breeding derived from open pollinations and continuous flux of genes in the relatively small geographical region where these cultivars have undergone differentiation (Susana *et* al., 2015). (He) ranged from 0.53 in mMiCIR_14 to 0.84 in LMMA_1 and MIAC_5with average value of 0.72, indicating high polymorphism. 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 large number of repeat units (SSR unites) tend to show high mutational rate. As a result, any mutations in any one of the alleles may create a heterozygous condition (Bharathi, 2011). The measure of level of heterozygosity across loci can be used as an indicator of the amount of genetic variability (Zulkifli *et al.,* 2012).

**Polymorphic Information Content (PIC)**

The PIC values in Table2, were quite high which thirty four markers (97 %) show a PIC value more than 0.5 and are considered informative markers (Botstein *et al.*, 1980). LMMA_6 had higher PIC value (0.85) than mMiCIR_13 (0.4) for the similar number of alleles (3). This result indicated that PIC values depend not only on the number of alleles but also shared frequencies of those alleles (Smith *et al*., 2000). However, the lower PIC value for mMiCIR_13 - 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 mean PIC value of 0.70 reflected the high level of polymorphisms of the used set of microsatellites and heterogeneity in 25 mango genotypes. 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 among 59 Florida cultivars and four related species from the USDA germplasm collection for mango. This may probably be due to the different number of analyzed samples and the different diverse genotypes analyzed. The broad range of PIC values in present study was indicative of the presence of unique alleles in some cultivars which facilitates their differentiation from another. Generally, PIC values increased proportionally with increasing heterozygosity at a locus.

**Fixation Index**

The F per locus ranged from -0.28 (LMMA_15) to 0.65 (MiSHRS_48) and the average value was 0.13. It means that only 13 % of the total genetic variation was explained by differences among populations and most of the genetic diversity (87%) corresponded to differences among individuals within populations. According to Archack *et al*. (2014), superior chance seedling selected as mango cultivars led fixation of very high degree of heterozygosity, leading to high within region variations. MIAC_3 and MiSHRS_48 possessed the highest F value. These values indicated the important role of these two loci in inter-population differentiation.

The F values for 10 markers out of 35microsatellites were slightly negative (excess of heterozygotes). In some cases heterozygosity may be slightly overestimated for microsatellites, because of uncertainties in assigning the status of homozygotes when slippage bands interfere with the main `allelic' bands. Problems arising from misinterpretation of slippage bands could explain the negative F values of microsatellites (Degen *et al.,* 1999). However, the negative F values for 10 markers suggested that the true F measures for those markers are probably not significant different from 0 and indicated a limited role for those markers in the genetic differentiation of the mango cultivars involved in this study.

**Gene flow (Nm)**

Gene Flow (Nm) represents the number of effective migrants per generation. Main effect of gene flow is the homogenization of allele frequencies

between populations; more gene flow between them is important, more so they are expected similar. The results showed the mean gene flow was 1.71 (Table**2**).

According to Wright (1978), a gene flow value greater than one, leads to homogenization of populations. As this gene flow was estimated on the basis of the Fst parameter it cannot not be considered contemporaneous, but a consequence of the genetic history of these cultivars (Begum *et al*., 2013). High gene flow is correlated with elevated levels of genetic diversity in populations (Ruiz-Garcia *et al*. 2006). In the case of mango, flying pollinators (mango is an allogamus, cross pollinated species) and, especially, human intervention by transferring specimens from one population to another (Nybom and Bartish, 2000; Kiambi *et al*. 2005; Ward *et al*. 2005) may explain the high levels of gene flow detected. These results are agreeing with Díaz *et al*. (2009).

**Marker index (MI)**

Marker index is a feature of a marker which elucidates the discriminatory power of a marker and therefore it was calculate for all the markers. The MI values for SSR ranged from 1.2 to 8.2 with an average of 4.46 per marker. Highest values (8.2) were scored with MIAC_5 and the lowest value (1.2) was scored with mMiCIR_13. Seven markers showed high MI values < 6 result from poly allelic character of SSR loci in these cultivars.

**Resolving Power (RP)**

Resolving power depended on the distribution of the alleles within genotypes. The RP values ranged from 2.4(LMMA_15) to 3.76 (LMMA_10) with a mean of 3.17 (Table2). Nineteen markers (54%), showed RP values greater than the mean value, and were able to distinguish all the 25 mango cultivars evaluated in this study. However, the exact number of cultivars distinguishable by any SSR primer pair was not solely correlated with its RP value, but rather a combination of RP, PIC and the number of detectable SSR alleles.

**Table2.** Various parameters related to efficiency of 35 markers for SSR analysis in 25 Mango cultivars

| locus | No. allele | effective No | Ho | He | PIC | F | Nm | MI | RP |
|---|---|---|---|---|---|---|---|---|---|
| LMMA_1 | 9 | 6.25 | 0.76 | 0.84 | 0.82 | 0.1 | 2.53 | 7.38 | 3.52 |
| LMMA_6 | 3 | 2.94 | 0.68 | 0.66 | 0.85 | -0.03 | -8.58 | 1.74 | 3.36 |
| LMMA_7 | 7 | 4.54 | 0.84 | 0.78 | 0.75 | -0.07 | -3.38 | 5.25 | 2.88 |
| LMMA_8 | 8 | 4 | 0.92 | 0.75 | 0.72 | -0.23 | -1.34 | 5.76 | 3.48 |
| LMMA_9 | 5 | 3.57 | 0.44 | 0.72 | 0.68 | 0.39 | 0.39 | 3.4 | 2.88 |
| LMMA_10 | 8 | 4.35 | 0.88 | 0.77 | 0.75 | -0.14 | 2.03 | 6 | 3.76 |
| LMMA_11 | 7 | 5 | 0.72 | 0.8 | 0.77 | 0.1 | 2.25 | 5.39 | 3.44 |
| LMMA_13 | 5 | 3.57 | 0.56 | 0.72 | 0.68 | 0.22 | 0.089 | 3.4 | 3.04 |
| LMMA_14 | 7 | 4.76 | 0.68 | 0.79 | 0.76 | 0.14 | 1.53 | 5.32 | 3.36 |
| LMMA_15 | 6 | 3.22 | 0.88 | 0.69 | 0.64 | -0.28 | -1.14 | 3.84 | 2.4 |
| LMMA_16 | 5 | 3.22 | 0.68 | 0.69 | 0.64 | 0.01 | 24.75 | 3.2 | 3.36 |
| MIAC_2 | 5 | 3.22 | 0.28 | 0.69 | 0.63 | 0.59 | 0.17 | 3.15 | 2.56 |
| MIAC_3 | 7 | 5.26 | 0.32 | 0.81 | 0.78 | 0.61 | 0.16 | 5.46 | 2.65 |
| MIAC_4 | 4 | 3.7 | 0.56 | 0.73 | 0.69 | 0.23 | 0.84 | 2.76 | 3.12 |
| MIAC_5 | 10 | 6.25 | 0.6 | 0.84 | 0.82 | 0.29 | 0.61 | 8.2 | 3.2 |
| MIAC_6 | 9 | 5.26 | 0.72 | 0.81 | 0.79 | 0.11 | 2.02 | 7.11 | 3.44 |
| MiSHRS_1 | 5 | 3.7 | 0.4 | 0.73 | 0.69 | 0.45 | 0.31 | 3.45 | 2.8 |
| MiSHRS_4 | 5 | 3.22 | 0.68 | 0.69 | 0.65 | 0.01 | 24.75 | 3.25 | 3.36 |
| MiSHRS_18 | 4 | 2.94 | 0.64 | 0.66 | 0.59 | 0.03 | 8.08 | 2.36 | 3.28 |
| MiSHRS_33 | 8 | 4.17 | 0.8 | 0.76 | 0.74 | -0.05 | -5.25 | 5.92 | 3.62 |
| MiSHRS_36 | 5 | 2.38 | 0.59 | 0.58 | 0.53 | 0.03 | 8.08 | 2.65 | 3.12 |
| MiSHRS_37 | 7 | 3.22 | 0.72 | 0.69 | 0.65 | -0.04 | -6.5 | 4.55 | 3.44 |
| MiSHRS_48 | 7 | 4.76 | 0.28 | 0.79 | 0.76 | 0.65 | 0.13 | 5.32 | 2.56 |
| mMiCIR_5 | 6 | 3.57 | 0.76 | 0.72 | 0.67 | -0.06 | -4.42 | 4.02 | 3.36 |
| mMiCIR_8 | 8 | 5.88 | 0.88 | 0.83 | 0.81 | -0.06 | -4.42 | 6.48 | 3.56 |
| mMiCIR_9 | 6 | 4.76 | 0.72 | 0.79 | 0.75 | 0.09 | 2.53 | 4.5 | 3.52 |
| mMiCIR_13 | 3 | 2 | 0.56 | 0.5 | 0.4 | -0.12 | -2.33 | 1.2 | 3.12 |
| mMiCIR_14 | 4 | 2.13 | 0.48 | 0.53 | 0.49 | 0.09 | 2.53 | 1.96 | 2.88 |
| mMiCIR_18 | 9 | 5.55 | 0.48 | 0.82 | 0.79 | 0.42 | 0.63 | 7.11 | 2.96 |
| mMiCIR_21 | 4 | 2.44 | 0.36 | 0.59 | 0.54 | 0.39 | 0.39 | 2.16 | 2.68 |
| mMiCIR_22 | 9 | 5.26 | 0.76 | 0.81 | 0.79 | 0.06 | 3.92 | 7.11 | 3.52 |
| mMiCIR_25 | 5 | 2.94 | 0.32 | 0.66 | 0.61 | 0.52 | 0.24 | 3.05 | 2.64 |
| mMiCIR0_27 | 4 | 2.7 | 0.56 | 0.63 | 0.56 | 0.11 | 2.02 | 2.24 | 3.12 |
| mMiCIR_29 | 9 | 5.26 | 0.76 | 0.81 | 0.78 | 0.06 | 3.93 | 7.02 | 3.52 |
| mMiCIR_30 | 6 | 4.76 | 0.72 | 0.79 | 0.76 | 0.09 | 2.53 | 4.56 | 3.52 |
| Mean | 6.26 | 4.02 | 0.63 | 0.73 | 0.70 | 0.13 | 1.72 | 4.46 | 3.17 |

**3-3. Cultivar – Specific bands**

In addition, the microsatellite assay generated cultivar-specific -unique allele/s (those present in only one cultivar) in mango cultivars is screened. Forty-five unique alleles were detected in 27 SSR loci across 17 mango cultivars. The highest number of such alleles (3) was found for LMMA_8, MIAC_5, MiSHRS_37, mMiCIR_18 and mMiCIR_29. These unique alleles were of 265,266 and 271 bp amplified by LMMA_8 in Zebda, Company and Mestkay cultivars, respectively. Similarly, unique alleles of 112, 121 and 128 bp amplified by MIAC_5 in Mesk, Hendi-B and langra. MiSHRS_37 amplified unique alleles of 363, 356 and 344bp in Zebda, Fagri and Succary. MMiCIR_18 amplified unique alleles of 211, 202, 216 bp in Zebda, Fagri and Succary. MMiCIR_29 amplified unique alleles of 180, 179, 161 bp in Hendi-B, Kent and Mesk. However, Zebda cultivar had the highest unique number (10 alleles) whereas Keitte, Succary, Mabrouk, Joolik, Dabsha, Bullock's Heart, Alphonse cultivar had only one unique allele (Table **3**). Therefore, the 17 different mango cultivars in Table3 with their unique alleles are most likely to be genetically distinct cultivars that could be the result of some mechanism generating *de novo* variation at the SSR loci in the original cultivar. Existence of unique cultivar-specific allele (s) suggest that due to the hypermutability caused by dinucleotide repeats, individual DNA microsatellite sequences may be expected in any isolated mango cultivar. It is thus conceivable that the random changes in the frequencies of the alleles over several generations in the cultivar will give rise to distinct sequences. These results have shown that even though the genome of mango is allotetraploid and relatively large, the microsatellite allelic patterns generated through PCR are capable of individualizing cultivars. Presences of unique alleles that are specific to single cultivar were reported in previous studies (Begum *et al*., 2013). Further, we suggest this discrimination of cultivars can be carried out with just these selected microsatellites. This would be of enormous assistance for the establishment of proprietary rights and the determination of cultivar purity. This suggests that SSR markers will be useful in germplasm identification and also in breeding programs through marker assisted selection (MAS).

**Table3**. Cultivars-Specific bands obtained with various SSR primers

| **Cultivar** | **Primer producing specific band (s)** | **Size of the specific band (bp)** |
|---|---|---|
| Zebda | **MIAC_3** | 176 |
| | **mMiCIR_5** | 159 |
| | **LMMA_8** | 265 |
| | **LMMA_13** | 194 |
| | **mMiCIR_8** | 156 |
| | **MiSHRS_33** | 216 |
| | **MiSHRS_37** | 363 |
| | **mMiCIR_13** | 335 |
| | **mMiCIR_18** | 211 |
| | **mMiCIR0_27** | 260 |
| Fagri | **mMiCIR_5** | 173 |
| | **MiSHRS_18** | 105 |
| | **LMMA_1** | 212 |
| | **MIAC_2** | 172 |
| | **MiSHRS_37** | 356 |
| | **mMiCIR_18** | 202 |
| langra | **MIAC_5** | 128 |
| | **mMiCIR_14** | 159 |
| | **MiSHRS_36** | 185 |
| | **mMiCIR_25** | 232 |
| | **MIAC_6** | 305 |
| Hendi -B | **LMMA_7** | 214 |
| | **MIAC_5** | 121 |
| | **LMMA_16** | 207 |
| | **mMiCIR_29** | 180 |
| Nabiel | **LMMA_15** | 219 |
| | **mMiCIR_9** | 149 |
| | **MIAC_6** | 270 |
| company | **LMMA_1** | 208 |
| | **LMMA_8** | 266 |
| kent | **LMMA_15** | 221 |
| | **mMiCIR_29** | 179 |
| Mestkay | **LMMA_8** | 271 |

| Cultivar | Primer producing specific band (s) | Size of the specific band (bp) |
|---|---|---|
| | **mMiCIR_29** | 161 |
| Mesk | **MIAC_5** | 112 |
| | **LMMA_10** | 147 |
| Naomi | **LMMA 11** | 237 |
| | **mMiCIR_22** | 162 |
| keitt | **MiSHRS_36** | 181 |
| Succary | **MiSHRS_37** | 344 |
| Mabrouka | **mMiCIR_18** | 216 |
| Joolik | **MiSHRS_33** | 231 |
| Dabsha | **MiSHRS_48** | 224 |
| Bullock's Heart | **MiSHRS_48** | 203 |
| Alphonse | **mMiCIR_22** | 170 |
| Total number of cultivars: 17<br>17/25=68% | Number of primers generating cultivars –specific bands: 27<br>27/35=77% | Total number of specific bands: 45<br>45/219=20% |

### 3-4. Construction of DNA barcode

Identification by SSR markers of allele size had the advantage that can be subjected to pair-wise comparison to detect genotypic differences (Galbacs *et al.,* 2009). The resulting numerical data can be converted to real fingerprints by the construction of barcodes (Jeffrey *et al.,* 1985). We converted the SSR results to DNA barcodes according to Galbacs *et al*., (2009) method, by uncoupling the allele size and the corresponding SSR locus information and then sorting the allele size data from lowest to highest. Figure shows the allele size bars drawn to a linear scale for 25 of mango cultivars included in this study. The resulting barcode system is a visual representation of the data, allowing easy detection of genotypic differences. Microsatellite allele size values generated in different laboratories are known to differ by 1 to 4 base pairs due to different analytical and rounding methods (This *et al.,* 2004). As such laboratory-specific deviations tend to be systematic; they will cause a minor shift in the position of the size bars, but leave the overall structure of the barcode unchanged. The barcode system is a visual representation of the data and can facilitate an easy detection of genotypic differences. The integration of such DNA barcodes into internationally coordinated databases could provide useful tools for cultivar identification, intellectual property protection, or resolution of commercial disputes. Earlier, similar work reported in grape accessions ((Galbacs *et al.,* 2009) was utilized for Hungarian *Vitis* germplasm database management.

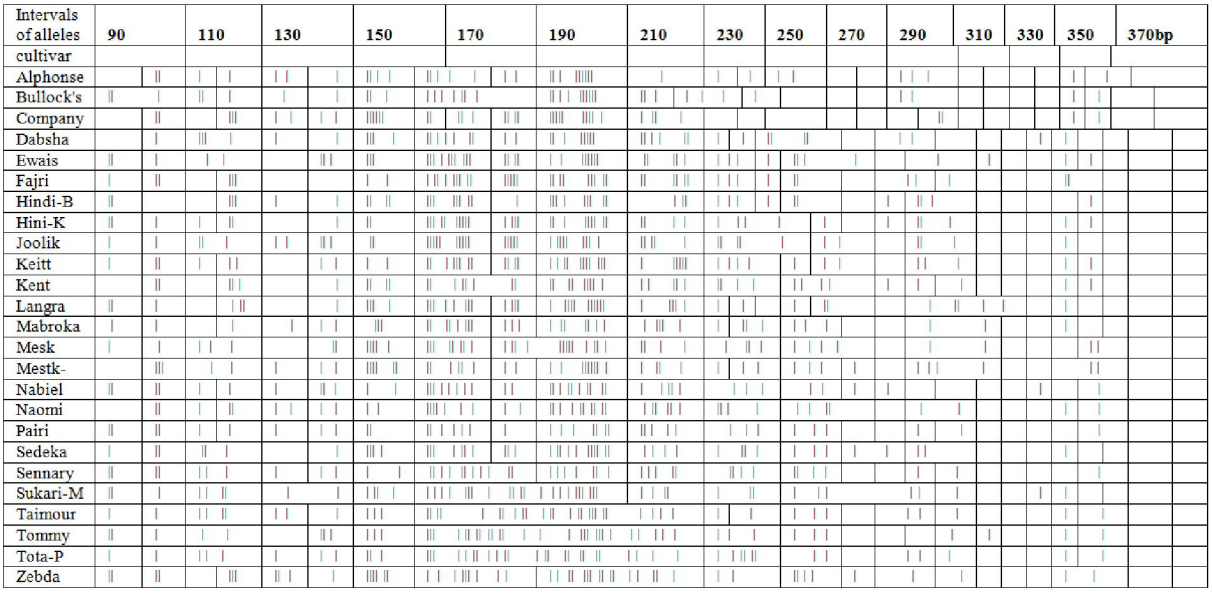


Figure. 1 Microsatellites based barcodes for 25 mango cultivars {Each row represents barcode for specific cultivar based on 35 microsatellite markers (SSR) and each bar indicates presence of allele at that locus}.

We concluded that the SSR marker was considered the most informative marker system because of its codominant and multiallelic nature. Other important properties of this marker system are the random distribution in the genome, high informativeness, robustness and reproducibility. In this study, Analyses of 35 SSR markers allow detailed parameters about genetic variation and characterization intra specific variation among mango cultivars. Additionally, the SSR data were converted to construct bar code, which according to Jeffrey *et al.* (1985) is the most important parameter for gene bank managers and curators, who want genotyping data that can be documented and handled easily in their database. The drawback of this technique is the hard work needed for marker development. However, this problem has been greatly simplified by the complete sequencing of the mango genome, and new primer sequences are frequently being added to the hundreds already available in the literature.

**Corresponding author:**
Dr. Manal Eid
Department of Botany
Faculty of Agriculture
New Ring Road-41522
Ismailia -Egypt
Email: eid_manl@hotmail.com

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