

MICROSATELLITE MARKERS FOR THE MOLECULAR CHARACTERISATION OF POTENTIALLY COMMERCIAL MANGO (*Mangifera indica*) PROGENIES

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ABSTRACT

Mango, or *Mangifera indica* as it is scientifically known, is a fruit crop that has significant importance to the global economy. This study characterised 10 commercially potential mango progenies using a total of 10 SSR markers. The analysis of the 10 SSR markers revealed the presence of 34 alleles. Each locus had 2–6 alleles equal to an average of 3.40 alleles per locus. The data also showed 0.115 (MiIHR21) to 0.692 (MiIHR28) polymorphic information content (PIC), equal to an average of 0.431. Each locus had heterozygosity and gene diversity values ranging from 0.044 (M1) to 0.957 (MiIHR29) and from 0.122 (MiIHR21) to 0.730 (MiIHR29), respectively, averaging 0.480 and 0.476 each. The range of the pairwise genetic distance was 0.000 (ND014↔ND019) to 0.611 (ND020↔SB003). The dendrogram analysis showed that each mango progeny was successfully differentiated using the 10 SSR markers except for ND014 and ND019. The allele combination using these 10 SSR markers could be used to develop a DNA profile for fingerprinting purposes for each progeny except for ND014 and ND019, which can only be differentiated with the addition of an extra marker. The developed DNA fingerprinting profile could be used for cultivar identification and authentication. Additionally, breeders could also use the developed DNA profiles for plant variety protection (PVP) purposes.

Key words: DNA fingerprinting, genetic relationship *Mangifera indica*, SSR marker

INTRODUCTION

Anacardiaceae is a family of predominantly tropical species, with a few temperate region representatives. It is also the family to which mango (*Mangifera indica* L. 2n=40) belongs. There are 600 species in this family. These species are further classified into 70 genera. Of these genera, pistachio (*Pistacia vera* L.) and cashew (*Anacardium occidentale* L.) are some of the cultivated species that have emerged. There are 70 species in the genus *Mangifera* and a further two subgenera under it (*Limus* and *Mangifera*). These species are mostly restricted to tropical Asia (Kostermans & Bompard, 1993). According to Singh *et al.* (2016), mango has an estimated genome size of 439Mb. It is also a diploid

(2n=40). In 2010, the world's largest producer of mango was India (42.2%), which produced 16.38 million metric tons in that period. China came in second at 4.35 million metric tons (11.3%) followed by Thailand at 2.55 million metric tons (6.6%) (Mitra, 2014).

Mango is a perennial crop that is hard to characterise. Phenotypic-based cultivar identification is used to characterise mangoes. In this method, similar to other fruit tree species, the morphological traits of mango based on descriptors are identified from its cultivars (IPGRI, 2006). This method is, however, limited to the number of discriminating traits and environmental influences, making it difficult to precisely assess the varietal variability of the fruits, thus rendering the method inaccurate. Further adding to the confusion is the nature of the mango, which is a cross-pollinated crop, so different

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sources of the mother tree could be used for seed and clonal propagation; therefore leading to intra- and inter-variety variability. Nevertheless, a powerful tool that could be used for crop species in crop breeding research has recently emerged, known as molecular technology (Surapaneni *et al.*, 2018).

Various molecular systems such as microsatellite markers (Viruel *et al.*, 2005), isozymes (Degani *et al.*, 1990), ISSRs (Pandit *et al.*, 2007) and AFLPs (Kashkush *et al.*, 2001) have been used to identify mango cultivars at the molecular level. Of these systems, microsatellites, also known as simple sequence repeats (SSRs) are codominant, allow reproducibility, and have a high level of polymorphism, making them the markers of choice for fingerprinting most plant species (Gupta & Varshney, 2000). Besides these advantages, SSRs perform particularly well in marker-assisted breeding and in the mapping of traits of interest of perennial crops. Therefore, this study aims to characterise and measure the genetic variability of ten potentially commercial mango progenies using microsatellite markers.

MATERIALS AND METHODS

Genomic DNA isolation and plant material

Ten potentially commercial mango progenies were selected for characterisation using microsatellite markers. The details of these progenies are summarised in Table 1. Five individuals of each progeny were collected before DNA extraction purposes. The Malaysian Agriculture Research and Development Institute (MARDI), Sintok, Malaysia (6° 28' 53"N, 100° 29' 00"E), containing the specific mango plots, supplied the progenies for this study. Young fresh leaves of each were collected and stored with silica gel in a zip-locked plastic bag for air-drying. A 96-well plate containing stainless steel beads (2.3 mm diameter) was prepared. Then, small fragments of the young leaves were punched onto the plate and were immediately frozen at -80°C for overnight. The frozen tissue was ground using

Tissue Lyser (Qiagen, Germany). After the tissue was ground, an extraction buffer (2% CTAB, pH8 of 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 0.05% β -mercaptoethanol) was immediately added. The protocol in Mace *et al.* (2003) was used to extract the total genomic DNA. Finally, 0.8% agarose gels were used to measure the DNA quality while Fluoraskan Ascent (Thermo Fisher Scientific, United States) was used to measure the DNA quantity.

SSR genotyping

The 10 SSR markers used in this study were taken from Ravishankar *et al.* (2011). These are listed in Table 2. The PCR was conducted as described by Schuelke (2000). First, a non-fluorescent labelled M13 sequence tail (TGTAACACGACGGCCAGT) and an M13 adapter labelled with a fluorescent dye (FAM, PET, NED or VIC) were used for ligating the primers (either forward or reverse). This was followed by preparing the PCR reaction mixture and making it up to a final volume of 10 μ L, containing 1 \times buffer (Invitrogen, United States), 10 μ M each of forward and reverse primer, 5 μ M fluorescence-labeled M13 adaptor, 2 μ M of each dNTP (Invitrogen, United States), 1U of Taq polymerase (Invitrogen, United States), and 0.1 μ L of bovine serum albumin (BSA) as a PCR enhancer. A GeneAmp® PCR System 9700 (Applied Biosystems, United States) was used to perform amplification. In the amplification process, initial denaturation was set to 94°C for 2 min. Then, 35 cycles were run according to the following setting: i) 4°C for 30 s ii) 41–65°C for 45 s, and ii) 72°C for 45 s. The final extension was set to 72°C for 5 min. Upon the completion of amplification, the PCR product was multiplexed using up to four primers with a combination of four different fluorescent dyes. Then, Hi-Di formamide and GeneScan 500 LIZ, standard molecular weight ladders (Applied Biosystems, United States), were mixed with the products. Finally, an ABI 3130xl Genetic Analyzer (Applied Biosystems, United States) was used to resolve the products.

Table 1. Characterization of ten progenies used in this study

Progeny ID	Mother Plant	Fruit Shape	Fruit Colour (ripe)
MK002	Mun Dian Kou	Oblong	Yellow
MM007	Masmuda	Elliptic	Yellow
ND001	Nam Dok Mai	Obovoid	Green
ND004	Nam Dok Mai	Oblong	Yellow
ND010	Nam Dok Mai	Elliptic	Yellow
ND014	Nam Dok Mai	Elliptic	Yellow
ND019	Nam Dok Mai	Elliptic	Yellow
ND020	Nam Dok Mai	Elliptic	Green
SB003	Strawberry	Obovoid	Red
SB019	Strawberry	Obovoid	Red

Statistical analysis

GeneMapper Version 5 (Thermo Fisher Scientific, United States) software was used to score the allele size. The methods described in Arif *et al.* (2010) were used as a basis for scoring and analysing the generated electropherograms. The number of alleles, the Major Allele Frequency, the Gene Diversity, the extent of heterozygosity, and the Polymorphism Information Content (PIC) of each microsatellite marker were calculated using PowerMarker software. The same program was also used to calculate the shared-allele based genetic-distance, as per Liu and Muse (2005). From the calculated genetic distance matrix of the shared microsatellite alleles, an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was constructed. Next, the UPGMA was used in the MEGA7 software (Kumar *et al.*, 2016) to generate the dendrogram for this study.

RESULTS AND DISCUSSION

Characterisation of microsatellite marker

After analysing the 10 microsatellite markers, the author observed 34 alleles in each locus, equal to 3.4 alleles per locus on average. There were two (MiIHR21) to six (MiIHR29) alleles and 0.115 (MiIHR21) to 0.692 (MiIHR28) PIC values, equal to an average of 0.431. The informativeness of the molecular markers can be assessed from the PIC value. As per Hildebrand *et al.* (1994), only five microsatellite markers (25%) were considered to be highly informative (PIC value > 0.7). Also, the value of heterozygosity per locus ranged from 0.000 (MiIHR10) to 0.8443 (MiIHR29). The details of the characterisation are summarised in Table 2. For the past 20 years or more, SSRs have remained the most trusted markers for genotyping plants, owing to their characteristics of informativeness, high polymorphic, multi-allele, co-dominant, experimentally reproducible, and transferability

across species (Vieira *et al.*, 2015). Moreover, microsatellites are greatly useful for diversity assessment measured in terms of genetic distance, cultivar identification through DNA fingerprinting, linkage and QTL analysis, and even in evolutionary studies (Kalia *et al.*, 2011). MiIHR29, which showed the presence of the highest number of alleles, is a simple dinucleotide perfect repeat motif microsatellite type with a high number of repeat motifs (n=10). Merrit *et al.* (2015) also found results consistent with this study, wherein the frequency of repeats were positively correlated with the number of alleles detected, suggesting that longer repetitive sequences tended to have higher chances and a greater extent of polymorphism irrespective of the mutation mechanism (Ellegren, 2004).

Molecular characterisation and genetic relationship among selected mango progenies

The UPGMA dendrogram (Figure 1), generated from shared allele matrix, indicate that there are two major clusters (Cluster A and Cluster B) for the studied progenies. There are two progenies, namely SB003 and SB009, in Cluster A. Both progenies are open-pollinated progenies from the same mother plant, namely the Strawberry cultivar. Meanwhile, the remaining eight progenies were clustered in Cluster B. Based on the results of the pairwise genetic distance (Table 3), the highest degree of variability between two progenies was observed for ND020 with SB003, as these had the highest genetic distance of 0.611. Meanwhile, the set of microsatellite markers used in this study was unable to differentiate the two progenies, ND014 and ND019, obtaining a value of 0.000 for the pairwise genetic distance between the two, which was also the lowest value. Both progenies were derived from the open-pollinated seed of the Nam Dok Mai variety. The limited number of microsatellite markers applied in this study resulted in its inability to differentiate the two mango progenies (ND014 and ND019). Further characterisation via the

Table 2. Characterization of ten microsatellite marker used in this study

Marker	Major Allele Frequency	Allele No	Gene Diversity	Heterozygosity	PIC
MiIHR01	0.413	3.000	0.651	0.044	0.577
MiIHR02	0.804	3.000	0.332	0.391	0.304
MiIHR12	0.818	3.000	0.313	0.364	0.289
MiIHR16	0.826	3.000	0.302	0.348	0.280
MiIHR20	0.685	3.000	0.464	0.630	0.401
MiIHR21	0.935	2.000	0.122	0.130	0.115
MiIHR26	0.620	3.000	0.544	0.500	0.486
MiIHR28	0.413	5.000	0.732	0.696	0.692
MiIHR29	0.402	6.000	0.730	0.957	0.690
MiIHR32	0.478	3.000	0.575	0.739	0.482
Mean	0.639	3.400	0.476	0.480	0.431

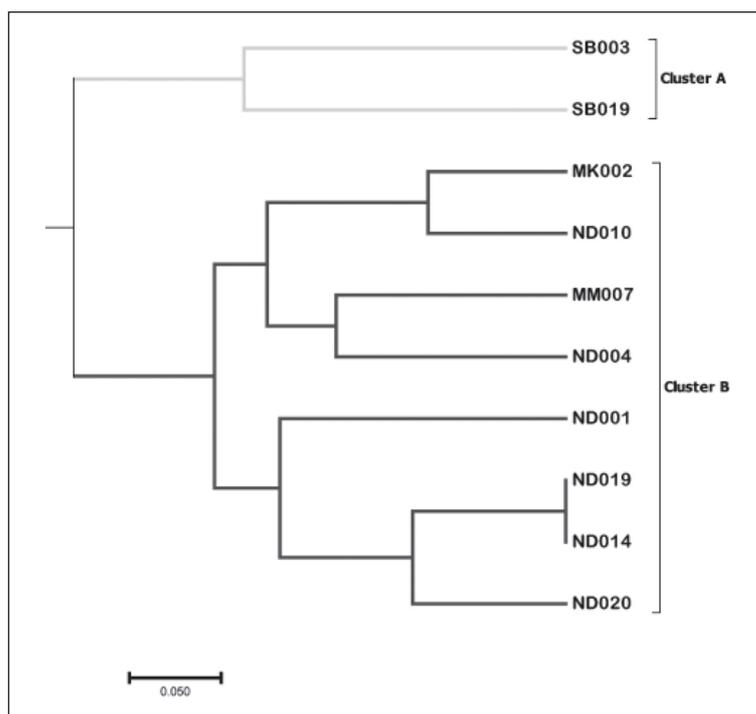


Fig. 1. Dendrogram analysis based on ten polymorphic microsatellite markers.

Table 3. Pairwise genetic distance of the studied progenies based on ten microsatellite markers

OTU	MK002	MM007	ND001	ND004	ND010	ND014	ND019	ND020	SB003	SB019
MK002		0.300	0.350	0.300	0.150	0.250	0.250	0.333	0.600	0.450
MM007	0.300		0.500	0.250	0.350	0.350	0.350	0.444	0.600	0.450
ND001	0.350	0.500		0.500	0.500	0.300	0.300	0.333	0.550	0.400
ND004	0.300	0.250	0.500		0.350	0.350	0.350	0.500	0.600	0.500
ND010	0.150	0.350	0.500	0.350		0.350	0.350	0.389	0.550	0.500
ND014	0.250	0.350	0.300	0.350	0.350		0.000	0.167	0.600	0.500
ND019	0.250	0.350	0.300	0.350	0.350	0.000		0.167	0.600	0.500
ND020	0.333	0.444	0.333	0.500	0.389	0.167	0.167		0.611	0.556
SB003	0.600	0.600	0.550	0.600	0.550	0.600	0.600	0.611		0.350
SB019	0.450	0.450	0.400	0.500	0.500	0.500	0.500	0.556	0.350	

addition of additional microsatellite markers is needed to differentiate both progenies. Smýkal *et al.* (2008) demonstrated that if an adequate number of microsatellite markers were used, the method could prove to have powerful discriminatory power and polymorphism information content, and could even perfectly identify all varieties, more so than the majority of biochemical, morphological, or DNA markers out there.

CONCLUSION

There are a few factors that contribute to the high degree of divergence observed among mango

hybrids. These include the high discriminatory power of the SSR markers, the different parental combinations of the mango hybrids, and the highly cross-pollinated nature of the mango crop. A cluster consists of the hybrids related to each other by descent. This study proved that SSR markers are not only useful for identifying the variety of mangoes, but also to estimate the heterosis for future mango breeding programmes.

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REFERENCES

- Arif, I.A., Khan, H.A., Shobrak, M., Al Homaidan, A.A., Al Sadoon, M., Al Farhan, A.H. & Bahkali, A.H. 2010. Interpretation of electrophoretograms of seven microsatellite loci to determine the genetic diversity of the Arabian Oryx. *Genetics and Molecular Research*, **9(1)**: 259-265.
- Degani, C., El-Batsri, R. & Gazit, S. 1990. Enzyme polymorphism in mango. *Journal of the American Society for Horticultural Science*. **115(5)**: 844-847.
- Ellegren, H. 2004. Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics*, **5(6)**: 435-445.
- Gupta, P.K. & Varshney, R.K. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, **113(3)**: 163-185.
- Hildebrand, C.E., David, C., Torney, C. & Wagner, P. 1994. Informativeness of polymorphic DNA markers. In: N.G. Cooper, NG (Ed.). *The human genome project: deciphering the blueprint of heredity*. University Science Books, Sausalito. 100-102 pp.
- International Plant Genetic Resources Institute (IPGRI). 2006. *Descriptors of Mango (Mangifera indica L.)*. International Plant Genetic Resources Institute, Rome, Italy. 60 pp.
- Kalia, R.K., Rai, M.K., Kalia, S., Singh, R. & Dhawan, A.K. (2011). Microsatellite markers: an overview of the recent progress in plants. *Euphytica*, **177(3)**: 309-334.
- Kashkush, K., Jinggui, F., Tomer, E., Hillel, J. & Lavi, U. 2001. Cultivar identification and genetic map of mango (*Mangifera indica*). *Euphytica*, **122(1)**: 129-136.
- Kostermans, A.J.G.H. & Bompard, J.M. 1993. *The mangoes: their botany, nomenclature, horticulture and utilization*. Academic Press, London.
- Kumar, S., Stecher, G. & Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33(7)**: 1870-1874.
- Liu, K. & Muse, S.V. 2005. PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics*, **21(9)**: 2128-2129.
- Mace, E.S., Buhariwalla, K.K., Buhariwalla, H.K. & Crouch, J.H. 2003. A high-throughput DNA extraction protocol for tropical molecular breeding programs. *Plant Molecular Biology Reporter*, **21(4)**: 459-460.
- Merritt, B.J., Culley, T.M., Avanesyan, A., Stokes, R. & Brzyski, J. 2015. An empirical review: characteristics of plant microsatellite markers that confer higher levels of genetic variation. *Applications in Plant Sciences*, **3(8)**: 1500025.
- Mitra, S.K. 2014. Mango production in the world – present situation and future prospect. In *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014)*, **IV 1111**: 287-296.
- 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(8)**: 1135-1141.
- 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(8)**: 1135-1141.
- Ravishankar, K.V., Mani, B.H.R., Anand, L. & Dinesh, M.R. 2011. Development of new microsatellite markers from Mango (*Mangifera indica*) and cross species amplification. *American Journal of Botany*, **98(4)**: e96-e99.
- Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*, **18(2)**: 233-234.
- Singh, N.K., Mahato, A.K., Jayaswal, P.K., Singh, A., Singh, S., Singh, N. & Rai, V. 2016. Origin, diversity and genome sequence of mango (*Mangifera indica L.*). *Indian Journal of History of Science*, **51(2)**: 355-368.
- Smykal, P., Horáček, J., Dostálová, R. & Hýbl, M. 2008. Variety discrimination in pea (*Pisum sativum L.*) by molecular, biochemical and morphological markers. *Journal of Applied Genetics*, **49(2)**: 155-166.
- Surapaneni, M., Vemireddy, L.R., Hameedunnisa, B., Reddy, B.P., Anwar, S.Y. & Siddiq, E.A. 2018. Genetic Variability and DNA Fingerprinting of Elite Mango Genotypes of India Using Microsatellite Markers. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, **89**: 1251-1258.
- Vieira, M.L.C., Santini, L., Diniz, A.L. & Munhoz, C.D.F. 2016. Microsatellite markers: what they mean and why they are so useful. *Genetics and Molecular Biology*, **39(3)**: 312-328.
- Viruel, M.A., Escribano, P., Barbieri, M., Ferri, M. & Hormaza, J.I. 2005. Fingerprinting, embryo type and geographic differentiation in mango (*Mangifera indica L.*, Anacardiaceae) with microsatellites. *Molecular Breeding*, **15(4)**: 383-393.

