DOI : 10.5958/0974-0112.2019.00001.X

Indian J. Hort. 76(1), March 2019: 1-7

Molecular fingerprints and genetic relatedness of traditional mango cultivars using SSR markers

Israr Ahmad*, **Muthukumar M, J. P. Verma, Anju Bajpai and S. Rajan**

Division of Crop Improvement and Biotechnology, Central Institute for Subtropical Horticulture, Lucknow 226101, Uttar Pradesh

ABSTRACT

Germplasm characterization is a prerequisite for conservation and utilization in breeding programmes. SSR markers were used to identify a set of polymorphic microsatellite loci and analysis of genetic diversity within mango cultivars. The present study was aimed to assess the intra-specific relationships among 37 diverse mango cultivars using 20 SSR markers. A total of 176 alleles were identified with an average of 8.8 allele per locus. About 19 primer pairs showed polymorphic bands with a number of alleles ranging from 2 to 18. Shannon diversity index and PIC varied in the ranges of 0.11-0.80 and 0.05-0.49, respectively. Marker index and resolving power ranged in the classes of 0.32-5.54 and 0.16-2.7, respectively. SSR markers are suitable for characterization and assessment across diverse mango cultivars and there is small difference in the genetic diversity with the species. Analysis of overall diversity of mango cultivars revealed the high intraspecies diversity (70%) between monoembryonic and polyembryonic mango cultivars. This high intraspecific diversity is due to low gene flow in population. The information of this research will be useful for selection and more efficient utilization of mango germplasm in breeding programs in the future.

Key words: *Mangifera indica*, microsatellite, genetic diversity.

INTRODUCTION

Mango (*Mangifera indica* L.), belonging to the family anacardiaceae, is called as the King of fruits. It has been evolved and cultivated in a wide range of climatic conditions of tropical and sub-tropical regions of the world and thus shows enormous level of adaptability. Globally, India is ranked first in production of fruits especially mango along with papaya and banana. It is commercially grown throughout the world with predominant area and production in countries like India, China, Australia, Philippines, Nigeria, Myanmar and Egypt (Singh *et al*., 12). The significance of the fruit is because of the variations exhibited in varieties due to attractive colours, savouring smell, delightful taste and high nutritional values (Mukherjee and Litz, 6). Every variety or cultivar of mango is characteristic of its own because they acquired any of these traits in combination. This diversity of characters with a continuous variation in each one creates complexity in the identification and classification of mango cultivars (Salvi and Gunjate, 10). India alone holds a vast diverse genetic pool of more than 1000 cultivars encompassing two major hot spot areas of primary centre of origin, North east India and southern peninsula (Mitra, 5).

Characterization of available cultivars is a prerequisite for their conservation as well as utilization in breeding programmes. Genetic analysis including assessment of genetic diversity, relatedness between or within species, population and individuals as well as genotype characterization, are central tasks for many disciplines of biological sciences. Conventionally, genetic analysis was dependent on morphological and/or biochemical markers. During the past few decades, classical strategies of genetic analysis have been increasingly complemented by molecular techniques. The most fundamental of these molecular techniques are DNA markers which portray genome sequence composition, enabling the detection of differences in the genetic information carried by the different indivi-duals. Therefore, these markers are powerful tools in genotype identification or fingerprinting and the estimation of relatedness between cultivars. Genetic characterization serves the twin purpose of the identification of cultivars and estimation of their genetic relatedness (Ravishankar *et al*., 7&8; Damodaran *et al*., 3). Characterization of genetic variation at the molecular level is possible using DNA based markers. SSR have gained considerable importance in genetic studies owing to their desirable attributes such as hypervariability, multiallelic nature, co-dominant inheritance and reproducibility. Based on informative and robustness the use of SSRs has been preferred to determine the genetic relationships among the mango cultivars. The present study was focussed on exploring SSR markers for assessment of genetic diversity in diverse mango cultivars.

*Corresponding author's Email: israr15ahmad@gmail.com

MATERIALS AND METHODS

Disease free fresh young leaves of 37 mango cultivars (Table 1) were collected from experimental farm of ICAR-CISH, Rehmankhera, Lucknow. DNA was extracted and purified using Qiagen DNeasy plant mini kit and purity of DNA was checked by running in 1% agarose-gel. PCR amplification was performed with 20 SSR primers (Table 2), in final volume of 10 µl containing 10x PCR buffer, 2 mM dNTPs, 0.5 unit of Taq DNA polymerase, 10 pmols/ reaction SSR primer and 50 ng of templet DNA. The PCR was performed by initial denaturation at 94°C for 5 min. followed by 40 cycles of denaturation at 94°C for 20 sec., annealing at 55/60°C for 30 sec., extension at 72°C for 30 sec. and final elongation at 72°C for 20 min. and the allele sizes were determined through three primer based PCR reaction followed by capillary electrophoresis coupled with ABI Biosystems DNA analyzer (ICRISAT, Hyderabad). In general, fluorescent-based analysis revealed single main peak in homozygotes and two different sized allelic peaks in heterozygotes in ABI Biosystems DNA analyzer. All the cultivars were scored for presence and absence of the SSR bands and the data were entered into a binary matrix as discrete variables. In order to avoid the genotyping errors of alleles, grouping of the SSR alleles per primer were grouped into distinct classes and transformed into a format that could be used for statistical analysis in NTSys 2.0 (Rohlf, 9). The 0/1 matrix was used to calculate similarity as Dice coefficient using SIMQUAL subroutine in similarity routine. The resultant similarity matrix was used to construct dendrogram by SAHN based UPGMA to infer genetic relationship (Rohlf, 9). The individual discriminative microsatellites bands at 18 loci were converted into binary matrix and used for barcode preparation using Crop DNA Fingerprint Database (NBPGR) (Bala, 1).

RESULTS AND DISCUSSION

Mango germplasm resources are enormous in India being a part of the primary centre of origin. as Morphological and molecular characterization of the germplasm resources in mango has been carried out using nuclear SSR markers for development of DNA fingerprint which serves as blueprints towards conservation and documentation of genetic resources (Bajpai *et al*., 2; Ravishankar *et al*., 7). Thus, germplasm collections and characterisation are important for genotypic and phenotypic analyses and as a genetic resource in breeding programs.

In the present study, about 20 SSR markers were used to amplify DNA fragments from 37 diverse mango cultivars which resulted in falling within the expected range of allele sizes (Table 2) as reported earlier. The allele number and size varied from data earlier reported by Ravishanker *et al* (8). In few primers, there was a deviation in the allelic configurations. The allele size ranges found in this study was slightly different found in Ravishanker *et al.* (8). The deviations in the observed allelic sizes over the earlier reported alleles may be due to the evolution of microsatellite alleles by slippages during replication (Hosseinzadeh-Colagar, 4). These alleles were found to be distinctly distinguishing the mango cultivars which is also evident in the SSR barcode.

SSR allelic data was transformed into different class intervals to minimize the genotyping errors.

Table 1. 37 mango cultivars studied.

Cultivar code	Cultivar	Cultivar code	Cultivar	Cultivar code	Cultivar
M-01	Munjjar Aamine	M-20	Vilas	M-35	Aamine Aabas
M-02	Matka Gola	M-21	Safeda Talukdar	M-36	Jawahari Safeda
M-03	Pan	M-22	Jamun	M-37	Bhagwant Khera
M-05	Allahabadi Chausa	M-23	Aamine abdul ahad Khan	M-39	Desi Gola
M-10	Markera	M-25	Taimuria	M-41	Vellai Kolamban
M-11	Safeda aamine	M-27	Ambika	M-42	Chandrakaran
M-12	Tukmi Heera	M-28	Aamine Khurd	M-43	Mylepelian
M-13	Sweta	M-30	Suraiya	M-44	Bappakai
M-15	Kacha Metha	M-31	Lucknowa Safeda	M-45	Olour
M-16	Desi Ramkela	M-32	Gole Bhadiyan	M-47	Moovandan
M-17	August	M-33	Samsul us samar	M-48	Nakkare
M-18	Aamine	M-34	Gilas	M-49	Goa
M-19	Zard aamine				

SSRs such as MiIIHR4, MiIIHR12, MiIIHR19, MiIIHR26, MiIIHR16 and MiIIHR36 (6 class intervals) were found to be the most variable, while MiIIHR09 was the least variable with only 2 allelic variants (293 and 294 bp). A total of 176 alleles were generated by 20 SSR markers with an average of 8.8 allele per locus. The cultivars studied revealed significant levels of DNA genetic diversity evident from the Shannon diversity index in the range of 0.11-0.80 and PIC values in the range of 0.05-0.49. Marker index and resolving power were ranged 0.32-5.54 and 0.16-2.7 (Table 3). Earlier, SDI and PIC values in mango using

Table 2. Description of SSR markers used in the present study.

SSR marker	Sequence (5'-3')	Expected band size	Observed Band size	Expected No of alleles	Observed No of alleles
MiIIHR04	F: CGTTTTTGACCCTCTTGAGC R: CCGCATACTTCCCTTCACAT	138-192	170-210	19	14
MiIIHR05	F: CTCTCCCTCACTTGCTCCAC R: AGACCACCGACAACGAAAAC	185-219	220-237	13	9
MiIIHR07	F: CCACTCAGCTAAATAGCCTCT R: TGCAGTCGGTAAAGTGATGG	159-185	176-188	6	12
MiIIHR09	F: GTTGTGACCGAGGCCTTAAA R: CTTTGACATCGCTGATCTGG	273-291	293-294	5	2
MiIIHR10	F: CGATTCAAGACGGAAAGGAA R: TTCAAGCACAGACGACCAAC	161-184	192-206	7	9
MiIIHR12	F: GCCCCATCAATACGATTGTC R: ATTTCCCACCATTGTCGTTG	154-188	175-199	9	11
MiIIHR13	F: CCCAGTTCCAACATCATCAG R: TTCCTCTGGAAGAGGGAAGA	169-194	189-209	9	11
MiIIHR15	F: CTAACCATTCGGCATCCTCT R: TGTGATAGAATGGCAAAAGAA	140-194	106-176	12	20
MiIIHR17	F: GCTTGCTTCCAACTGAGACC R: GCAAAATGCTCGGAGAAGAC	236-268	248-285	12	12
MiIIHR18	F: TCTGACGTCACCTCCTTTCA R: ATACTCGTGCCTCGTCCTGT	155-174	170-190	9	14
MiIIHR19	F: TGATATTTTCAGGGCCCAAG R: AAATGGCACAAGTGGGAAAG	177-208	159-224	13	11
MiIIHR23	F: TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC	132-154	131-175	13	18
MiIIHR24	F: GCTCAACGAACCCAACTGAT R: CCAGCATTCAATGAAGAAGTT	237-260	253-279	9	12
MiIIHR26	F: GCGAAAGAGGAGAGTGCAAG R: TCTATAAGTGCCCCCTCACG	131-167	152-186	16	19
MiIIHR28	F: GCGGTCGCAGACAAATTCTATAT R: CAACTCGAGATTGTCACATCTTT	101-124	121-143	18	12
MiIIHR30	F: AGCTATCGCCACAGCAAATC R: GTCTTCTTCTGGCTGCCAAC	190-209	211-235	11	9
MiIIHR31	F: TTCTGTTAGTGGCGGTGTTG R: CACCTCCTCCTCCTCCTCTT	211-230	230-249	8	9
MiIIHR32	F: TGGTGGTGTTTGTTTGCAGT R: ACCACCCGCAGTATTGAAAG	176-196	199-217	15	11
MiIIHR34	F: CTGAGTTTGGCAAGGGAGAG R: TTGATCCTTCACCACCATCA	222-244	241-263	8	12
MiIIHR36	F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAAGTAG	214-247	233-268	12	13

Table 3. Comparative analysis of diversity parameters based on 20 SSR markers.

SSR marker	Polymorphic information content	Marker index	Shannon diversity Index	Gene diversity	Resolving power	Average bands per cultivar
MillHR04	0.23	4.46	0.64	0.97	2.7	1.54
MillHR05	0.05	0.82	0.11	0.66	0.32	1.16
MillHR07	0.27	2.49	0.40	0.89	1.68	1.51
MillHR09	0.49	0.32	0.16	0.81	0.65	0.68
MillHR10	0.08	2.97	0.11	0.83	0.16	1.03
MillHR12	0.16	4.62	0.37	0.93	1.41	1.38
MillHR17	0.21	3.7	0.42	0.95	2.05	1.30
MillHR18	0.19	3.9	0.44	0.97	2.0	1.08
MillHR19	0.18	5.54	0.64	0.98	2.65	1.46
MillHR23	0.24	3.65	0.54	0.97	2.54	1.35
MillHR24	0.22	3.62	0.45	0.95	2.11	1.38
MillHR26	0.24	4.32	0.65	0.96	3.14	1.68
MillHR32	0.26	3.41	0.54	0.94	2.38	1.59
MillHR34	0.36	1.73	0.44	0.94	2.54	1.27
MillHR13	0.41	1.22	0.41	0.81	1.51	1.78
MillHR15	0.33	3.65	0.80	0.94	4.11	2.35
MillHR28	0.38	1.54	0.42	0.89	2.0	1.46
MillHR30	0.39	1.27	0.41	0.81	1.89	2.73
MillHR31	0.28	2.46	0.44	0.90	1.78	1.54
MillHR36	0.22	4.3	0.52	0.93	2.0	1.70

SSR markers have been reported falling in similar lines earlier reported by Bajpai et al., 2016. Similarly, the marker index and resolving power of the same primers were reported to be on par with the earlier reports (Ravishankar *et al.*, 7).

The dendrogram generated by UPGMA (Fig. 1) could differentiate among all the cultivars of mango with 28% similarity and was grouped into two different clusters with cluster I having three sub-cluster *i.e.* Ia, Ib and Ic. Mango cultivars falling in sub-cluster Ia included Munnajar Amine, Matka Gola, Gole Bhadiyan, Allahabadi Chausa, Safeda Talukdar, Pan, Tukmi Heera, Safeda amine, Lucknowa safeda, Amine, Suraiya, Zard amine, Samsul Samar, Sweta, Amine Khurd, Vilas, Jawahari Safeda, Amine Abas, Jamun, Amine abdul ahad khan and Taimuria differentiating at 43% similarity coefficient. Sub-cluster Ib contains Desi Ramkela, Bhagwant Khera, Ambika and Gilas with 41% similarity. Sub-cluster Ic contains Kacha Metha, August and Desi Gola with 31% similarity. Cluster II includes all the cultivars of polyembryony mango *i.e.* Olour, Chandrakaran, Nakkare, Bappakai, Moovandan, Mylepelian, Goa and Vellaicolamban with 30% similarity.

The same SSR allelic data was used to generate the SSR based barcode for the 37 mango cultivars (Fig. 2) which is a comprehensive presentation of alleles at multiple loci. Similar SSR based barcodes have been earlier reported in mango as DNA fingerprints for popular north, south, east and west indian cultivars of mango by several researchers (Bajpai *et al.*, 2; Ravishankar *et al.*, 7). Unique fingerprints of a genotype based on marker specific alleles serve as indicators of a particular region of the genome specific to a particular trait of horticultural importance. DNA fingerprinting has been thus used for individual identification of cultivars or rootstock, genetic diversity analysis and identification of suitable parents in crossing programs (Schnell *et al.*,11; Viruel *et al.*, 13). As with other vegetative propagated clonal crops, the differences among mango cultivars can result from epigenic modifications in response to the environment. The cultivars carrying the unique alleles may prove useful for introducing diversity in the future mango breeding programmes.

The use of genetic distance among cultivars is important for plant breeding programmes and the understanding of intra-specific genetic variation

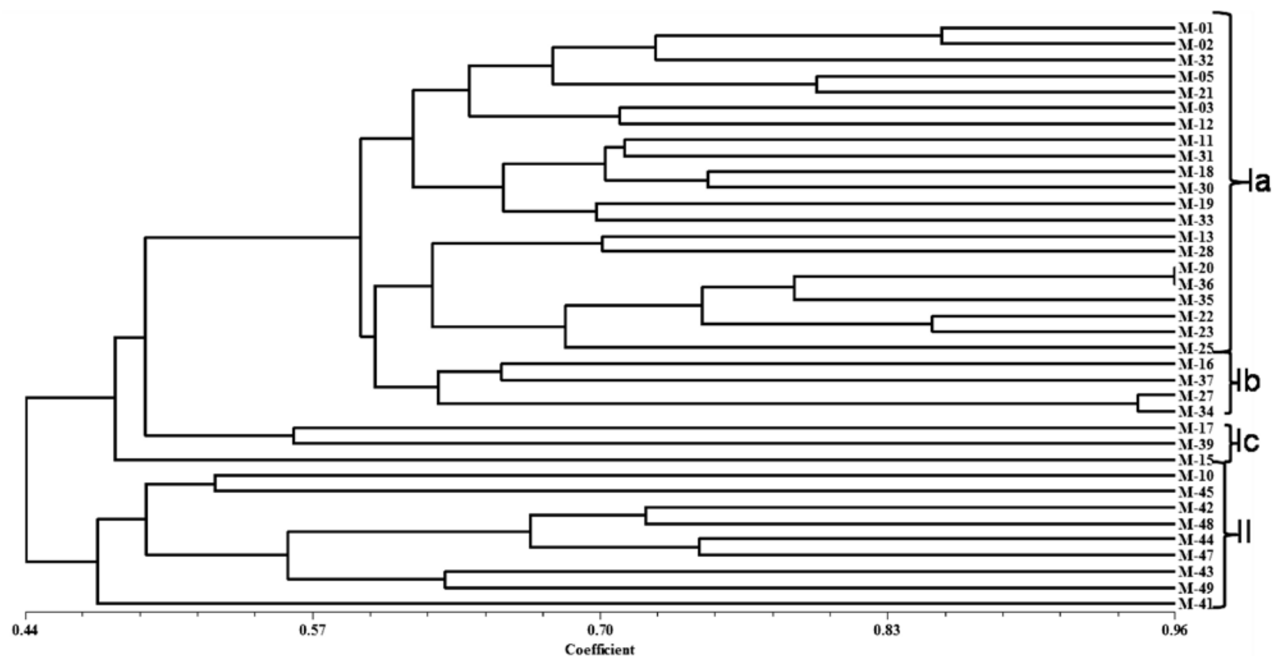

Fig. 1. Dendrogram generated by 20 SSR marker data based on UPGMA method.

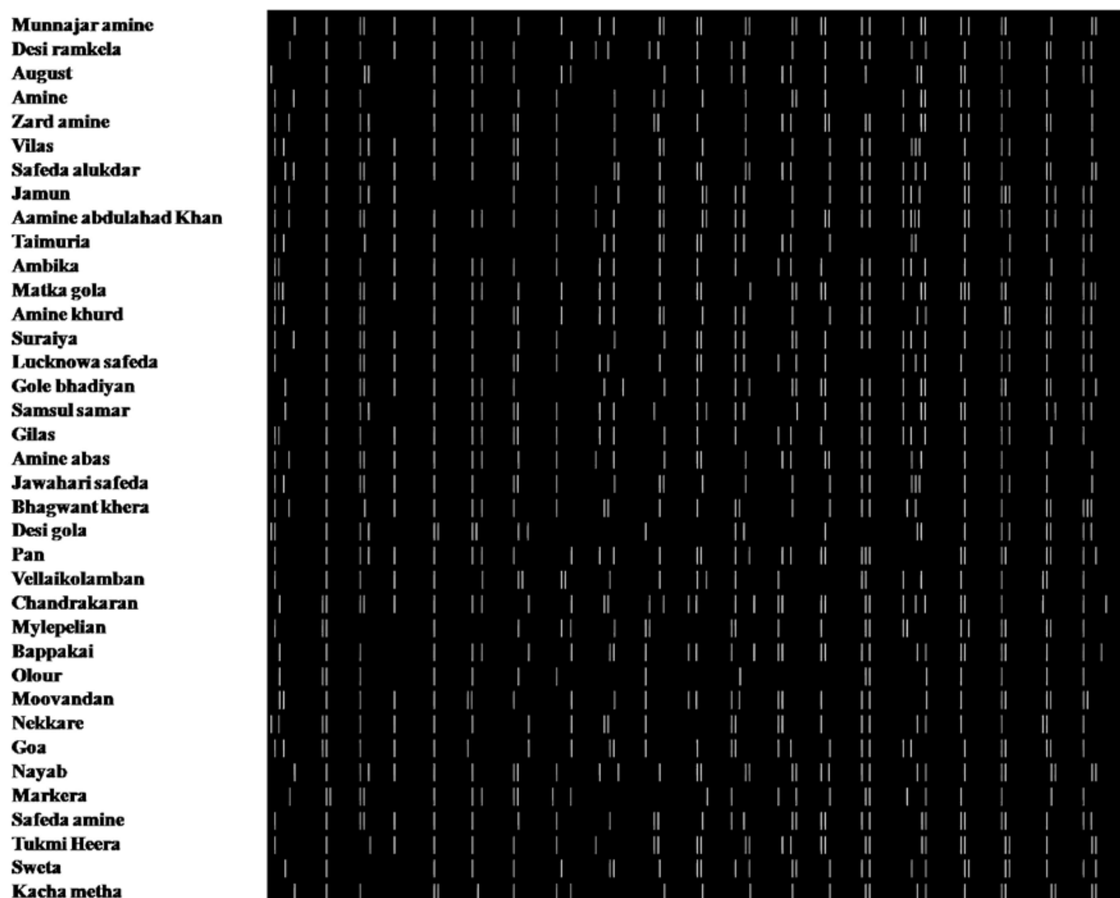

Fig. 2. SSR based barcode generated by 20 SSR markers among 37 mango cultivars.

patterns is also needed for effective genetic resource conservation and management. The SSR allelic data of the 37 mango cultivars were analyzed using NTSys 2.0 to understand the phylogenetic grouping. The dendogram generated by UPGMA method, grouped the 37 genotyped into two distinct clusters (cluster I and II) (Fig. 1). UPGMA cluster analysis revealed significant genetic diversity as evident from the Dice similarity coefficient ranging from 0.44 to 0.96 (Fig. 1). It is well known that autopolyploidy, out breeding, wide range of agro-climatic conditions prevailing in different mango growing regions have contributed immensely to the existing variability in mango. Yet, in the present study, the diversity among the 37 mango cultivars studied seems to be moderate. Moreover, the high genetic diversity within populations is explained by the breeding system since mango is an allogamous species (Viruel *et al*., 13; Ward *et al*., 14; Ravishankar *et al*., 8). Results indicated that two polyembryony mango cultivars were found to be far distant of the genetic relationship from the monoembryony cultivars. The analysis present evident distinction between the polyembryonic and monoembryonic varieties. This clustering did not only allow understanding the genetic relationship between the 37 mango cultivars but also help in selection of the pollen parents with desired traits for crossing and selection of elite cultivars in breeding programs.

From the present study it is evident that SSR markers are suitable for characterization and establishing genetic relationship across diverse mango cultivars as there was moderate to high genetic diversity within the species detected among the 37 mango cultivars assessed. Analysis of overall diversity of mango cultivars revealed the high intra-species diversity (70%). This diversity could be attributed to no gene flow in the population proving Hardy-Weinberg equilibrium. The close relationship across the species might be explained by either historical relationship in sharing common ancestor or more likely geographical proximity and large population size which favour genetic interchange. On the whole, the present study has given a brief insight into the genetic relatedness and the power of SSR markers in establishing the genetic divergence among mango cultivars. The study revealed that existing mango varieties are diverse, based on allele richness and genetic dissimilarity. Furthermore, clustering of the varieties based on UPGMA assembled these into two groups, the clustering pattern aligning well with the known fruit morphology. As mango is one of the priority crops for genetic conservation in India these mango varieties requires conservation and multiplication. Documentation of the diversity and distribution of these varieties for planting in community orchards will ensure sustainable conservation.

ACKNOWLEDGEMENTS

The authors are grateful to Indian Council of Agricultural Research, New Delhi for financial support.

REFERENCES

1. Bala, M. 2007. Software for DNA fingerprinting analysis. *Current Sci.* **93**: 1488-89.

2. Bajpai, A., Muthukumar, M., Ahmad, I., Ravishankar, K.V., Parthasarthy, V.A., Sthapit, B., Rao, Ramanatha., Verma, J.P. Rajan, S. 2016. Molecular and morphological diversity in locally grown non-commercial (heirloom) mango varieties of north India *J. Environ. Bio*. **37**: 221-28.

3. Damodaran, T., Kannan, R., Ahmed, I., Srivastava. R.C., Rai, R.B. and Umamaheshwari, S. 2012. Assessing genetic relationships among mango accessions of andaman islands using inter simple sequence repeat markers. *New Zealand J. Crop Hort. Sci.* **40**: 229-40.

4. Hosseinzadeh-Colagar, A., Haghighatnia, M.J., Amiri, Z., Mohadjerani, M., Tafrihi, M. 2016. Microsatellite (SSR) amplification by PCR usually led to polymorphic bands: Evidence which shows replication slippage occurs in extend or nascent DNA strands. *Mol. Bio. Res. Com.* **5**: 167-74.

5. Mitra, S.K. 2016. Mango production in the world – present situation and future prospect. *Acta Hort.* **1111**: 287-96.

6. Mukherjee, S.K. and Litz, R.E. 2009. Introduction: Botany and Importance. In: Litz RE (ed) The mango botany, production and uses, 2nd edn. CBI International, Wallingford, pp. 1-18.

7. Ravishankar, K.V., Bommisetty, P., Bajpai, A., Srivastava, N., Mani, B.H. and Vasugi, C. 2015. Genetic diversity and population structure analysis of mango (*Mangifera indica*) cultivars assessed by microsatellite markers. *Trees,* **29**: 1-9.

8. Ravishankar, K.V., Reddy Mani, B.H., Anand, L., Dinesh, M.R., 2011. Development of new microsatellite markers from mango (*Mangifera indica*) and cross-species amplification *American J. Bot*. **98**: 96-99.

9. Rohlf, F. J. 1998. NTSYS: numerical taxonomy and multivariate analysis system, 2nd ed. Exeter Software, State University of New York, Stony Brook, N.Y.

10. Salvi, M. J., and Gunjate, R. T. 1988. Mango breeding work in the Konkan region of Maharashtra state. *Acta Hort*. **231**: 100-102.

11. Schnell, R.J., Brown, J.S., Olano, C.T., Meerow, A.W., 2006. Mango genetic diversity analysis and pedigree inferences for Florida cultivars using microsatellite markers. *J. American Soc. Hort. Sci.* **131**: 1-11.

12. Singh, N K., Mahato, A.K., Jayaswal, P.K., Singh, A., Singh, Sangeeta., Singh, N., et al. 2016. Origin, Diversity and Genome Sequence of Mango (*Mangifera indica* L.). *Indian J. Hist. Sci*. **51**: 355-68.

13. Viruel, M.A., Escribano, P., Barbieri, M., Ferri, M. and Hormaza, J.I. 2005. Fingerprinting, embryo type and geographic differentiation in mango (*Mangifera indica* L.) Anacardiaceae with microsatellites. *Mol. Breed*. **15**: 383-93.

14. Ward, M., Dick, C.W., Gribel, R., Lemes, M., Caron, H. and Lowe, A.J. 2005. To self, or not to self. A review of outcrossing and pollen-mediated gene flow in neo tropical trees. *Heredity,* **95**: 246-54.

Received : July, 2017; Revised : February, 2019; Accepted : February, 2019