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Assessment of genetic diversity in mango (*Mangifera indica* L.) based on RAPD markers

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ABSTRACT

Assessment of genetic diversity in 24 Indian mango cultivars was carried out with eight Random Amplified Polymorphic DNA (RAPD) markers. A total of fifteen RAPD markers were screened out of which eight primers produced polymorphism for finally selected for diversity analysis. Eight primers produced a total of 20 bands with an average 2.5 bands per primer thus generated 100% polymorphism. Heterozygosity varied from 0.278-0.49 while PIC was in ranged from 0.363 to 0.449. The highest Polymorphic Information content (PIC) value (0.449) was observed for primer OPA 16 while lowest PIC value was observed in primer OPC 6 (0.363). Effective multiplex ratio was maximum (4.083) for the primer OPAB 11 and minimum effective multiplex ratio (0.163) showed by OPA 16 primer. Arithmetic means of H was maximum (0.014) for primer OPA 2 while minimum Arithmetic mean of H (0.003) exhibited by OPAB 11 primer. Maximum marker index (0.012) exhibited by the primer OPA 18, OPA 11 and OPAB 10 while minimum marker index (0.002) observed with OPA 16 primer. Maximum discriminating power of marker (0.978) showed by OPA 16 and minimum discriminating power (0.397) observed with OPA 3 primer. Resolving power of markers varied in the range of 0.333 to 2.75. Maximum resolving power of markers (2.75) noted with OPC 6 while minimum marker resolving power (0.333) gave by OPA 16 primer. Jaccard's similarity between pairs of cultivars ranged between 0.33 and 0.96. The cluster dendrogram of RAPD revealed 2 major clusters and cluster I was the small and included 3 genotypes while clusters II comprising 20 genotypes. Highest genetic similarity was found between genotype Gulab Jamin and genotype Surkha, while the lowest genetic similarity was observed between genotype Manjeera and Alphonso and between Meetha Malda and Ramkela. Molecular data produced by RAPD markers clearly indicates that genotypes like Malda Meetha, Rataul and Berma Surakh was found more divergent and can be used for further improvement programme.

Key words: *Mangifera indica, RAPD markers, Genetic diversity.*

Introduction

Mango (*Mangifera indica* L.) and closely related genera (*Mangifera* spp.) belong to the family Anacardiaceae that consists of dicotyledonous trees and shrubs. Mango has been cultivated for more than 4000 years and a wide genetic diversity exists in this crop in the subcontinent. Mango (*Mangifera*

indica) is originated from the Assam valley in Myanmar and domesticated to several areas to the southwest and southeast of the centre of origin. Domestication in the Indian region (southwest) gave rise to the monoembryonic varieties and domestication in the Indochina, Thailand and Myanmar regions (southeast) gave rise to the polyembryonic varieties (Bompard and Schnell, 1997). Cultivation of mangoes in India is as old as civilization and has been cultivation over 4000 years. Now in world gene pool having 1000 named varieties of mango (Kumar *et al.*, 2001). Spreading of common mango throughout the tropical and subtropical world has been associated with the migration of people and trade within and between regions (Duval *et al.*, 2006). Today mangoes are grown throughout the tropics on all continents and in many subtropical areas. Allopolyploidy (2n = 40), out-breeding and a wide range of agroclimatic conditions prevailing in this country continue to contribute to the diversity of this crop.

In India, tremendous efforts have been made for germplasm collection and screening of mango genotypes (; Naik *et al*., 2000). However, the information for higher fruit yield and yield contributing characters is limited. Therefore, it is required to estimate the genetic variation in mango genotypes for selecting diverse parents for complementing various breeding programmes. Present study was designed to select suitable plant material based on the genetic diversity estimation conducted on a small set of mango genotypes.

Materials and Methods

Plant materials and DNA isolation

A representative set of 24 accessions of mango which are maintained at Horticultural Block, SVPUAT, Meerut, UP, India were used for present study (Table 1). Young leaves of the various cultivars were collected from the mango plants for isolation of DNA. Total genomic DNA was extracted from young leaves of mango genotypes by the standard CTAB method as described by (Doyle and Doyle, 1990) with minor modifications

Molecular analysis

Molecular analysis was done by online Marker Efficiency Calculator (*iMEC* software) as described by (Amiryousefi *et al*., 2018) which is a simple computation of seven basic measures polymorphism indices for individual markers such as, *iMEC* calculates heterozygosity index (H), polymorphism information content (PIC), discriminating power (D), effective multiplex ratio (E), marker index (MI), arithmetic mean heterozygosity (Havp), and resolving power (R). The source code used to develop *iMEC* is available on GitHub (https://github.com/Limpfrog/iMEC). *iMEC* software is also available at https:// irscope.shinyapps.io/iMEC/

Results and Discussion

Markers efficiency

Polymorphic genetic markers have wide potential applications in plant improvement programmes as a means for varietal and parentage identification, evaluation of polymorphic genetic loci affecting quantitative economic traits, and genetic mapping of plants (Baliyan *et al*., 2014). DNA based molecular markers have been proved as potential tool for assessment of genetic diversity and parental analysis of horticultural crops (; Kumar *et al*., 2018; Kumar *et*

Table 1. List of 24 mango genotypes with their parents used for diversity study.

Ambika (Amrapali x Janardan Pasand)	Alphanso(Hapoos x Tamy Atkins)
Pusa Arunima (Amrapali x Sensation)	Surkha(Local Selecton)
Dasheri-51(Selection from Dasheri)	Gulab Jamun (Local Selecton)
Kesar (Local selection)	Totapuri (Fibrood Anderson x Brooks)
Arunika (Amrapali x Vanraj)	Neelam
Rataul (Local selection)	Dasheri (Change seedling)
Langra (Change seedling)	Mallika (Neelum x Dasheri)
Chausa	Meetha Malda (Local Selecton)
Suvarnarekha	Berma Surkh (Local Selecton)
Baramasi (Local selection)	Ketki Bihar (Local Selecton)
Manjeera (Rumani x Neelum)	Ramkela
Surya (Selection from eldon)	Amrapali (Dashari x Neelum)

al., 2019; Ahmad *et al*., 2021) including mango (Razak *et al*., 2019). In the present study diversity analysis based on RAPD fingerprinting showed by number of bands detected through 8 different RAPD primers ranged from one to four bands with an average of 2.5 bands per primer (Table 2 and Fig. 1). There was a total of 20 bands were produced by 8 primers, which generated 100% polymorphism (Table 4.1). The maximum number of bands 4 bands produced by OPC 9 and OPAB 10 primers followed by 3 bands with the primers OPA 3 and OPA 2 while minimum number of 1 band generated by OPA 16 and OPA 18 primers. Our results are superior to other studies carried out by Eiadthong *et al*., (2000) where they reported 77% of polymorphisms in 14 *Mangifera species* by analysis with AFLPs, and Yamanaka *et al.* (2006) obtained 96% of polymorphism among and within four *Mangifera species*. According to Amiryousefi *et al.* (2018), there are two major dimensions of genomic marker polymorphism efficiency and informativeness as heterozygosity (H) and the polymorphic information content (PIC). These indices were measured based on data gained from RAPD primers using the *iMEC* (online marker efficiency calculator). The range of H and PIC value for a binary or dominant marker is maximum as 0 (monomorphic) to 0.5 (highly judicial, with multiple alleles in an identical frequency) due to assume of two alleles per locus and both are influenced by the number and frequency of alleles (Ahmad *et al*., 2018). Considering the ranges of PIC and H values indicate the moderate discriminatory capacity of markers. Heterozygosity index for all primers showed variable differences and its ranged

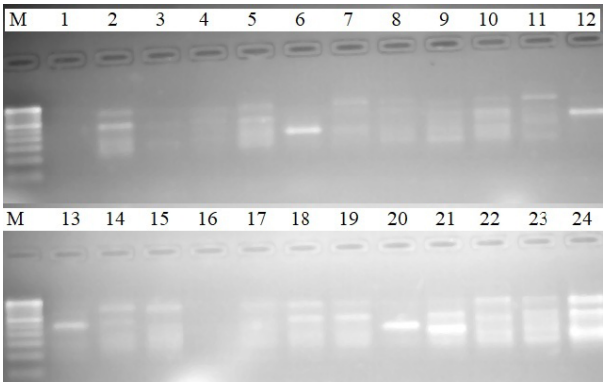

Fig. 1. Representative gel image depicting PCR amplifications using primers OPAB-10 primer in 24 selected genotypes of mango. M represents marker ladder.

Table 2. Details of primers and their efficiency in diversity study on 24 mango genotypes.

Primer	Total no. alleles	No. of polymorphic allele	No. of monomorphic allele	Heterozygosity Index	Polymorphic Information Content	Effective multiplex ratio	Arithmetic mean of H	Marker Index	Discriminating power	Resolving power
OPA 03	3	3	0	0.346	0.428	2.333	0.005	0.011	0.397	1.333
OPA 16	1	1	0	0.278	0.449	0.167	0.012	0.002	0.978	0.333
OPA 18	1	1	0	0.478	0.373	2.417	0.005	0.012	0.638	2.500
OPC 6	2	2	0	0.499	0.363	2.625	0.004	0.011	0.726	2.750
OPC 9	4	4	0	0.413	0.402	0.583	0.009	0.005	0.919	1.000
OPAB 11	2	2	0	0.435	0.393	4.083	0.003	0.012	0.538	2.333
OPAB 10	4	4	0	0.460	0.382	3.208	0.004	0.012	0.590	2.417
OPA 02	3	3	0	0.330	0.433	0.208	0.014	0.003	0.964	0.417
Total	20	20	0	3.29	3.22	15.624	0.056	0.068	5.750	13.083
Average	2.5	2.5	0	0.044	0.402	1.953	0.007	0.008	0.718	1.635

varied from 0.278-0.49. The maximum heterozygosity index (0.49) showed by OPC 6 primer while minimum heterozygosity index (0.278) observed with the primer OPA 16. In the present study, PIC values, derived from allelic diversity and frequency among genotypes were not uniform for all of the RAPD primers. The PIC value for RAPD primer loci was in ranged from 0.363 to 0.449 with an average of 0.402. The highest Polymorphic Information Content (PIC) value (0.449) was observed for primer OPA 16 while lowest PIC value was observed in primer OPC 6 (0.363) which sowing moderate value of PIC. The PIC value in present study was higher than the previous studies as conducted by Kumar *et al*. (2014) but lower than other previous studies carried out by Begum *et al*., (2014); Azam *et al*., (2019) and Razak *et al*., (2019). Markers with PIC value of 0.5 or higher are known to be highly informative for genetic studies and extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (Botstein *et al*., (1980). Effective multiplex ratio was maximum (4.083) for the primer OPAB 11 and minimum effective multiplex ratio (0.163) showed by OPA 16 primer. Arithmetic mean of H showed variable results among the primers and maximum Arithmetic mean of H (0.014) observed with OPA 2 primer followed by (0.012) for the primer OPA 16 and minimum Arithmetic mean of H (0.003) exhibited by OPAB 11 primer. The greater the Resolving Power (RP) and marker index (MI) indices refer to the greater efficacy of the respective primer (Zarei and Erfani-Moghadam, 2021). In our study marker index showed variation among the primers and varied from 0.002-0.012. Maximum marker index (0.012) exhibited by the primer OPA 18, OPA 11 and OPAB 10 while minimum marker index (0.002) exhibited with OPA 16 primer. Maximum discriminating power of marker (0.978) showed by OPA 16 followed by (0.964) with the primer OPA 02 while minimum discriminating power (0.397) observed with OPA 3 primer. Resolving power of markers varied in the range of 0.333 to 2.75 with an average of 1.635. Maximum resolving power of markers (2.75) noted with OPC 6 followed by (2.50) for the primer OPA 18 and minimum marker resolving power (0.333) gave by OPA 16 primer (Table 4.1). Depending upon the value of PIC and RP, most of the RAPD primers are moderate efficient in analysis of mango genotypes. Similar observations were reported by (Ravishankar *et al*., 2011. Bajpai *et al*., 2016. Ahmad *et al*., 2019) in mango.

Table 3. Jaccard's similarity matrix among the genotypes of mango using RAPD markers

Variety	Ambika	Pusa-Arunima	Dasheri-51	Kesar	Arunika	Rataul	Langra	Chausa	Suvarnarekha	Baramasi	Manjeera	Surya	Alphanso	Surkha	Gulab-Jamun	Totapuri	Neelum	Dasheri	Mallika	Meetha-Malda	Berma-Surkh	Ketki-Bihar	Ramkela	Amrapali
Variety																								
Ambika	1.000																							
Pusa-Arunima	0.630	1.000																						
Dasheri-51	0.630	0.630	1.000																					
Kesar	0.630	0.704	0.778	1.000																				
Arunika	0.630	0.926	0.704	0.778	1.000																			
Rataul	0.593	0.667	0.519	0.519	0.667	1.000																		
Langra	0.630	0.704	0.778	0.778	0.778	0.667	1.000																	
Chausa	0.630	0.630	0.630	0.704	0.704	0.593	0.852	1.000																
Suvarnarekha	0.741	0.815	0.667	0.667	0.815	0.778	0.741	0.667	1.000															
Baramasi	0.630	0.852	0.630	0.704	0.852	0.519	0.704	0.778	0.741	1.000														
Manjeera	0.444	0.593	0.667	0.593	0.593	0.556	0.741	0.741	0.630	0.593	1.000													
Surya	0.667	0.741	0.741	0.593	0.667	0.556	0.519	0.444	0.778	0.667	0.556	1.000												
Alphanso	0.370	0.593	0.519	0.519	0.593	0.556	0.444	0.370	0.481	0.519	0.333	0.630	1.000											
Surkha	0.556	0.778	0.556	0.630	0.704	0.593	0.704	0.630	0.741	0.704	0.741	0.741	0.519	1.000										
Gulab-Jamun	0.519	0.815	0.593	0.667	0.741	0.630	0.741	0.667	0.778	0.741	0.778	0.778	0.556	0.963	1.000									
Totapuri	0.815	0.519	0.593	0.741	0.593	0.481	0.593	0.519	0.630	0.519	0.407	0.630	0.481	0.519	0.481	1.000								
Neelum	0.704	0.852	0.704	0.778	0.852	0.667	0.778	0.704	0.815	0.778	0.741	0.741	0.519	0.778	0.815	0.667	1.000							
Dasheri	0.667	0.815	0.741	0.815	0.889	0.630	0.815	0.741	0.778	0.815	0.704	0.704	0.556	0.741	0.778	0.704	0.963	1.000						
Mallika	0.407	0.704	0.556	0.556	0.704	0.667	0.630	0.556	0.593	0.704	0.519	0.593	0.741	0.704	0.741	0.444	0.704	0.741	1.000					
Meetha-Malda	0.556	0.407	0.630	0.481	0.481	0.593	0.630	0.630	0.593	0.481	0.667	0.370	0.370	0.481	0.444	0.519	0.481	0.519	0.407	1.000				
Berma-Surkh	0.556	0.704	0.481	0.704	0.704	0.370	0.556	0.481	0.593	0.704	0.444	0.593	0.519	0.704	0.667	0.741	0.704	0.741	0.630	0.407	1.000			
Ketki-Bihar	0.556	0.704	0.704	0.704	0.704	0.593	0.704	0.556	0.741	0.630	0.815	0.667	0.444	0.704	0.741	0.593	0.852	0.815	0.556	0.556	0.630	1.000		
Ramkela	0.407	0.704	0.556	0.630	0.704	0.593	0.630	0.556	0.593	0.704	0.519	0.593	0.741	0.704	0.741	0.519	0.704	0.741	0.926	0.333	0.704	0.630	1.000	
Amrapali	0.519	0.889	0.519	0.667	0.815	0.630	0.593	0.519	0.778	0.741	0.630	0.778	0.630	0.815	0.852	0.556	0.815	0.778	0.667	0.370	0.741	0.815	0.741	1.000

Diversity analysis

Genetic similarities (GS) were calculated using the Nei-Li similarity co-efficient. Significant genetic variation was found among all mango genotypes with the GS value ranging from 0.33 to 0.96 (Table 3). Of the 24 pair wise combinations so generated, the highest genetic similarity was found between genotype Gulab Jamin and genotype Surkha, while the lowest genetic similarity was observed between genotype Manjeera and Alphonso and genotype Meetha Malda and Ramkela. The dendrogram generated through RAPD revealed 2 major clusters at at Jaccard's similarity coefficient (GS=0.50) which showed narrowed base of diversity. In the present study a dendogram was constructed using RAPD data (Fig. 2) and cluster analysis done based on Jaccard's similarity coefficient using UPGMA, which grouped all the 24 mango varieties into the 2 two major groups (Group 1 and Group 2) at the co-efficient of GS=0.95. Group 1 at Jaccard's similarity coefficient (GS=0.407) consisted of 23 mango genotypes which varied from Ambika to Ramkela while Group 2 at Jaccard's similarity coefficient (GS=0.556) contained only one mango genotype like as Meetha Malda. Group 1 was further sub divided in to 2 sub groups (1 & 2), sub group 1 at Jaccard's similarity coefficient (GS=0.556) consists only three genotypes viz., Ambika, Totapuri and Berma-Surkh however, sub group 2 at Jaccard's similarity coefficient (GS=0.704) divided in to 7 clusters (2a, 2b, 2c, 2d, 2e, 2f& 2g), cluster 2a at (GS=0.815) contained a total of six genotypes viz., Pusa Arunima, to Suvarnrekha, cluster 2b at (GS=0.815) consisted of three genotypes Surkha to Amrapali, cluster 2c at genetic similarity coefficient (GS=0.667) showed only one genotypes like Surya, cluster 2d at genetic similarity coefficient (GS=0.815) consists only two genotypes namely Manjeera and Ketki Bihar while cluster 2e at (GS=0.63) consisting of four genotypes which can be seen in dendogram as Dashari 51 to Chausa. Cluster 2f at (GS=0.593) consisted only one genotype like Rataul and cluster 2g at genetic similarity coefficient (GS=0.741) comprised of three genotypes Alphanso, Malika and Ramkela. Similar results were also observed by Ravishankar *et al*., (2000) who formed two main clusters when analyzed 18 commercial cultivars of mango using RAPD markers. In cluster analysis one genotype as Meetha-Malda exits in different cluster and showed more divergence as compared to other mango genotypes. Among the clustering pattern, two cultivars as Pusa Arunima a hybrid of (Amrapali x Sensation) an Arunika a hybrid of (Amrapali x Vanraj) clustered together and rest of the hybrid cultivars did not show any resemblance together. The results are partial close conformity with Jena and Chand, (2016) in mango. In present study genotype Neelam showed 100 percent

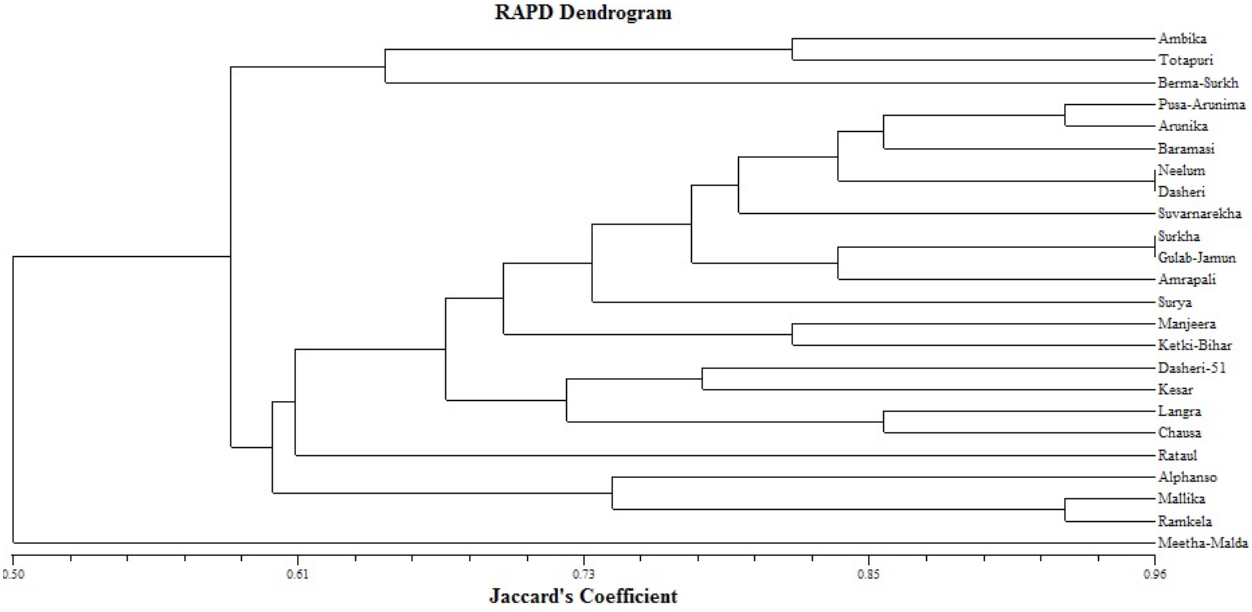

Fig. 2. Dendrogram showing clustering of 24 mango genotypes constructed using UPGMA based on Jacquard's similarity coefficient obtained from RAPDs analysis.

similarity with Desheri and Surkha and Gulab Jamun also exhibited 100 percent similarity. Similar findings were also reported by Rajwana *et al*., (2008) in mango.

Conclusion

It may be concluded that cultivars like Meetha Malda, Rataul and Berma Surkhh were found more genetically diverse by using RAPD analysis and may be potential sources for further breeding purpose.

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