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Validation of genome-data based simple sequence repeats for ascertaining hybridity in mango (*Mangifera indica*) hybrids

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ABSTRACT

The present study was carried to identify polymorphic SSRs derived from the whole draft genome data of mango (*Mangifera indica* L.) for their polymorphism between Amrapali and Sensation genotypes (parents) and their validation on the derived hybrid progenies. Fifty new hypervariable mango SSRs (HMSSRs) were screened against the parental genotypes. Out of which, 11 HMSSRs were polymorphic, 18 were monomorphic and 21 did not amplify at all. These polymorphic HMSSRs were then used for ascertaining the hybridity of the hybrid progenies. The genetic diversity indices of each SSR locus among mango hybrids were calculated using Power Marker ver. 3.25. The genetic diversity indices such as the number of alleles ranged from 2 (HMSSR35) to 5 (HMSSR57), gene diversity from 0.36 (HMSSR54) to 0.71 (HMSSR57 & HMSSR58), and PIC values from 0.35 (HMSSR69) to 0.66 (HMSSR57). The dendrogram generated using Neighbor joining tree divided the 22 genotypes into three distinct clades, viz. Clade 'A', Clade 'B' and Clade 'C'. The hybrids, viz. H-3-1, H-3-2, and H-7-4 were identified as true hybrids, since they were genetically more close to female parent (Amrapali), while hybrids H-3-3, H-3-11, H-11-1, H-12-5, H-1-6, H-1-13, H-12-8, H-12-10, H-3-12, H-1-1 and H-4-10 were more close to the male donor parent (Sensation) and also had red peel colour. In the present study, the 20 hybrids were confirmed to be the true hybrids since the bands unique to the two parents were present in them.

Keywords: Genetic relatedness, Genomic resources, Hybridity confirmation, Hypervariable SSRs

Mango (*Mangifera indica* L.) referred as king of fruits, is the most economically important fruit crop grown in tropics as well as sub-tropical regions across the globe. Since, mango is highly heterozygous, allopolyploid in nature, having a complex genetic makeup, meagre information available on the inheritance patterns of several agronomically important traits and almost negligible pre-selection criteria makes its breeding very difficult (Litz 2003). The breeding process is further complicated due to the existence of long juvenile period, heavy fruit drop, besides, requirement of large progeny population and acreage for making a meaningful evaluation of the hybrid progenies. Furthermore, it is possible that the pistil of a flower might have got cross-pollinated by several other undesirable pollens from different genotypes under a particular circumstance resulting in production of hybrids, which otherwise may not be the desired ones and result as a progeny of any random out-crossing. As a consequence, assessing the hybridity of progenies using a precise marker system is very important. Among the different molecular marker types, simple sequence repeats (SSRs) which are made up of 1–6 nucleotide repeats are most efficient owing to their desirable characteristics such as multi-allelic nature, codominant inheritance, hyper-variability, and high degree of reproducibility (Kalia *et al*. 2011). Earlier, SSRs have been used for ascertaining the hybridity in mango (Singh *et al*. 2015, Kumar *et al*. 2016, Lakhotia *et al*. 2016) and also in other crops (Subashini *et al*. 2014). The advancements made in genome resource generation in mango in terms of identification of large number of new SSRs (hypervariable mango SSRs) derived from the whole genome sequencing and transcriptomic data may be utilized for identification of polymorphic SSRs among parental genotypes. Therefore, the main objective of the investigation was to identify polymorphic SSRs between Amrapali and Sensation, i.e. the parental genotypes and accordingly validating it in the resultant hybrid (full-sib) progenies. This would further assist in understanding the segregation pattern of a particular locus in the bi-parental progenies, thereby aiding in genetic linkage mapping and identification of QTLs associated with the important horticultural traits in mango.

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MATERIALS AND METHODS

The study was carried at the Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi, during 2017–21. Twenty mango hybrids resulting from the cross Amrapali × Sensation, were used for validation of the hypervariable SSRs such as H-1-1, H-1-6, H-1-8, H-1-11, H-1-13, H-3-1, H-3-2, H-3-3, H-3-11, H-3-12, H-4-10, H-6-2, H-11-1, H-12-5, H-12-8, H-12-9, H-12-10, H-13-1, H-13-4 and H-7-4.

Plant material: The most prominently utilized parental mango genotypes at the ICAR-IARI hybridization programme, viz. Amrapali and Sensation and their 20 F_1 hybrids were selected for the study. Over 50 hypervariable SSRs designed from the whole genome draft data developed under the ICAR Network Project on Transgenic Crops-Mango Functional Genomics were utilized for validation in the present study (Supplementary Table 1).

DNA isolation and quantification: Fully grown green leaves were excised separately from the healthy plants. The leaves were wiped off with tissue paper and wrapped in aluminium foil. The samples were then brought to the laboratory in ice-box and maintained in deep freezer at -80°C for further use. Genomic DNA from the leaf samples was isolated by using cetyl trimethylammonium bromide (C-TAB) method developed by Doyle and Doyle (1987) with some modifications. The quality of the extracted DNA was estimated using agarose gel electrophoresis and quantified using Nanodrop® (Thermo-Scientific). The genomic DNA was electrophoresed on 0.8% agarose gel at 80–100 V and 70 mA for two hours in 1X TAE buffer. The gel was stained with ethidium bromide (0.33 μg/ml) and then observed on UV trans-illuminator. After assessment of quality and quantity, the DNA from each sample was diluted (20 ng/μl) in TE buffer.

PCR amplification: The PCR reaction for each marker was set using diluted DNA samples. The PCR reaction mixture (10 μl) comprised of 2 μl of 20 ng genomic DNA and 5 μl of Ready PCR Mix (One PCR™, GeneDireX), 1 μl (0.5 μl forward and 0.5 μl reverse) of 100 pmol primer and 2 μl of molecular grade water. The thermal profile followed for the SSR amplification comprised of initial denaturation at 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 57.4°C for 1 min and 72°C for 1 min. The final extension was done at 72°C for 10 min. The amplified DNA was thoroughly mixed with loading dye and then electrophoresed in 3.0% agarose gel in 1X TAE buffer. The DNA profiles were visualised on UV trans-illuminator and photographed on gel documentation system.

Scoring of bands: Number of polymorphic bands generated by each primer were scored. The dendrogram was constructed to know genetic relatedness between the individual hybrids using Neighbor joining tree method. High degree of genetic diversity was depicted among the hybrids. The genetic diversity indices such as, major allelic frequency, gene diversity, heterozygosity and polymorphism information content of each SSR locus were calculated using the software Power Marker ver. 3.25.

RESULTS AND DISCUSSION

Identification of polymorphic SSRs: In the present study, a total of 50 new hypervariable SSRs (HMSSRs) were screened against the parental mango genotypes (Amrapali and Sensation). Out of these, 11 HMSSRs (HMSSR35, 36, 53, 54, 55, 56, 57, 58, 60, 62, 69) were polymorphic; 18 HMSSRs (HMSSR31, 32, 33, 34, 40, 41, 44, 45, 47, 59, 61, 63, 64, 65, 66, 67, 68, 74) were monomorphic, while 21 markers did not amplify (HMSSR25, 26, 27, 28, 29, 30, 34, 37,38, 39, 42, 43, 46, 48, 49, 50, 51, 52, 71,72,73) at all. The identified polymorphic HMSSRs were further used for ascertaining the hybridity of F_1 hybrid progenies obtained from the Amrapali × Sensation cross. Primers, viz. HMSSR35, HMSSR36, HMSSR55, HMSSR56, HMSSR57, HMSSR60 and HMSSR62 were more informative and could be used for ascertaining hybridity of the progenies. Earlier, Srivastava *et al.* (2004) used various marker systems including SSR and successfully confirmed the parentage of commercial mango hybrids, viz. Mallika (Neelum × Dashehari), Amrapali (Dashehari × Neelum), Sindhu (Ratna × Alphonso) and Ratna (Neelum × Alphonso). Similarly, Lakhotia *et al*. (2016) used SSR markers to validate the hybridity of mango derived from cross between Alphonso × Vanraj.

Validation of primers: The informative polymorphic primers were validated on hybrid progenies (Amrapali × Sensation). Out of 11 polymorphic primers, only three primers (HMSSR35, HMSSR55, and HMSSR56) were efficient in confirming the hybridity in the progenies. The primer HMSSR35 showed good amplification, while rest of the polymorphic primers showed poor amplification in hybrids. Two specific bands were identified in hybrids (300 bp and 240 bp), while unique band for Amrapali was 300 bp and 240 bp for Sensation. Earlier also, SSRs have been widely used for such validation in several mango genotypes (Olano *et al*. 2005, Shamili *et al*. 2012, Kumar *et al*. 2016, Lakhotia *et al*. 2016).

Diversity statistics of HMSSRs loci: The genetic distance of hybrids was estimated using the Power Marker and used for cluster analysis. For each SSR locus amplified among the mango hybrids and parents, diversity indices such as the number of alleles, major allelic frequency, heterozygozity, and polymorphism information content were calculated (Table 1) using the Power Marker and used for cluster analysis. Eleven polymorphic SSR primers were used for generating banding pattern. The number of alleles detected varied from 2–3 (HMSSR35, 36, 53, 54, 55, 56), and 4–5 (HMSSR57, 58, 60, 62, 69). The average number of alleles per primer pair was 3.27. The allele size ranged from 150 bp (HMSSR62) to 300 bp (HMSSR36).

The gene diversity value ranged from 0.3615 (HMSSR54) to 0.3788 (HMSSR69), 0.5000–0.6933 (HMSSR35, 53, 55, 56, 60, 62) and 0.7130 (HMSSR57, 58) with mean value of 0.5802. The present set of SSR markers gave Polymorphism information content (PIC) values ranging from 0.3538 (HMSSR69) to 0.6648 (HMSSR57) with a mean value of 0.5097. The heterozygosity of SSR

Table 1 SSR loci and primer details including allele size, polymorphism, gene diversity and heterogyzosity validated on 20 mango hybrid progenies.

Marker	Major allele frequency	Allele	Gene diversity	Hetero-zygosity	PIC
HMSSR35	0.5000	2.0000	0.5000	0.8182	0.3750
HMSSR36	0.5000	2.0000	0.5000	0.0000	0.3750
HMSSR53	0.3636	3.0000	0.6653	0.6364	0.5912
HMSSR54	0.7632	2.0000	0.3615	0.4737	0.2962
HMSSR55	0.5588	3.0000	0.5900	0.5294	0.5242
HMSSR56	0.5313	3.0000	0.5957	0.8125	0.5220
HMSSR57	0.3611	5.0000	0.7130	0.8889	0.6648
HMSSR58	0.3462	4.0000	0.7130	1.0000	0.6605
HMSSR60	0.3889	4.0000	0.6713	0.9444	0.6094
HMSSR62	0.4000	4.0000	0.6933	0.5333	0.6350
HMSSR69	0.7750	4.0000	0.3788	0.3500	0.3538
Mean	0.4989	3.2727	0.5802	0.6352	0.5097

PIC, Polymorphism information content.

markers ranged from 0.3500 (HMSSR69) to 1.0000 (HMSSR58) with mean value of 0.6352. In present experiment, some of the SSR primers detected multiple loci (HMSSR57), which can be attributed to the allopolyploid nature of mango. The diversity indices obtained in present study are in conformity with the results obtained by Begum *et al*. (2014) where high mean PIC value (0.47) was noted for all loci indicating their high discriminatory power. Recently, Razak *et al*. (2020) characterized 10 commercially potential mango progenies using 10 SSR markers. It was shown that the PIC values ranged from 0.115 (MiIIHR21) to 0.692 (MiIIHR28) with an average of 0.431. Markers with higher PIC values are said to possess higher potential to reveal allelic variation. The average PIC value of SSR markers confirmed by different researchers varied with number of SSR markers used and also the number of genotypes tested. These highly polymorphic markers can also aid in mango breeding by allowing true F_1 hybrids to be identified more easily.

Genetic diversity analysis: The genetic relationship among mango hybrids was determined based on Neighbor joining tree method, which depicted high degree of genetic diversity amongst them. The hybrids and their parents were differentiated based upon the genetic distance with respect to their dissimilarity towards each other. The data of 11 SSR loci was used for cluster analysis and tree generation. The dendrogram generated from Neighbor joining tree distributed the 22 genotypes into three distinct clades (Fig 1). The clade 'A' consists of Amrapali and its hybrids such as H-3-1, H-3-2, and H-7-4, clade 'B' consist of H-13-4, and H-12-9 and Clade 'C' was again bifurcated into two sub-clades, viz. clade 'C' 1 and clade 'C' 2 consisting of H-13-1, H-1-11, H-6-2, H-1-8 and clade 'C' 2, which consists of parent Sensation and 11 hybrids. This genetic relatedness of the hybrids with parent genotypes confirmed their closeness or uniqueness due to their distinct recombination. The hybrids namely, H-3-1, H-3-2, and H-7-4 were identified as true hybrids, which were noted more close to female parent (Amrapali), while hybrids H-3-3, H-3-11, H-11-1, H-12-5, H-1-6, H-1-13, H-12-8, H-12-10, H-3-12, H-1-1 and H-4-10 were more close to the donor parent (Sensation) and also had red peel colour. While other hybrids, namely, H-1-8, H-6-2, H-1-11 and H-13-1 grouped into different clades but were proven to be hybrids. Earlier, Singh *et al*. (2012) generated a dendrogram of 48 mango hybrids using 17 SSR markers based on UPGMA cluster analysis. Recently, 20 mango genotypes were classified to establish the dendrogram among genotypes using the cluster analysis based on 20 SSR loci (Ravishankar *et al*. 2017). Several others reports also showed that SSR markers can be effectively used for confirmation of hybridity, establishment of polymorphism

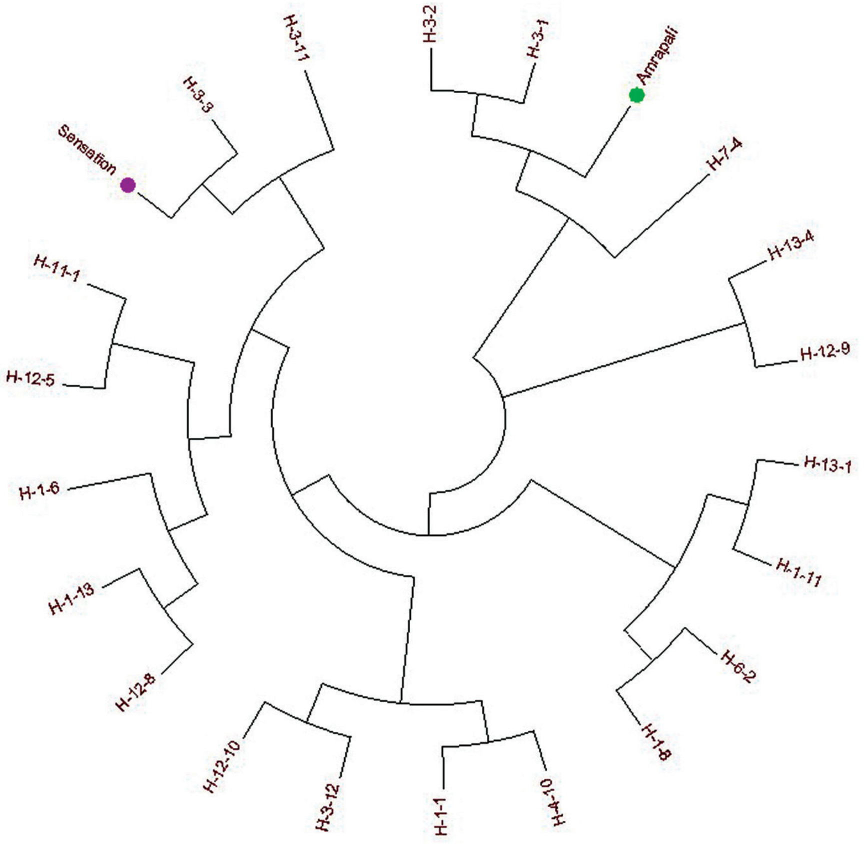

Fig 1 Dendrogram of parent mango genotypes (Amrapali and Sensation) and their hybrids based on Neighbor Joining (NJ) tree method.

and other diversity information in mango (Tang *et al*. 2006, Rao *et al*. 2008, Singh *et al*. 2012, Dillon *et al*. 2014, Subashini *et al*. 2014, Ravishankar *et al*. 2015, Yamanaka *et al*. 2019, Razak 2020).

Marker-assisted selection (MAS) has the potential to increase selection performance, by allowing for pointed and earlier selection, reducing the plant population size under evaluation and is cost effective. Markers can also help in the improvement of mango breeding by allowing real F_1 hybrids to be easily identified. Thus, these SSRs may help mango researchers for diverse molecular applications like marker-assisted selection, cultivar identification, DNA fingerprinting of the commercial varieties, selection of appropriate parents, maintaining the genetic purity of germplasm by discarding the duplicates. In the present study, 11 polymorphic HMSSRs markers were identified that could be effectively used for ascertaining the genetic purity of hybrids. Of these 11 markers, a set three (HMSSR35, HMSSR55, HMSSR56) were very effective in hybrid validation and thus found to be highly useful in diversity analysis (full-sib). The 20 hybrids were confirmed to be the true hybrids by using whole genome sequence derived hypervariable SSR markers.

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REFERENCES

Begum H, Reddy M T, Malathi S, Reddy P, Narshimulu G, Nagaraju J and Siddiq E A. 2014. Morphological and microsatellite analysis of intravarietal variability in ‘Cherukurasam’ cultivar of mango (*Mangifera indica* L.). *Jordan Journal of Agricultural Sciences* **10**(3): 452–71.

Dillon N L, Innes D J, Bally I S E, Wright C L, Devitt L C and Dietzgen R G. 2014. Expressed sequence tag-simple sequence repeat (EST-SSR) marker resources for diversity analysis of mango (*Mangifera indica* L.). *Diversity* **6**(1): 72–87.

Doyle J J and Doyle J L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* **19**: 11–15.

Gálvez-López D, Hernández-Delgado S, González-Paz M, Becerra-Leor E N, Salvador-Figueroa M and Mayek-Pérez N. 2009. Genetic analysis of mango landraces from Mexico based on molecular markers. *Plant Genetic Resources* **7**(3): 244–51.

Kalia R K, Rai M K, Kalia S, Singh R and Dhawan A K. 2011. Microsatellite markers: an overview of the recent progress in plants. *Euphytica* **177**(3): 309–34.

Kumar K, Srivastava M, Singh S K and Kumar V. 2016. Ascertaining hybridity of progenies in mango (*Mangifera indica* L.) using microsatellite (SSR) markers. *Journal of Agriculture and Ecology* **2**: 1–10.

Lakhotia P, Dinesh M R and Ravishankar K V. 2016. Validation of hybridity in mango (*Mangifera indica* L.) through markers. *BIOINFOLET-A Quarterly Journal of Life Sciences* **13**(1b): 145–48.

Litz R E. 2003. The mango: botany, production, and uses. Tropical Research and Education Centre, University of Florida, USA. CAB International, Wallingford, United Kingdom, pp 587.

Olano C T, Schnell R J, Quintanilia W E and Campbell R J. 2005. Pedigree analysis of Florida mango cultivars. (*In) Proceedings of the Florida State Horticultural Society*, pp. 192–97.

Rao M N, Soneji J R, Chen C, Huang S and Gmitter F G. 2008. Characterization of zygotic and nucellar seedlings from sour orange-like citrus rootstock candidates using RAPD and EST-SSR markers. *Tree Genetics and Genomes* **4**(1): 113–24.

Ravishankar K V, Bommisetty P, Bajpai A, Srivastava N, Mani B H, Vasugi C and Dinesh M R. 2015. Genetic diversity and population structure analysis of mango (*Mangifera indica*) cultivars assessed by microsatellite markers. *Trees* **29**(3): 775–83.

Ravishankar K V, Padmakar B, Lavanya B, Mani B H and Dinesh M R. 2017. Development and characterization of microsatellite loci from mango (*Mangifera indica* L.). *Indian Journal of Biotechnology* **16**(2): 250–53.

Razak S A B, Ariffin M A T, Mohamad S M S, Azman N H E N, Hassan M A and Sarip J. 2020. Microsatellite markers for the molecular characterization of potentially commercial mango (*Mangifera indica*) progenies. *Malaysian Applied Biology* **49**(3): 81–85.

Samal K C, Jena R C, Swain S S, Das B K and 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**(2): 195–13.

Shamili M, Fatahi R and Hormaza J I. 2012. Characterization and evaluation of genetic diversity of Iranian mango (*Mangifera indica* L.) genotypes using microsatellites. *Scientia Horticulturae* **148**: 230–34.

Singh A, Singh A K and Singh S K. 2012. SSR markers reveal genetic diversity in closely related mango hybrids. *Indian Journal of Horticulture* **69**(3): 299–05.

Singh A. 2015. ‘Bio-hardening and hybridity analysis of *in vitro* embryo cultured mango (*Mangifera indica* L.) plantlets’. PhD Thesis, Division of Fruits and Horticultural Technology ICAR-Indian Agricultural Research Institute New Delhi.

Srivastava A P, Chandra R and Ranade S A. 2004. Applicability of PCR based molecular markers for parentage analysis of mango (*Mangifera indica* L.) hybrids. *Indian Journal of Genetics and Plant Breeding* **64**(4): 275–80.

Subashini V, Shanmugapriya A and Yasodha R. 2014. Hybrid purity assessment in *Eucalyptus* F_1 hybrids using microsatellite markers. *Biotechnology* **4**(4): 367–73.

Tang J, Zhou S, Cheng M, Lin Q and Zhou Z. 2006. Identification of the F_1 hybrids of *Malus xiaojinensis* using RAPD and SSR molecular markers. *Chinese Agricultural Science Bulletin* **2**: 1–10.

Yamanaka S, Hosaka F, Matsumura M, Onoue-Makishi Y, Nashima K, Urasaki N and Yamamoto T. 2019. Genetic diversity and relatedness of mango cultivars assessed by SSR markers. *Breeding Science* **69**(2): 332–44.