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Molecular and morphological diversity in locally grown non-commercial (heirloom) mango varieties of North India

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Abstract

Mango (*Mangifera indica* L.) has been cultivated and conserved in different agro-ecologies including Malihabad region in northern part of India, that is well known for housing diverse types (heirloom and commercial varieties). In the present study, 37 mango types comprising of 27 heirloom varieties from Malihabad region and 10 commercial varieties grown in North and Eastern India were assessed for morphological attributes and molecular diversity. The employed SSR markers amplified 2-13 alleles individually, cumulatively amplifying 124 alleles. These were studied for allelic diversity and genetic dissimilarity ranged from 0.035 to 0.892 arranging the varieties in three major clusters. The results revealed that majority of unique heirloom mangoes from Malihabad were different from the eastern part of the country. It is interesting to note Dashehari, a commercial variety from Malihabad was not aligned with heirloom varieties. Commercial varieties like Gulabkhas and Langra were placed in a separate group including Bombay Green, Himsagar, Dashehari, etc., indicating their dissimilarity with heirloom varieties at molecular level and thus, indicating importance for later from conservation point of view. Furthermore, the hierarchical clustering of varieties based on fruit morphology, assembled these into four groups largely influenced by fruit size. The maximum agreement subtree indicated seemingly good fit as thirteen varieties were arrayed in common grouping pattern. Appreciable dissimilarity among the heirloom varieties demonstrated by molecular analysis, underlines the importance for their on-farm conservation.

Key words

Allele richness, Heirloom varieties, Mango varieties, On farm conservation, SSR markers

Introduction

Mango (*Mangifera indica* L.) has been cultivated and conserved in different agro-ecologies including Malihabad region in Lucknow-Saharanpur mango belt of the country (Prakash and Dinesh, 2007). Malihabad falls in the northern belt of mango growing area in Uttar Pradesh, where Dashehari is main variety and home also to a wide range of traditional mango varieties and seedling populations. Traditional mango varieties of Malihabad are recognized for their unique characteristics and many of them have originated as open pollinated seedlings from the varieties introduced from different parts of country as, well as, selection of superior seedlings (Ram and Rajan, 2003). Open-pollinated seedling progenies have yielded development of many important varieties throughout the world *viz.,* 'Ceriese', 'Heidi' and 'Neldawn' in South Africa (Marais, 1992); 'Paiyur-1' from 'Neelum'; 'Dashehari-51' from 'Dashehari' in

India (Yadav, 1997; Negi, 1997); 'Rumang 'a chance seedling of 'Xiangmang' in China (Luo and He, 1996); 'Ataulfo' in Mexico (Galan Sauco, 2011). Similarly, seedling population of 'Chausa' is progenitor of most of the mango cultivars is grown in Pakistan Rajwana *et al.* (2011). Hence, regional top varieties of mango in India, such as Alphonso in Maharasthra, Malda in Bengal, Banganapalli in Andhra Pradesh and Tamil Nadu, exhibit unique quality attributes and accordingly mango varieties are specific in their climatic requirements and necessitate conscious *in situ* conservation programme within their area of origin and 'comfort zone'.

Some of the preferred varieties from North and Eastern parts of the country are Dashehari, Langra, Bombay Green, Fazli, Chausa, Langra, Kishen Bhog, Zardalu, Himsagar and Bombay being successfully grown in Malihabad. Besides these, many novel and unique cultivars are conserved in the public germplasm repositories, as well as, private orchards and homestead gardens in important mango growing belts of Uttar Pradesh, Punjab and Orissa (Rabbani and Singh, 1989; Parida and Rao, 1989; Singh *et al*., 2012). Rapid loss of diversity due to urbanization, industrialization and resultant felling of trees has been well documented (Khan *et al.*, 2015), highlighting the need for collection and conservation of non-commercial, heirloom varieties. Heirloom varieties are defined as local or regional varieties, passed down from generation to generation of gardeners/farmers, maintained by asexual means, also include old commercial varieties/antiques, presently not grown on commercial scale and limited to few in orchards. Historically, these are important non-commercial varieties that do not limit or restrict to a particular family or community. Pre-occupation of the agricultural research sector, mango growers and market sector, which are mainly concerned with few established commercial varieties might overlook and underestimate the potential of heirloom varieties. Conservation of genetic diversity is important for combating climate change challenges. Subsequently on-farm conservation of traditional varieties is being highly advocated (Rajan *et al*., 2014).

The present study was carried out to assess diversity pattern in locally grown, non-commercial (heirloom) varieties of Malihabad and their comparison with important north and east Indian cultivars. Furthermore, collating molecular data with quantitative fruit descriptors would

Table 1 : SSR loci and primer details including allele size and polymorphism

SSR Locus	Primer sequences (5´ -> 3´) F: forward, R: reverse	Allele size (bp)	Number of alleles	Polymorphic bands	Percent polymorphism	PIC
*Mi*IIHR17	F: GCTTGCTTCCAACTGAGACC					
	R: GCAAAATGCTCGGAGAAGAC	236-268	9	6	66.66667	0.536
*Mi*IIHR18	F: TCTGACGTCACCTCCTTTCA					
	R: ATACTCGTGCCTCGTCCTGT	155-174	12	7	58.33333	0.7684
*Mi*IIHR24	F: GCTCAACGAACCCAACTGAT					
	R: TCCAGCATTCAATGAAGAAGTT	238-260	9	5	55.55556	0.233
*Mi*IIHR19	F: TGATATTTTCAGGGCCCAAG					
	R: AAATGGCACAAGTGGGAAAG	177-208	13	11	84.61538	0.457
*Mi*IIHR23	F: TCTGACCCAACAAAGAACCA					
	R: TCCTCCTCGTCCTCATCATC	132-154	8	4	50	0.779
*Mi*IIHR15	F: CTAACCATTCGGCATCCTCT					
	R: TCTGTGATAGAATGGCAAAAGAA	186-209	13	11	84.61538	0.4711
*Mi*IIHR30	F: AGCTATCGCCACAGCAAATC					
	R: GTCTTCTTCTGGCTGCCAAC	190-210	12	9	75	0.3688
*Mi*IIHR31	F: TTCTGTTAGTGGCGGTGTTG					
	R: CACCTCCTCCTCCTCCTCTT	211-230	8	8	100	0.6179
*Mi*IIHR26	F: GCGAAAGAGGAGAGTGCAAG					
	R: TCTATAAGTGCCCCCTCACG	131-167	11	10	90.90909	0.3988
*Mi*IIHR32	F: TGGTGGTGTTTGTTTGCAGT					
	R: ACCACCCGCAGTATTGAAAG	150-194	7	4	57.14286	0.3097
*Mi*IIHR13	F: CCCAGTTCCAACATCATCAG					
	R: TTCCTCTGGAAGAGGGAAGA	169-194	5	3	60	0.0430
*Mi*IIHR36	F: TCTATAAGTGCCCCCTCACG					
	R: ACTGCCACCGTGGAAAGTAG	214-247	6	2	33.33333	0.3747
*Mi*IIHR34	F: CTGAGTTTGGCAAGGGAGAG					
	R: TTGATCCTTCACCACCATCA	223-245	9	5	55.55556	0.3666
*Mi*IIHR12	F: GCCCCATCAATACGATTGTC.					
	R: ATTTCCCACCATTGTCGTTG	169-175	2	0	0	0.4156
	Total / Average	124/ 8.85	85/ 6.07	Av.62.26623		

ascertain similarity in pattern of differentiation of the cultivars. This study would evaluate the molecular and morphological characteristics of selected heirloom varieties along with commercial types for establishing on-farm their conservation needs.

Materials and Methods

A total of 37 mango varieties including 10 commercial (Bombay Green, Chausa, Dashehari, Gulab Khas, Himsagar, Husneara, Kishan Bhog, Langra Banarsi, Malihabad Safeda, Fazri) and 27 heirloom (Alif Laila, Amin Abdul Ahad Khan, Amin Angoori, Amin Bulandbagh, Amin Dofasla, Amin Khurd Bulandbagh, Amin Prince, Amin Tehsil, Benzeer Sandila, Bhoodia, Fakirwala, Gilas, Hardil Aziz, Jafrani Shahbad, Kalan I, Karwa Sagar, Khas Ul Khas, Markea, Nasewala, Nayab, Nisar Pasand, Sadaphal, Sadaphal Malihabad, Sheredar, Shorab Sah, Surkha and Taimura) varieties were studied. Evaluation was carried out by taking fruit samples of each variety in five replicates for fruit characteristics *viz*., fruit weight, fruit length, fruit width, skin weight, stone weight, TSS and pulp percentage using standard descriptors and methodology described earlier (Rajan *et al*., 2009).

Genomic DNA isolation, PCR amplification and SSR based DNA barcode : Mango leaves were collected from identified locations and DNA was extracted and purified using modified cetyl trimethyl ammonium bromide (CTAB) method from 2 g of fresh leaf tissue as described by Dellaporta *et al.* (1983). Purity of DNA was checked by UV spectrophotometer and running in 1% agarose gel. Quantification of DNA in RNA free sample was done using UV spectrophotometer. Purity of DNA sample was determined by calculating A_{260}/A_{280}.

PCR amplification was performed on genomic DNA samples with 14 SSR primers (Table 1, Ravishankar *et al*., 2011). All PCR reactions were carried out in a final volume of 20 µl reaction mixture containing 50 ng DNA, 100 M of each dNTP, 2 mM $MgCl_2$, 400 nmoles primers, 1X *Taq* polymerase buffer, 0.5 unit of *Taq* DNA polymerase (Merck). Amplification was performed in programmable thermal cycler (Bio Rad) initial denaturation at 94 °C for 2 min followed by 35 cycle of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec, extension at 72 °C for one min and final elongation of at 72 °C for 5 min. The amplified DNA was separated in 2.5% agarose gel containing ethidium bromide in 1X TBE buffer at constant voltage ($5Vcm^{-1}$) for 3 hrs. These were photographed under UV excitation using Alpha Digi Doc system (Alpha Innotech Corporation). The amplified products were sequence characterized to arrive at correct allele size. The Gene Scan analysis was performed on

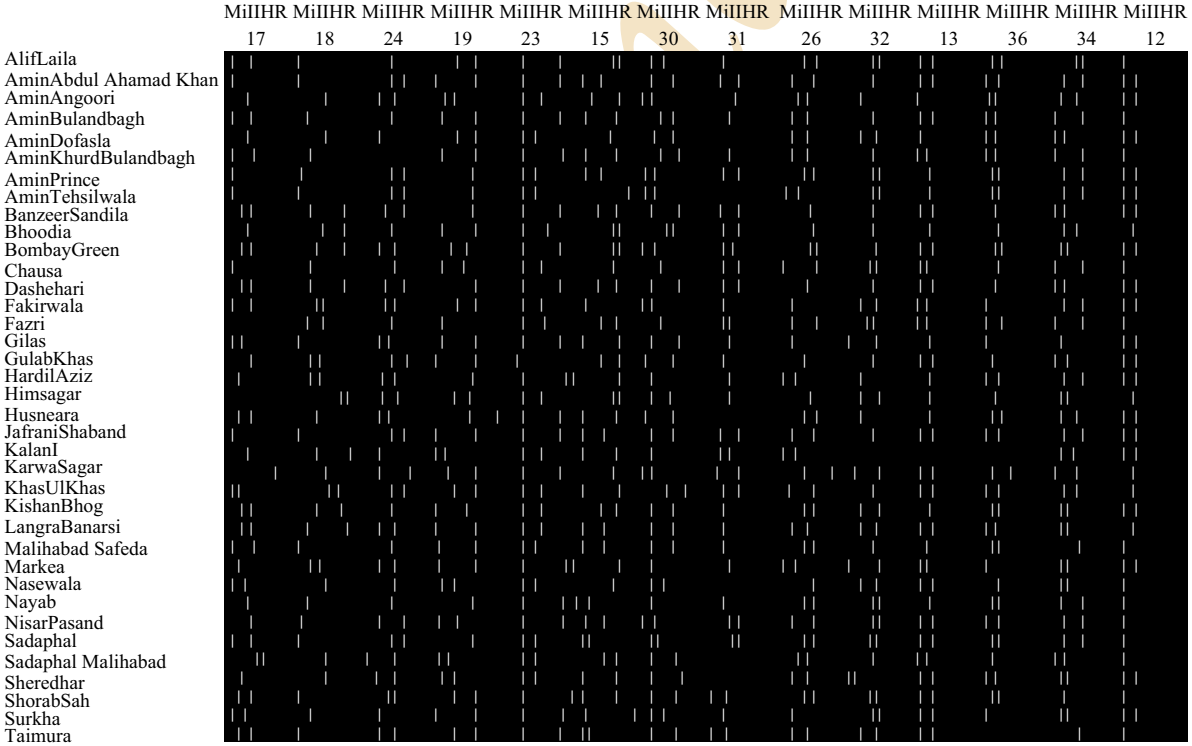

Fig. 1 : SSR barcode of thirty seven mango cultivars

amplified sample in Gene Mapper of Applied Biosystems.

The fluorescent-based analysis revealed single main peak in homozygotes and two different sized allelic peaks in heterozygotes in ABI Biosystems DNA analyzer. The individual discriminative microsatellites bands at 14 loci were converted into binary matrix using Crop DNA Fingerprint Database (NBPGR) (Bala, 2007) which was subsequently used for barcode generation. The individual alleles arranged in ascending order (with in a column) represent barcode specific to that locus. Cumulate arrangement of 14 loci make up barcode of particular accession.

Statistical analysis : Clustering analysis was carried out based on genetic distances using Hierarchical clustering method using DARwin v 5.0.158 software, while tree construction method utilized Wards minimum variance (Perrier *et al.,* 2003).

Results and Discussion

Mango accessions comprising of traditional farmers' varieties from Malihabad region and commercial varieties grown in North and Eastern India were analysed for molecular diversity using 14 polymorphic SSR markers. The employed SSR markers amplified 2-13 numbers of alleles individually, cumulatively amplifying 124 alleles (Table 1). The loci displayed 2-13 alleles, many of these corresponding to indels. Average bands per primer were 8.85, out of which 6.07 were polymorphic yielding an average polymorphism of 62.26. Based on allelic diversity and polymorphism, *Mi*IIHR 26 and 31 were most informative. Mangoes were analysed and characterized using SSR markers extensively, *viz.,* Duval (2005) amplified 4-14 alleles from 207 mango samples from Caribbean islands; while Honsho (2005) used 6 SSR markers for testing 36 cultivars amplifying 2-6 alleles at each marker locus, Dillon *et al.* (2014) recording 5.38 alleles per locus

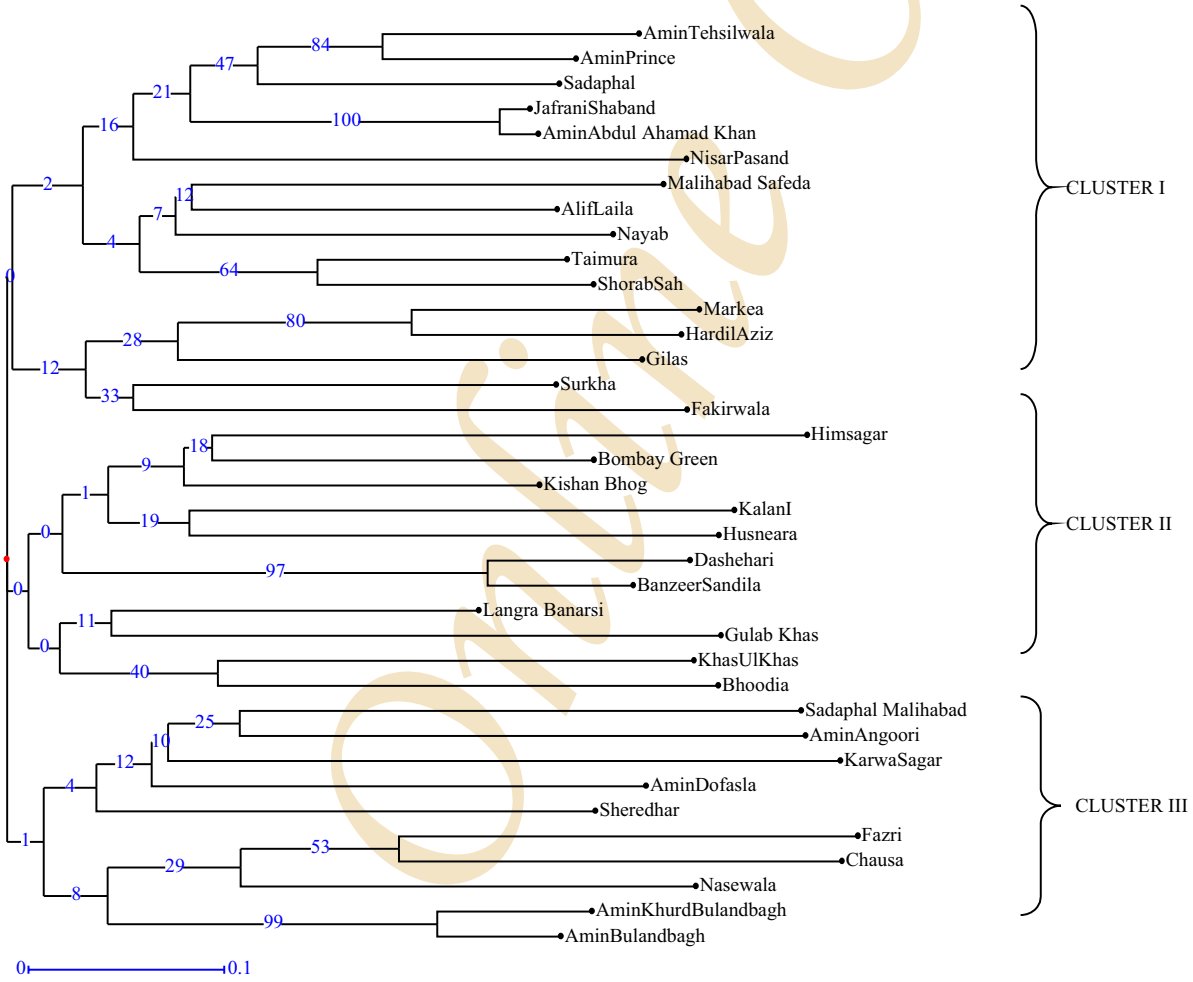

Fig. 2 : Dendogram depicting relationship among 37 mango cultivars based on molecular markers

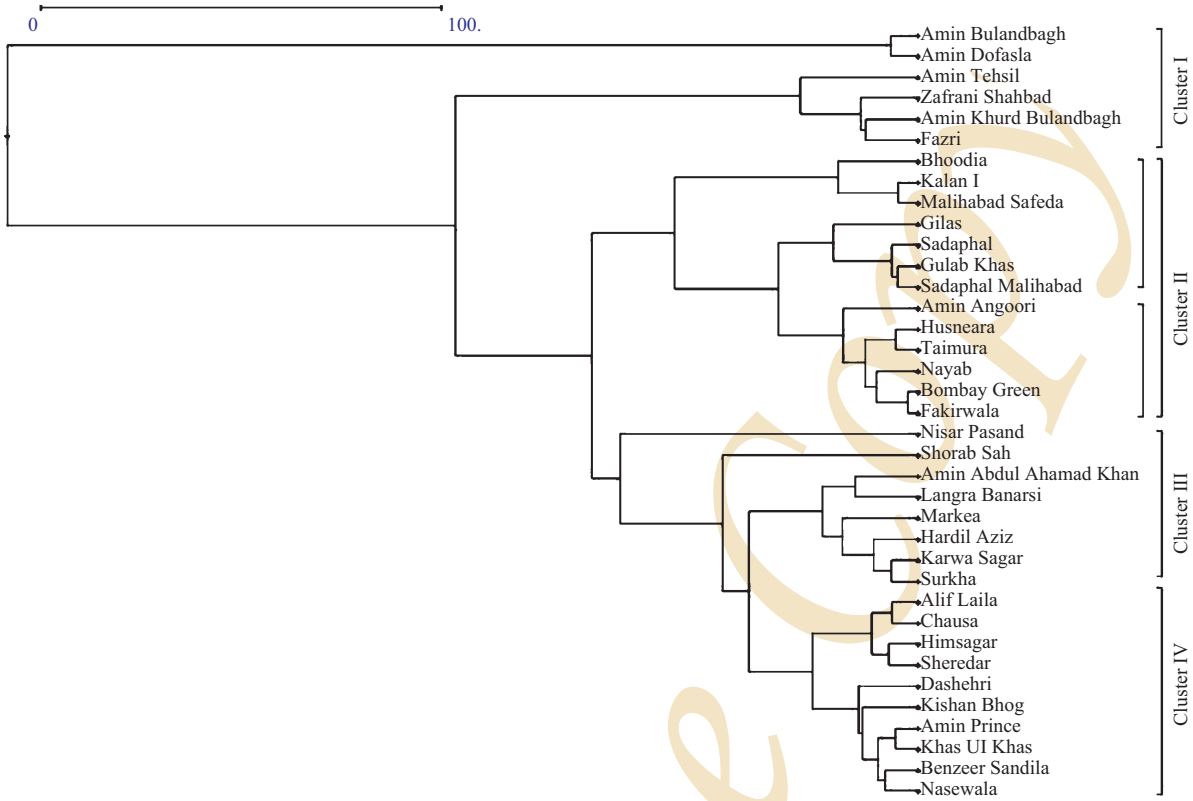

Fig. 3 : UPGMA Dendogram depicting relationship among 37 mango cultivars based on morphological characteristics

based on EST-SSR. Earlier, PCR based dominant marker system used for genetic analysis of mango cultivars from eastern and northern parts of the country, established genetic similarity in the range of 0.378 to 0.88 (Srivastava *et al.*, 2012).

PIC values ranged from 0.04030 in *Mi*IIHR 13 to 0.779 for *Mi*IIHR 23. Allelic data was computed for estimating allelic diversity and exposing the relationship. Genetic dissimilarity coefficient, ranged from 0.0357 (Amin Abdul Ahmad Khan and Jafrani Shaband), between two heirloom varieties from Malihabad region to 0.9230 (Fazri and Himsagar), it is evident that the region represents noticeable variability which could be assessed in small sample of 37 cultivars mainly from Malihabad and commercial varieties from Northern and Eastern parts of India. Uttar Pradesh, Andhra Pradesh and Telangana are leading mango producing states in India (Saxena and Gandhi, 2014), and important for mango varietal development as seedling selections (Yadava and Rajan, 1993) and home to a number of local cultivars/ heirloom varieties. Recent studies by Surapaneni *et al.* (2013) showed diversity limited by 44-88% similarity among mangoes from Andhra Pradesh, however, higher diversity exhibited in Malihabad indicating role played by on-farm conservation of non-commercial heirloom types in orchards and backyard gardens of Malihabad.

Furthermore, it is well established that SSR data lends itself well to digitalization and can be converted to real fingerprints by generation of barcodes (Jeffrey *et al*, 1985). Genotyping data of 37 mango samples with 14 SSR markers is presented in the form of barcode (Fig. 1), which is a comprehensive and cumulative presentation of alleles at 14 marker loci, arrangement of alleles is based on increasing molecular size (left to right). Earlier, similar work reported in grape accessions (Galbacs *et al.,* 2009) was utilized for Hungarian *Vitis* germplasm database management.

Phylogenetic dendrogram using NJ method resulted in grouping of 37 cultivars into three major clusters (Fig. 2). Majority of mangoes from Malihabad were clustered in separate groups (Cluster I and III) different from the commercial varieties. It is interesting to note that Dashehari, a commercial variety was placed in separate cluster II, along with Banzeer Sandila having high bootstrap value when

Fig. 4 : Dendogram depicting relationship among 37 mango cultivars based on molecular markers

compared to clustering pattern of traditional Malihabad varieties. Varieties from West Bengal and Bihar *viz*., Gulabkhas Langra, Bombay Green and Himsagar were grouped with Dashehari and Khas-Ul-Khas which are not native to eastern India. In spite of close genetic relationships of few of the cultivars studied, a relatively high average number of alleles per locus (8.85) and expected heterozygosity (0.65) were obtained in the present study, which also indicated high allele richness in population. Petit *et al*. (1998) advocated allele richness as better indicator of past demographic changes, and thus of interest in the context of diversity conservation. This view was further acknowledged by population geneticists as the most relevant criteria for measuring diversity should be allelic richness particularly in the context of genetic conservation (Jean-Michel *et al*. 2006). Previous studies by Schnell *et al.* (2005) utilizing 15 markers in 59 Florida mango cultivars and four related species reported amplification of two to seven alleles per locus Viruel *et al.* (2005) used 16 microsatellite primer pairs in collection of 28 mango cultivars of different origins reported 88 fragments with 16 SSRs, averaging 5.5 bands/SSR. In comparing earlier studies, utilizing SSR markers for mango diversity assessment, high polymorphism and allele richness (2-13 alleles per locus) was noted in the present study. Recent studies involving Indian varieties utilizing RAPD and ISSR markers precisely arranged 60 mango accessions from India into eight clusters, which correspond well with their pedigree relationship (Samal *et al.,* 2012) and the results indicated no clear-cut geographical separation, among East, West, North and South Indian mango cultivars, thereby supporting common genetic origin of mango. However, the present study suggested conflicting view as majority of Malihabad mangoes were arranged in separate clusters of two supporting regional nature of distribution of varieties. Rich reserves of genetic variability in Malihabad heirloom varieties depict large number of alleles that are important for long-term response to selection and survival of populations and species (Leberg, 2002).

Analysis of variance revealed that weight of fruit, pulp, stone and peel, pulp: stone ratio, length of fruit and stone had high heritability associated with high genetic

coefficient of variation, suggesting that these seven traits provided greater scope for further selection (Rajan *et al.,* 2009). The dendrogram generated from standardized fruit descriptors is presented Fig. 3, wherein the hierarchcal clustering based on UPGMA assembled these into four groups, the clustering pattern fitting very well with the known fruit morphology (fruit weight and size). The heirloom variety "Fakirwala" and commercial cultivar "Bombay Green" were clustered together in group II, having maximum distance (692.774) from "Bhoodia" and "Amin Dofasla" (Fig. 3). Maximum agreement sub tree drawn by comparing molecular and morphological distance matrices indicated seemingly good fit as thirteen varieties were arrayed with same grouping pattern (Fig. 4).This suggested pivotal role of SSR markers in estimating genetic similarity, perhaps due to random distribution of markers in the genome (genome coverage). As fruit parameters are most important traits for divergence studies and heritability (Preisigke *et al*., 2013), diversity structure description based on morphological distance is ostensibly dependable method.

Based on allele richness and genetic dissimilarity it can stated that existing heirloom varieties in Malihabad were diverse from commercial varieties. As mango is one of the priority crops for genetic conservation in India, the heirloom varieties of the region warrant multiplication for on-farm conservation. Cataloguing/documentation of diversity through community efforts and distribution of these varieties for planting in community orchards would ensure sustainable conservation because of their rare occurrence in the ecosystem.

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References

Bala, M.: Software for DNA fingerprinting analysis. *Curr. Sci.,* **93**, 1488-89 (2007).

Dellaporta, S.L., J., Wood and J.B. Hicks: A plant DNA minipreparation. *Plant Mol. Bio. Rep.,* **1**, 19-21 (1983).

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

Duval, M.F., J. Bunel, C. Sitbon and A.M. Risterucci: Development of microsatellite markers for mango (*Mangifera indica* L.) *Mol. Ecol. Notes,* **5,** 824–826(2005).

Galan Sauco V.: Current situation and future prospects of worldwide mango production and market. In: Global Conference on Augmentation Production and Utilization of Mango: Biotic and Abiotic Stresses, Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow(UP), India, pp. 27-39(2011).

Galbacs, Z., S. Molnár, G. Halász, P. Kozma, S. Hoffmann, L. Kovács, A. Veres, Z. Galli, A. Szőke, L.Heszky and E. Kiss: Identification of grapevine cultivars using microsatellite-based DNA barcodes, *Vitis,* **48**, 17–24(2009).

Honsho, C., K. Nishiyama, W. Eiadthong and K. Yonemori: Isolation and characterization of new microsatellite markers in mango (*Mangifera indica*). M*ol. Eco. Notes*, **5,** 152-154(2005).

Jean-Michel,C., J. Parrotta, E. Brockerhoff, M. Arbez, H. Jactel, A. Kremer, D. Lamb, K. O'Hara and B. Walters: Planted forests and biodiversity *J. For*., **104,** 65-77(2006).

Jeffrey, A.J., V. Wilson and S.L. Thein: Hypervariable 'minisatellite' regions in human DNA. *Nature,* **314**, 67-73(1985).

Khan, A.S., S. Ali, and I.A. Khan: Morphological and molecular characterization and evaluation of mango germplasm: An overview. *Scientia Horticulturae,* **194,** 353–366 (2015).

Leberg, P.L.: Estimating allelic richness: Effects of sample size and bottlenecks *Mol. Ecol.* **11**, 2445–2449(2002).

Luo, G. and P. He: Rumang-a promising new mango cultivar. *China Fruits*, **4**, 6-7(1996).

Marais, Z.: Mango cultivation for breeding. *Ingligtings Bull.,* N. **234,** 7-8 (1992).

Negi, S.S.: Dasheheri-51-A regular bearing, high yields mango clone, *ICAR News,* **3**, 14(1997).

Parida, G.N. and D.P. Rao: Classification and selection of some mangoes in Orissa. *Acta Hort.,* **231,** 93-96(1989).

Perrier, X., A. Flori and F. Bonnot: Data analysis methods. In: Genetic diversity of cultivated tropical plants Montpellier (France) (Ed.: P. Hamon, M. Seguin, X. Perrier, J.C. Glaszmann): Enfield, Science Publishers; 43-76 (2003).

Petit, R.J., A.El. Mousadik and O. Pons: Identifying populations for conservation on the basis of genetic markers. *Conser. Biol.,* **12,** 844–855(1998).

Prakash, G.S. and M.R. Dinesh: Tropical fruits. In: Biodiversity in Horticulture Crops (Ed.: K.V. Peter and Z. Abraham).Vol **1**, Daya Publishing House, pp. 39-56(2007).

Preisigke, da Costa, A. Sandra, C.L. de, S.N. Seraglio, N.L. Grillo, B.M.A. Aparecido, P.B. da Luz, K.L. Araújo and S. de P. Sobrinho: Genetic divergence in mango and obtaining minimum efficient descriptors. *Am. J. Plant Sci.,* **4**, 2318-2322 (2013).

Rabbani, A. and I.S. Singh: Evaluation of local sucking mango trees of Punjab. *Acta Hort.,* **291**, 99-106 (1989).

Rajan, S., L.P. Yadava, R. Kumar and S.K. Saxena: Genetic divergence in mango (*Mangifera indica* L.) varieties and possible use in breeding *Indian J. Horti.,* **66,** 7-12 (2009).

Rajan, S., M.R. Dinesh, K.V. Ravishankar, A. Bajpai, I. Ahmad, A. Singh, S.K. Singh, I.P. Singh, R. Vasudeca, B.M.C. Reddy, V.A. Parthasarthy and B. Sthapit: Heirloom varieties of important tropical fruits:A community initiative to conservation, ICAR-IIHR, p 34 (2014).

Rajwana, I.A., I.A. Khan, A.U. Malik, B.A. Saleem, A.S. Khan, K. Ziaf, R. Anwar and M. Amin: Morphological and biochemical markers for varietal characterization and quality assessment of potential indigenous mango (*Mangifera indica*) germplasm. *Int. J. Agric. Biol.,* **13**, 151-58 (2011).

Ram, S. and S. Rajan: Status Report on Genetic resoruces of mango in

Asia-Pacific Region, International Plant Genetic Resource Institute, New Delhi, pp 196 (2003).

Ravishankar, K.V., B.H. Mani, L.Anand and M.R. Dinesh: Development of new microsatellite markers from Mango (*Mangifera indica*) and cross-species amplification. *Am. J. Bot.,* **98**, e96-9(2011).

Ribeiro, I.C.N.S., Santos, C.A.F. and Lima Neto, F.P. : Morphological characterization of mango (*Mangifera indica*)accessions based on Brazilian adapted descriptors. Journal of Agricultural Science and Technology, **3**, 798-806,(2013).

Samal, K.C., R.C. Jena, S.S. Swain, B.K. Das and P.K. Chand: 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**, 195-213(2012).

Saxena, M. and C.P. Gandhi: Indian Horticulture database-2014. NHB, Ministry of Agriculture, Govt. of India. p. 302 (2014)

Schnell, R.J., C.T. Olano, W.E. Quintanilla and W.E. Meero: Isolation and characterisation of 15 microsatalite loci from mango and cross species amplification in closely related taxa. *Mol. Ecol. Notes,* **5**, 625-627 (2005).

Singh, N.P., N. Jerath, G. Singh and P.P.S. Gill: Physico-chemical characterization of unexploited mango diversity in sub-mountane zone of Northern India. *Indian J. Plant Genet. Resour.,* **25**, 261–269 (2012).

Srivastava, N., A. Bajpai, R. Chandra, S. Rajan, M. Muthukumar and M.K. Srivastava: Comparison of PCR based marker systems for genetic analysis of mango *J. Environ. Biol.,* **33**, 159-166(2012).

Surapaneni, M., L.R.Vemireddy, H. Begum, B.P. Reddy, C. Neetasri, J. Nagaraju, S.Y. Anwar and E.A. Siddiq: Population structure and genetic analysis of different utility types of mango (*Mangifera indica* L.) germplasm of Andhra Pradesh state of India using microsatellite markers. *Plant Syst. Evol*. **299**, 1215-1229 (2013).

Viruel, M., P. Escribano, M. Barbieri, M. Ferri and J. Hormaza: Fingerprinting, embryo type and geographic differentiation in mango (*Mangifera indica* L., Anacardiaceae) with microsatellites *Mol. Breed.,* **15,** 383–393(2005).

Yadav, I.S.: Mango Research in India: the past 50 years. *Indian Hort.,* **42**, 10-17 (1997).

Yadav, I.S. and S. Rajan: Genetic Resources of *Mangifera* In: Advances in Horticulture (Eds.: K.L. Chadha and O.P. Pareek). Vol. 1 Part 1 Malhotra Publishing House, New Delhi pp 77-93(1993).