Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7425

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **7 (2):** 382-395 (2019)

Research Article

Mango (*Mangifera indica* L.): Morphological and Genetical Diversity in India

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Received: 3.03.2019 | Revised: 7.04.2019 | Accepted: 20.04.2019

ABSTRACT

Mango (Mangifera indica L.) is known as the 'ruler of natural products' for its rich taste, season, shading, creation volume and various end use. It has a place with plant family Anacardiaceae and has a little genome size of 439 Mb (2n = 40). Old writing shows inception of developed mango in India. Albeit wild types of sort Mangifera are circulated all through South and South-East Asia, recuperation of Paleocene mango leaf fossils close Damalgiri, West Garo Hills, Meghalaya point to the beginning of class in peninsular India before joining of the Indian and Asian mainland plates. India delivers in excess of 50% of the world's mango and develops in excess of thousand assortments. Notwithstanding its enormous financial criticalness genomic assets for mango are restricted and hereditary qualities of helpful plant characteristics are ineffectively comprehended. Here we present a short record of our ongoing endeavors to create genomic assets for mango and its utilization in the examination of hereditary assorted variety and populace structure of mango cultivars.

Sequencing of leaf RNA from mango cultivars 'Neelam', 'Dashehari' and their mixture 'Amrapali' uncovered generously more elevated amount of heterozygosity in 'Amrapali' over its folks and created genic basic grouping rehash (SSR) and single nucleotide polymorphism (SNP) markers. Sequencing of twofold processed limitation site-related genomic DNA (ddRAD) of 84 assorted mango cultivars recognized 1.67 million top notch SNPs and two noteworthy sub-populaces. We have collected 323 Mb of the exceedingly heterozygous 'Amrapali' genome utilizing long succession peruses of PacBio single atom ongoing (SMRT) sequencing science and anticipated 43,247 protein coding qualities. Mangifera indica L. is the most prevalent and broadly developed natural product crop in the tropics. As indicated by verifiable information, mango trees are personally associated with Indian culture and old stories since 4000 years prior.

Key words: *Mango (Mangifera indica L.), SSR, RAPD, SNP markers, Genetic diversity.*

INTRODUCTION

Mango (*Mangifera indica* L.) is the choicest fruit of India and occupies a prominent place among the best fruits of the world. It is widely grown in the tropical and subtropical regions world over.

Cite this article: Singh, A.K., Charmkar, N.K., Singh, R., Mango (*Mangifera indica* L.): Morphological and Genetical Diversity in India, *Int. J. Pure App. Biosci.* **7(2):** 382-395 (2019). doi: http://dx.doi.org/10.18782/2320-7051.7425

India has rich mango varietal wealth and the country has the richest wealth of mango germplasm in Southeast Asia. Reportedly, there are over 1,000 varieties of mango found in India. However, there is a lot of confusion in nomenclature of the mango cultivars noted that lack of systematic approach in naming of mango cultivars in the past has resulted in a great confusion in their nomenclature due to many synonyms and duplication of names in the absence of any rules governing nomenclature[27,33].

Indian mango breeders have realized this recent trend in mango breeding to catch the emerging export market as well as to meet the demands of domestic consumers. A notable feature of Indian mango indusry is the negligible presence of Indian exporters in world mango market. Subrahmanyam noted that although the international trade in mango was increasing rapidly, India continued to lag behind in total mango exports as compared to other exporting countries like Mexico, Philippines and Venezuela. Though there is a vast potential for export to western countries, constraints like suitability of a few varieties for export, pests and disease problems have restricted the expansion of the exports from India. Removal of some of the constraints will increase the potential for exports to USA and Japan. Research efforts are also needed for prolonging the shelf life of fruit so that it could be made available for longer period in the international market. The existing scenario reminds us the importance of frontier sciences like marker assisted breeding and their integration with conventional fruit breeding for obtaining desirable results with more precision.

The term DNA fingerprinting in its original sense refers to the method developed in 1985 by Sir Alec Jeffreys and his associates for the detection of highly variable DNA fragments by hybridization of specific multilocus probes to electrophoretically separated restriction fragments. The DNA fingerprints, resembling barcodes, are unique to the individual and hence can be utilized in much the same way as conventional fingerprints to identify individuals with absolute certainty. Fingerprinting a vast number of mango cultivars is a significant contribution to mango cultivation, as presently several mango cultivars have many synonyms in different regions, which make identification difficult and create confusion. Simple Sequence Repeat (SSR) markers, also known as microsatellites, are tandemly repeated motifs of 1-6 nucleotides found in all prokaryotic and eukaryotic genomes Microsatellites, with a polymorphism based on different numbers of repeated motifs at a given locus, are becoming markers of choice in many fruit breeding programmes since they are multi-allelic and amenable to automation. In addition to their usefulness in mapping and breeding, have become the markers of choice for fingerprinting purposes in most plant species due to their high polymorphism, co-dominancy and reproducibility[14].

History of Mango – 'King of Fruits':

The history of Mango began thousands of years ago on the Indian sub-continent. The Mango is the national fruit of India, Pakistan and the Philippines. It is also the national tree of Bangladesh. Not only is it one of the most highly prized fruits of South Asia, it is also intimately connected with folklore and legends across many religions. There is consensus among the historians and horticulturists that the cultivated mango has originated in India. Vavilov has suggested Indio – Burma region as the centre of origin of Mango based on the observed level of genetic diversity. Mukherjee[28] considered origin of genus Mangifera probably in the South – East Asia but the origin of cultivated Mango in the Assam – Burma region. Scientists of the Birbal Sahni Institute of Palaeibitany, Lucknow, have traced the origin of genus Mangifera from 60 million years old fossil compressions of carbonized mango leaves in the Palaeocene sediments near Damalgiri, west Garo Hills, Meghalaya and named it Eomangiferop-hyllum damalgiriensis.

History of Mangoes in India:

Mango is native to India and is one of the most important fruit crops world-wide. Its botanical

name is *Mangifera indica L.* and is the most important species of the genus *Mangifera*, which produces the most delicious fruit called the mango. The genus *Mangifera* contains about 49 species, of which 8 are of doubtful status and 41 are valid species. Morphologically the genus could be separated under two sections based on the character of the flower disc: the first, with 34 species, has flowers with well developed swollen disc, and the second, with 7 species, has obsolete or pedicellate disc. The cultivation of mango in India is as old as 4,000 to 6000 years. Hsüan-tsang appears to be the first person to bring the mango to the notice of people outside India. Geographic spread of mango was essentially completed in the last half of the 19th Century with its introduction to such far flung places as Florida, Hawaii, Fiji, Queensland and Natal. The Portuguese are said to introduce vegetative propagation methods in India for the first time to clone superior mono-embryonic trees in the 15th Century. The most important mango cultivars of India (Alphonso, Dashehari, Langra etc.), are selections made at the time of Akbar (1542–1605 AD) and have been propagated by vegetative method for several hundred years. Though, a tropical fruit, it is now cultivated under subtropical conditions in 89 countries of the world. The major mango growing countries are India, Pakistan, Bangladesh, Myanmar, Sri Lanka, Florida and Hawaii of USA, Australia, Brazil, Thailand, the Philippines, Malaysia, Vietnam, Indonesia, Fiji Islands, Egypt, Israel, South Africa, Sudan, Somalia, Kenya, Uganda, Tanzania, Niger, Nigeria, Zaire, Madagascar, Mauritius, Venezuela, Mexico, West Indies Islands, Cambodia, etc[47].

Global distribution of Mango:

Distribution of Mango Presently, besides India, it is being cultivated in Pakistan, Bangladesh, Burma, Sri Lanka, Thailand, Vietnam, Malaysia, the Philippines, Indonesia, the Fiji Islands, Tropical Australia, Egypt, Israel, Sudan, Somalia, Kenya, Uganda, Tanzania, South Africa, Nigeria, Zaire, Madagascar, Mauritius, the USA (Florida, Hawaii, Puerto and Rico), Venezuela, Mexico, Brazil, Australia, West Indies Islands and Cambodia.98 The mango, which has been under cultivation in India for 4000 years or more, forms now a commercial crop in many countries. The account of the introduction and distribution of the mango in other countries of the world is interesting. Perhaps Malaya was one of the earliest to obtain the material from India. The size of the seeds is too great to allow carriage by birds or other animals and suggest dispersal by human agency. The dissemination of Mango throughout the world started with the commencement of trade between Asia and Europe. The Portuguese were the first to come to India, and they seized the opportunity of trading in spices and other vegetable products of the East.100 It had become established in Somaliland on the eastern coast of Africa before 1331.

Fig. 1: Worldwide distribution of mango[21]

Indian distribution of Mango:

Varieties of Mango for successful mango growing, it is necessary that the varieties planted in a commercial orchard are productive, of good quality and adaptable to the climate of the tract. Different varieties are suitable for growing in different climatic conditions. The number of varieties of mango found in India is great. In fact, there are far too many varieties. The number of commercial varieties is estimated at over 1000104 each differing in size, shape, colour, texture and taste. The popular varieties grown in Tamil Nadu are Neelum, Bangalara, Banganapalli, Rumani and Mulgoa. Pickle varieties are Butty, Natty and Gaddemar. The name of the mango variety is varies from region to region. The same variety may be known by a different name in a different location. For example, Himsagar of South Bengal area is known as Khirsapati in Malda. There are nearly 1000 mango varieties in India. However, only the following varieties are grown on a commercial scale in different States: Alponso Bangalora Banganapalli Bambai Bambay Green Dashehari Fajri Fernandiein Himsagar Kasar Kishenbhug Langra Mankurad Mulgao Neelum Sambarbehisht Chausa Rumani and Raspuri[3].

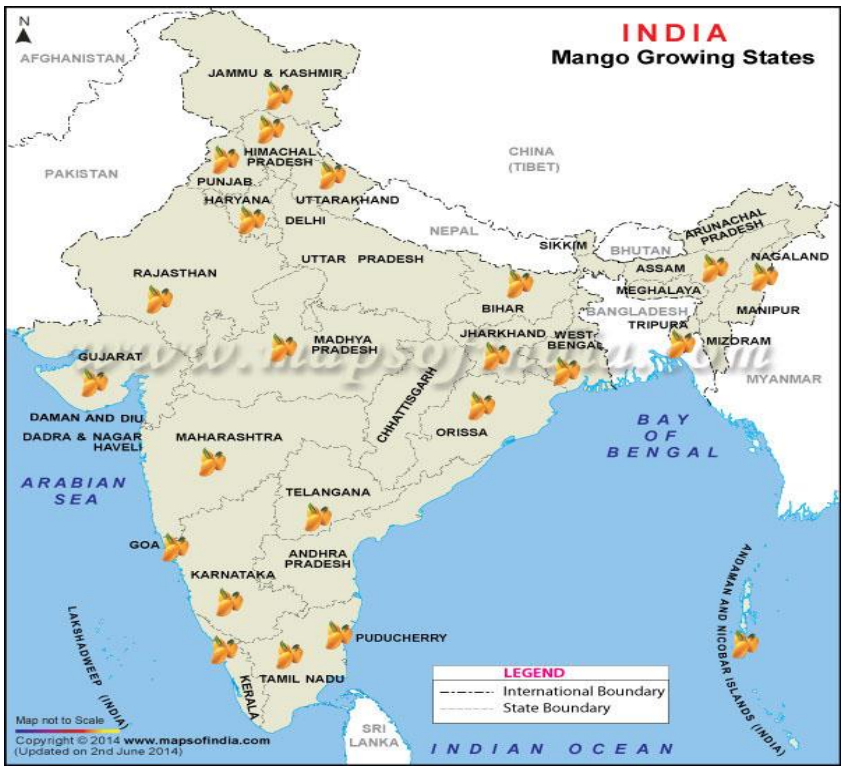

Fig. 2: Indian distribution of mango[3]

Morphological Diversity:

Molecular diversity analysis and fingerprinting of mango cultivars has been carried out using different types of DNA markers, including random amplified polymorphic DNA (RAPD) by Schnell *et al*., However, most of these studies were carried it on small sets of genotypes. Recently, Ravishankar *et al*., using 14 carefully selected SSR markers on a comprehensive set of 367 Indian mango cultivars identified two main sub-populations representing mango cultivars from the North-East and South- West regions of India. We identified 1.67 million high quality SNPs by sequencing double digested restriction site associated DNA (ddRAD) from 84 diverse mango cultivars from different regions of India and abroad and a database of SNPs was created.

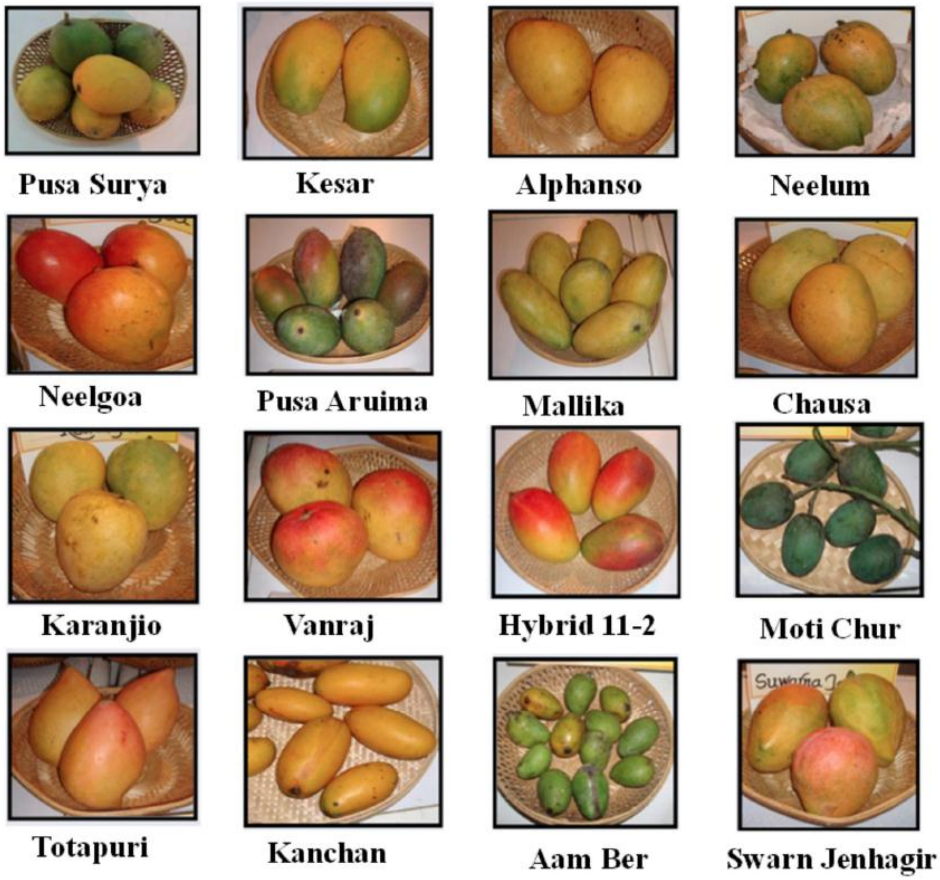

Fig. 3: A sample of the diversity in the color, size and shape of mature mango fruits of Indian mango cultivars

Varieties : Though there are nearly 1000 varieties of mango in India, only following varieties are grown in different states : Alphonso, Bangalora, Banganpalli, Bombai, Bombay Green, Dashehari, Fazli, Fernandin, Himsagar, Kesar, Kishen Bhog,Langra, Mankhurd, Mulgoa, Neelam, Samarbehist, Chausa, Suvarnarekha, Vanaraj and Zardalu. Recently some mango hybrids have been released for cultivation by different institutes / universities. A brief introduction to such varieties is presented below :

Mallika - It is a cross between Neelam and Dashehari. Fruits are medium sized cadmium coloured with good quality, reported to be a regular bearer.

Amrapali - It is a cross between Dashehari and Neelam. It is a dwarf vigorous type with regular and late bearing variety. It yields on an average 16 t/ha and about 1600 plants can be accommodated in one hectare.

Mangeera : It is a cross between Rumani and Neelam. It is a semi vigorous type with a regular bearing habit. Fruits are medium sized with light yellow coloured skin, firm and fibreless flesh and sweet to taste.

Ratna : It is a cross between Neelam and Alphonso. It is a regular bearer and free from spongy tissue. Fruits are medium sized with excellent quality. Flesh is firm and fibreless, deep orange in colour with high TSS (19-21 Brix).

Arka Aruna : It is a hybrid between Banganapalli and Alphonso with regular bearing habit and dwarf in stature. About 400 plants can be accommodated per hectare. Fruits are large sized (500- 700 gm) with attractive skin colour. Pulp is fibreless, sweet to taste (20-22 Brix). Pulp percentage is 73 and the fruits are free from spongy tissue.

Arka Puneet : It is a regular and prolific bearing hybrid of the cross between Alphonso and the Banganapalli. Fruits are medium sized (220-250 gm) with attractive skin colour, having red blush. Pulp is free from fibre, pulp percentage being 70 percent. Fruits are sweet

to taste (20-22 Brix) with good keeping quality and free from spongy tissue. It is a good variety for processing also.

Arka Anmol : It is a semi-vigorous plant type from the cross between Alphonso and Janardhan Pasand. It is also a regular bearing and free from spongy tissues. Fruits ripen to uniform yellow colour. Keeping quality of the fruit is very good and it is suitable for export. It has got excellent sugar and acid blend and fruits weigh on an average about 300 g Pulp is orange in colour.

Morphological marker:

Commercially grown mango cultivars have been identified on the basis of vegetative and reproductive parameters such as leaf size, leaf shape, shoot length, panicle length, fruit size, fruit shape, peel colour, stone size and stone weight. These parameters, based on morphological traits, constitute the morphological markers, which are the oldest and most widely used genetic markers for the germplasm characterization and cultivar identification. Extensive studies have been carried out on the morphological diversity of Indian mangoes and scores of morphological markers have been reported in mango by several workers for the purpose of evaluation of cultivars and hybrids[40].

Variation of mango cultivars for qualitative raits

Tree and leaf characters

The cultivars were largely non-grafted seedlings, irregular (alternate) bearing behavior, tree height range from medium to very tall group, broadly pyramidal to semi-circular crown shape, and spreading growth habit. The alternate bearing which is dependent on agronomic practices environmental conditions and genetic makeup is a common phenomenon of mango. Most cultivated mango trees are between 3 and 10 m in height when fully matured depending on the way of pruning. However, mango trees can reach a height of 40 m or more while grafted ones are usually shorter Tree canopies vary in genotypes, propagation method, the density of plantation, and prevailing agro-climatic conditions Intermediate foliage density, oblong leaf blade shape, semi-erect to horizontal leaf attitude in relation to branch, a medium category in the angle of secondary veins to the midrib, acuminate apex, acute base shape, and mild leaf fragrance were observed in the majority of the cultivars. These characters are among the important attributes that could be utilized for classification of the cultivars.

Fruit, stone and seed characters

The predominant fruit shape of the cultivars was oblong followed by roundish, obtuse fruit apex, absent fruit stalk cavity, absent to slightly neck prominence and perceptible beak type. The majority of the cultivars had orange, greenish yellow to yellow skin color and orange to yellow pulp color when ripe. The cultivars fruit attractiveness was from average to good though there were excellent attractive cultivars (26.1%). Most had low to intermediate fiber in fruit pulp, very juicy, intermediate aroma, and very good to excellent eating quality. This indicated the potential of cultivars for the table as well as processing purpose if further studied. Most cultivars in Shendi, Sudan also reported oblong fruit shape followed by round and obtuse fruit apex.

Alphonso (Happus) Fruit is medium in size, ovate oblique in shape, orange yellow in colour. Juice is moderate-abundant, excellent keeping quality, good for pulping and canning. Mainly exported as fresh fruit. Flesh develops spongy tissue. Bangalora (Totapuri) The fruit is medium-large, oblong shaped with pointed base with golden yellow colour. Good keeping quality; used for processing; heavy and regular bears variety, Susceptible to bacterial spot. Banganapalli (Baneshan, Safeda) Fruit is large sized, obliquely oval in shape, golden yellow in colour, good keeping quality, and good for canning, biennial in habit. Variety suited for dry areas Bombai (Malda) Variety is alternate bearer. Fruit is medium, ovate and yellow in colour. Keeping quality is medium. Bombay Green Fruit size is medium, shape ovate oblong with spinach green colour. Keeping quality is medium. Early seasoning variety. Biennial in habit highly susceptible to both vegetative and floral malformation. Dashehari

Best varieties of the country. Mankur The variety develops black spots on the skin in rainy season. Fruit is medium ovate and yellow in colour. Fruit quality is very good but keeping quality is poor. Mulgoa Fruit is large roundish-oblique in shape and yellow in colour. High fruit quality and good keeping quality. Neelum Fruit is medium ovate-oblique in shape and saffron yellow in colour. Good keeping quality

Table 1: Studies on fruit morphological characteristics of mango varieties

Variety	Fruit Shape	Fruit Surface	Presence of Neck	Presence of Sinus
1	2	3	4	5
Amin	Medium Elliptic	Rough	Absent	Present
Amrapali	Medium Elliptic	Smooth	Present	Present
Bara Malda	Broad Elliptic	Smooth	Absent	Present
Banarasi Betali	Medium Elliptic	Smooth	Absent	Absent
Baramasi	Medium Elliptic	Smooth	Present	Present
Bathui	Broad Elliptic	Smooth	Present	Absent
Bijoragarh	Broad Elliptic	Smooth	Present	Absent
Bombay Green	Broad Elliptic	Smooth	Absent	Present
Bride of Russia	Broad Elliptic	Rough	Absent	Absent
Chausa	Medium Elliptic	Smooth	Absent	Present
Dashehari	Medium Elliptic	Smooth	Absent	Present
Dashehari-51	Medium Elliptic	Smooth	Absent	Present
Duddha Peda	Broad Elliptic	Smooth	Absent	Absent
Gulabkhas	Broad Elliptic	Smooth	Absent	Absent
Gulabkhas Green	Medium Elliptic	Smooth	Present	Present
Gurwani	Broad Elliptic	Smooth	Absent	Present
Haathijhool	Broad Elliptic	Rough	Present	Absent
Husn-a-ra	Medium Elliptic	Smooth	Absent	Present
Kaitki Bihar	Broad Elliptic	Smooth	Absent	Absent
Kesar (Basti)	Broad Elliptic	Smooth	Present	Present
Khas-ul-Khas	Circular	Smooth	Absent	Absent
K.O.-07	Broad Elliptic	Rough	Absent	Present
Langra	Broad Elliptic	Smooth	Present	Present
Langra Gorakhpur	Circular	Smooth	Present	Present
Langra Rampur	Circular	Smooth	Absent	Absent
Mallika	Medium Elliptic	Rough	Present	Present
Mithua Malda	Circular	Rough	Absent	Absent
Mulgoa Deshi	Circular	Smooth	Present	Absent
Neelum	Medium Elliptic	Rough	Absent	Present
Neelum×Chausa	Medium Elliptic	Smooth	Absent	Present
Pulgoa Darbhanga	Circular	Smooth	Absent	Present
Rahman Pasand	Broad Elliptic	Smooth	Absent	Absent
Rataul	Broad Elliptic	Smooth	Absent	Absent
Rumani	Broad Elliptic	Smooth	Absent	Present
Safeda Lucknow	Medium Elliptic	Smooth	Absent	Absent
Sensation	Broad Elliptic	Smooth	Absent	Present
Suvarnrekha	Broad Elliptic	Smooth	Present	Present
Tamancha	Broad Elliptic	Smooth	Absent	Absent
Thanking Amadi	Medium Elliptic	Rough	Present	Present
Totapari Red Small	Broad Elliptic	Smooth	Present	Present
Vanraj	Circular	Smooth	Absent	Absent
Zafrani Gola	Circular	Rough	Absent	Absent
Zardalu	Medium Elliptic	Smooth	Absent	Absent

Table 2: Studies on fruit weight and size of different mango varieties

Variety	Fruit weight (g)			Fruit length (mm)			Fruit breadth (mm)		
	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled
Amin	204.81	205.83	205.32	107.26	118.79	113.03	61.26	60.09	60.68
Amrapali	189.52	204.38	196.95	99.00	106.82	102.91	58.34	62.12	60.23
Bara Malda	137.74	156.58	147.16	78.21	82.99	80.60	65.42	72.02	68.72
Banarasi Betali	336.34	370.67	353.50	121.67	129.98	125.83	65.67	74.92	70.30
Baramasi	225.06	221.49	223.28	110.38	107.53	108.96	61.55	59.18	60.37
Bathui	223.86	202.92	213.39	107.80	98.16	102.98	54.13	54.85	54.49
Bijoragarh	298.41	316.67	307.54	97.83	104.87	101.35	83.72	87.12	85.42
Bombay Green	235.67	255.69	245.68	92.23	97.94	95.09	67.76	73.05	70.40
Bride of Russia	266.92	281.92	274.42	93.55	94.47	94.01	75.07	79.21	77.14
Chausa	330.01	343.33	336.67	104.02	115.53	109.78	71.47	76.62	74.05
Dashehari	161.81	174.00	167.91	91.93	100.42	96.18	54.52	58.41	56.47
Dashehari-51	171.53	185.41	178.47	98.99	101.27	100.13	54.76	58.21	56.48
Duddha Peda	140.54	162.92	151.73	84.78	101.25	93.02	54.20	60.30	57.25
Gulabkhas	219.76	242.72	231.24	102.34	103.13	102.73	67.67	66.93	67.30
Gulabkhas Green	162.39	139.28	150.84	92.09	88.79	90.44	60.64	57.95	59.30
Gurwani	226.51	254.17	240.34	91.21	92.15	91.68	71.33	76.28	73.80
Haathijhool	1906.30	1754.10	1830.20	203.19	194.93	199.06	133.80	124.07	128.95
Husn-a-ra	146.92	132.08	139.50	100.11	89.29	94.70	51.21	51.02	51.11
Kaitki Bihar	230.41	200.26	215.33	83.98	75.19	79.59	73.72	65.70	69.71
Kesar (Basti)	237.78	267.08	252.43	124.17	135.37	129.77	58.73	63.36	61.05
Khas-ul-Khas	222.78	242.47	232.63	93.48	100.68	97.08	66.32	70.76	68.54
K.O.-07	235.71	252.93	244.32	87.36	96.39	91.88	67.46	70.77	69.12
Langra	295.66	311.43	303.55	103.36	106.26	104.81	68.47	74.22	71.35
Langra Gorakhpur	334.58	293.75	314.17	137.11	130.35	133.73	67.56	67.77	67.66
Langra Rampur	274.36	260.83	267.60	109.88	98.95	104.42	77.91	73.35	75.63
Mallika	431.58	419.91	425.75	154.36	147.49	150.93	82.78	76.41	79.60
Mithua Malda	209.95	220.83	215.39	95.62	102.72	99.17	68.20	67.77	67.99
Mulgoa Deshi	331.99	357.52	344.76	87.41	100.07	93.74	79.83	85.10	82.47
Neelum	123.89	132.48	128.18	78.54	76.97	77.76	56.67	61.48	59.07
Neelum×Chausa	278.89	314.17	296.53	117.81	125.96	121.89	66.40	71.85	69.13
Pulgoa Darbhanga	269.57	250.18	259.88	101.59	94.62	98.11	71.58	65.50	68.54
Rahman Pasand	279.44	291.39	285.42	97.02	109.20	103.14	80.18	71.34	75.76
Rataul	172.36	145.28	158.82	85.70	77.60	81.65	67.12	54.86	60.99
Rumani	160.69	179.67	170.18	68.64	72.83	70.74	70.53	71.73	71.13
Safeda Lucknow	125.11	135.83	130.47	78.33	80.81	79.57	50.06	58.34	54.20
Sensation	247.75	265.42	256.58	103.43	114.72	109.08	69.52	72.51	71.02
Suvarnrekha	402.67	392.22	397.45	111.15	107.08	109.12	80.34	79.23	79.78
Tamancha	113.64	104.59	109.12	67.16	62.96	65.06	52.50	55.12	53.81
Thanking Amadi	250.53	254.50	252.51	103.44	105.95	104.69	71.38	69.20	70.29
Totapari Red Small	118.36	119.58	118.97	88.10	89.16	88.63	53.50	54.33	53.92
Vanraj	302.99	310.20	306.60	96.80	98.31	97.56	78.09	80.50	79.29
Zafrani Gola	221.69	220.42	221.05	77.82	78.56	78.19	75.27	71.85	73.56
Zardalu	203.45	226.73	215.09	106.98	104.73	105.86	62.37	65.97	64.17
S.Em.±	4.22	9.69	5.04	1.92	1.88	1.41	1.04	1.21	0.81
C.D. at 5%	11.86	27.25	14.18	5.41	5.29	3.98	2.92	3.39	2.29

Table 3: Studies on fruit physical parameters of different mango varieties

Variety	Pulp : stone ratio			Waste index			Fibre content (%)			Lenticel density (%)		
	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled
Amin	2.41	2.78	2.60	1.68	1.89	1.79	2.28	2.35	2.31	21.50	23.34	22.42
Amrapali	4.31	5.00	4.65	2.44	2.52	2.48	2.35	2.81	2.58	14.12	13.55	13.84
Bara Malda	3.47	3.57	3.52	2.63	2.72	2.68	2.89	3.14	3.01	11.52	11.48	11.50
Banarasi Betali	6.31	6.22	6.26	3.45	3.27	3.36	2.58	2.54	2.56	8.13	8.32	8.22
Baramasi	3.46	3.52	3.49	2.09	2.16	2.12	2.14	1.93	2.04	14.45	15.36	14.91
Bathui	3.07	2.41	2.74	1.75	1.65	1.70	2.98	1.89	2.44	11.07	10.68	10.87
Bijoragarh	5.57	4.92	5.24	2.99	2.64	2.82	2.42	3.33	2.87	13.06	12.85	12.96
Bombay Green	3.13	2.75	2.94	1.94	1.67	1.80	0.94	0.94	0.94	7.15	7.74	7.44
Bride of Russia	2.53	3.34	2.94	1.46	1.69	1.57	2.63	2.85	2.74	13.70	13.43	13.57
Chausa	3.96	4.77	4.37	2.46	2.70	2.58	1.57	1.57	1.57	31.86	28.54	30.20
Dashehari	2.99	2.82	2.90	1.57	1.43	1.50	1.61	1.98	1.79	19.29	19.22	19.26
Dashehari-51	4.96	4.94	4.95	2.06	2.06	2.06	0.87	0.77	0.82	21.33	19.46	20.40
Duddha Peda	4.01	3.79	3.90	2.45	1.93	2.19	0.79	0.82	0.81	8.09	8.26	8.18
Gulabkhas	2.89	3.26	3.08	1.80	1.78	1.79	2.20	2.82	2.51	21.90	22.06	21.98
Gulabkhas Green	2.51	2.56	2.53	1.45	1.59	1.52	0.89	0.68	0.79	11.43	11.43	11.43
Gurwani	2.59	2.32	2.46	1.63	1.45	1.54	2.46	2.80	2.63	15.83	15.14	15.48
Haathijhool	10.60	10.30	10.50	5.30	5.24	5.27	5.27	5.43	5.35	21.49	23.96	22.72
Husn-a-ra	1.98	1.90	1.94	1.36	1.46	1.41	0.94	0.80	0.87	34.66	37.68	36.17
Kaitki Bihar	4.33	4.37	4.35	2.50	2.84	2.67	0.76	0.47	0.62	18.34	18.28	18.31
Kesar (Basti)	1.79	2.12	1.95	0.69	0.79	0.74	1.94	2.39	2.17	10.13	11.06	10.60
Khas-ul-Khas	2.86	2.49	2.67	1.28	1.08	1.18	1.22	1.41	1.31	6.52	7.51	7.02
K.O.-07	1.93	1.89	1.91	1.04	0.98	1.01	2.17	2.82	2.50	10.18	10.48	10.33
Langra	4.66	4.24	4.45	2.87	2.53	2.70	1.64	1.72	1.68	21.51	22.38	21.95
Langra Gorakhpur	6.99	7.20	7.09	3.51	3.89	3.70	2.14	2.65	2.40	17.34	16.76	17.05
Langra Rampur	6.01	5.92	5.97	2.67	2.66	2.67	2.18	1.78	1.98	18.53	19.88	19.21
Mallika	7.37	8.90	8.13	3.93	4.64	4.29	1.25	1.72	1.48	29.08	24.76	26.92
Mithua Malda	3.40	3.81	3.61	1.76	1.84	1.80	1.33	1.15	1.24	9.49	8.95	9.22
Mulgoa Deshi	4.29	4.53	4.41	2.16	2.14	2.15	2.18	2.68	2.43	24.47	24.64	24.56
Neelum	3.78	3.69	3.73	2.56	2.28	2.42	1.21	1.33	1.27	13.94	14.54	14.24
Neelum×Chausa	3.72	4.08	3.90	2.17	2.26	2.22	1.67	2.15	1.91	15.12	16.57	15.85
Pulgoa Darbhanga	3.48	3.35	3.42	2.20	2.20	2.20	2.79	2.45	2.62	32.50	33.81	33.16
Rahman Pasand	2.58	2.57	2.58	1.65	1.63	1.64	3.63	3.72	3.68	24.56	24.10	24.33
Rataul	2.50	2.11	2.31	1.53	1.35	1.44	2.01	1.44	1.73	18.99	18.64	18.82
Rumani	3.42	4.96	4.19	2.64	3.46	3.05	0.77	0.77	0.77	49.57	51.58	50.57
Safeda Lucknow	2.94	3.23	3.08	2.23	2.34	2.28	1.32	1.05	1.19	13.38	13.34	13.36
Sensation	3.49	3.54	3.51	1.92	1.92	1.92	1.85	1.59	1.72	8.77	8.54	8.66
Suvarnrekha	7.13	7.18	7.15	3.68	3.72	3.70	1.53	0.88	1.21	12.03	10.83	11.43
Tamancha	2.88	2.87	2.87	1.64	1.83	1.74	0.90	0.97	0.93	8.06	8.24	8.15
Thanking Amadi	4.10	4.50	4.30	2.19	2.15	2.17	1.84	1.83	1.83	15.17	15.06	15.12
Totapari Red Small	3.17	3.13	3.15	2.21	2.06	2.14	0.72	0.73	0.72	23.21	25.51	24.36
Vanraj	7.63	7.91	7.77	4.00	3.91	3.96	1.65	1.86	1.76	21.90	18.60	20.25
Zafrani Gola	6.18	6.36	6.27	2.55	2.47	2.51	1.59	1.55	1.57	31.71	35.39	33.55
Zardalu	2.13	2.68	2.40	1.59	1.85	1.72	1.05	1.32	1.19	10.46	10.55	10.51
S.Em.±	0.10	0.11	0.12	0.06	0.11	0.06	0.10	0.07	0.06	2.15	1.94	1.58
C.D. at 5%	0.29	0.31	0.33	0.17	0.31	0.17	0.29	0.19	0.17	6.06	5.47	4.44

Genetic Diversity:

DNA markers

The importance of genetic variations in facilitating plant breeding and/or conservation strategies has long been recognized Molecular markers are useful tools for assaying genetic variation and provide an efficient means to link phenotypic and genotypic variation. In recent years, the progress made in the development of DNA based marker systems has advanced our understanding of genetic resources. These molecular markers are classified as: (i) hybridization based markers, i.e., restriction fragment length polymorphisms (RFLPs), (ii) PCR-based markers i.e. random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), inter simple sequence repeats (ISSRs) and microsatellites or simple sequence repeats (SSRs), and (iii) sequence based markers, i.e., single nucleotide polymorphisms (SNPs). Majority of these molecular markers have been developed either from genomic DNA library (e.g. RFLPs or SSRs) or from random PCR amplification of genomic DNA (e.g. RAPDs) or both (e.g. AFLPs) Availability of an array of molecular marker techiques and their modifications led to comparative studies among them in many crops including fruit crops Molecular markers provide an attractive and more reliable alternative to morphological and biochemical markers. In mango, phenotypic markers have been the commonly used method of describing cultivars. Although this approach is useful to distinguish distantly related cultivars, its reliable application has proven more difficult when it comes to differentiating closely related lines or off types of a particular cultivar. This is because phenotypically indistinguishable trees can be genotypically different and therefore variants of a given cultivar cannot be easily detected by phenotypic assessment. Consequently, a more refined technique, such as molecular markers that are highly polymorphic is required. DNA-based markers can be gainfully used in mango breeding for marker assisted selection (MAS) and for cultivar identification.

Random Amplified Polymorphic DNA (RAPD)

The potential of RAPD markers to establish genetic relationships among *Mangifera* species, in the identification of *Mangifera indica L.* cultivars and in the validation of genetic relationships among them has been demonstrated by many authors[1,35].

Variable Number Tandem Repeat Sequence (VNTRS)

Adato *et al*., analyzed DNA fingerprint information of some mango genotypes using minisatellite multilocus probes. Genomic DNA of 26 mango cultivars and 14 mango rootstocks probed with 33.6, R18.1, 22.3, (GGAT)4, (GTG)5, and (GATA)**4** revealed resolvable and complex band pattern. DNA fingerprinting pattern of mango cultivar and rootstocks showed high polymorphism between unrelated cultivars. Each cultivar or rootstock can be identified by its DSP pattern. The probability of two unrelated cultivars and rootstocks having the same pattern is 8 x 10-6 and 1.2 x 10-5**,** respectively. The presence of many specific bands and low levels of band sharing between cultivars reflects the high level of informativeness of probe 33.6. The loci detected by other microsatellites were of low polymorphism. Based on DNA fingerprint information, genetic distances between 20 mango cultivars were evaluated and an evolutionary tree was established. Analysis of DNA fingerprint band patterns of 12 progeny resulting from a cross between Tommy Atkins and Keitt mango revealed neither linked or nor allelic bands. The results suggested that DNA fingerprints in mango are likely to be useful for identification and breeding purposes

Restriction Fragment Length Polymorphism (RFLP)

Description of Restriction Fragment Length Polymorphism (RFLP) as a DNA profiling technique and elucidation of its potential use in varietal identification by Botstein *et al*., opened new avenues in genetic studies and varietal improvement programmes. Capote *et al*., and Ravishankar *et al.*[35], have employed the RFLP technique for genetic studies in mango.

Amplified Fragment length Polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is a PCR-based technique which allows inspection of polymorphism at fairly a large number of loci within a very short span of time and at the same time requires a very small amount of DNA. The usefulness of AFLP marker system in genetic diversity analysis and fingerprinting of mango cultivars/genotypes has been reported by Eiadthong *et al*[10],.

Inter Simple Sequence Repeat markers (ISSR)

Amplification of inter-simple-sequence-repeats (ISSRs) is a relatively novel technique and has proven to be a powerful, rapid, simple, reproducible and inexpensive way to assess genetic diversity or to identify closely related cultivars in many species, including fruit trees[12]. A number of reports are available regarding the potential application of ISSR markers in genetic diversity estimation and fingerprinting in mango[10,12,17,34,38,40].

Simple Sequence Repeat (SSR) markers

Among the molecular markers available for genetic studies, SSR markers have gained considerable importance owing to many desirable attributes including hypervariability, multiallelic nature, codominant inheritance, reproducibility, relative abundance, extensive genome coverage (including organellar genomes), chromosome specific location, amenability to automation and high throughput genotyping. In contrast, RAPD assays are not sufficiently reproducible, whereas RFLPs are not readily adaptable to high throughput sampling. AFLP is complicated as individual bands are often composed of multiple fragments mainly in large genome templates. SSRs have been succesfully used for genetic diversity estimation and DNA fingerprinting in mango[44].

A dendrogram was constructed from the 304 accessions. These accessions displayed 207 different genotypes. Some suspected identification errors in the germplasm bank were confirmed. Eight accession identities could be corrected according to morphological observations confirmed by molecular pattern comparisons with the original Floridian varieties. Galvez-Lopez *et al*., investigated genetic relationships among 112 mango (*Mangifera indica*) plants native to 16 states of Mexico and four controls [three mango cultivars (Ataulfo, Manila and Tommy Atkins) and one accession of *Mangifera odorata*] based on simple sequence repeat (SSR) markers.

Despite the low degree of genetic differentiation among accessions, Myanmar's accessions were distinguishable from mango accessions from Florida, India, and Southeast Asia in a principal coordinates plot. Genetic differentiation of the Myanmar accessions from other groups was also observed in a Bayesian cluster analysis. No population structure among Myanmar accessions was revealed by a neighbor-joining tree. The results revealed a broad genetic background and genetic distinctiveness of mango in Myanmar. Olano *et al*., used 25 SSR markers to evaluate the genetic background of 63 Florida mango varieties, as well as cultivars from India, Asia and other locations. The parentage analysis, performed in a multistage process resulted in four parent-offspring sets. The results indicated that as few as four Indian cultivars, and the 'Turpentine' land race were involved in the early cultivar selections. Sixty-three of the 85 parents identified across the four generations were other Florida cultivars. Results of the study indicated that the Florida types are more closely related to Indian than to Southeast Asian types. It was also confirmed that the Florida group is not more diverse than either of the parental groups. Schnell *et al.*, reported the development and characterization of 15 microsatellite loci isolated from *Mangifera indica L.*

SSR Amplification Polymerase chain reaction (PCR) amplification was performed in a Perkin Elmer Thermocycler (PCR-Gene Amp PCR System 9700) as per the protocol suggested by Williams *et al*., using 109 mango-specific microsatellite markers (Table 2). Amplified products were separated by electrophoresis in a 3% metaphor-agarose gel

using Tris-acetate EDTA Tris-acetate EDTA (TAE) buffer at pH 8.0. The amplified fragments were observed and photographed under UV light in Gel Doc System (Syngene, Cambridge, United Kingdom). 198 alleles were chosen for their clear pattern and high allele numbers to study diversity within the entire sample.

Table 4:

List of polymorphic microsatellite primers used in this study.

Primer	Sequence (5'-3')	Annealing Temperature (°C)	Allele size range (bp)
SSR-18	F: CGTCATCCTTTACAGCGAACT R: CATCTTTGATCATCCGAAAC	56	100-115
SSR-20	F: CGCTCTGTGAGAATCAAATGGT R: GGACTCTTATTAGCCAATGGGATG	58	295-310
SSR-23	F: AAACAAAGAATGGAGCA R: TGGACTGAATGTGGATAG	50	240-270
SSR-28	F: GACCCAACAAATCCAA R: ACTGTGCAAACCAAAAG	52	160
SSR-41	F: ATCCCCAGTAGCTTTGT R: TGAGAGTTGGCAGTGTT	53	210–244
SSR-50	F: ATGGAGACTAGAATGTACAGAG R: ATTAAATCTCGTCCACAAGT	52	202
SSR-51	F: AAATAAGATGAAGCAACTAAAG R: TTAGTGATTTTGTATGTTCTTG	52	287
SSR-52	F: AAAAACCTTACATAAGTGAATC R: CAGTTAACCTGTTACCTTTTT	52	207
SSR-59	F: TTCTTTAGACTAAGAGCACATT R: AGTTACAGATCTTCTCCAATT	56	191
SSR-62	F: CACAGCTCAATAAACTCTATG R: CATTATCCCTAATCTAATCATC	53	172
SSR-68	F: GGTCAGCTGTGTGTGTGTG R: CAATTCAATGCTTTGGATGCT	56	158
SSR-78	F: CCTTGGGTTCATTCGCTAAA R: GGACGCCACACACACACAC	55	165
SSR-80	F: TGGTATTCAAGCATGGTCCTC R: TCCCATCACACACACACAC	57	244
SSR-81	F: TCTCCCTTCATCGATTGTCC R: GGAGCGTCTCTCTCTCTCCA	55	122
SSR-82	F: TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC	57	108-155
SSR-85	F: GCTTGCTTCCAACTGAGACC R: GCAAAATGCTCGGAGAAGAC	58	229-269
SSR-87	F: GCCCCATCAATACGATTGTC R: ATTTCCCACCATTGTCGTTG	55	153-187
SSR-89	F: CGCCGAGCCTATAACCTCTA R: ATCATGCCCTAAACGACGAC	55	92-122
SSR-90	F: TGATATTCAGGGCCCAAG R: AAATGGCACAAGTGGGAAAG	54	167-209
SSR-91	F: GCTCAACGAACCCAACTGAT R: TCCAGCATTGAATGAAGAAGTT	60	237-260
MngSSR-14	F: TCATTAAGCTGTGGCAACCA R: CATTGCATAGATGTGGTCATT	59	160-192
MngSSR-26	F: ACCTTGGTCAGGACAAAATCC R: GACTTCATAAGAAGAGGCGTC	60	135-150
MngSSR-27	F: CGAAACCGACTGCCTATTTT R: CCATTAATAAAGTTGTGGCCA	57	158-172

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