

Morphological and Microsatellite Analysis of Intravarietal Heterogeneity in 'Beneshan' Mango (*Mangifera indica* L.)

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Abstract

'Beneshan' is the choicest table cultivar of mango (*Mangifera indica* L.) that has been under cultivation for more than a century in Andhra Pradesh state, India. Through an eco-geographic survey covering the three regions of the state, 31 accessions of 'Beneshan' (BN Acc-1 to BN Acc-31) were selected and their fruit and leaf samples were collected to study intracultivar heterogeneity based on morphological fruit traits and microsatellite markers, respectively. *In-situ* characterization and evaluation of fruit samples revealed phenotypic variations among 'Beneshan' accessions. Of the 109 mango-specific simple sequence repeats (SSRs) validated, 23 were polymorphic. Polymorphic microsatellites produced a total of 58 alleles, of which 30 were polymorphic (51.72%). The polymorphic information content values varied from 0.03 (SSR-59) to 0.72 (SSR-87). Highly polymorphic microsatellites like SSR-80, SSR-87, SSR-28, and SSR-89 were more useful in differentiating the 'Beneshan' accessions. Microsatellites SSR-91 and MngSSR-26 produced unique alleles of 280 and 140 bp in BNacc-8 and BNacc-9 accessions, respectively. Jaccard's similarity coefficient varied from 0.50 to 1.00. There was a wide range of intravarietal heterogeneity (up to 50%) indicating that 'Beneshan' whatsoever cultivated throughout the state is not pure clone, which allows the genetic breeding of this cultivar by means of mass selection.

Keywords: Eco-geographic survey, fruit morphology, fruit quality, intracultivar diversity, microsatellites polymorphism

1. Introduction

Mango (*Mangifera indica* L.) is one of the most important fruit crops of the Anacardiaceae family. It originated as an allopolyploid from eastern India, Assam and Burma (Popenoe, 1920). It occurs as a domesticated or wild entity in the complex biotic community of the ecosystem. It has been undoubtedly under cultivation for more than 4000 years in Eastern India and Burma (Mukherjee,

1951). It is now cultivated pantropically throughout the world. It is one of the most popular fruits in the tropics (Lakshminarayana, 1980). In India, mangos are cultivated in an area of 2.31 million ha with an annual production of 12.75 million tons. Indian mangos occupy 45.59% of the total world's mango area and contribute 35.70% to the total world's mango production. India is the largest

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mango producer in the world (NHB, 2010). Mango has become the third most important fruit crop in Andhra Pradesh at the turn of the 20th century. In Andhra Pradesh, mangos are cultivated in an area of 0.50 million ha with an annual production of 2.52 million tons. Andhra Pradesh mangos occupy 21.55% of the total country's mango area and contribute 19.78% to the total country's mango production. Andhra Pradesh is the second largest mango producer in the country (NHB, 2010).

Mango has rich germplasm diversity and there are about 1600 varieties in the world (Pandey, 1998). In India, there are hundreds of mango cultivars, of which only some 25 to 40 are of commercial importance (Chadha and Pal, 1986). 'Beneshan' is the choicest table cultivar of mango in Andhra Pradesh. It originated in Banaganapalle (Rajagopal, 1974), which is a definite geographical territory in Kurnool district, Andhra Pradesh. The folk talk and the existing plantations in 'Kausar Bagh' stand indelible witness to emphasize 'Beneshan' is the native of Banaganapalle. While mango is called as the 'King of fruits', 'Beneshan' is called as the 'King of mangoes' (Begum *et al.*, 2010) owing to its appearance, taste and flavor. 'Beneshan' is tolerant to gall midge (Jhaka *et al.*, 1987), tolerant to stone weevil (Rao *et al.*, 1971) and less susceptible to hopppers (Vijayalakshmi *et al.*, 2010). The 'Beneshan' production originates from 'Banaganapalle' of Rayalaseema region, but also has been spread over Coastal Andhra and Telangana regions of Andhra Pradesh. It is in demand domestically as well as universally for its attractive color, appealing shape, unique flavour and soft, stringless, ample, sweet and low fiber containing pulp and long shelf life. The Indian mango business has been predominantly based on this cultivar. It is the most popular and most exported mango cultivar of India. It is also the flavor of choice for the mango lovers all over the world. Andhra Pradesh, the largest producer of 'Beneshan' mango in the country wants to increase its participation in the international market. Despite the still growing economic importance of 'Beneshan' mango in India, its potential has not yet been fully utilized. Further, 'Beneshan' mango production struggles with several constraints including intravarietal variability. 'Beneshan' cultivar is troublesome for gardeners because of its locality dependent variation in the fruit size, shape and quality. It was shown that genetic variability

could occur within the same cultivated populations, which could justify heterogeneity in production and quality. Moreover, at present, 'Beneshan' has numerous vernacular names in different regions, which makes identification difficult and in turn causes confusion among farmers and breeders. Characterization of intravarietal variability of 'Beneshan' is thus important to provide scientists, producers and all interested people, with information about the distinct and unique characteristics of this cultivar. Therefore, it becomes necessary to establish the phylogenetic relationship and investigate any possible differences at molecular level in the 'Beneshan' mangoes from different pockets. Simultaneously, it may also help to assess the extent of variability for morphological traits according to geographical variability.

Genomic polymorphism between individuals can arise through several different mechanisms, which include single nucleotide changes, deletions and insertions and above all, through variable numbers of simple sequence repeats. Microsatellites or simple sequence repeats (SSRs) are particularly informative because they are evenly spread throughout the genomes and typically are highly polymorphic. In an attempt to study genomic polymorphism within the 'Beneshan' cultivar from several orchards and stocks, we have carried out a preliminary set of experiments using previously described SSR primers. This work was focused on validation of microsatellite markers, their polymorphic potential and assessment of intravarietal polymorphism among 31 accessions of 'Beneshan', as a fundamental base for breeding programs and conservation.

2. Background or Literature Review

Mango is a clonally propagated fruit crop in Andhra Pradesh. The cultivar 'Beneshan' mango is highly popular in all three regions of the state, where a large number of public and private nurseries are involved in clonal propagation and supply of plant material (grafts). This ensured the spread of 'Beneshan' variety on commercial scale. Even though the planting material has been multiplied vegetatively by veneer grafting or epicotyl grafting, often clonal variation is routinely observed under field conditions (Gurudatta *et al.*,

2004; Islam *et al.*, 2004; Prasanth *et al.*, 2007; Rosetti *et al.*, 2004). Clonal variations are manifested many times in fruit characteristics besides other vegetative characteristics and yield attributes (Gan *et al.*, 1981; Naik, 1948; 1971; Oppenheim, 1956; Pandey, 1998; Singh *et al.*, 2009).

There are different methods to characterize intravarietal heterogeneity, such as morphological, biochemical and molecular markers. Several procedures have been employed for the identification and characterization of intracultivar heterogeneity of mango based on morphological (Gan *et al.*, 1981; Naik, 1948; 1971; Oppenheim, 1956; Pandey, 1998; Singh *et al.*, 2009), biochemical (Gan *et al.*, 1981) and genetic traits (Bally *et al.*, 1996; de Souza and Lima, 2004; Diaz-Matallana *et al.*, 2009; Manchekar, 2008; Rocha *et al.*, 2012; Singh *et al.*, 2009). In mango, conventional methods of intracultivar heterogeneity identification are based on objective description of leaf, fruit and stone characteristics. Previously, morphological characteristics such as characteristics of fruits were used to identify intravarietal variability in mango. It was observed that considerable variation exists among trees of the same clone in an orchard of mango with respect to fruit shape, size, colour and quality. Morphological traits, in general, are subjective and are visually evaluated in most cases. They can improve characterizations for defining the potential use of any genotype. There are few reports on variability among the trees of the same variety of mango in an orchard with respect to fruit size, shape, colour and quality (Gan *et al.*, 1981; Naik, 1948; 1971; Oppenheim, 1956; Pandey, 1998; Singh *et al.*, 2009). Naik (1948) observed significant variation among the trees of the same variety in an orchard with regard to fruit shape, size, color and quality of the fruits which was ascribed to bud mutations. Oppenheimer (1956) reported a wide variability in the performance of the trees belonging to same variety in same orchard, after surveying many orchards in India. Further, there are few reports on variability among the trees of the same variety in an orchard with respect to fruit size, shape, color and quality (Naik, 1971), which are highly influenced by environment. Morphologically, all the six 'clones' of mango studied viz., 'Harummanis', 'Apple', 'Irwin', 'Kent', 'Malgoa' and 'Hj Bakar' are very distinct

with respect to the morphology and the taste of the fruits (Gan *et al.*, 1981). Singh *et al.* (1985) isolated two high yielding clones from the orchards of 'Langra' with improved resistance to bacterial black spot. Whaley *et al.* (1993) identified certain strains within 'Kensington' clone, having improved resistance to bacterial black spot disease. Pandey (1998) studied different clones of 'Alphonso' and found that they differ from one another in more than one character. Morphological analysis based on 17 fruit characters detected prominent variation in the landraces 'Banganapalli', 'Langra', and 'Dashehri' and some variation in the cultivar 'Mallika' (Singh *et al.*, 2009). In the above cases, the identification of intravarietal variability is based on morphological traits. But number of these traits is limited, they are unstable and they do not always enable distinguishing between closely related accessions or cultivars (Konarev, 2000). The disadvantages of morphological characterizations are their low polymorphism, heritability, and sensitivity to changes in environmental conditions.

As in other vegetatively propagated crop species (Meneghetti *et al.*, 2011; Pissard *et al.*, 2008; Zdunic *et al.*, 2009) the genetic intravarietal variability of mango has been analyzed by PCR-derived marker systems. Molecular characterization of intravarietal variability of mango cultivars has been carried out with different molecular systems such as isozymes (Gan *et al.*, 1981), randomly amplified polymorphic DNA-RAPDs (Bally *et al.*, 1996; de Souza and Lima, 2004; Diaz-Matallana *et al.*, 2009; Manchekar, 2008;), inter simple sequence repeats-SSRs (Rocha *et al.*, 2012; Singh *et al.*, 2009). A genetic dissimilarity of 0.05% was observed among 27 accessions of 'Kensington Pride', a polyembryonic cultivar of mango using RAPD markers (Bally *et al.*, 1996). A genetic dissimilarity of 55% was observed among 25 accessions of 'Rosa', a polyembryonic cultivar of mango tree using RAPD markers (de Souza and Lima, 2004). Molecular analysis of nine clones of 'Alphonso' collected from different locations of Maharashtra (Devgad, Dapoli, Ratnagiri and Vengurla) and Karnataka (Belgaum and Dharwad) using seven RAPD primers proved that RAPD is a promising technique to detect intracultivar genetic diversity and to study the genetic relationship among the nine clones of 'Alphonso' mango (Manchekar,

2008). In addition, there are few reports on intracultivar variability in 'Langra', 'Dashehri' and 'Neelum' cultivars of mango detected using ISSRs (Singh *et al.*, 2009). Diaz-Matallana *et al.* (2009) observed a small genetic differentiation among the mango 'Hilacha' populations studied using 60 RAPD markers. 102 accessions of 'Uba' mango tree were characterized using ISSR markers and found that accessions with 100% of similarity were not found, which indicates the absence of duplicates (Rocha *et al.*, 2012). Overall, these researches indicated that various molecular genetic markers have proven to be valuable tools for identifying intracultivar heterogeneity in mango. Microsatellites are widely used as a versatile tool in plant breeding programmes because of their high ability for showing diversity among the genotypes. Microsatellites are considered as the marker of choice for fingerprinting and breeding in both plant and animal species. In mango, although microsatellites have been successfully used for genetic diversity analysis (Begum *et al.*, 2012; Duval *et al.*, 2005; Eiadthong *et al.*, 1999; Hirano *et al.*, 2010; Honsho *et al.*, 2005; Lopez *et al.*, 2009; Schnell *et al.*, 2005; 2006; Vasugi *et al.*, 2012; Viruel *et al.*, 2005; Wahdan *et al.*, 2011), no attempt has been made on intracultivar genetic diversity analysis. Morphological markers combined with molecular characterisation are essential for better understanding of genetic diversity in mango (Singh *et al.*, 2009; Begum *et al.*, 2012).

3. Materials and Methods

Survey and Documentation

Following the review of relevant literature and consultation with mango experts, 9 districts covering three eco-geographical regions of Andhra Pradesh were selected as target sites for eco-geographic survey and were assumed rich in 'Beneshan' mango diversity. Eco-geographic survey was conducted during summer 2009. The team visited at least 1-5 target sites within each district. The target sites represented the farmers' mango orchards and mother blocks of the nurseries. In the process, due consideration was

also given to visiting old mango orchards grown by communities. The survey sites are in diverse geography covering the Coastal Andhra, Rayalaseema and Telangana regions of Andhra Pradesh. A total of 31 accessions of 'Beneshan' (accession IDs starting with BN; BN Acc-1 to BN Acc-31) were selected for the present study (Table1). One 'Beneshan' tree was sampled from each of the 26 farmers' mango orchards (BN Acc-4 to BN Acc-29), while two and three mango trees were sampled from mother blocks of the Fruit Research Station (FRS), Sangareddy (BN Acc-1 to BN Acc-3) and the Horticultural Research Station (HRS), Anantharajupeta (BN Acc-30 to BN Acc-31), respectively through simple random sampling.

Fruit Sampling, Characterization, Evaluation and Analysis

Following simple random sampling strategy, ten tree-ripe fruits spread over all the sides of the tree canopy were collected from each of the 31 selected trees (accessions) of 'Beneshan'. Collected fruit samples were characterized and evaluated in-situ for variation in 7 qualitative and 8 quantitative traits of fruits. In each sample, quantitative traits like fruit length (cm), fruit width (cm), fruit thickness (cm), fruit weight (g), peel (%), pulp (%), stone (%) and total soluble solids (TSS) (°Brix) were recorded. Percent peel, pulp and stone were calculated by the weight of the peel, pulp and stone, respectively divided by total weight of the fruit multiplied with 100. TSS was recorded with hand refractometer. In addition, the fruit samples were also characterized for qualitative traits like fruit shape, skin colour of mature fruit, skin thickness, skin texture, pulp colour, quantity of fiber and eating quality. Eating quality was assessed qualitatively through organoleptic evaluation by a panel of mango tasters with combined assessment of flavour, acidity, sweetness, aroma and astringency and quantitative measurement of TSS of the pulp of the fruit samples when ripe. The mean data of each of the random fruit sample of 31 'Beneshan' accessions was analysed for 9 quantitative traits following 'Descriptive Statistics' for mean, standard error, standard deviation, sample variance and coefficient of variation.

Table 1

List of 'Beneshan' mango accessions and their collection sites

Accession	Sampling unit	Collection site		
		Village	District	Region
BN Acc-1	Mother block, FRS	Sangareddy	Medak	Telangana
BN Acc-2	Mother block, FRS	Sangareddy	Medak	Telangana
BN Acc-3	Mother block, FRS	Sangareddy	Medak	Telangana
BN Acc-4	Farmers' orchard	Pithapuram	East Godavari	Coastal Andhra
BN Acc-5	Farmers' orchard	Bobbili	Vizainagaram	Coastal Andhra
BN Acc-6	Farmers' orchard	Tuni	East Godavari	Coastal Andhra
BN Acc-7	Farmers' orchard	Nandivampu	East Godavari	Coastal Andhra
BN Acc-8	Farmers' orchard	Nandivampu	East Godavari	Coastal Andhra
BN Acc-9	Farmers' orchard	Nuziveedu	Krishna	Coastal Andhra
BN Acc-10	Farmers' orchard	Reddygudam	Krishna	Coastal Andhra
BN Acc-11	Farmers' orchard	Rangapuram	Krishna	Coastal Andhra
BN Acc-12	Farmers' orchard	Reddygudam	Krishna	Coastal Andhra
BN Acc-13	Farmers' orchard	Mylavaram	Krishna	Coastal Andhra
BN Acc-14	Farmers' orchard	Pondugala	Krishna	Coastal Andhra
BN Acc-15	Farmers' orchard	Panyam	Kurnool	Rayalaseema
BN Acc-16	Farmers' orchard	Panyam	Kurnool	Rayalaseema
BN Acc-17	Farmers' orchard	Banaganapalle	Kurnool	Rayalaseema
BN Acc-18	Farmers' orchard	Banaganapalle	Kurnool	Rayalaseema
BN Acc-19	Farmers' orchard	Konampeta	Kadapa	Rayalaseema
BN Acc-20	Farmers' orchard	Konampeta	Kadapa	Rayalaseema
BN Acc-21	Farmers' orchard	Konampeta	Kadapa	Rayalaseema
BN Acc-22	Farmers' orchard	Konampeta	Kadapa	Rayalaseema
BN Acc-23	Farmers' orchard	Sanampudi	Prakasam	Coastal Andhra
BN Acc-24	Farmers' orchard	Sanampudi	Prakasam	Coastal Andhra
BN Acc-25	Farmers' orchard	Tandarpalli	Medak	Telangana
BN Acc-26	Farmers' orchard	Tandarpalli	Medak	Telangana
BN Acc-27	Farmers' orchard	Siddarthnagar	Krishna	Coastal Andhra
BN Acc-28	Farmers' orchard	Lakkireddypalle	Chittoor	Rayalaseema
BN Acc-29	Farmers' orchard	Kollapur	Mahboobnagar	Telangana
BN Acc-30	Mother block, HRS	Anantharajupet	Kadapa	Rayalaseema
BN Acc-31	Mother block, HRS	Anantharajupet	Kadapa	Rayalaseema

BN Acc = Beneshan accession; FRS= Fruit Research Station; HRS= Horticultural Research Station

Leaf Sampling

Two to three young leaves per accession (individual tree) were collected from the field and kept in paper bags from the 31 randomly sampled 'Beneshan' accessions. The leaves were wrapped in moist tissue paper and were refrigerated at -50°C when not subjected to immediate use. Molecular analysis was carried out in the Institute of Biotechnology (Formerly Biotechnology Unit), ANGRAU, Rajendranagar, Hyderabad.

Extraction of Genomic DNA

Total DNA was extracted from leaf samples of each accession according to the modified CTAB method described by Porebski *et al.* (1997).

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.* (1990) 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 2

Quantitative fruit characteristics of 'Beneshan' accessions

Genotype	Fruit length (cm)	Fruit width (cm)	Fruit thickness (cm)	Fruit weight (g)	Peel (%)	Pulp (%)	Stone (%)	Total soluble solids (°Brix)
BN Acc-1	12.00	7.50	6.50	390.00	10.20	77.00	12.80	17.00
BN Acc-2	10.00	6.20	6.50	314.00	11.10	75.60	13.30	16.20
BN Acc-3	11.00	6.50	7.50	352.00	14.20	71.10	14.70	19.00
BN Acc-4	11.20	9.10	6.50	382.00	14.30	70.00	15.70	16.60
BN Acc-5	8.00	6.80	7.00	214.00	15.80	68.40	15.80	16.00
BN Acc-6	8.20	6.00	5.00	190.00	15.70	63.80	20.50	18.00
BN Acc-7	9.50	7.00	5.50	222.00	20.20	57.80	22.00	16.60
BN Acc-8	9.00	6.50	5.00	178.00	23.00	53.50	23.50	17.40
BN Acc-9	11.40	7.80	6.20	330.00	24.20	60.60	15.20	15.00
BN Acc-10	12.00	8.00	7.00	410.00	21.90	66.00	12.10	15.60
BN Acc-11	12.50	7.80	7.40	434.00	18.40	67.80	13.80	15.00
BN Acc-12	12.00	8.50	7.50	430.00	17.50	70.90	11.60	15.00
BN Acc-13	11.50	7.00	5.80	292.00	17.10	69.30	13.60	16.00
BN Acc-14	10.50	8.00	7.50	390.00	25.80	58.90	15.30	15.20
BN Acc-15	12.50	9.00	7.00	392.00	10.30	77.00	12.70	15.60
BN Acc-16	13.50	8.30	7.90	520.00	15.30	75.10	9.60	17.00
BN Acc-17	16.20	7.20	7.20	342.00	11.60	78.50	9.90	20.60
BN Acc-18	14.00	8.80	7.50	520.00	13.40	74.30	12.30	20.00
BN Acc-19	11.50	8.00	7.00	390.00	10.20	79.40	10.40	16.20
BN Acc-20	12.50	8.00	6.50	320.00	10.90	78.50	10.60	15.20
BN Acc-21	12.50	9.00	6.50	370.00	10.80	77.80	11.40	15.80
BN Acc-22	11.00	8.90	6.70	360.00	11.10	77.30	11.60	15.80
BN Acc-23	10.50	8.30	7.50	454.00	8.80	77.90	13.30	22.00
BN Acc-24	14.00	7.50	6.50	466.00	10.70	78.60	10.70	17.00
BN Acc-25	12.20	7.50	7.20	474.00	14.70	72.50	12.80	18.00
BN Acc-26	11.00	6.50	7.50	352.00	11.30	74.00	14.50	19.00
BN Acc-27	12.00	7.90	6.50	400.00	17.50	72.50	10.00	19.00
BN Acc-28	9.50	6.00	6.50	252.00	15.80	70.40	13.80	19.60
BN Acc-29	13.00	9.50	6.50	470.00	17.00	74.40	8.60	19.00
BN Acc-30	10.00	7.50	6.00	280.00	14.20	71.60	14.20	18.00
BN Acc-31	10.50	6.60	5.50	280.00	10.80	75.00	14.20	18.00
Mean	11.46	7.65	6.67	360.32	14.96	71.47	13.56	17.24
Range	8.20	3.50	2.90	342.00	17.00	25.90	14.90	7.00
Standard Error	0.31	0.18	0.14	16.45	0.81	1.21	0.61	0.33
Standard Deviation	1.75	0.98	0.76	91.59	4.51	6.74	3.41	1.83
Sample Variance	3.07	0.96	0.57	8389.63	20.38	45.43	11.60	3.37
CV(%)	15.28	12.78	11.34	25.42	30.18	9.43	25.11	10.64

BN Acc = Beneshan accession

Data Scoring and Statistical Analysis

One hundred and nine mango-specific SSR primer pairs were evaluated from a group of 31 accessions of 'Beneshan'. On the basis of their capacity to generate polymorphic bands only 23 pairs of highly polymorphic SSRs were used to estimate the intracultivar heterogeneity among all the 31 accessions (Table 2). Only clear and reproducible

bands were scored. The banding patterns obtained by each SSR primer were scored as absent (0) or present (1). From the banding patterns obtained with each primer, the accession-specific band(s), if any, along with their sizes were recorded. All computations were carried out using the numerical taxonomy and multivariate analysis system (NTSYS)-pc, Version 2.1 package (Rohlf, 2000).

The resultant similarity matrix data was employed to construct a dendrogram based on unweighted pair group of arithmetic means algorithm (UPGMA) to infer genetic relationships among accessions.

4. Results and Discussion

The mango crop is one of the most significant agribusiness in India. Mango business in India is based largely on 'Beneshan'. It is one of the most cultivated table varieties of mango in India. It is believed to have originated in Banaganapalle, Andhra Pradesh, India, where it has been under cultivation for more than hundred years. The Andhra Pradesh State, India, stands out as a major producer and supplier of 'Beneshan' mango, a cultivar with excellent conditions for domestic as well as export markets due to its superior characteristics, such as pulp color and flavor, besides the great appreciation for fresh consumption. In spite of possessing so many virtues, 'Beneshan' cultivar is troublesome for gardeners because of its locality dependent variation in the fruit size, shape and quality resulting in heterogeneity in production and quality. Data on the regional polymorphism of this important cultivar is scarce or non-existent. This demands conceivable clarification for genetic purity and relativity among 'Beneshan' mangoes from different regions in the state.

Morphological Characterization

Morphological traits are visually evaluated in most cases and are thereby subjective morphological characteristics that can improve characterizations for defining the potential use of any genotype. These traits have long been the means of studying variability among populations in fruit crops. In fruit tree species, quantitative and qualitative fruit traits have been found useful in identification and assessment of intravarietal heterogeneity and selection of elite forms for fruit production on a large scale as these traits help in developing the ideotypes. In mango, the oldest and most widely used markers were the morphological traits, which may still be optimal for certain cases, where the cultivars were identified based on leaf, panicle, fruit and other physical characteristics. In spite of it, actual identity of some cultivars is still in question, because similar cultivars grown in

different areas often have various names (Lakshminarayana, 1980).

From the results of mean, standard error, standard deviation, sample variance and coefficient of variation (Table 2), it is evident that there was significant variation in 9 quantitative fruit traits among 31 accessions of 'Beneshan' under study. The fruit length ranged from 8.00 to 16.20 cm. The fruit width ranged from 6.00 to 9.50 cm. The fruit thickness ranged from 5.00 to 7.90 cm. The fruit weight ranged from 178.00 to 520.00 g. The peel, pulp and stone contents ranged from 8.80 to 25.80%, 53.50 to 79.40% and 8.60 to 23.50%, respectively. The TSS ranged from 15.00 to 22.00 °Brix with an average of 16.75 °Brix. In the present study, fruits of all accessions of 'Beneshan' were found variable in size, weight, fiber length, peel, pulp and stone content and total soluble solids. There were some differences among 31 accessions of 'Beneshan' (Table 3) with respect to certain qualitative traits like fruit shape, color of skin of mature fruit, skin thickness, pulp color, quantity of fibre and eating quality. The present findings are in consonance with those of Singh *et al.* (2009) who also detected prominent variation in the cultivar 'Banganapalli' based on morphological analysis of 17 fruit characters. The present findings are also in agreement with those of Bally *et al.* (1996), who also observed phenotypic variation in the type of fruit in 15 accessions of 'Kensington Pride', a polyembryonic cultivar of mango. From the results of this morphological study, a vision is obtained about the range of intravarietal heterogeneity in 'Beneshan' mango conserved in farmers' orchards in the collection sites. Conventionally also, the intracultivar heterogeneity of mango has been characterized mostly at the morphological level by several researchers (Gan *et al.*, 1981; Naik, 1948; 1971; Oppenheim, 1956; Pandey, 1998; Singh *et al.*, 2009). The prime advantages of morphological traits are simplicity and rapid, inexpensive assays, even from herbarium specimens and other dead tissues. Although morphological traits are very useful, they have several disadvantages. They are often limited in number. They suffer from lack of decisiveness. They face heritability problems as they may be controlled by epistatic and pleiotropic gene effects. Morphological characterizations are error prone due to environmental variations affecting expression of these characteristics. In addition, these

observations are time consuming and this mode of identification is slow because of long juvenile periods. Thus, these morphological characters may not adequately represent the genetic heterogeneity among accessions of a cultivar. Hence, characterization of intravarietal heterogeneity based on morphological traits needs complementation with molecular markers as they can contribute greatly to the utilization of intravarietal heterogeneity through descriptive information of structure of genotypes, analyses of relatedness, the study of identity and location diversity.

Microsatellite Allele Polymorphism

The study of genetic heterogeneity has been greatly facilitated by the advent of DNA marker technology in the 1980s, which offered a large number of environmentally-insensitive genetic markers that could be generated to follow the inheritance of important morpho-agronomic traits. A number of DNA fingerprinting techniques are available for detection of genetic polymorphism (Semagn *et al.*, 2006). Microsatellites are among the most commonly used DNA markers for studying genetic polymorphism. Microsatellites are tandem repeats of 1 to 6 nucleotides long DNA motifs, present in eukaryotic genome.

Table 3

Qualitative traits of 'Beneshan' accessions

Accession	Fruit shape	Color of skin of mature fruit	Skin thickness	Skin texture
BN Acc-1	Obliquely oval	Deep yellow	Thin	smooth
BN Acc-2	Obliquely oval	Deep yellow	Medium	Smooth
BN Acc-3	Obliquely oval	Deep yellow	Thin	Smooth
BN Acc-4	Oblong oval	Light yellow	Thin	Smooth
BN Acc-5	Obliquely oval	Yellow	Thin	Smooth
BN Acc-6	Oblong oval	Yellow	Thin	Smooth
BN Acc-7	Obliquely oval	Yellow	Thin	Smooth
BN Acc-8	Obliquely oval	Yellow	Thin	Smooth
BN Acc-9	Ovate oblong	Yellowish green	Thin	Smooth
BN Acc-10	Ovate oblong	Yellow	Thin	Smooth
BN Acc-11	Oblong oval	Yellow	Thin	Smooth
BN Acc-12	Obliquely oval	Yellow	Thin	Smooth
BN Acc-13	Obliquely oval	Yellowish green	Thin	Smooth
BN Acc-14	Oblong oval	Dark yellow	Thin	Smooth
BN Acc-15	Obliquely oval	Golden yellow	Thin	Smooth
BN Acc-16	Obliquely oval	Golden yellow	Thin	Smooth
BN Acc-17	Ovate reniform	Golden yellow	Thin	Smooth
BN Acc-18	Obliquely oval	Yellow	Thin	Smooth
BN Acc-19	Obliquely oval	Red blush over shoulder	Thin	Smooth
BN Acc-20	Obliquely oval	Red blush over shoulder	Thin	Smooth
BN Acc-21	Obliquely oval	Golden yellow	Thin	Smooth
BN Acc-22	Obliquely oval	Yellowish green	Thin	Smooth
BN Acc-23	Obliquely oval	Yellowish green	Thin	Smooth
BN Acc-24	Obliquely oval	Yellow	Thin	Smooth
BN Acc-25	Obliquely oval	Yellow	Thin	Smooth
BN Acc-26	Obliquely oval	Golden yellow	Thin	Smooth
BN Acc-27	Obliquely oval	Yellowish green	Thin	Smooth
BN Acc-28	Obliquely oval	Greenish yellow	Thin	Smooth
BN Acc-29	Obliquely oval	Golden yellow	Thin	Smooth
BN Acc-30	Obliquely oval	Red blush on yellow	Thin	Smooth
BN Acc-31	Obliquely oval	Yellow with red blush	Thin	Smooth

Table 3 (Contd.)

Accession	Pulp color	Quantity of fibre	Eating quality
BN Acc-1	Golden yellow	Scarce	Good
BN Acc-2	Golden yellow	Scarce	Good
BN Acc-3	Golden yellow	Scarce	Good
BN Acc-4	Yellow	Scarce	Good
BN Acc-5	Yellow	Scarce	Good
BN Acc-6	Light yellow	Scarce	Good
BN Acc-7	Yellow	Scarce	Good
BN Acc-8	Yellow	Scarce	Good
BN Acc-9	Yellow	Absent	Good
BN Acc-10	Light yellow	Scarce	Good
BN Acc-11	Golden yellow	Scarce	Good
BN Acc-12	Yellow	Absent	Good
BN Acc-13	Yellow	Scarce	Good
BN Acc-14	Yellow	Scarce	Excellent
BN Acc-15	Yellow	Abundant	Excellent
BN Acc-16	Golden yellow	Abundant	Excellent
BN Acc-17	Golden yellow	Abundant	Excellent
BN Acc-18	Golden yellow	Abundant	Excellent
BN Acc-19	Dark yellow	Abundant	Excellent
BN Acc-20	Yellow	Abundant	Good
BN Acc-21	Yellow	Abundant	Excellent
BN Acc-22	Dark yellow	Abundant	Excellent
BN Acc-23	Yellow	Scarce	Good
BN Acc-24	Yellow	Abundant	Excellent
BN Acc-25	Yellow	Abundant	Excellent
BN Acc-26	Yellow	Absent	Excellent
BN Acc-27	Yellow	Absent	Excellent
BN Acc-28	Yellow	Absent	Excellent
BN Acc-29	Golden yellow	Scarce	Excellent
BN Acc-30	Yellow	Absent	Excellent
BN Acc-31	Yellow	Absent	Excellent

SSR analysis is performed by using pairs of specific primers flanking tandem arrays of microsatellite repeats. Microsatellites are abundant in plant systems (Condit and Hubbel, 1991). The first report of length polymorphisms of microsatellites in soybean (Akkaya *et al.*, 1992) opened up a new source of PCR-based molecular markers for other plant genomes. Microsatellite markers are consistently found to be highly polymorphic, easily visualized, stable, and co-dominant (Mc Couch *et al.*, 1997; Powell *et al.*, 1996). In addition, they have hyper-variability, wide genomic distribution, reproducibility, multi-allelic nature, and chromosome specific location. At present, SSRs are the most preferred marker types because they are highly polymorphic even between closely related lines, require low amounts of DNA, can be easily automated and allow high-throughput screening, can be exchanged between laboratories and are highly transferable between

populations. SSR markers are efficient, time consuming and cost-effective approaches for diversity analysis.

Molecular marker analysis is an efficient method of assessing genetic heterogeneity within the cultivars of mango and PCR-based genomic polymorphism has been detected in several cultivars of mango (Bally *et al.*, 1996; de Souza and Lima, 2004; Diaz-Matallana *et al.*, 2009; Rocha *et al.*, 2012). However, studies on intra-varietal heterogeneity in 'Beneshan' cultivar are very limited. Here, a total of 109 mango-specific microsatellite markers were utilized to characterize and assess intracultivar genetic diversity among 31 accessions of 'Beneshan' mango. Of these 4 SSRs (SSR-69, SSR-70, SSR-73, and SSR-75) could not amplify. Among the amplified primers, only 23 markers showed clear and consistent banding patterns (Table 2). Amplification profile revealed

by one of the highly polymorphic marker (SSR-87) across 'Beneshan' accessions is depicted in Figure 1. Each of the 23 loci differed significantly in their ability to determine variability among the accessions. A total of 58 polymorphic alleles were detected across 31 accessions using 23 SSR markers (Table 4). Of these, 30 amplification fragments were polymorphic, yielding a polymorphism rate of 51.72%. In previous study by Bally *et al.*, (1996) following RAPD analysis of 30 Kensington mangoes comprising of 27 'Kensington Pride' accessions, 2 'R2E2' and 1 seedling with 10 oligonucleotide primers allowed the scoring of 107 alleles. This is in line with the findings of (Manchekar, 2008), wherein nine clones of Alphonso screened with seven RAPD primers generated a total of 36 bands, out of which twelve were polymorphic revealing 36.38 per cent mean polymorphism. The level of polymorphism present in the microsatellites was variable ranging from 2 alleles (SSR-18, SSR-23 etc.) to 4 alleles (SSR-81) with an average of 2.48 alleles per SSR. The analysis of 23 SSRs revealed that the PCR product size (bp) ranged from 100 (SSR-52) to 310 (SSR-20) in 31 accessions. Polymorphic information content (PIC) value is the reflection of allele diversity and frequency among the accessions, and varied greatly for all the SSR loci tested. The PIC values varied widely among loci and ranged from 0.03 (SSR-59) to 0.72 (SSR-87) with an average of 0.37 per locus (Table 5). The microsatellites with high PIC values (SSR-80, SSR-87, SSR-28, and SSR-89) were found to be more useful in differentiating the 'Beneshan' accessions. Over all, these data extends the knowledge of SSR application as a molecular tool in intravarietal improvement of mango as reported by Bally *et al.* (1996), de Souza and Lima (2004), Diaz-Matallana *et al.* (2009) and Rocha *et al.* (2012), who have used ISSR and RAPD markers for molecular characterization of intravarietal heterogeneity in different cultivars of mango. The present work provides evidence that the SSRs appear to be effective to explore the molecular polymorphism and to assess the intravarietal heterogeneity in the 'Beneshan' mango.

However, further studies with many more SSR markers need to be continued for efficient use of this intravarietal heterogeneity in 'Beneshan'.

Intravarietal Genetic Diversity and Heterogeneity

In the present study, the highest genetic dissimilarity coefficient of 0.50 was observed among 31 accessions of 'Beneshan'. From this, it is concluded that 'Beneshan' whatsoever cultivated throughout the state is not pure clone. de Souza and Lima (2004) also observed similar level of genetic dissimilarity coefficient of 0.45 among 25 accessions of 'Rosa' cultivar of mango using RAPD markers and concluded that they are not pure clones and it is possible to breed this cultivar. Bally *et al.* (1996) observed absolutely low level of genetic dissimilarity coefficient of 0.05 among 15 accessions of 'Kensington Pride', a polyembryonic cultivar of mango using 10 RAPD markers and concluded that they are pure clones. Rocha *et al.* (2012) while studying intravarietal heterogeneity in 'Uba', a polyembryonic cultivar of mango using ISSR markers, no duplicates (clones) were found among the 102 accessions at the Zona da Mata of Minas Gerais State, Brazil and concluded that it is possible to select elite accession of 'Uba' mango tree adapted to the conditions of soil and climate of the cultivation region. This high genetic dissimilarity, as observed in this work, could seriously affect the varietal homogeneity of 'Beneshan' mango. This could be the probable cause for the heterogeneity in production and quality of 'Beneshan' mango in the state of Andhra Pradesh. It is probably caused by both insect pollinators and human intervention by repeatedly transferring materials from one population to another. Moreover, the high intravarietal heterogeneity among accessions is explained by the breeding system. Being highly cross-pollinated and owing to the fact that most of superior clones of mango including 'Beneshan' are monoembryonic, propagation through sexual means does not ensure true-to-the type plant reproduction.

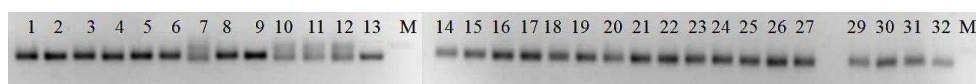


Figure 1: Amplification profiles of SSR-87 marker in the 31 'Beneshan' accessions. Lanes 1-31 corresponds to line ID in Table 1. M = 200 bp DNA ladder.

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: ATTAATCTCGTCCACAAGT	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: CAATTCATGCTTTGGATGCT	56	158
SSR-78	F: CCTTGGGTTTCATTGCTAAA 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: ATTTCCCACCATTTGTCGTTG	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

Source: eurofins mwg/operon (www.Eurofinsdna.com)

Table 5

Polymorphism detected with 23 SSR primers in 31 'Beneshan' accessions.

Primer ID	No. of alleles	PIC values
SSR-18	2	0.61
SSR-20	3	0.04
SSR-23	2	0.09
SSR-28	2	0.69
SSR-41	3	0.31
SSR-50	2	0.49
SSR-51	2	0.06
SSR-52	3	0.52
SSR-59	2	0.03
SSR-62	2	0.06
SSR-68	2	0.56
SSR-78	2	0.71
SSR-80	2	0.71
SSR-81	4	0.44
SSR-82	2	0.06
SSR-85	3	0.06
SSR-87	3	0.72
SSR-89	3	0.64
SSR-90	3	0.50
SSR-91	2	0.50
MngSSR-14	2	0.06
MngSSR-26	3	0.35
MngSSR-27	3	0.33

A broad morphological and genetic heterogeneity of 'Beneshan' mango that emerged in Andhra Pradesh state could be due to free sexual recombination, continuous grafting of outstanding plants produced by stones from 'Beneshan' trees cultivated in the state. The characterization and identification of outstanding 'Beneshan' plants can be useful for intravarietal diversity conservation as well as the use for mango breeding. Intravarietal heterogeneity of 'Beneshan' mango under cultivation in different eco-geographical regions is a valuable resource for further improvement of this cultivar. The SSR markers revealed considerable intravarietal genetic diversity among the accessions, a finding which strongly agrees with the great morphological variability observed among the accessions in this study. SSR marker system proved to be useful for analyzing the intravarietal genetic diversity of 'Beneshan' mango. The knowledge of the diversity of this germplasm will facilitate its use in intravarietal breeding programs and the improvement of management of large collections of the mango. Intracultivar improvement is mostly based on the selection of elite trees

made for not only fruit yield but also fruit quality. Elite forms of a cultivar should have attractive, good sized and quality fruits and improved shelf life.

Cluster Analysis and Genetic Relationships

The information obtained from intravarietal diversity analysis will be utilized in making the crosses and selection of divergent parents to maximize heterosis in future intravarietal breeding programmes. A UPGMA cluster diagram grouped the 31 'Beneshan' accessions into two major clusters (cluster I and II), effectively differentiating the accessions collected from different regions with additional sub-clusters (Figure 2). Cluster-I consisted of 23 accessions, while cluster-II comprised of 8 accessions. Cluster-I was divided into three sub-groups (cluster IA, IB and IC) and cluster II was divided into two sub-groups (cluster IIA and IIB). Of the three subclusters (cluster-IA, IB, IC) of cluster-I, the subcluster IA and IC are solitary comprising of single genotype each. Most of the accessions from a single collection site were grouped together; however, some exceptions were also observed. All of the three accessions from Sangareddy were grouped together in one subcluster IIB. All of the two accessions from Nandivampu (BNAcc-7 and BNAcc-8) were grouped together in one subcluster IIA. The subcluster-IIA included 5 accessions (BN Acc-4 to BN Acc-8) of which one accession each was from Pithapuram (BN Acc-4), Bobbili (BN Acc-5) and Tuni (BN Acc-6) and 2 accessions were from Nandivampu (BN Acc-8 and BN Acc-8). The subcluster-IA included only one (BN Acc-12) of the two accessions (BN Acc-10 and BN Acc-12) collected from Reddygudem. This is in line with the findings of Manchekar (2008), wherein UPGMA cluster analysis of nine clones of Alphonso (DVG-I, DPL-I, RTN-I, BGM-I, BGM-II, VEN-I, DWR-I, DWR-II and DWR-III) on the basis of seven RAPD data revealed that the selected nine location specific 'Alphonso' clones of both Maharashtra and Karnataka state formed two major clusters. Here, a suggested explanation for this division is that these accessions probably had common genomic components that originated from other mother trees. From the above results, the relationships among various 'Beneshan' accessions could be well resolved by the SSR markers, strongly verifying their effectiveness in identify-

cation of 'Beneshan' accessions. Possibly, this finding implies a potential relation between the genetic markers and 'Beneshan' accessions.

Genetic relationships would be useful in utilization and management of the accessions during intra-cultivar breeding programs. The genetic relationships among the 'Beneshan' accessions were assessed by a cluster analysis of the similarity matrix. A similarity matrix based on the proportion of shared SSR alleles was used to establish the level of relatedness between the accessions sampled. All of the three accessions collected from the mother block of the nursery, FRS, Sangareddy (BN Acc-1 to BN Acc-3) are genetically dissimilar. Two accessions (BN Acc-1 and BN Acc-2) from a single collection site of Sangareddy with a similarity coefficient of 0.98 had 98% genetic similarity with each other. All of the two accessions collected from the mother block of the nursery, HRS, Anantharajupeta (BN Acc-30 and BN Acc-31) are genetically dissimilar (6%) with a similarity coefficient of 0.94. From these genetic similarity values of less than 100% among the multiple accessions of both of the mother blocks under sampling, it is evident that there is heterogeneity within the mother block at the DNA level. Further, from the separate grouping pattern of the multiple accessions of the mother blocks under sampling, it is evident that there is heterogeneity between the mother blocks at the DNA level. The genetic dissimilarity of the multiple accessions collected from the mother block of the nursery, FRS, Sangareddy (BN Acc-1 to BN Acc-3) and HRS, Anantharajupeta (BN Acc-30 and BN Acc-31) denotes heterogeneity among the multiple accessions, which might have arisen due to the genetically variable scions used in the establishment of mother blocks. Accessions from different collection sites like Konampeta (BN Acc-22), Sanampudi (BN Acc-24), Kollapur (BN Acc-29) and Anantharajupeta (BN Acc-31), were the closest accessions having the highest similarity index of 100%. Two accessions each from the single collection site of Konampeta (BN Acc-20 and BN Acc-21) and Banaganapalle (BN Acc-17 and BN Acc-18) were the closest accessions having the highest similarity index of 100%. Here, genetic diversity was observed among the accessions of 'Beneshan' obtained from mother blocks of the nurseries, which might be assumed to have a high level of cultivar fidelity. Based on this study, the

wide range of similarity values for related accessions using SSRs provides greater confidence for the assessment of genetic diversity and relationships. Dendrogram revealed that the accessions that are derivatives of genetically similar type clustered more together. Existence of the 26 individual accessions (BN Acc-4 to BN Acc-29) collected from the 26 individual orchards in different groups and sub groups indicate that there was heterogeneity among the orchards under sampling. However, this study could not give any insight about the homogeneity and/or heterogeneity within the orchards sampled because of the fact that only one tree (accession) per orchard was sampled. For having better insight about the homogeneity and/or heterogeneity of orchards, multiple trees must be sampled from the individual orchards to be sampled. This information can be extremely useful to the mango nurseries for helping in the correct choice of the mango multiplication materials.

Accession Identification

Selection and correct identification of elite trees of a cultivar, is difficult, inefficient and inaccurate when based on morphological traits only. Due to the availability of various varieties of mango saplings in market, which look similar, it is difficult to identify a pure variety on the basis of morphological markers alone. Since the mango plant will take four years to grow, fruit and only then the quality and purity of the variety can be identified. This problem can be solved by comparing the genetic profile with the recorded gene profile of the mango varieties within two weeks. Therefore, in a crop improvement program based on mass selection, there would be unnecessary spending of time and financial resources if genetically similar plants selected based on phenotypic characteristics were reproduced and cultivated in the same location and evaluated for several years. The selection of mother plants must be performed considering the genetic similarity based on the information about DNA molecular markers. Further, the nomenclature of mango cultivars has been complicated by the widespread use of synonyms (Lakshminarayana, 1980). Many clonally propagated mango cultivars have unique local and regional names and the spelling and name variants have been translated to the Roman alphabet.

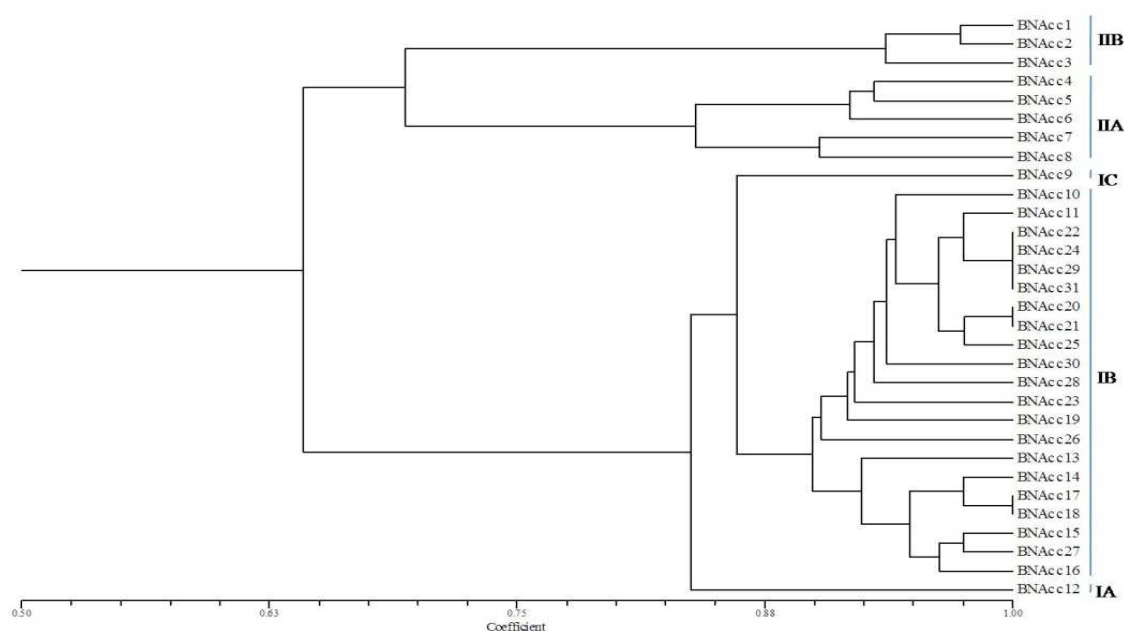


Figure 2: UPGMA cluster analysis showing the diversity and relatedness among 31 'Beneshan' accessions of mango using 58 alleles generated by 23 microsatellite markers.

Table 6

List of SSR primers producing unique alleles for specific accessions of 'Beneshan'

Accession	SSR producing specific band(s)	Size of the specific bands (bp)
BNacc-1, BNacc-2 and BNacc-3	SSR-87	170
BNacc-4 and BNacc-6	MngSSR-27	195
BNacc-8	SSR-91	280
BNacc-9	MngSSR-26	140
BNacc-12 and BNacc-13	SSR-81	135

At present, 'Beneshan' has numerous vernacular names in different regions (Beneshan, Baneshan, Benishan, Chappatai, Safeda, Banganapalli, Banaganapalle and Banginapalli), which makes identification and further multiplication difficult and in turn causes confusion among farmers and breeders.

In this study, the appearance, size and shape of fruits are highly variable in different 'Beneshan' accessions. These fruit characters play an important role in accession identification. In addition, the microsatellite assay generated accession-specific rare allele(s) in some of the accessions is screened; these may be used as DNA fingerprints for accession identification. An allele that was observed in only one or two of the 31 accessions was considered rare. Nine (29.03%) of the

'Beneshan' accessions had unique SSR alleles. These unique alleles were of 280 and 140 bp amplified by SSR-91 and MngSSR-26 primers in BNacc-8 and BNacc-9 accessions, respectively (Table 6). Similarly, unique allele of 170 bp amplified by SSR-87 primer in BNacc-1, BNacc-2 and BNacc-3 accessions. Similarly, unique allele of 195 bp amplified by MngSSR-27 primer in BNacc-4 and BNacc-6 accessions. Similarly, unique allele of 135 bp amplified by SSR-81 primer in BNacc-12 and BNacc-13 accessions. This study has shown that even though the genome of mango is allotetraploid and relatively large, the microsatellite allelic patterns generated through PCR are capable of individualizing cultivars or accessions. This would be of enormous assistance

for the establishment of proprietary rights and the determination of cultivar purity.

Management of Intravarietal Heterogeneity

The wide range of dissimilarity values (0.00-0.05) suggests that 'Beneshan' accession collection represent a genetically diverse population. In this study, the amplitude of genetic dissimilarity (50%) indicated considerable intracultivar variability in 'Beneshan' in *in-situ* conditions. Majority of the accessions under study are genetically heterogeneous. This is because of the fact that the farmers are not supplied with the most productive and uniform planting material. Although the use of homogenous, well documented material as scion is highly recommended for mango grafting, some of the surveyed nurseries are using genetically variable scion from their own mother blocks, while other nurseries are using scion from the farmers' orchards of unknown genetic identity. On the other hand, the evident intracultivar heterogeneity in 'Beneshan' mango is important from breeding point of view. This intracultivar polymorphism would offer good scope for breeding within the cultivar for intracultivar improvement. Here, majority of the accessions can be considered as distinct genotypes exhibiting variation in different morphological characters. To generate precise information on the intracultivar heterogeneity within such cultivar, it is essential to test the accessions under replicated trials to compare them against standard commercial table varieties to confirm the distinctiveness and superiority. The production and quality of 'Beneshan' in this state can thus be

improved by using the most productive and uniform planting material for different climate and soil conditions. This process can harmonize both quantity and quality of fruit production across the state. Development of an efficient and sustainable system for supplying interested farmers with high quality uniform planting material of the most elite form of the variety together with information on good management practices is urgently needed to harmonize 'Panchadarakalasa' mango production and quality in the state.

5. Conclusions

Characterization and evaluation based on morphological fruit traits revealed phenotypic variations among 31 accessions of 'Beneshan'. PCR-based SSR analysis confirmed genomic polymorphism in 'Beneshan' mango. Our results here strongly suggest that the 'Beneshan' samples collected in Andhra Pradesh state do not belong to the same clone. Hence, it is proposed to use the term 'variety' instead of 'clone'. Morphological markers combined with molecular characterization are essential for better understanding of intravarietal heterogeneity of 'Beneshan' mango. This study has shown that even though the genome of mango is allotetraploid and relatively large, the microsatellite allelic patterns generated through PCR are capable of individualizing accessions. Microsatellite markers have proven to be a valuable tool for identifying intracultivar heterogeneity in mango.

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