

**आम की आनुवंशिक विविधता और  
लक्षणीकरण का अध्ययन**

**GENETIC DIVERSITY AND  
CHARACTERIZATION STUDIES  
IN MANGO**

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# GENETIC DIVERSITY AND CHARACTERIZATION STUDIES IN MANGO

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## CERTIFICATE

This is to certify that the thesis entitled “**Genetic diversity and characterization studies in mango**” submitted to the Faculty of Post Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfilment of the requirements for the degree of **Doctor of Philosophy in Horticulture** by **Mr. Donald Mree Sangma, Roll No. 10473** embodies the results of a *bona fide* research work carried out by him under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or source of information, as has been availed for this work, has been duly acknowledged by him.

Date : 29.10.19

Place : ICAR-IIHR, Bengaluru

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Chairman, Advisory Committee

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## ABBREVIATIONS USED AND THEIR EXPANDED FORM

Symbols & Abbreviation	Expansion
%	: per cent
@	: at the rate
°B	: Degree brix
°C	: Degree celsius
µg	: microgram
ANOVA	: Analysis of variance
AOAC	: Association of officials analytical chemists
bp	: basepair
C.D.	: Critical difference
C.V.	: Coefficient of variation
cm	: centimetre
CTAB	: Cetyl Trimethyl Ammonium Bromide
cv.	: cultivar
DNA	: Deoxyribo nucleic acid
dNTP	: Deoxyribonucleotide triphosphate
e.g.	: exempli gratia (for example)
EDTA	: Ethylene Diamine Tetra Acetic acid
<i>et al.</i>	: et alia (and others)
Fig.	: Figure
g	: gram
GA	: Gallic Acid
GCV	: Genotypic coefficient of variation
<i>i.e.</i>	: id est (that is)
ICAR	: Indian Council of Agricultural Research
IIHR	: Indian Institute of Horticultural Research
IPGRI	: International Plant Genetic Resources Institute
GC-MS	: Gas chromatography-Mass Spectrometry

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OD	:	Optical Density
mg	:	milligram
ml	:	millilitre
mm	:	millimetre
NHB	:	National Horticulture Board
nm	:	nanometer
PCA	:	Principal Component Analysis
PCR	:	Polymerase Chain Reaction
pH	:	Potential of hydrogen
PIC	:	Polymorphic Information Content
PVP	:	Poly Vinyl Pyrrolidone
rg	:	Genotypic correlation coefficient
rp	:	Phenotypic correlation coefficient
rpm	:	Revolutions per minute
SEm±	:	Standard Error of mean
SSR	:	Simple Sequence Repeats
TAE	:	Tris-acetate-EDTA
Taq	:	<i>Thermus aquaticus</i>
TSS	:	Total Soluble Solid
viz.,	:	videlicet (namely)

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# *INTRODUCTION*



Mango (*Mangifera indica* L.) is a member of the dicotyledonous family Anacardiaceae, in the order Sapindales and is believed to be originated in the Indo-Burma region (De Candole, 1904; Popenoe, 1927). It is the most important fruit crop in India having a great cultural, socio-economic and religious significance since ancient times. Its long period of domestication in India is well evidenced from its mention in ancient scriptures.

Mango is produced in about 90 countries across the globe and India ranks first in production among the mango producing countries, where it occupies an area of 2.26 million ha with an annual production of 19.68 MT (NHB, 2018). By virtue of its excellent flavor, delicious taste, attractive color, delicious fruit quality with richness in vitamins and minerals, accessibility to common man, liking by the masses, mango has been assigned the status of the 'King of the fruits' in the tropical world and it is the 'National Fruit of India'. Mughal emperor Babar called it the "choicest fruit of Hindustan" and his descendant's nurtured mango by evolving grafting techniques, growing technologies and making huge collection of variability.

Mukherjee (1953) opines that mango has been under cultivation for at least 4000 years with over 1000 varieties in cultivation. Mango culture gradually spread to different parts of the world. It is imperative to note that selection by man from seedlings of unknown parentage has played the most significant role in the development of new mango cultivars. Kostermans and Bompard (1993) has listed more than 60 species world-wide, the highest diversity being found in the heart of the distribution area of the genus *Mangifera* i.e. the Malayan Peninsula, Borneo and Sumatra. Many of the *Mangifera* species are known to possess important horticultural traits that may be transferred into *M. indica*. Moreover, screening of the different *Mangifera* species needs to be carried out on priority for pest and disease resistance and other important horticultural traits. Large diversity is seen in the case of monoembryonic mango genotypes. Almost all of them are selections made from naturally occurring open-pollinated seedlings. There are seven centres of diversity for *Mangifera indica* L. in India (Yadav and Rajan, 1993) and five wild species (*M. indica*, *M. andamanica*, *M. camptosperma*, *M. khasiana* and *M. sylvatica*) are native to India. There is a need to study this large diversity, which is present in different parts of India, so that varieties with better traits can be commercialized directly or these can be used in the breeding programme. Mango being perennial and highly heterozygous, there is a need to select the progenies early using markers so that only those desirable ones can be evaluated.

The intended improvement in ideal plant type needs greater attention for making its cultivation more profitable by increasing production with low inputs. Greater attention is thus needed for the collection of *Mangifera* species, wild types, cultivars, clones and land races and their characterization and evaluation for immediate use and posterity. Success of any crop improvement program depends essentially on nature and magnitude of genetic variability available in crop germplasm. Enormous genetic diversity of mango exists in India, which is the primary center of domestication. There are more than 1000 monoembryonic and polyembryonic mango cultivars in India (Negi, 2000). This genetic variability of mango can be exploited in breeding programs to produce high quality mangoes suitable for a variety of purposes.

Mango is considered to be an allopolyploid, most probably amphidiploid and outbreeding species having chromosome number  $2n=40$  (Mukherjee, 1950). It is highly heterozygous as performance varies with the climate which resulted in a high level of genetic diversity. Further, confusion exists in the nomenclature of mangoes due to different local names for the same variety. Characterization and assessment of diversity is essential to utilize these unique cultivars in crop improvement programmes and also for better conservation of genetic resources. Utilization of the conserved germplasm in the breeding programme requires precise information on the genetic relationships among the cultivars while information on the genetic distance among the cultivars will also be of help in avoiding duplicates, thus, clearing the ambiguity in nomenclature, widening the genetic base of the core collections and ultimately help in preserving the valuable diversity. However, due to the changes in socio-economic situation, land use pattern and the shrinking homesteads, area under mango cultivation has been reduced. Urbanization and industrialization paved a way to large scale destruction of mango germplasm. Moreover, there was a shift in the preference of people towards new varieties and grafts. This has resulted in the genetic erosion of traditional mango germplasm of the country. Therefore, there is an urgent need to catalogue and conserve at least the available traditional genetic resources, which are on the verge of extinction. Further, proper assessment of existing genetic diversity is important in view of the emerging patent rules. The assessment of diversity based on morphological parameters has been often constrained by lack of adequate data on distinguishable morphological characters and confusion due to the wide variations for a particular trait in a given population.

Though, morphological markers have been in use to assess the genetic diversity, they had limited application in breeding as they are few in number and phenotype is influenced by the environment. Biochemical markers offer greater diversity as compared to the morphological markers, but they also suffer from some limitations like insufficient polymorphism among closely

related cultivars and dependence on developmental stage of tissue for expression. Molecular markers, on the other hand, are practically unlimited in number, remain unaffected by environment and growth conditions and are simply inherited. Hence, molecular approaches overcome the limitations in other methods and offer an efficient alternative tool to identify and develop genetically unique germplasm that complements existing cultivars (Begum *et al.*, 2012).

DNA marker technologies are extensively used in several crop plants not only for genetic diversity analysis but also for a wide range of molecular genetic studies. Different DNA markers *viz.*, Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Variable Number of Tandem Repeats (VNTR), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeats (SSRs) or Microsatellites have been employed in mango to study monogenic and polygenic traits (Powell *et al.*, 1996; Parsons *et al.*, 1997; Saxena *et al.*, 2005 and Krishna and Singh, 2007). Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSRs) are of particular importance because of amplification based assay and only nanogram quantities of DNA are required. Simple Sequence Repeats (SSRs), also known as microsatellites are efficient type of molecular markers based on tandem repeats of short DNA sequence and have advantages over other types of molecular markers, *i.e.*, their abundance in most genomes, uniform distribution, highly polymorphic, high reproducibility, co-dominance, analytically simple and readily transferable (Weber, 1990; He *et al.*, 2003).

The improvement of mango has been a challenge for many years. The success in mango improvement primarily depends on the nature and magnitude of variation present in the germplasm. Considerable variations are still being generated owing to the diverse climatic and edaphic conditions and allopolyploid nature of the crop. For the improvement programme a well described, characterized and evaluated germplasm is a prerequisite. The need of proper selection criteria has always been felt. Moreover, the assessment of the genetic variability, heritability, expected genetic advance and development of association among growth and yield characters are of immense value for the selection. As with other field and horticultural crops, precise estimation of genetic variability is an important pre-requisite for the genetic improvement of mango. It provides the fruit breeder with an accurate description of the germplasm material which is essential for their identification, conservation, management and utilization in genetic improvement programmes. Knowledge about the extent of genetic diversity/relatedness in mango germplasm is vital for developing coherent strategies for future gains in productivity and quality.

Nomenclature ambiguity is one of the hurdles in the improvement of the crop as it comes in the way of selection of parents with wider genetic distance. Information on the genetic distance between the varieties is lacking for most of the centres. The diversity studies would help in generating this information, which further would be useful in developing new recombinants with desirable traits by exploiting heterosis. Selection of parents for utilization in the hybridization programme would also become much easier.

Keeping in view the above facts, the present investigation is proposed with the following objectives:

- Study the diversity existing in *M. indica* varieties from different regions.
- Characterization of the genotypes using molecular tools.
- Attempting marker assisted selections in the progeny population.
- Estimation of volatiles in certain progenies and their parents.

# *REVIEW OF LITERATURE*



An important way to increase productivity in any fruit crop is to first select desirable cultivars from existing variation and to use the superior types for crop improvement programmes. Morphological traits such as fruit and leaf characteristics, branching habit of trees are influenced by environmental factors and cause numerous synonyms. Now it is possible to track out differences present at molecular level which is more authentic and less affected by environmental factors. The literatures pertaining to morphological, biochemical and molecular characterization in fruit crops have been reviewed. The brief account of available information along with supporting evidences in mango have been presented as under.

#### 2.1 Genetic variability studies

#### 2.2 Coefficient of variation, heritability and genetic advance

#### 2.3 Correlation studies

#### 2.4 Genetic divergence studies

#### 2.5 Molecular characterization

#### 2.6 Estimation of volatiles

### **2.1 Genetic variability studies**

The first scientific description of mango varieties including those from India was attempted by Maries (1901). A system of description mainly based on fruit characters was suggested by Woodhouse (1909), who described mango varieties from Bhagalpur (Bihar). Special importance to fruit characters, particularly shape, was given in the classification of mango varieties grown in Florida (USA) by Ralphs (1915). Eighty nine mango varieties from erstwhile Bombay Presidency were classified based on fruit characters by Burns and Prayag (1920).

According to Naik and Gangolly (1950), no other single structure in mango gives so many morphologically important characters as the fruit. In the key developed by the authors for identification of varieties, form of fruit and form of beak were the primary characters for variety identification. Fruit characters alone were used by Sturrock (1951) for development of artificial key for identification of mango varieties grown in Florida (USA).

### 2.1.1 Fruit weight and size

Tripathi (2001) evaluated 93 accessions of mango germplasm under Tarai conditions and reported that the fruit weight of Mallika, Nariyal, Fazli and Dadamiyo were more than 420 g.

Mitra *et al.* (2001) evaluated local mango strains grown in West Bengal and noted wide range of variations for average fruit weight from 70.9 g/fruit (Subza) to 372.5 g/fruit (Surya Pasand) and length of fruits varied from 6.76 cm (Subza) to 12.27 cm (Surya Pasand).

Sarkar *et al.* (2001) evaluated 10 mango hybrids and reported the longest fruit (10.70 cm) in Amrapali, while the maximum fruit weight (285 g/fruit) and breadth (7.95 cm) was obtained in A.U. Rumani.

Kher and Sharma (2002) reported that the maximum fruit weight (440 g) and size (length 14.85 cm and breadth 8.26 cm) among five mango cultivars in subtropical rain fed regions of Jammu. Banglora fruits recorded maximum weight (242 g/fruit) followed by Langra out of twenty-one mango varieties evaluated at Bhagalpur, Bihar (Singh, 2002).

Anila and Radha (2003) concluded that Ratna had all the desirable characteristics in terms of maximum fruit length, breadth and weight. The minimum contribution of stone to fruit weight was in Ratna and the maximum in Muvandan under Kerala conditions. Hoda *et al.* (2003a) evaluated twenty mango cultivars at Sabour in Bihar and reported that Fazli produced the heaviest fruits (450.98 g).

Dinesh (2004) evaluated 130 mango varieties for fruit characteristics and reported that Maharaja Pasand, Sora and Tenneru had higher fruit length (>20 cm), whereas the least was found in the varieties Chandrakaran and Pacharasi (<6 cm). The fruit breadth was observed to be maximum in the variety Arka Aruna (10.93 cm), whereas, the varieties Sora, Tenneru and Maharaja Pasand had higher fruit breadth. The fruit weight was found maximum in Maharaja Pasand and Sora, with more than 1000 g each.

Serry (2010) evaluated the mango strains under Ismailia conditions and observed that different strains produced higher fruit weight ranging from 440-610 g per fruit and classified them medium to bigger on the basis of their fruit weight, length & width. The length of the fruit ranged from 10.1 cm to 13.2 cm and width from 7.6 cm to 9.2 cm.

Majumder *et al.* (2011) studied the characteristics of mango fruits and observed the heavier fruit weight (365.33 g), with more length (11.50 cm) in MI 1 mango cultivar. The average

breadth of different mango germplasm was found to vary from 10.96 cm to 5.37 cm. The maximum thickness (9.71 cm) was reported in MI 16, whereas lower thickness (4.29 cm) was found in MI 60.

Significant variations in length and width of fruit were noted in 30 mango varieties by Singh *et al.* (2011). The higher length of fruit was noted in Mallika, Fazli and Kakaria, while the minimum length was observed in Kelam, Rumani, Himsagar and Dashehari Chhotee. On the other hand maximum width of fruit was noted in Baneshan and Fazli, whereas minimum width of fruits was obtained from Dashehari Chhotee, Safeda and Kelam.

Iqbal *et al.* (2012) evaluated mango cultivars and observed that the highest fruit weight was found in Fazli (455.90 g), followed by Safed Chaunsa (374.36 g). The minimum fruit weight was found in Anwar Ratul (129.57 g).

An experiment was conducted on physico-chemical quality parameters of mango fruits grown in Spain by Pleguezuelo *et al.* (2012). The results revealed that the fruits of cultivar Osteen had higher average weight (697 g), while Australian cultivar Kensington had lower fruit weight (171 g). Maximum fruit length was noted in Osteen (126 mm) followed by Tommy Atkins (114 mm) and lower (80.8 mm) in Kensington Pride.

Begum *et al.* (2012) conducted a trial for genetic distinctiveness and relationship of indigenous landraces. Fruit length varied from 8.00 cm to 14.80 cm and fruit width from 5.00 cm to 10.50 cm. Fruit weight ranged from 164.00 g to 552.00 g. Barua *et al.* (2013) studied the fruit characteristics of mango accessions in Bangladesh and found higher fruit weight in BARI Aam-4 (373 g) and lower fruit weight of 172 g in BARI Aam-3. The longer fruit (12.30 cm) was obtained from BARI Aam-1. The higher fruit breadth (9.18 cm) and fruit thickness (7.04 cm) were recorded in BARI Aam-4, while its lower value were observed in BARI Aam-3 (6.24 cm and 5.50cm, respectively).

Begum *et al.* (2013) screened 31 accessions of 'Beneshan' (BN Acc-1 to BN Acc-31) and reported that the fruit length ranged from 8.00 to 16.20 cm, the fruit width from 6.00 to 9.50 cm and the fruit weight from 178 to 520 g. Singh *et al.* (2013) assessed 20 mango varieties from Kymore plateau of Madhya Pradesh and reported that the fruit weight ranged from 126.68 g (Neelam) to 401.7 g (Vanraj).

Wang *et al.* (2013) studied 16 Australian varieties of mango in China. The average fruit weight ranged from 200 g to 700 g, out of which, 'R2E2' had the biggest fruit size with an average weight of 686 g while 'Ono' had the smallest fruit size with an average weight of 207 g.

Begum *et al.* (2014) assessed 30 accessions of 'Cherukurasam' (CKR Acc-1 to CKR Acc-30) and reported that fruit length ranged from 7.60 to 14.00 cm with an average of 11.20 cm. The fruit width ranged from 5.40 to 8.00 cm with an average of 6.31 cm while the fruit weight ranged from 130 to 380 g with an average of 266.55 g.

Kaur *et al.* (2014) characterized 14 mango genotypes and reported maximum fruit weight in Chausa. Eight mango cultivars were evaluated by Naz *et al.* (2014) who observed that Fajri produced the maximum green and ripe fruit weight, fruit length and perimeter (453.0 g, 403.0 g, 13.80 cm, and 21.57 cm, respectively).

Shi *et al.* (2015) in their evaluation of 28 mango genotypes for physico-chemical characters reported highest fruit weight (771.73 g) in Valencia Pride, whereas Yuexi No. 1 had the lowest fruit weight (138.06 g).

Azmat *et al.* (2016) in their assessment of elite commercial mango cultivars reported maximum fruit weight in Sufaid Chaunsa (540.2 g) and minimum in Malda (173 g). Maximum fruit length (18.16 cm) and fruit diameter (31.54 cm) were observed in New Sindhri and Langra, respectively. According to Kheshin *et al.* (2016), Sukkary-4 had the highest fruit weight compared with the other accessions. Sukkary-1 recorded the highest fruit length and diameter.

Galal *et al.* (2017) observed the maximum fruit weight in Keitt (713.97 g) and minimum in Ewais (262.17 g). Sedeeq had the highest fruit length (15.03 cm) as compared to the other cultivars, while Keitt recorded the highest fruit breadth (9.70 cm).

In a study conducted by Bora *et al.* (2017), Mallika showed the highest (340.17 g) fruit weight while the lowest fruit weight was observed in Dashehari Clone (94.17 g). Fruit length ranged from 7.21 cm to 12.55 cm with the highest recorded in cv. Mallika (12.55 cm). The mean value of fruit width showed a range of 5.07 cm to 8.85 cm, with the highest being recorded in Vanraj (8.85 cm).

Hada and Singh (2018) in their investigation of the physical characteristics and quality parameters of different mango cultivars observed maximum fruit length (14.38 cm), fruit breadth

(8.08 cm) and fruit weight (382.78 g) in cv. Mallika. Minimum fruit weight (167.62 g), fruit length (7.32 cm) and fruit breadth (6.12 cm) was observed in cv. Sepiya.

Significant variation in fruit length and fruit breadth among the mango cultivars was observed by Singh and Pathak (2018). The maximum fruit length was recorded in Samarbahist Chausa (9.37 cm), whereas, the minimum fruit length was recorded in Gaurjeet (5.63 cm). The maximum fruit breadth was noted in Bombay Green (6.57 cm) and minimum in Gaurjeet (4.47 cm). The highest fruit weight was recorded in Banarasi Langra (186.67 g) followed by Langra Kukori (178.67 g), while the lowest fruit weight was noted in Gaurjeet (96 g).

### **2.1.2 Fruit skin characters**

Qualitative characters of local mango varieties grown in Kerala were analyzed by Satyawati *et al.* (1972) who reported that peel content varied from 13.7 to 18.1 per cent. Moti and Gangwar (1973) observed that peel content varied from 9.2 to 16.9 per cent.

Kulkarni and Rameshwar (1981) stated that Vanraj had the minimum peel per cent (6.8%) at Sangareddy, Andhra Pradesh. Kalra *et al.* (1982) reported the maximum peel content in cv. Markeera and Gaurjit at Lucknow.

Under West Bengal conditions, Ghosh *et al.* (1985) reported the maximum peel weight in Jangale, Sorikhos, Safdar Pasand, Bombay Green and Bombay Yellow as compared to Piarafully, Meghlantan, Amriti, Sardamani Bhog and Rani Pasand. The variety Safdar Pasand, however, showed the maximum peel percentage compared to Bombay Yellow and Meghlantan.

Srivastava *et al.* (1987) observed maximum peel percentage in Neelum, Banglora, Kesar, S.B. Chausa, Alphonso and peel per cent varied from 14.3 to 28.8 per cent under Rewa, Madhya Pradesh conditions.

Ali *et al.* (1992) evaluated the fruits of 32 varieties of mango grown at Shujabad and found the minimum peel per cent (10.6% to 10.8%) in Banganapalli, Wadia Muna Syed and Swarnarekha.

Gowda and Ramanjaneya (1994) studied the physico-chemical characteristics of eleven mango varieties and reported that the peel content was lowest in Suvarnarekha.

Kumar (1998) reported the minimum peel percentage in cv. Langra. Vasquez *et al.* (2002) studied nine Thai mango cultivars and reported that peel content varied from 15 to 17.5% and peel thickness ranged from 0.63 to 1.23 mm.

Evaluation of mango genotypes for their fruit characters was conducted in Bihar during 1997 and 1998 by Singh and Singh (2003) who reported that Hathi Jhula produced the highest peel percentage (22.83%).

Shafique *et al.* (2006) found Fazli fruit with only 12.2% peel content, whereas Khirsapat, Ranipasand, Mohanbhog, Kishanbhog and Langra had higher peel content. Pradeepkumar *et al.* (2006) studied the physico-chemical characteristics of mango genotypes in northern Kerala and observed that the cultivar Chotta Jehangir had maximum peel weight (75.5 g) followed by Mulgoa (45.0 g), Cherukurasam (43.5 g) and Hybrid-56 (39.0 g), while minimum peel weight was noted in cultivars Gomanga, Kalapady and Heralappa (7.0, 9.2 and 11.5 g, respectively).

Jilani *et al.* (2010) noted maximum peel weight in cultivar Farzi (84.99 g), followed by Alphonso (71.33 g) and Sanghlakhi (65.28 g). Statistically, similar peel weight was recorded in Langra and Gulabkhas (56.72 and 54.72 g, respectively). The lower peel weight was noted in Anwar Ratual (21.87 g).

Pleguezelo *et al.* (2012) reported that the peel weight was found minimum in cultivar Glenn (6.30%) followed by Valencia Pride (6.90%) and it was reported maximum in cultivars Kensington (13.4%), Sensation (12.0%) and Tommy Atkins (11.2%).

Begum *et al.* (2013) screened 31 accessions of 'Beneshan' (BN Acc-1 to BN Acc-31) and reported that the peel content ranged from 8.80 to 25.80 per cent. Begum *et al.* (2014) assessed 30 accessions of 'Cherukurasam' (CKR Acc-1 to CKR Acc-30) and reported that peel content ranged from 14.70 to 23.10 per cent.

Singh *et al.* (2014) conducted a field experiment at College of Agriculture, Rewa (MP) and found maximum (16.96%) peel percentage in Dashehari and minimum (12.11%) in Langra.

Bora *et al.* (2017) noted peel weight to be ranging from 13.5 g to 40.50 g. The maximum peel weight was exhibited by Ratna (40.50 g) and minimum by Neeluddin (13.5 g). With respect to the peel thickness, it ranged from 0.67 mm to 1.53 mm with minimum peel weight being recorded in Gulabkhus (21.80 g) and maximum in Mallika (34.39 g).

### 2.1.3 Fruit pulp characters

Pulp content of thirteen mango varieties investigated by Desai and Dhandar (2000) varied from 67.56% (Bemcorado) to 83.21% in RCMS-1 (Bemcorado selection). High pulp content (above 75%) was recorded in Surkhuru-2, Langra, Kesar and Au-Rumani (Tripathi, 2001).

Mitra *et al.* (2001) reported that the average pulp weight in twenty local mango strains of West Bengal varied from 304.75 g (Surya Pasand) to 456.25 g (Talabi). The high pulp to stone ratio in Rugh Sheeradhar and Surya Pasand which indicated their suitability for fruit processing.

Kher and Sharma (2002) reported the maximum pulp per cent (78.78%) in Mallika among the five mango cultivars in sub tropical rain fed regions of Jammu. Banglora fruits had maximum pulp content (67.17%) among twenty-one mango varieties evaluated at Bhagalpur, Bihar (Singh, 2002).

Hoda *et al.* (2003a) evaluated twenty mango cultivars at Sabour, Bihar for quality attributes and reported that Mallika recorded the maximum pulp percentage (68%).

Dinesh (2004) evaluated 130 mango varieties for fruit characteristics and reported that pulp per cent was found maximum in variety Tenneru (81%). Kumar and Singh (2005) reported that the pulp percentage was found maximum in Mallika (69.00%) and minimum in Bangalora (57%).

Chatterjee *et al.* (2005) observed maximum pulp percentage (72.91%) in Amrapali, followed by Mallika (71.50%) and minimum in Ratna (61.79%) followed by Mahmood Bahar (61.83%). Ratio of pulp and stone was found maximum in Langra (4.63) followed by Mallika (4.27) and Amrapali (3.90), whereas, minimum was observed in Sundar Langra (2.73). Chanana *et al.* (2005) observed that the per cent pulp content was maximum in Langra (71.66%) followed by Mallika (70.75%), while it was minimum in Amrapali (64.80%).

Shafique *et al.* (2006) determined pulp content at three maturity stages, which varied from 66.4 to 73.5%, 67.4 to 75.3% and 68.7 to 76.6% for immature, mature and ripe mangoes, respectively. While evaluating the mango strains under Ismailia conditions, Serry (2010) recorded the higher pulp percentage in strain 6 (78.5%) and the lower in strain 7 (64.5%). In general, the various strains which had the pulp percentage more than 70% are desirable for fresh consumption and processing.

Jilani *et al.* (2010) noted that the cv. Fazli ranked first (329.63 g) followed by Suvarnarekha (326.20 g) with regard to pulp weight. Sanglakhi, Alphonso, Langra and Sinduri also showed good pulp weight. The cultivar Anwar Ratual had the lower pulp weight due to smaller fruit size. Mango cultivar Suvarnarekha had the higher pulp percentage (92.40 %) followed by Fazli (74.49%) and Langra (79.40%).

Majumdar *et al.* (2011) observed that per cent edible portion pulp varied significantly from 45.22 to 79.83% in different cultivars of mangos. It was found maximum in MI 09 (79.83%) and minimum in MI 39 (45.22%). The higher percentage of non-edible portion (54.78%) was obtained from MI 39, whereas the germplasm MI 09 had the lower percentage of non-edible portion (20.17%).

Pleguezelo *et al.* (2012) reported that the higher ratio of pulp and seed was found in cultivar Osteen (20.2) and the lower ratio in cultivar Kensington (6.3%) and Sensation (7.6%). The maximum pulp weight was found in Glenn (85.5 g) followed by Osteen (85.4 g), while minimum pulp weight was found in cultivar Sensation (77.6 g).

Begum *et al.* (2012) conducted an experiment in Andhra Pradesh, to study the relationship of indigenous landraces of mango and observed per cent pulp varied from 48.30 to 74.40. 31 accessions of 'Beneshan' (BN Acc-1 to BN Acc-31) screened by Begum *et al.* (2013) were found to have pulp content ranging from 53.50 to 79.40 per cent, while pulp color varied from yellow to dark yellow.

Begum *et al.* (2014) assessed 30 accessions of 'Cherukurasam' (CKR Acc-1 to CKR Acc-30) and reported that pulp content ranged from 54.30 to 67.40 per cent. Kaur *et al.* (2014) characterized 14 mango genotypes and reported maximum pulp percentage and pulp stone ratio in Chausa and Langra Baramasi.

Maximum pulp weight per fruit was recorded in Mallika (316.99 g) and minimum in Sepiya (104.32 g). Maximum pulp percentage (82.71%) was recorded in Mallika and minimum (61.51%) in Sepiya (Hada and Singh, 2017).

Bora *et al.* (2017) found that the mango cultivars differed significantly with respect to their pulp weight. Mallika ranked first in pulp weight (257.91 g) followed by Langra (226.09 g) and Neelgoa (224.68 g). The lower pulp weight was recorded in cv. Dashehari Clone (32.59 g) which was statistically *at par* with Amrapali (55.25 g).

Singh and Pathak (2018) observed significant variations in pulp weight among different cultivars of mango. The maximum pulp weight was recorded in Banarasi Langra (135.50 g) followed by Samarbahist Chausa (124.80 g) and Zardalu (121.20 g), whereas the lowest value was recorded in Gaurjeet (63.67 g).

#### **2.1.4 Stone characters**

The form, size and shape of stone and also the pattern of stone venation provide useful information for varietal identification (Gangolly and Ranjith, 1957).

High stone percentage in Rasgulla, Banglora, Safeda Sharbati, Chausa Dwarf and Pathre was reported by Tripathi (2001). Sarkar *et al.* (2001) evaluated ten mango hybrids and reported the maximum stone percentage (20%) in Amrapali. Nine Thai mango cultivars were assessed by Vasquez *et al.* (2002) and reported that stone content varied from 12.8 to 29%.

Anila and Radha (2003) conducted a research on physico-chemical analysis of mango varieties under Kerala conditions and concluded that the stone weight ranged from 22.55 g to 47.76 g and was maximum in Ratna, but the percentage contribution to fruit weight by stone was the minimum in this variety (12%). With regard to these parameters, Muvandan was at the other extreme with minimum pulp, maximum stone and peel percentage.

Mannan *et al.* (2003) reported that the higher stone length (13.66 cm) was found in the variety Madrazi Tota but the stone width (4.75 cm) and depth (1.61 cm) were maximum in Sharmai Fazli and minimum stone length (5.43 cm), width (3.44 cm) and depth (1.29 cm) were observed in Indian Lota.

Dinesh (2004) evaluated 130 mango varieties for stone characteristics and found that stone length was maximum in the variety Tenneru (20 cm) followed by the varieties Sora and Maharaja Pasand. The weight of the stone was observed to be maximum in the variety Tenneru (89 g) followed by the variety Sora. Kumar and Brahmachari (2004) evaluated fifty mango cultivars for stone characteristics in a trial conducted in Bihar. The lowest stone percentage (5.51%) was observed in cv. Pansera. The lowest breadth of 2.48 cm was observed in Aman Prince and the highest length (11.10 cm) was observed in Pansera cultivar.

Chatterjee *et al.* (2005) observed that the stone per cent was maximum (25.40%) in Alfazli followed by Sundar Langra (24.55%) and minimum in Langra (14.77%).

In an experiment on the identification of superior clones and elite seedlings of mango from Uttar Pradesh, Pandey *et al.* (2006) noted minimum (18.3 g) stone weight in Clone V-1 of Langra, followed by Clone V-3 (22.4 g), and maximum stone weight in Chausa seedling (39.0 g) followed by cultivar Janisahab Karkan (36.3 g) and Dashehari clone K-1 (31.0 g).

Pradeepkumar *et al.* (2006) studied the physico-chemical characteristics of mango genotypes in northern Kerala. The results showed that smaller stone was found in Ratnagiri Alphonso (19.9 g) followed by Kallu Neelum (20 g) and Gomanga (21.5 g), whereas larger stone was found in variety Chotta Jehangir (66.5 g) followed by Chinnarasam (61.5 g) and Panakkalu (59.0 g).

Jilani *et al.* (2010) reported that Fazli and Alphonso excelled in stone weight (48.67 g and 47.07 g, respectively) with a non-significant difference. The minimum stone weight (22.99 g) was recorded in Dashehari followed by Anwar Ratual (30.98 g).

Majumdar *et al.* (2011) studied the physico-morphology, floral biology and fruit characteristics of mango and found that the MI 39 produced the heavier stone (111.61 g) followed by MI 82 (96.11 g) and MI 98 (92.07 g) while the lighter was observed in MI 54 (21.33 g). MI 16 had the longer stone (12.93 cm) but MI 58 produced the smaller stone (5.34 cm). The wider stone (5.84 cm) was noted in MI 16 and narrower stone (1.42 cm) was found in MI 01.

Singh *et al.* (2011) found that the percentage weight of stone was highest in the variety Banglora followed by Taimuriya and Safeda and lowest in Baneshan and Fazli.

In a study conducted on physico-chemical characteristics of some mango fruit cultivars growing under a Mediterranean subtropical climate in Spain, Pleguezuelo *et al.* (2012) reported that the seed weight of the fruits of cultivar Osteen proved to be the lowest (4.2%) among all the cultivars. On the other hand, the percentage of seed contribution to the total fruit weight for cultivar Tommy Atkins and Irwin were acceptable (6.9 and 5.7%, respectively). Cultivar Kensington and Sensation showed the highest percentage of seed to total fruit weight (11.8 and 10.4%, respectively).

Begum *et al.* (2013) screened 31 accessions of 'Beneshan' (BN Acc-1 to BN Acc-31) and reported that the stone content ranged from 8.60 to 23.50 per cent. Thirty accessions of 'Cherukurasam' (CKR Acc-1 to CKR Acc-30) were assessed by Begum *et al.* (2014) and reported that stone content ranged from 13.10 to 25.00 per cent.

Shi *et al.* (2015) in their evaluation of 28 mango genotypes for physicochemical characters reported Valencia Pride had the highest stone weight (60.22 g) whereas Yuexi No. 1 had the lowest stone weight (16.71 g).

Maximum stone weight was observed in Sufaid Chaunsa (55.27 g) and minimum in Faiz Kareem (22.25 g) by Azmat *et al.* (2016). Regarding the stone weight (%), Sedeek had the highest value (13.08%) and Naomi, the lowest (4.26 %) among the studied cultivars (Galal *et al.*, 2017).

Outcome of the research work conducted by Hada and Singh (2017) revealed that minimum stone length (5.72 cm) and stone breadth (1.92 cm) was recorded in Sepiya and maximum stone length (12.49 cm) and breadth (4.25 cm) in Mallika. Minimum stone weight was recorded in Gulabkhus (25.26 g), while maximum weight was found in Chausa (42.96 g).

Bora *et al.* (2017) observed that the stone weight varied significantly, ranging from 12.49 g (Dashehari Clone) to 35.06 g (Langra). Minimum stone width was noticed in cv. Dashehari (2.33 cm) while maximum was observed in Neelgoa (4.11 cm). The lowest stone thickness was observed in Neeleshan (1.51 cm), while higher stone thickness was observed in Sabri (2.32 cm).

Among the cultivars evaluated, the minimum stone weight was recorded in Gaurjeet (13.5 g) followed by Banarasi Langra (20.33 g) and Rataul (21.08 g). The maximum (28.36 g) value was recorded in Dashehari (Singh and Pathak, 2018).

### **2.1.5 Bio-chemical characters**

Mitra *et al.* (2000) studied bio-chemical characteristics of twenty one mango varieties grown in West Bengal and reported that Jagannath Bhog had the highest total soluble solids (22.33%). The lowest titratable acidity (0.14%) and the highest ascorbic acid content (123.33 mg/100g) were observed in fruits of Jahanara and Kashir Langra, respectively.

Reddy *et al.* (2000) observed the highest TSS (24.20 °Brix) in Mallika, whereas Chausa recorded the highest TSS: acid ratio and was adjudged the most palatable mango cultivar among twenty cultivars evaluated at Ranchi, Bihar. Mitra and Mitra (2001) studied the fruit composition and quality of 19 mango cultivars grown in West Bengal on the basis of evaluation from 1995 to 1997. The higher total soluble solids (22.66 °B) and the lower titratable acidity (0.10%) were recorded for Misti Bhog. Lohajang had the highest content of ascorbic acid (104.66 mg/100g).

Tripathi (2001) reported high TSS in Bijoragarh, Amrapali, Surkhuru-2, Lucknow Safeda, Dashehari, Mallika, Sepia, Dadha Peda and Surkhuru-1. Minimum acidity was recorded in Chausa Dwarf, Langra, Dashehari, Chausa, Alphonso and high ascorbic acid content in Kazalio, Pulihora and Langra.

Out of twenty local mango strains grown in West Bengal, the TSS of the fruits was maximum in cv. Rani and minimum in Tephala. The lowest titratable acidity (0.09%) was observed in Saradamani Bhog and Sita Bhog, whereas the highest ascorbic acid content was reported in Shah Pasand (Mitra *et al.*, 2001).

Singh (2001) evaluated thirty-one genotypes of mango, based on higher TSS, total sugars and ascorbic acid content during ripening. Collection number 20/80 was observed most suitable for processing purpose. Singh (2002) studied the performance of 21 mango cultivars in an experiment conducted in Bihar, during 1997-1998 and observed maximum TSS (23 °B) and total carotenoids content (8.17 mg/100g) in Amrapali followed by Langra and Zardalu.

Kher and Sharma (2002) observed that Dashehari fruits were superior in TSS and TSS: acid ratio than other four cultivars taken for performance study and recommended the cultivation of Dashehari in rain fed region of Jammu.

Vasquez *et al.* (2002) studied nine Thai mango cultivars and reported that total phenol index varied from 35 to 117.1 mg/100g and  $\beta$ -carotene from 0.66 to 15.72 mg/kg fresh weight. TSS varied from 15 to 18.7 °Brix and TSS/ TA ratio from 47.6 to 114.1. Titratable acidity ranged from 0.16 to 0.36 g/100g.

Anila and Radha (2003) studied bio-chemical characteristics of four cultivars and two hybrids under Kerala conditions and recorded the highest values of total soluble solids, sugars and ascorbic acid in Ratna and H-151. They concluded that Ratna had all the desirable characteristics in terms of TSS and sugar content.

Hoda *et al.* (2003a) studied the quality attributes of twenty mango cultivar under Bihar condition and observed that cultivar Dashehari had the maximum percentage of TSS (21.90 °B).

Hoda *et al.* (2003b) recorded the highest total soluble solids and ascorbic acid content for Dashehari among twenty cultivars evaluated. They further observed that the cultivars from the Southern and Western zones were inferior to the cultivars from the Northern and Eastern zones

and based on the overall performances, Mallika had the greatest potential for cultivation under Bihar conditions which recorded the highest total carotenoids (4.95 mg/100g) contents.

A study was conducted by Abirami *et al.* (2004) to evaluate the chemical characteristics and fruits quality attributes of 13 polyembryonic cultivars and compared them to the monoembryonic cultivar Totapuri. The maximum total soluble solids (27.97 °Brix) was observed in Chandrakaran followed by starch (20.17 °B) and Mylepelian (17.83 °B). The maximum values for acidity (0.34%) were observed in Olour.

Muhammad *et al.* (2004) conducted an experiment to determine the physico-chemical characteristics of Pakistani mango cultivars. Anwar Rataul gave the highest soluble solid content (25.20%) while Supar Badam gave the highest acidity (0.84%). Dhillon *et al.* (2004) evaluated various characteristics of eight mango cultivars and found that Summer Bahist Chausa had the highest TSS (18.7 °B) and lowest (12.1%) in Summer Bahist Rampur. The juice of Summer Bahist Rampur and Summer Bahist Chausa contained the highest (0.46%) and lowest (0.27%) acid content, respectively.

Bhowmick and Banik (2005) studied the performance of nine mango cultivars and observed that TSS was highest in cultivar Neelum (16.05 °B). The study showed that Neelum was most acidic (0.44%) and Langra the least (0.19 %). Kumar and Singh (2005) evaluated mango genotypes for flowering, fruiting and fruit quality attributes. The results showed that Total Soluble Solid was found maximum (22.90%) in Mallika followed by Langra, Kesar and Alphonso, while Dadamian recorded minimum TSS content. Titrable acidity was found minimum in Kesar (0.20%) which was *at par* with Mallika (0.23%). Bagbahar had maximum titrable acidity (0.89%) followed by Banglora.

Chanana *et al.* (2005) reported higher total soluble solid (20.04 °B) in the fruits of Dashehari which was closely followed by those of Langra, Amrapali and Mallika, whereas it was the minimum (17.50 °B) in Alphonso. Chatterjee *et al.* (2005) observed maximum TSS in Amrapali (25.00 °B) and minimum in Neelgoa (18.43 °B). The maximum acidity was obtained in Neeleshan (0.26%) and the minimum (0.17%) in Prabhashankar. The higher ascorbic acid (42.82 mg/100g) content was recorded in Langra followed by Sundar-Langra (38.75 mg/100 g) and the lower (19.13 mg/100g) in Alfazli.

An experiment was conducted to study the biochemical characteristics of different mango germplasm grown in climatic condition of Mymensingh by Uddin *et al.* (2006). The result

revealed that Pahlam contained the higher TSS (26.27 °B) followed by Langra (25.20 °B), Khirsapat (25.17 °B) and Rad (25.50 °B). The lower total soluble solids (19.73 °B) were recorded in Tommy Atkins. The higher titrable acidity (0.53%) was found in Mixed Special while Amrapali had the lower content of titrable acidity (0.24%).

Pradeepkumar *et al.* (2006) found that the TSS in 31 north Kerala mango genotypes ranged between 12.7 and 25.20 °B. The genotype 'Heralappa' (25.20 °B) and 'Kalapadi' (24.70 °B) were the two to have more TSS content. Fruits of 'Ratnagiri Alphonso' showed higher acidity (0.92 %) and lower TSS (17.2 °B) than the popular variety 'Alphonso'. The variability in ascorbic acid ranged from 9.2 to 164.64 mg/100g. Higher ascorbic acid was found in Heralappa (164.4 mg/100g) followed by Karpuram (139.1 mg/100g), while it was noted lower in Kallunellam (9.2 mg/100g).

During identification of superior clones and elite seedlings of mango from Uttar Pradesh, Pandey *et al.* (2006) reported that Langra clone V-3 possessed 18.0 °B TSS and 0.26% acidity. Dashehari clone K-12 showed good TSS (17.5 °B) and low acidity (0.16%). The TSS varied from 10.0 °B in Janisahab Karkan to 18.50 °B in Naudah. The lower acidity was measured in Abdullah Great (0.06%) and the higher in Zanipasand Karkan (0.35%). They reported that Dashehari clone K-1 possessed maximum ascorbic acid (12.3 mg/100g) and lowest in Abdullah Great (5.7 mg/100g).

Uddin *et al.* (2006) observed highly significant difference with respect to ascorbic acid, ranging from 5.32 to 52.14 mg/100g. Sindhu had the maximum content of ascorbic acid (52.14 mg/100g), while the minimum was recorded in Rad (5.32 mg/100g).

Shafique *et al.* (2006) studied the physiological and biochemical composition of different mango cultivars at various maturity levels. The higher titrable acidity was found in cultivar Khirshapat (0.48%) followed by Fazli (0.43%). Maximum ascorbic acid content was observed in Khirshapat (48.5 mg/100g), whereas Ranipasand contained lower ascorbic acid content (35.0 mg/100g).

Kabir *et al.* (2007) found TSS varying from 16.25 to 27.65 °B in different mango varieties, while studying 12 mango germplasm under Mymensingh condition. The phenol content ranged from 48.40 mg/100g in cv. Haden to 208.70 mg/100 g of edible portion in cv. Uba (Ribeiro *et al.*, 2007).

Zaied *et al.* (2007) reported higher TSS (13.63 °B) in Langra followed by Alphonso, while the lower TSS (7.90 °B) was detected in Mabrouka. The acidity percentage of Dabsha fruit was the highest (1.89%) as compared to all other varieties. They noted maximum vitamin C content in cultivar Dabsha (27.04 mg/100g).

Saleh *et al.* (2009) observed that the acidity percentage of Ganofia fruit (1.91%) was the highest in comparison with other mango accessions, while the lower acidity percentage was detected in Zebra fruit (1.73%). Total soluble solid per cent was noted higher in Gamelan (10.43 °B) and Ganofia (10.00 °B), whereas, lower TSS was noted in cultivar Zebra (8.83 °B). Higher Vitamin-C content was observed in El-Madam fruits and lowest in variety Gemela (14.60 mg/100g).

Five varieties of mangoes from four countries were evaluated to compare the  $\beta$ -carotene, total phenol, and ascorbic acid levels of the fruit pulp. Ascorbic acid ranged from 11 to 134 mg/100g pulp puree and  $\beta$ -carotene varied from 5 to 30 mg/kg among the five varieties. Total phenolic content ranged from 19.5 to 166.7 mg gallic acid equivalents (GAE)/100 g puree (John *et al.*, 2009).

Rajwana *et al.* (2010) observed higher acidity (1.90%) in Anwar Ratual followed by Faiz Kareem (1.43%) and Chausa (0.60%). The comparable values found in this study ranged between 0.27 and 1.90. Higher TSS was noted in cultivar Chausa (26.72 °B) followed by Anwar Ratual (26.47 °B). Vitamin-C content in different cultivars ranged from 12 to 108.0 mg/100g. With respect to total carotenoids content, it ranged between 1.77 to 6.99 mg/100g.

Serry (2010) reported that titrable acidity ranged from 0.3 to 1.0% while evaluating different strains of mango. With respect to vitamin-C, it ranged from 38.7 to 62.7 mg/100ml juice. Jilani *et al.* (2010) reported maximum ascorbic acid in the fruit of Gulab-e-Khas (179 mg/100g), whereas minimum content was found in fruit of Anwar Ratual (131 mg/100g).

Majumdar *et al.* (2011) observed that the variation in TSS among the germplasm was highly significant. The cultivar MI 28 was found to have higher TSS (28.26 °B), while lower in MI 20 (16.90 °B).

The maximum TSS was found in Dashehari Chhotee followed by Dashehari and Amrapali, while the minimum was noted in Banglora followed by Baneshan and Fazli. The titratable acid in the fruits was maximum in Amrapali, Kelam and Dashehari Chhotee and lowest in Kakaria, Rumani and Krishnabhog (Singh *et al.*, 2011).

In a study on physico-chemical characteristics of mango grown in Mediterranean subtropical climate (South Spain), Pleguezuelo *et al.* (2012) reported higher acidity being showed by Valencia Pride, with an average acidity of 0.22% and lower for Kent and Lippins, with 0.04 and 0.06% respectively. In relation to the total soluble solids, it ranged from 19.5 °B for cultivar Osteen to 15.7 °B for cultivar Glenn.

Fiyas *et al.* (2012) reported that freshly extracted mango pulp from 'Sindura' and 'Mallika' showed significant amount of TSS (19.4 and 21.8 °B) and titrable acidity (0.27 and 0.48%). 'Sindura' and 'Mallika' cultivars contained significant amount of total carotenoids (7.154 and 4.80 mg/100g, respectively). The amount of bio-accessible beta-carotene was higher in 'Mallika' (0.89 mg/100g), followed by 'Badami' (0.79 mg/100g).

Sogi *et al.* (2012) studied the total carotenoids content in Tommy Atkins and detected a range from 1.13 to 1.66 mg/100g. Nehra and Sharma (2012) studied the comparison of total carotenoids content in three mango cultivars namely, Dashehari, Chausa and Fazli. The maximum carotenoids contents was observed in Chausa (6.09 mg/100g) followed by Dashehari (5.12 mg/100g) and Fazli (4.80 mg/100g).

A study was conducted to assess the physico-chemical quality characteristics of 9 mango fruit cultivars grown under a Mediterranean subtropical climate in Spain and reported the highest acidity (0.22%) in Valencia Pride while Lippens contained the highest total soluble solids (Pleguezuelo *et al.*, 2012).

Begum *et al.* (2013) screened 31 accessions of 'Beneshan' (BN Acc-1 to BN Acc-31) and reported that the TSS ranged from 15 to 22 °Brix with an average of 16.75 °Brix. Singh *et al.* (2013) assessed 20 mango varieties in Kymore plateau of Madhya Pradesh and reported that total soluble solid content was maximum in Dashehari (23.6 °Brix), followed by Langra (23.45 °Brix), Mallika (22.1 °Brix) and Bombay Green (22.1 °Brix).

Wang *et al.* (2013) studied 16 varieties (all introduced from Australia) of mango in China. In 12 varieties the total soluble solids content was between 15.0 to 18.9 °Brix, which were medium and high concentration levels. In the titratable acid content, except 'Valencia' and 'KRS' which had an acidity above 0.5 which was high acid, the others have medium low acidity. Ascorbic acid content ranged from 3.51 to 44.37 mg/100g.

Afifa *et al.* (2014) evaluated 5 Bangladeshi ripe mango (*Mangifera indica* L.) varieties and found maximum amount of total soluble solids (20.13%) in Amrapali. Highest titratable

acidity (0.80%) was observed in Ashwina and lowest (0.15%) was found in Fazli. Ashwina and Langra showed significantly higher ascorbic acid content (65.66 and 59.17 mg/100g) whereas Himsagor and Fazli showed lower ascorbic acid content (10.83 and 12.5 mg/100g) compared to those of other varieties. Significantly higher levels of total phenol contents were found in Langra followed by Ashwina, Amrapali, Fazli, and Himsagar.

Begum *et al.* (2014) assessed 30 accessions of 'Cherukurasam' (CKR Acc-I to CKR Acc-30) and reported that TSS ranged from 14.00 to 23.90 °Brix with an average of 17.26 °Brix.

Kaur *et al.* (2014) characterized 14 mango genotypes and reported a high range of variability for TSS content (11.35-28.95 °Brix). Minimum TSS of 11.35 °Brix was recorded in Local Selection-II, while Malda (T10) ranked at the top with TSS content of 28.95 °Brix. The acidity ranged from 0.20 to 7.86% in evaluated genotypes.

Naz *et al.* (2014) in their evaluation of 8 mango cultivars found highest titratable acidity (0.49%) in Sindhri followed by Anwar Ratual Late-12 (0.41%). The fruits of Samar Bahisht Chaunsa and Fajri had the lowest content of titratable acidity, 0.12% and 0.14% respectively. The highest vitamin C content was obtained from pulp of mango cv. Langra (165 mg/100g) while the lowest was found in cv. Anwar Ratual (126 mg/100g).

The highest titratable acidity content was identified in Guixiang (2.35 g/100g) and the lowest in Nam Dok Mai (0.14 g/100g). The highest TSS (17.00-17.40%) were found in Kensington, Carabao, and Nam Dok Mai, whereas the lowest (7.60-8.60 %) were seen in Zihua and Jinshui (Shi *et al.*, 2015). Maximum TSS in Anwar Ratole (26.36 °Brix) and minimum in Sindhri (17.26 °Brix) was reported by Azmat *et al.* (2016).

Bora *et al.* (2017) reported that mango cultivar Mallika (22.41 °B) possessed highest amount of TSS and Langra (16.90 °B), the least. The maximum titratable acidity was observed in Langra (0.29%), whereas minimum content was found in Ratna (0.14 %) & Sabri (0.14 %). Langra possessed the higher amount of ascorbic acid content (55.62 mg/100g). Maximum carotenoids content was observed in Amrapali (8.38 mg/100g) and minimum in Mahmood Bahar (1.53 mg/100g).

Two essential chemical parameters, TSS and acidity were recorded in support of quality of mango fruits by Galal *et al.* (2017). Their findings revealed that the highest TSS content was found as 21.68 °Brix in Ewais. On the other hand, the lowest TSS content was recorded for

Sedeeq cultivar (9.88 °Brix). The acidity content ranged from 0.20% for Naomi to 0.63% for Ewais.

Hada and Singh (2018) in their investigation of the physical characteristics and quality parameters of different mango cultivars observed maximum TSS (24.10 °Brix), minimum acidity percentage (0.18%) and maximum value of TSS/acid ratio (137.38) in cv. Chausa. Maximum ascorbic acid was recorded in cultivar Langra (51.78 mg/100g) and minimum in cv. Alphonso (13.59 mg/100g), while maximum total carotenoids content was found in cultivar Amrapali (8.78 mg/100g) and minimum value was noted in cv. Fazli (1.82 mg/100g).

Himabindu *et al.* (2018) characterized thirty four mango cultivars using bio-chemical fruit markers in subsequent years to know the genetic diversity in mango. Significant differences were observed among the cultivars for all the characters studied. TSS content ranged from 15.05 °Brix to 25.44 °Brix, the highest TSS being recorded in cultivar Cherukurasam and the lowest in Sora Mamidi. The cultivars Chinnarasam and Banganapalli-1 recorded maximum  $\beta$ -carotene content. The highest ascorbic acid content was found in Baramasi (88.40%) and the lowest in Elamandala (22.14%).

Significant variation in TSS, acidity and ascorbic acid were recorded among different cultivars of mango (Singh and Pathak, 2018). The TSS content was recorded highest in Samarbahist Chausa (20.78 °B) while, the lowest TSS was recorded in Rataul (16.19 °B). The maximum acidity was recorded in Rataul (0.49%) followed by Lucknow Safeda (0.43%) and Gaurjeet (0.37%), whereas minimum acidity was recorded in Samarbahist Chausa (0.24%). Ascorbic acid was recorded highest in Banarasi Langra (74.28 mg/100g) and lowest in the cultivar Himsagar (22.61 mg/100g).

## **2.2 Co-efficient of variation, heritability and genetic advance**

Genetic variability within the population is a pre-requisite for any crop improvement programme. It is determined using certain genetic parameters *viz.*, genotypic co-efficient of variation (GCV), phenotypic co-efficient of variation (PCV), heritability in broad sense ( $H^2$ ) and genetic advance as per cent of mean.

The degree to which variability is a quantitative trait may be transmitted to the progeny is referred to as heritability. Johnson *et al.* (1955a) pointed out that heritability is the ratio of genotypic to phenotypic variance. In strict sense, it is a fraction of the total variance that is due to

additive effect of genes known as heritability in narrow sense. For predicting the effective selection, heritability estimates along with genetic advance are more useful rather than heritability estimates alone. Panse (1957) studied quantitative traits and partitioned the phenotypic variability into heritable and non-heritable components.

The role of genetic variability, its transmissibility into the progeny and extent of the inheritance are very important in selecting the suitable breeding methods for crop improvement. The estimates of heritability and the genetic advance expected after selection indicate the feasibility and the extent to which the improvement was possible. A brief review of the research work done so far in mango with respect to coefficient of variation, heritability and genetic advance is presented in this part of the chapter.

Significant varietal differences for all the fruit characters at genotypic level were observed by Desai and Dhandar (2000). They reported highest genotypic coefficient of variation for pulp weight and fruit size for mango.

Studying the genetic variability of 17 different characters in 18 mango genotypes at Anand, Patel (2002) observed wide range of phenotypic variability for fruit yield and its component traits. He also observed that phenotypic and genotypic variance were high for number of fruits per tree, fruit weight, fruit yield per tree, ascorbic acid, panicle length and total sugar. Genotypic variance was greater than the environmental variance for all the characters.

Singh (2002) studied the genetic variation among 36 early and regular bearing clones of mango. The genetic and phenotypic coefficients of variance, heritability, genetic advance and coefficient of correlation were estimated for different characters in mango. Significant variability characterized by high broad sense heritability and low to moderate genetic variance, was observed among the clones for all characters

In a study carried out using half-sib analysis, the fruit characters like fruit weight, fruit volume, peel weight, stone weight, TSS content and pulp content were found controlled by non-additive factors and that the heritability of these characters were low (Dinesh, 2003).

Mango fruit quality attributes were evaluated in hybrids developed by the Australian National Mango Breeding Program (NMBP). Analysis of the data indicated that many important fruit quality aspects such as fruit weight, fruit shape, ground skin colour, fruit width and pulp depth have high heritabilities, and can therefore be readily selected in a breeding programme. (Brettell *et al.*, 2004).

Singh *et al.* (2004a) studied genetic variability for 5 traits (yield per plant, cumulative yield per plant, fruit weight, total soluble solid (TSS) and acidity) in 43 genotypes of mango in Bihar. A wide range of variability was observed for all characters, except for TSS. The magnitude of phenotypic coefficient of variation was higher than that of genotypic coefficient of variation.

Singh (2005) assessed 20 genotypes of mango and found high phenotypic coefficient of variation along with high genotypic coefficient of variation for peel color, fruit weight and ascorbic acid content. High heritability along with high expected genetic advance was noticed for fruit weight and ascorbic acid content. Pradeepkumar *et al.* (2006) studied variability in physico-chemical characteristics of 31 mango genotypes in northern Kerala. Maximum variability was noted for acidity and minimum for fruit perimeter.

Rathod (2007) found that fruit weight and ascorbic acid showed high to moderate genotypic and phenotypic variance. The higher estimates of GCV and PCV were obtained for ascorbic acid, fruit weight, fruit length, acidity, peel per cent, stone per cent, non reducing sugar and reducing sugar. The characters like acidity, ascorbic acid, non reducing sugar, reducing sugar, fruit length, fruit weight, peel per cent and stone per cent exhibited high heritability coupled with high genetic advance, indicated better scope for improvement of these traits by an effective selection programme.

Bhowmick and Banik (2008) conducted an experiment with 9 mango cultivars at BCKV, West Bengal to determine the genetic variability and correlation coefficient for different fruit physico chemical characteristics. Phenotypic co-efficient of variation was more than that of genotypic counter part for each of the characteristics. The estimated heritability showed a wide range of variation, *i.e.*, 37.10–95.41 per cent. The genetic advance as percentage of mean also revealed high degree of variation (9.03–33.93).

Rajan *et al.* (2009) studied 42 cultivars to assess the heritability and divergence in fruit characters. Weight of pulp, fruit, stone, and peel, length of fruit and stone and pulp: stone ratio had high genotypic coefficient of variation and heritability accompanied with greater genetic advance as per cent of mean indicating important role of these traits for selection of parents in hybridization programme. Pulp weight contributed maximum towards the genetic divergence (34.03%) followed by peel weight, TSS, stone weight and stone width.

Lopez *et al.* (2010) analyzed 41 local mango accessions in the state of Chiapas, Mexico using morphological traits from leaves, fruits and seeds and found significant morphological

variability in mango accessions. Seven fruit traits (length, width, and weight of fruits, pulp thickness and weight, fiber content and fiber length) and two leaf traits (length and width) were used to index mango morphology. Quantitative traits with the highest CVs (>35%) were number of fertile stamens, pulp thickness, inflorescence length and fruit weight.

Dinesh *et al.* (2013) studied the heritability in mango cultivars and observed that heritability was >0.9 for the fruit characters *viz.*, fruit weight, length, width, thickness and total soluble solids (TSS). The phenotypic coefficient of variability was lower than the genotypic coefficient of variability implying greater role of environment in the expression of characters *viz.*, fruit weight, fruit length, fruit breadth, fruit thickness, TSS, skin weight, stone weight and pulp percentage.

Nayak *et al.* (2013) studied the genetic variability of fruit quality in mango F1 progenies and genetic parameters of fruit quality traits in mango hybrid population. Value of phenotypic coefficients of variation was higher than genotypic coefficients of variation but minimum difference was noticed between them. Comparatively high degree of genotypic coefficients of variation (GCV) along with phenotypic coefficients of variation was observed in quality traits like, fruit weight, fruit volume, pulp: stone ratio and total carotenoids. High to moderate broad-sense heritability was estimated for different fruit quality traits.

Himabindhu *et al.* (2016a) assessed the genetic variability, heritability and genetic advance for different characters in 34 diverse cultivars of mango. All the characters showed very small difference between genotypic coefficient of variation (GCV) and respective phenotypic coefficient of variation (PCV), indicating that all the characters were least affected by environment.

Patel *et al.* (2016) evaluated 20 mango genotypes at Anand during 2012-13 to study genetic variability, heritability, genetic advance and correlation coefficient. Wide range of variability was observed among twenty genotypes of mango for different plant characters. The narrow difference between genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) along with high heritability estimates for all the characters except tree canopy, indicated that these characters were largely under genetic control and environment had least influence on the expression of these traits. High heritability estimates coupled with high genetic advance were observed for all the characters except tree canopy.

Sridhar *et al.* (2018) carried out a study to assess the genetic variability, heritability and genetic advance for different characters in 16 diverse cultivars of mango. All the characters showed very small difference between genotypic coefficient of variation (GCV) and respective phenotypic coefficient of variation (PCV), indicating that all the characters were least affected by environment. High heritability coupled with high genetic advance were observed for all characters indicating these characters are governed by additive gene action and phenotypic selection may be more fruitful.

### **2.3 Correlation studies**

The concept of correlation was first put forward by Galton (1889) and later elaborated by Fisher (1918) and Wright (1921). In plant breeding correlation measures the mutual relationship between yield and its component characters. Selection was done on phenotypic basis by a breeder. Correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters on which selection can be based for genetic improvement in yield and other yield attributes and thus helps in the selection of superior genotypes from genetic population. However, in quantitative characters the genotypes are influenced by the environment and phenotypic expressions of traits, hence it was essential to increase the correlation of phenotypic and genotypic levels.

Significant correlations among different characters suggested the scope of direct and indirect effective selections for further improvement. Most of the quantitative and qualitative characters of economic importance show correlated response. Hence, the correlation studies are important in framing selection programme.

Attri *et al.* (1999) reported highly significant positive correlations in fourteen collections of mango from South Andaman for the characters like fruit length with weight, fruit breadth with weight, fruit weight with volume, specific gravity, stone and pulp percentage, TSS, total sugars and overall quality were positively correlated with each other. On the other hand, high negative correlation was observed for peel per cent with pulp per cent and for stone per cent with pulp per cent.

Karibasappa *et al.* (1999) noticed very high positive correlations for fruit weight with fruit volume, ripe fruit width with fruit volume, total sugars with reducing sugars. Conversely, significant negative correlations were observed for fruit yield and fruit weight with total sugars.

An experiment was conducted for 2 consecutive years in 12 genetically diverse strains or varieties of mango to determine their inter-relationship among different characteristics and their effects on fruit weight (Kumar, 2000). The genotypic and phenotypic correlation coefficient values revealed that genotypic correlation coefficients were higher than their corresponding phenotypic correlation coefficients, suggesting a strong inherent relationship among the different genotypes. Fruit weight had highly significant and positive correlation with fruit length, fruit diameter, fruit volume and seed stone length during both years. Shoot diameter, leaf length and reducing sugar also exhibited positive but non-significant association with fruit weight.

Patel (2002) studied the correlation of 17 different characters in 18 mango genotypes at Anand. He observed that genotypic correlations were higher than the phenotypic correlations in all the trait pairs indicating inherent association between different characters. The fruit yield per tree exhibited significant and positive correlation with number of fruit per tree, tree height, plant spread (N-S), plant spread (E-W), non-reducing sugar and ascorbic acid at both genotypic and phenotypic level, whereas, it was significantly and negatively correlated with stone, peel: pulp ratio and stone: pulp ratio at both the levels.

Jha *et al.* (2003) carried out association study for pickle purpose mangoes under Ranchi conditions and revealed that all the traits taken for study except stone weight were significantly and positively correlated. Stone weight was significantly and positively correlated with fruit weight only. Moreover, they also mentioned the highest positive correlation for pericarp weight and fruit weight (0.991) followed by fruit weight and diameter (0.909) and pericarp weight and diameter of fruit (0.904).

Yadav *et al.* (2003) studied correlation of fruit characteristics of 69 types of mango cv. Langra. Results revealed that correlation at the genotypic level was higher than at the phenotypic level, indicating a strong genetic correlation of characters and the lowering of effect of environmental interaction at the phenotypic level. Fruit length, fruit diameter, stone weight, peel weight, pulp weight and pulp percentage showed highly positive and significant genotypic and phenotypic correlation with fruit weight. Stone weight showed a strong and positive correlation with peel weight. Fruit length was negatively and significantly correlated with total soluble sugar and total sugar content, but was positively correlated with fruit acidity, pulp weight and peel weight.

Pradeepkumar *et al.* (2006) studied variability in physico-chemical characteristics of 31 mango genotypes in Northern Kerala. Highest significant correlation was noted between fruit

length and pulp weight, while ascorbic acid exhibited negative correlation with fruit length and peel weight.

Bhowmick and Banik (2008) conducted an experiment with 9 mango cultivars at BCKV, West Bengal to determine the genetic variability and correlation coefficient for different fruit physico chemical characteristics. There was a significant positive correlation of fruit weight with pulp content, breadth and significant negative co-relation with peel and acid content. Total soluble solids (TSS) showed high positive correlation with total sugar and non reducing sugar. Whereas, acidity showed high negative correlation with non reducing sugar, fruit weight, pulp content, TSS, sugar and reducing sugar content.

Gajera *et al.* (2011) analyzed the genetic variability and relationships among 20 *Mangifera indica* genotypes representing 15 endangered genotypes and 5 cultivars, obtained from Indian Gir forest region. It was evident that total sugar was inversely correlated with fruit weight and volume and smaller the fruit size, the higher the sugar content.

Majumder *et al.* (2012a) studied the correlation and path coefficient analysis of mango in sixty diverse genotypes of mango. They recorded that the percent flowering shoot had significant positive correlation with inflorescence per shoot, percent perfect flower, percent initial fruit set, number of fruits per plant and fruit weight both at phenotypic and genotypic levels.

Patel *et al.* (2016) evaluated 20 mango genotypes at Anand during 2012-13 to study genetic variability, heritability, genetic advance and correlation coefficient. The correlation study revealed significant and positive correlation of fruit yield per tree with number of fruits per tree at both genotypic and phenotypic level. Hence, yield and number of fruits could be given due weightage during selection process for yield improvement of mango.

Correlation studies in mango with different physical parameters were carried out during 2015-16 at Fruit Research Station (FRS), Sangareddy by Krishna *et al.* (2017). The data on correlation coefficient of yield and its component characters reveals that yield per tree was significantly and positively correlated with percent flowering and number of fruits per tree, where as negatively correlated with vegetative buds per cent and with number of days taken for panicle initiation. Among the quality parameters, the data showed that yield is positively correlated with total sugars and TSS: acidity ratio, whereas negatively correlated with ascorbic acid.

## 2.4 Genetic divergence studies

Genetic divergence helps in the selection of genetically diverse parents for their exploitation in hybridization programme. Genetic diversity was a basic criterion for the crop plants, either through natural selection or hybridization. Several methods have been developed for measuring divergence between populations using multivariate analysis, such as, multiple regression (Hotelling, 1936), discriminant function (Fisher, 1936) and  $D^2$  statistic (Mahalanobis, 1936). Out of these methods,  $D^2$  statistic was a powerful tool in specifying the degree of divergence among population at genotypic level and to assess the relative contribution of different components to the total divergence.

Karibasappa *et al.* (1999) used numerical taxonomic approach of unweighted pair group method using arithmetic average (UPGMA) for 37 quantitative and 6 qualitative characters in sixty seven genotypes of mango and identified eleven clusters. They reported that clusters 2, 4, 3 and 10 were most homogenous, whereas cluster 9, 7 and 11 were highly heterogenous. Cluster 7 (Neelum, Baramasi, Kalepad) and Cluster 11 (Batlimavu and Cowasji Patel) were the most divergent followed by cluster 10 (Dophasla, nl.him-46, Neeluddin, Local-4, KO-11, Creeping) and cluster 11, while cluster 3 (Dashehari, Pahutan, nl.him-32, nl.him-33, Local-1, csr.nl, Nekkare-2, Nekkare-1) and cluster 10 were the least divergent.

Singh (2005) assessed 20 genotypes of mango at IARI, New Delhi for cluster analysis and dendrogram study resulted in the identification of six clusters and region specific separation of genotypes of North Indian, South Indian and exotic cultivars.

Pradeepkumar *et al.* (2006) evaluated physico-chemical properties of the fruits of 31 mango genotypes from North Kerala. Based on the physical and chemical characteristics, these genotypes formed four homogenous clusters. Large sized table fruit varieties like Mulgoa, Phirangiladua and Banganapalli formed a single group. Conversely the low quality large fruited varieties like Banglora, Gudad and Chotta Jehangir deviated considerably from other mango cultivars.

Rathod (2007) assessed genetic diversity by Mahalanobis's  $D^2$  statistics of 35 mango genotypes. These genotypes were distributed in seven different clusters and the genotypes grouped in each cluster showed different behaviour irrespective of their origin, suggesting that geographical diversity was not related to genetic diversity. Genotypes with high fruit weight and high inter cluster distance are pinpointed for their utilization in a crossing programme to realize

the broad spectrum of genetic variability in segregating generations to effect selection for fruit weight improvement.

Rajan *et al.* (2009) studied about 42 cultivars to assess the heritability and divergence in fruit characters. Using group constellation, cultivars were grouped into three distinct clusters. Cultivar with higher mean for weight of fruit and peel, fruit length, width and thickness of fruit and stone, fruit length and width ratio (FLWR) and pulp: stone ratio were grouped in cluster III and high pulp weight and per cent, TSS, weight, length and width of stone were grouped in cluster II. Highest intra-cluster distance was observed in cluster I, while highest inter-cluster distance was between cluster I and II. Pulp weight contributed maximum (34.03%) towards the genetic divergence followed by peel weight (22.65%), TSS (10.22%) stone weight (7.90%) and width (5.46%).

Rufini *et al.* (2011) evaluated the genetic dissimilarity in 67 mango 'Uba' accessions by means of biometrical and physico-chemical characterization of the fruits. Fruits of accessions were characterized by evaluation of fruit mass, endocarp mass, pulp/endocarp ratio, longitudinal diameter, transversal diameter, total soluble solids (TSS), titratable total acidity (TTA), TSS/TTA ratio and pH. Statistical analysis was performed using the clustering techniques and dissimilarity measures. Cluster analysis demonstrated the formation of two groups of accessions.

By the application of clustering technique, Barhate *et al.* (2012) classified the twelve mango genotypes studied into four clusters. Among the four clusters, cluster I was the biggest one, consisting of six genotypes and cluster II contained four genotypes, while cluster three and four had one genotype each.

Silva *et al.* (2012) studied the genetic diversity of fifteen mango cultivars, eight from Brazil and seven from Florida (USA). The cultivars that showed more similarities were 'Kent' and 'Palmer'. 'Extrema' was not grouped with the others by the UPGMA clustering method. This analysis was also used for the separation of Brazilian and USA cultivars. The principal component analysis of the chemical characteristics did not group the cultivars 'Extrema' and 'Tommy Atkins' with the others. The physical characteristics showed the same separation obtained with UPGMA method, except for the cultivar 'Extrema' that was grouped with other cultivars.

Majumder *et al.* (2013) assessed the genetic divergence in 60 mango genotypes through  $D^2$  statistics and principal component analysis. The genotypes were grouped into eight clusters,

with diversity being influenced by the morphological characters, not by the geographical distribution of the genotypes. The clustering pattern revealed that the genotypes collected from the same region did not fall in the single cluster. The first nine characters of the principal component axes with eigen values above unity accounted for 88.3% of the total variation among the fifteen characters.

20 mango accessions in Caceres, Brazil were evaluated to assess genetic divergence as well as to identify the minimum efficient descriptors for that crop. The accessions were clustered using the methods of Tocher, Ward and UPGMA. The study observed that it was possible to reduce the number of descriptors from 64 to 35, and that the clustering methods were compatible with the study of the genetic diversity in mango (Sandra *et al.*, 2013).

Barholia and Sangeetha (2014) tested 48 genotypes for physical fruit characters in subsequent years to know the genetic diversity in mango. These genotypes were grouped in 5 clusters in two subsequent years based on  $D^2$  values. Number of genotypes varied from cluster to cluster due to existence of genotype-environment interactions. Fruits/tree, fruit yield/tree, weight per fruit, length and width of fruit and percentage weight of pulp contributed more towards genetic divergence. Hence, selection of parents based on these physical fruit traits in form of selection indices can be advantageous in the genetic improvement of physical fruit quality with high yield in mango.

Thirty-eight local mango trees in eastern and central Kenya were sampled and cluster analysis conducted with 56 selected descriptors (33 qualitative and 23 quantitative ones) resulted in the formation of six distinct clusters. Cluster 1 grouped eight mango samples with small and eggshaped fruits. In cluster 2, four mango samples with round and completely green fruits were grouped together. Thirteen mango samples with round, but yellow-green fruits were grouped in cluster 3. In cluster 4, only three mango samples with the biggest and heaviest fruits of yellow-green skin color were included. Four samples with long fruits having curved fruit shapes and a typical deep sinus were grouped in cluster 5. Cluster 6 grouped five samples characterized by oblong, almost quadratic shaped fruits without sinus (Sennhenn *et al.*, 2014).

Himabindu *et al.* (2016b) analyzed the genetic diversity among thirty four cultivars using Mahalanobis  $D^2$  statistic for thirty quantitative characters in mango. Total phenols followed by fruit skin thickness, fruit weight and pulp weight contributed maximum towards genetic divergence. The thirty four mango cultivars were grouped into 6 clusters. Among all the clusters, clusters I was the largest containing 29 genotypes, the clusters II, III, IV, V and VI were mono

genotypic. The high intra and inter cluster distances indicates the presence of wide genetic diversity among the genotypes present within cluster and between the clusters respectively.

Himabindu *et al.* (2017) conducted an experiment to analyze the genetic diversity among thirty four cultivars using principal component analysis for thirty quantitative characters in mango. The first seven principal components with eigen values more than one contributed to 82.04 per cent of cumulative variability among the 34 mango cultivars. The thirty four mango cultivars were grouped into 6 clusters. Among all the clusters, clusters I was the largest containing 19 cultivars followed by cluster II with 8 cultivars. Cluster III, IV and V contained 2 cultivars each, while cluster VI was mono genotypic. The high intra and inter cluster distances indicated the presence of substantial amount of genetic diversity in the genetic material.

## **2.5 Molecular characterization**

Mango cultivars were identified based on morphological characters, which lack high degree of accuracy, as these are often modified by the environment. Biotechnological methods have a definite role in understanding the genetics, crop improvement, germplasm characterization and removing confusion in the nomenclature of cultivars. With the advent of molecular biology techniques, DNA based markers have replaced enzyme markers in germplasm identification and characterization as well as in gene tagging. Owing to its plasticity, ubiquity and stability, DNA was the ideal molecule for such analysis (Caetano-Anolles *et al.*, 1991). Various types of molecular markers were utilized to evaluate DNA polymerase chain reaction (PCR) based markers (Joshi *et al.*, 1999).

Information regarding genetic variability at the molecular level could be used to help identify and develop genetically unique germplasm that complements existing cultivars. Molecular markers are useful for identifying cultivars and landraces and for studying genetic similarities among them (Duneman, 1994). 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 through put screening, can be exchanged between laboratories and are highly transferable between populations. Simple sequence repeats (SSRs) or microsatellites (Honsho *et al.*, 2005; Duval *et al.*, 2005; Schnell *et al.*, 2005; Hirano *et al.*, 2010; Wahdan *et al.*, 2011) have been employed in mango to study monogenic and polygenic traits.

Ravishankar *et al.* (2011) developed microsatellite markers to characterize and assess the genetic diversity among mango (*Mangifera indica*) cultivars and to test their amplification in

closely related species. A total of 36 microsatellite loci were isolated by a microsatellite-enriched partial genomic library method. Primers designed for these loci were characterized using 30 diverse mango cultivars. The number of alleles ranged from 3 to 19 with an average of 9.2 alleles per locus. PIC values ranged from 0.185 to 0.920 with a mean of 0.687. Loci from five related species, *M. odorata*, *M. anadamanica*, *M. zeylanica*, *M. camptosperma*, and *M. griffithii*, were successfully amplified using these SSR primers, showing their potential utility across species.

Begum *et al.* (2012) selected 20 indigenous landraces of mango according to fruit morphology and their fruit and leaf samples were collected for morpho-physiological and molecular characterization, respectively. Morpho-physiological characterization and evaluation based on fruit characteristics revealed that six landraces *viz.*, DM Acc-3, 4, 7, 15, 17 and 18 were elite with respect to fruit characteristics, which were further characterized for their genetic distinctiveness and relationships with the choicest juicy cultivars of mango in Andhra Pradesh ('Peddarasam', 'Chinnarasam', 'Cherukuram', 'Panchadarakalasa' and 'Suvarnarekha') at the molecular level, using 109 mango-specific microsatellite markers (SSRs). Of the 109 SSR markers validated, 57 were polymorphic, of which 10 were highly polymorphic.

Allelic patterns and genetic distances were examined in a collection of 103 foreign and Brazilian mango (*Mangifera indica*) accessions in order to develop a reference database to support cultivar protection and breeding programs (Ribeiro *et al.*, 2012). An UPGMA dendrogram was generated using Jaccard's coefficients from a distance matrix based on 50 alleles of 12 microsatellite loci. Three groups were observed in the UPGMA dendrogram; the first group was formed predominantly by foreign accessions, the second group was formed by Brazilian accessions, and the Dashehari accession was isolated from the others.

Shamili *et al.* (2012) used 16 Simple Sequence Repeat (SSR) markers to analyze 41 mango genotypes present in Iran. A total of 56 alleles were detected from 15 polymorphic loci ranging from 2 to 6 alleles per locus with an average of 3.7 alleles per locus. Mean expected and observed heterozygosities over the 15 polymorphic SSR loci averaged 0.57 and 0.63 respectively. UPGMA and Bayesian cluster analysis suggested a clear separation between the Iranian cultivars originated from India and Pakistan.

Singh *et al.* (2012) analyzed forty eight mango hybrids using 17 simple sequence repeat (SSR) markers which detected 59 scorable loci, of which 45 were polymorphic. SSR markers were highly polymorphic with an average of 3.47 alleles per primer. The genetic relationship among mango hybrids, based on Jaccard's Similarity Coefficient values ranged from 0.38

(between H-1-13 and H-6-8) to 0.97 (between H-13-4 and H-13-7). The dendrogram, based on UPGMA cluster analysis, grouped the mango hybrids into three major groups. The Principal Coordinate Analysis also exhibited more or less similar distribution of mango hybrids. The tendency of clustering among mango hybrids revealed that they had stronger affinity towards female parent Amrapali.

Microsatellites were successfully used by Vasugi *et al.* (2012) for genetic diversity analysis of the indigenous 'Appemidi' type and the major compounds that contribute to the unique aroma of these types were also estimated. The materials used in the study consisted of 43 accessions and 14 SSRs developed at IIHR, Bangalore. The analysis of 211 bands detected by the 14 Simple Sequence Repeats (SSRs) markers showed unambiguous discrimination of the 43 mango genotypes. The dendrogram resulted in the grouping of accessions into two major clusters, *viz.*, cluster I with highly acidic types and cluster II with less acidic and high TSS group.

Begum *et al.* (2013) selected 31 accessions of 'Beneshan' (BN Acc-1 to BN Acc-31) and their fruit and leaf samples were collected to study intracultivar heterogeneity based on morphological fruit traits and microsatellite markers, respectively. 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.

Kumar *et al.* (2013) conducted a study to show genetic variation and investigate inter-relationship between ten mango genotypes. Twenty SSR markers were tested with 10 genotypes. PCR amplification of the isolated DNA produced a total of 240 amplified products, of which 184 were polymorphic and 56 monomorphic. SSR markers were highly polymorphic with an average of 2.70 alleles per primer. The dendrogram generated from the unweighted pair group arithmetic average (UPGMA) cluster analysis broadly placed 10 mango cultivars into three major clusters at co-efficient similarity of 0.65.

Genetic analysis of 90 mango genotypes including juicy, table, dual and pickle types from different parts of Andhra Pradesh of India was carried out by Surapaneni *et al.* (2013) employing 143 mango-specific microsatellite markers. Characterization of the 90 genotypes resulted in the detection of 301 alleles from 106 polymorphic loci with an average of 2.87 alleles

per locus and polymorphism information content of 0.67. UPGMA cluster analysis grouped all the genotypes into 2 major groups with a genetic similarity range of 47–88%.

Tsai *et al.* (2013) examined the genetic relationship of twenty-two mango (*Mangifera indica* L.) cultivars/lines based on 37 SSR markers. Numbers of alleles per locus of the 37 SSR markers ranged from 2 to 11, and a total of 182 alleles with an average of 4.86 alleles per locus. Banding patterns obtained from 37 SSR primers allowed for each cultivar/line to be distinguished from the others with the exception of vegetative clones. The result indicates that SSR analysis is an efficient method for cultivar identification.

Vasugi *et al.* (2013) used morphological and molecular methods (SSR) to identify duplicates in the mango germplasm, resulting in the identification of duplicate accessions *viz.*, ‘Thumbebeedu’ and ‘Isagoor Appe’, ‘Dorganikayi’ and ‘Shahjahan’, ‘Peter’ and ‘Lal Pairi’ and clarified the ambiguity in nomenclature. However, molecular characterization using SSR markers of these synonyms differ with respect to a few loci which could be due to the changes in their locus.

Dillon *et al.* (2014a) conducted a study wherein a collection of 24,840 expressed sequence tags (ESTs) generated from five mango (*Mangifera indica* L.) cDNA libraries was mined for EST-based simple sequence repeat (SSR) markers. Of these, 25 EST-SSRs in genes involved in plant development, stress response, and fruit color and flavor development pathways were selected, developed into PCR markers and characterized in a population of 32 mango selections including *M. indica* varieties and related *Mangifera* species. Twenty-four of the 25 EST-SSR markers exhibited polymorphisms, identifying a total of 86 alleles with an average of 5.38 alleles per locus, and distinguished between all *Mangifera* selections.

Eleven microsatellites or simple sequence repeat (SSR) markers were evaluated for their usefulness to identify varieties, validate progeny and parents, and estimate genetic diversity of populations (Dillon *et al.*, 2014b). The markers proved ideal for fingerprinting of varieties, with an average of 8.36 alleles per locus identified to distinguish between all 105 accessions tested. Among breeding progeny, SSR markers identified the parents of hand- and open-pollinated mango hybrids with 95% confidence.

Dinesh *et al.* (2015) conducted genetic diversity studies utilizing 44 *naati* (indigenous) types from Chittoor district in Andhra Pradesh. The 8-labeled SSR markers employed in the study detected 97 alleles in 44 genotypes. The number of alleles for each locus ranged from 9 to 19,

with a mean number of alleles per locus being 12.13. Observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ) for each locus ranged from 0.364 to 0.674 and 0.693 to 0.889, respectively.

Ravishankar *et al.* (2015) assessed the genetic diversity and population structure of mango cultivars by employing fourteen simple sequence repeat markers, with high polymorphic information content. A set of 387 mango accessions from different regions of India was used. Modelbased structure analysis revealed the presence of two subpopulations comprising the cultivars from 'South-West' region and 'North-East' region. A similar clustering pattern was observed in the dendrogram analysis, with two major groups identified that were further sub-grouped based on their genetic relatedness. The extent of genetic diversity was found to be higher in case of 'South and West' population than 'North and East' population.

Azmat *et al.* (2016) assayed 13 premium quality commercial mango cultivars grown in Pakistan with seven SSR primer series *viz.*, mMICIR, MiSHRS, MIAC, MITGIT, LMMA, UBC and MiIHR. Among the 120 primers used, 101 produced bands and revealed a narrow genetic base ranging from 0.62-0.8 with maximum similarity among the cvs. 'Anwar Ratole' and 'Sensation' while the maximum divergence was between cvs. 'Sindhri' and 'Sensation'. The UPGMA based Euclidian dendrogram constructed with similarity indices placed all the cultivars according to regions of their origin and magnitude of diversity among them.

Bajpai *et al.* (2016) assessed 37 mango types comprising of 27 heirloom varieties from Malihabad region and 10 commercial varieties grown in North and Eastern India for morphological attributes and molecular diversity. The employed SSR markers amplified 2-13 alleles individually, cumulatively amplifying 124 alleles. These were studied for allelic diversity and genetic dissimilarity ranged from 0.035 to 0.892 arranging the varieties in three major clusters. The results revealed that majority of unique heirloom mangoes from Malihabad were different from the eastern part of the country.

In their investigation of mango cultivars, Thumar *et al.* (2016) employed five physiological parameters, six quality parameters and three molecular markers (RAPD, ISSR and SSR) to measure the discriminatory power of marker and to select lines that can be used as potential parents in a future breeding program. Three principal components extracted (PC1, PC2 and PC3) from both physiological and qualitative characters showed that all physiological characters such as fruit weight, fruit volume, peel weight, pulp weight and stone weight had maximum contribution (83.28%) to total variance. SSR marker was found to be a good choice for estimating variability as it revealed 71.86 per cent polymorphism.

Alves *et al.* (2016) conducted a study to develop new microsatellite markers as additional tools in genetic studies on mangoes (*Mangifera indica* L.). Twenty accessions were analyzed using 27 microsatellite markers, of which 16 were developed during the study. The clusters, based on the morphological descriptors by Ward-MLM strategy and the microsatellite markers, suggested that Brazilian mango cultivars have extensive genetic diversity and are related to cultivars with different provenances, demonstrating their different enhanced breeding histories.

Lal *et al.* (2017) mapped 17 critical pomological traits in mango through genome-wide association mapping using the generalized linear model (GLM) and mixed linear model (MLM) approaches. Significant phenotypic variability in the selected traits was observed in 60 mango varieties, which indicated the suitability of these varieties for association studies. Genotyping of the varieties was performed using 87 polymorphic genic-simple sequence repeats (SSR) markers. Population structure analysis performed using the genotyping data revealed three distinct sub-populations that corroborated with the geographical origin of the varieties.

Nazish *et al.* (2017) used microsatellites to determine the genetic relatedness and variability among 15 indigenous mango cultivars (*Mangifera indica* L.). Overall, 181 bands were produced using 12 simple sequence repeat (SSR) primers. Out of the 12 primers used, 10 were polymorphic and two were monomorphic. Genetic relatedness among cultivars was assessed by constructing a dendrogram using the unweighted pair group method of arithmetic means. The accessions exhibited coefficients of similarity ranging from 75 to 100%, indicating the frequent use of only a few parent cultivars and the presence of inbreeding.

The genetic diversity of 60 Myanmar mango accessions from 21 orchards within three locations in Kyaukse District were studied by Htway *et al.* (2018). A total of 9 SSR markers were used to study the genetic diversity and phylogenetic relationships among the collected mango accessions. Total of 48 scorable bands were observed on amplification with the sizes ranging between 110 bp and 369 bp. Polymorphic information content (PIC) of 9 SSR markers were 0.265 to 0.74 with an average of 0.421 per marker. By using UPGMA cluster analysis, it grouped all the accessions from three locations with a genetic similar coefficient between 0.68-0.96.

Surapaneni *et al.* (2018) studied the genetic variability of 20 promising Indian mango genotypes, including juicy and table types, employing 143 simple sequence repeats (SSRs). Of 143 SSRs, 80 were found to be polymorphic among the genotypes. Characterization of the 20 genotypes resulted in the detection of 236 alleles from 80 polymorphic loci with an average of 2.87 alleles per locus and polymorphism information content (PIC) of 0.63. UPGMA cluster

analysis grouped all the genotypes into two major groups with a genetic similarity range of 37–66%. The genotypes were grouped into clusters based on the utility type.

## 2.6 Estimation of volatiles

Andrade *et al.* (2000) obtained the aromas of 15 varieties of mango (*Mangifera indica* L.) cultivated in Brazil by simultaneous distillation-extraction and analyzed by GC/MS. The data analysis of volatile compounds has identified three distinguishable aroma groups. The first group, rich in  $\alpha$ -terpinolene, was composed of the following varieties: Cheiro (66.1%), Chana (62.4%), Bacuri (57.0%), Cametah (56.3%), Gojoba (54.8%), Carlota (52.0%), Coquinho (51.4%) and Comum (45.0%). The second group, rich in  $\Delta^3$ -carene, comprised the following varieties: Haden (71.4%), Tommy (64.5%) and Keith (57.4%). The third group, rich in myrcene, was dominated by the following varieties: Cavalo (57.1%), Rosa (52.4%), Espada (37.2%) and Paulista (30.3%).

‘Kensington Pride’ mangoes were harvested (*Mangifera indica* L.) at the mature green, half ripe and ripe stages by Lalel *et al.* (2003a) to investigate glycosidically-bound aroma volatiles in their skin and pulp. The composition and concentrations of glycosidically-bound aroma compounds were strongly influenced by fruit part and maturity stages. Glycosidically-bound aroma volatile compounds produced via aromatic amino acid metabolites were the most abundant class of compounds found in the skin, whilst in the pulp, terpenes were found to be the most abundant compounds, accounting for 41.18 and 38.04% of the total number of compounds in skin and pulp, respectively.

Lalel *et al.* (2003b) studied ‘Kensington Pride’ mango aroma volatile compounds emitted during ripening using headspace solidphase microextraction as a sampling method and gas chromatography with a flame ionisation detector as well as gas chromatography mass spectrophotometry for analysis. Sixty-one aroma volatile compounds were identified, of which 35 compounds have not been reported previously in ‘Kensington Pride’ mango. (+)-Spathulenol and  $\beta$ -maaliene were found for the first time in mango fruit. The most abundant group of volatile compounds was hydrocarbons, accounting for about 59% of the total identified compounds, followed by esters (20%).

Volatile compounds were extracted from the pulp of mango cultivar Tommy Atkins through a simultaneous solvent distillation and extraction process using Likens and Nickerson’s apparatus. Identification of volatile organic compounds was achieved in a system of high resolution gas chromatograph coupled with mass spectrometer. A total of 104 compounds were

identified in the pulp of ripe fruit. The major classes of organic compounds identified in the flavor profile of Tommy Atkins fruit were hydrocarbons, esters, terpenes, lactones and aromatic compounds (Narain and Galvao, 2004).

Singh *et al.* (2004b) identified sixty one aroma volatile compounds from the 'Kensington Pride mango fruit pulp, using a head space solid phase microextraction (SPME) technique with gas chromatography (GC) and GC combined with mass spectrophotometry (GC-MS). Low temperatures during storage induced chilling injury and reduced the production of aroma volatile compounds during fruit ripening and in fully ripe fruits. The composition of CA storage as well as storage period affected production of aroma volatile compounds in the pulp of the ripe Kensington Pride and Delta R2E2 mangoes.

Pino *et al.* (2005) investigated the volatile components of 20 mango cultivars by means of simultaneous distillation-extraction, GC, and GC-MS. Three hundred and seventy-two compounds were identified, of which 180 were found for the first time in mango fruit. The total concentration of volatiles was ~18-123 mg/kg of fresh fruit. Terpene hydrocarbons were the major volatiles of all cultivars, the dominant terpenes being  $\delta$ -3-carene (cvs. Haden, Manga Amarilla, Macho, Manga Blanca, San Diego, Manzano, Smith, Florida, Keitt, and Kent), limonene (cvs. Delicioso, Super Haden, Ordonez, Filipino, and La Paz), both terpenes (cv. Delicia), terpinolene (cvs. Obispo, Corazon, and Huevo de toro), and R-phellandrene (cv. Minin).

Zhang *et al.* (2006) used manual headspace Solid Phase Microextraction (SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS) to study the aroma characteristics of three kinds of Chinese mango cultivars (Xiangya, Xiaoxiang and Jidan mangoes). In total, twenty-four aroma volatiles, including alkene, alkane, alcohol, aldehyde, ketone and aromatic compounds were identified. Aroma characteristics of different mango cultivars were specified by Principal Component Analysis (PCA).

The head space volatiles from flowers, as well as green and ripe mango fruit of cv. Aaulfo from Soconusco, Chiapas was collected by Sandoval *et al.* (2007) using Solid Phase Microextraction (SPME). Identification of chemical species was carried out by Gas Chromatography-Mass Spectrometry (GC-MS). A complex mixture of monoterpenes and sesquiterpenes was found. 3-carene,  $\alpha$ -pinene, myrcene, limonene, terpinolene,  $\beta$ -selinene and the sesquiterpene tentatively identified as germacrene D were the major constituents.

Mango fruit (*Mangifera indica* L.), cv. 'Cogshall', 'Kent' and 'Keitt' were harvested at different maturities and at different sizes by Lebrun *et al.* (2008). Immediately after harvest (green) or after 1 week of ripening at room temperature (ripe), fruit were homogenized or left intact and evaluated by electronic nose (enose) or by gas chromatography (GC) for aroma and other volatiles as well as for soluble solids and acids. Both the enose and GC were able, in most cases, to separate fruit from different harvest maturities. Solids and acids data indicated that later harvest maturities resulted in sweeter fruit and later-harvested fruit had a different volatile profile from earlier-harvested fruit.

Pandit *et al.* (2009) assessed volatile blends of 22 Indian and five non-Indian cultivars using solvent extraction and gas chromatography. Totally 84 volatiles belonging to various chemical classes were detected. Based on the cumulative occurrence of members of these classes, cultivars were grouped as monoterpene or sesquiterpene dominant.  $\alpha$ -pinene,  $\beta$ -myrcene and  $\beta$ -caryophyllene were found in all 27 cultivars. For ordination, common compounds with high (relative) concentration provided quantitative characters, whereas the rare and lesser ones provided qualitative (binary) characters; non-Indian cultivars separated from Indian ones but displayed close relations within their groups.

The volatile components in ripening fruit peel and pulp of mango (*Mangifera indica* L. var Zihua) were extracted with 100 $\mu$ m PDMS or 75  $\mu$ m Carboxen/ PDMS fibres prior to GC-MS analysis (Wei *et al.*, 2010). Twenty-six volatile components were identified in mango peel extracted with 75 $\mu$  m Carboxen/ PDMS fibre, accounting for 98.71% of total volatile components. Totally 21 components were detected in mango pulp, occupying 99.29% of total volatile components. However, only 22 volatile components were identified in mango peel extracted with 100  $\mu$ m PDMS fibre, which accounted for 98.51% of total volatile components. Fifteen volatile components were found in mango pulp, the total relative content 97.68%, and the content of terpenes 96.69% with the dominant terpinolene of 79.18%.

Gebara *et al.* (2011) investigated volatile compounds present in fruits and leaves of *Mangifera indica* var. coquinho by headspace solid phase microextraction (HS-SPME) and hydrodistillation (HD). Fruits and leaves were analysed in both mature and immature stages.

Alphonso is known to exhibit geographic variation in the flavour of ripe fruits. To get chemical insight into this difference, volatiles were studied in the ripening fruits of Alphonso mangoes from three cultivation locations in India by Kulkarni *et al.* (2012). Ripe fruits from Deogad had lower content of mono- and sesqui-terpenes and higher content of lactones and

furanones as compared to the fruits from Dapoli; whereas fruits from Vengurle had average quantities of these chemicals in comparison with Deogad and Dapoli fruits. This variation was clearly reflected as separate clustering of the localities in the Principal Component Analysis.

The aroma volatiles of four varieties of mango (*Mangifera indica* L.) cultivated in Zhanjiang, Guangdong province were obtained by solid-phase microextraction (SPME) and analyzed by GC/MS in the skin and pulp at the fully ripe stage (Wei *et al.*, 2013). The aroma volatiles with the highest content in the four varieties were: (*Z*)- $\beta$ -ocimene (43.02%), Terpinolene (68.37%), D-Limonene (54.91%) and 3-Carene (49.951%) in the skin and (*Z*)- $\beta$ -ocimene (82.32%), Terpinolene (67.84%), Cinene (68.33%) and 3-Carene (67.86%) in the pulp.

Benevides *et al.* (2014) analyzed the volatile compounds of three mango varieties (Tommy Atkins, Rosa and Espada) using the static headspace technique with SPME coupled to GC-MS. The data were evaluated by using principal components analysis (PCA) and hierarchical grouping analysis, in order to visualize grouping tendencies of volatile compounds. Thirty-seven volatile compounds belonging to different chemical classes, such as esters, terpenes, alcohols and others, were tentatively identified in the 3 varieties of mango. Amongst them, 23 presented chromatographic peaks with relative areas larger than 2%.

Preethi *et al.* (2014) undertook a laboratory study to determine the volatile profile of mango fruits using hexane extract and GCMS technique. The number of aroma components identified from Neelum and Banganapalli were 24 and 31, respectively. Neelum had esters as principal constituent and alkanes, ketones, alcohols, lactone and acid as associates. In Banganapalli, alkanes were the dominant constituent followed by esters, alcohols, ethers, fatty acid, amino acid triterpene and sulfur. The presence of mineral components such as sulfur and nitrogen were found to constitute the aroma of mango fruits.

The physicochemical properties and volatile flavor compounds in Taiwan Apple mango (TAM) and Philippines Carabao mango (PCM) were investigated by An *et al.* (2015). The volatile flavor compounds were extracted using solid-phase microextraction (SPME) and analyzed by GC/MS. They identified 56 and 59 volatile flavor compounds in TAM and PCM, respectively. Terpenes and their derivatives comprised 94.42% of the volatile flavor compounds in TAM, but only 63.79% of those in PCM.  $\delta$ -3-Carene was the dominant flavor compound in these two mango cultivars..

White *et al.* (2016) demonstrated real-time profiling of mango ripening based on proton transfer reaction-time of flight-mass spectrometry (PTR-ToF-MS) of small molecular weight volatile organic compounds (VOCs) headspace measurements of ‘Tommy Atkins’ mangoes. VOC metabolites produced during the ripening process were sampled directly, which enabled simultaneous and rapid detection of a wide range of compounds. A principle component analysis of the results indicated that several mass channels were not only key to the ripening process but could also be used to distinguish between mango cultivars.

Li *et al.* (2017) studied the volatile diversity of 25 mango cultivars from China, America, Thailand, India, Cuba, Indonesia, and the Philippines. The volatile compositions, their relative contents, and the intervarietal differences were detected with headspace solid phase microextraction tandem gas chromatography-mass spectrometer methods. In total, 127 volatiles were found in all the cultivars, belonging to various chemical classes. The highest and lowest qualitative abundances of volatiles were detected in ‘Zihua’ and ‘Mallika’ cultivars, respectively. Based on the cumulative occurrence of members of the classes of volatiles, the cultivars were grouped into monoterpenes (16 cultivars), proportion and balanced (eight cultivars), and nonterpene groups (one cultivar).

San *et al.* (2017) reported a high throughput method to quantify in a single analysis, the key volatiles that contribute to the aroma of commercially significant mango cultivars grown in Australia. The method constitutes stable isotope dilution analysis (SIDA) in conjunction with headspace (HS) solid-phase microextraction (SPME) coupled with gas-chromatography mass spectrometry (GCMS). Seven volatiles, hexanal, 3-carene,  $\alpha$ -terpinene,  $\beta$ -cymene, limonene,  $\alpha$ -terpinolene and ethyl octanoate, were targeted. The method was applied to identify the key aroma volatile compounds produced by ‘Kensington Pride’ and ‘B74’ mango fruit and by ‘Honey Gold’ mango sap. This method represents a marked improvement over current methods for detecting and measuring concentrations of mango fruit and sap volatiles.

The composition and relative content of volatiles in 37 cultivars was investigated by Ma *et al.* (2018) using headspace solid phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC-MS). Distinct differences in the components and content of volatile compounds among and within cultivars was observed. In total, 114 volatile compounds, including 23 monoterpenes, 16 sesquiterpenes, 29 non-terpene hydrocarbons, 25 esters, 11 aldehydes, five alcohols and five ketones, were identified. The total volatile content among cultivars ranged from 211 to 26,022  $\mu\text{g}/\text{kg}$  fresh weight (FW), with 123-fold variation.

Zakaria *et al.* (2018) conducted a study to analyze the volatile organic compounds (VOCs) of three mango varieties (Harumanis, Tong Dam and Susu) for the discrimination of authentic Harumanis from other mangoes. The VOCs of these mangoes were extracted and analysed nondestructively using Head Space-Solid Phase Micro Extraction (HS-SPME) coupled to Gas Chromatography-Mass Spectrometry (GC-MS). Chemometric techniques, such as principal components analysis (PCA), hierarchical clustering analysis (HCA), and discriminant analysis (DA) were successful in identifying the grouping tendencies of the mango samples according to the presence of their respective volatile compounds, thus enabling the identification of the groups of substances responsible for the discrimination between the authentic and unauthentic Harumanis mangoes.

# *MATERIALS & METHODS*



The research work entitled “**Genetic diversity and characterization studies in mango**” was conducted during 2015-18 at the Division of Fruit Crops, Division of Biotechnology and Division of Physiology and Biochemistry, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru. The experimental site is located 13° N and 17°37' E at an altitude of 890 m above mean sea level. Soil is red sandy loam with pH 5.2 – 6.4. The average rainfall of this area is about 890 mm, distributed over a period of six to eight months (April to November) with peaks during September.

The research work was carried out by studying the morphological and biochemical characters to assess the extent of variability and the genetic diversity by using SSR markers. Parentage analysis was also carried out in Amrapali × Vanraj and Amrapalli × *M. odorata* hybrids using eight SSR primers. The volatile profile of leaves of 39 mango hybrids and their parents were also analyzed by headspace-solid phase micro-extraction (HS-SPME) technique using capillary GC and GC–MS/MS. The materials and methods adopted during the course of investigation are presented in this chapter under the following heads.

- Study the diversity existing in *M. indica* varieties from different regions
- Characterization of the genotypes using molecular tools
- Attempting marker assisted selections in the progeny population
- Estimation of volatiles in certain progenies and their parents

### **3.1 Study the diversity existing in *M. indica* varieties from different regions**

#### **3.1.1 Experimental material**

In the present study 156 mango cultivars from the Field Gene Bank at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru were used. These mango cultivars have been collected from different regions of India and are maintained in field gene banks. Except for a few recently bred hybrids, all other cultivars represent land races which originated as chance seedlings and have been maintained by vegetative propagation. These accessions were planted at a spacing of 8 x 8 m with three individual trees per accession. Standard horticultural practices

were followed including irrigation, weeding, protection from pests and diseases. All the morphological observations were taken as per the IPGRI descriptor for mango (IPGRI, 2006).

### **3.1.2 Fruit morphological characters**

#### **3.1.2.1 Fruit size**

Fruit weight (g), fruit length (cm), fruit width (cm) and fruit thickness (cm) were recorded.

##### **3.1.2.1.1 Fruit weight (g)**

Fruits were weighed with the help of electronic balance separately and expressed in grams.

##### **3.1.2.1.2 Fruit length (cm)**

Fruit length from base of the fruit to the top of the groove at the calyx end was recorded with the help of vernier caliper and expressed in centimetres.

##### **3.1.2.1.3 Fruit width (cm)**

Fruit width was measured at the broadest part of fruit and recorded in centimetres.

##### **3.1.2.1.4 Fruit thickness(cm)**

Fruit thickness was measured with the help of vernier caliper and expressed in centimetres.

#### **3.1.2.2 Skin characters**

Fruits at fully ripe stage were taken to observe the skin characters such as skin thickness and skin weight.

##### **3.1.2.2.1 Skin weight (g)**

The peel of fruits were taken and weighed with the help of electronic balance separately and expressed in grams.

##### **3.1.2.2.2 Skin thickness (cm)**

The peel thickness was measured with the help of vernier caliper and expressed in centimetres.

### **3.1.2.3 Pulp characters**

#### **3.1.2.3.1 Pulp weight (g)**

The peel and stone were separated from the fruit and weighed. The pulp weight was calculated by subtracting the stone and peel weight from the total fruit weight.

#### **3.1.2.3.2. Pulp per cent (%)**

Pulp per cent was calculated by using the following formula.

$$\text{Pulp per cent} = \frac{\text{Pulp weight}}{\text{Fruit weight}} \times 100$$

#### **3.1.2.4 Stone weight (g)**

The stone was weighed with the help of electronic balance separately and expressed in grams.

### **3.1.3 Bio-chemical characters**

#### **3.1.3.1 Total soluble solids (°Brix)**

The percentage of total soluble solids was determined by using ERMA hand refractrometer by placing a drop of filtered juice on the prism of the refractrometer and observing the coincidence of shadow of the sample with the reading on the scale and expressed as °Brix. Before taking the reading, the refractrometer was tested for its error with distilled water, corrected accordingly and TSS content was recorded (Ranganna, 1986).

#### **3.1.3.2 Titratable acidity (%)**

Acidity was determined by titration method (AOAC, 1965). Mango samples were homogenized in a blender to a fine puree. 10 grams of mango puree was mixed with distilled water, squeezed through a muslin cloth and volume was made up to 50 ml. A known volume of the filtrate (10 ml) was titrated against 0.01N NaOH using phenolphthalein as indicator. Acidity was calculated as percentage of citric acid equivalents using citric acid standard curve.

$$\text{Acidity (\%)} = \frac{\text{Titre value} \times \text{Std. value } (\mu\text{g}) \times \text{Total vol. of extract} \times \text{Correction factor} \times 100}{\text{Assay volume} \times \text{Wt. of the sample (g)} \times 1000}$$

#### **3.1.3.3 Ascorbic acid (mg/100ml)**

##### **Reagents**

- 4% Oxalic acid

- 2, 6-Dichlorophenol indophenol sodium salt ( $C_{12}H_6Cl_2N NaO_2 \cdot 2H_2O$ ) dye solution: Dissolve 50 mg of 2, 6-dichlorophenol indophenol in distilled water, mix with 42 mg of anhydrous sodium bicarbonate and make up the volume to 200 ml.
- Standard ascorbic acid ( $C_6H_8O_6$ ) solution: 0.01% in 4% oxalic acid.

Ascorbic acid content was determined by 2, 6-Dichlorophenol indophenol (DCPIP) method (AOAC, 1965). Ten grams of mango puree was mixed thoroughly with 4% oxalic acid solution, squeezed through a muslin cloth and volume was made up to 50 ml. Vitamin C content present in the solution was estimated by titrating a known quantity of the extract against DCPIP. Vitamin C content was calculated as mg of ascorbic acid equivalents per 100 g fresh weight using a standard curve of L-Ascorbic acid.

$$\text{Vitamin C (mg/100g)} = \frac{\text{Titre value} \times \text{Std. value } (\mu\text{g}) \times \text{Total vol. of extract} \times 100}{\text{Assay volume} \times \text{Wt. of the sample (g)} \times 1000}$$

#### 3.1.3.4 Total carotenoids (mg/100g)

Total carotenoids were analyzed by spectrophotometric method (Lichtenthaler, 1987). 5 g of sample was taken in a mortar, one spatula of  $CaCO_3$  was added and grind with acetone. Extract the residue with more solvent until the supernatant becomes colourless. All the extractions should be carried out under low light or red light. The extract was taken in a separating funnel, 15 ml hexane was added along with 100 ml water. Shake well, allow to stand for a few minutes. The two phases formed were separated and the lower aqueous phase was re-extracted with additional hexane, until the aqueous phase was colourless. Collect the upper layer and repeat the same by taking 5 ml hexane. Dry with anhydrous  $Na_2SO_4$  and make up the volume to 25 ml with hexane and read absorbance at 470 nm. The carotene content was calculated using standard  $\beta$ -carotene and expressed in mg/100g fresh weight using standard curve.

$$\text{Total carotenoids (mg/100g)} = \frac{OD_{470nm} \times \text{Std. value } (\mu\text{g/OD}) \times \text{Total vol. of extract} \times 100}{\text{Wt. of the sample (g)} \times 1000}$$

#### 3.1.3.5 Total phenols (mg gallic acid equivalents/100g)

##### Reagents required

- 80% Methanol
- Folin-Ciocalteu's phenol reagent (1N)
- 20% Sodium Carbonate ( $Na_2CO_3$ )

- Standard phenol (Gallic acid) solution (20-100 µg/ml) prepared in 80% methanol

5 g of sample was homogenized with 20 ml of methanol (80%) in a pestle and mortar 2-3 times. Pool the extracts and make up the volume to 50 ml. Take 0.5 ml of the extract in test tubes, add 0.2 ml of Folin-Ciocalteu's phenol reagent followed by 3.3 ml of distilled water and mix well. After 2 min., add 1 ml of sodium carbonate solution and mix. The solution was allowed to stand at room temperature for 30 minutes and read in a spectrophotometer at 700 nm. A standard curve for phenols was prepared using gallic acid (GA) as standard.

$$\text{Total phenol content (mg/100g)} = \frac{\text{OD}_{700\text{nm}} \times \text{Std. value } (\mu\text{g/OD}) \times \text{Total vol. of extract} \times 100}{\text{Assay volume} \times \text{Wt. of tissue (g)} \times 1000}$$

### 3.1.3.6 Total flavonoids (mg catechin equivalents/100g)

#### Reagents required

- 80% methanol
- 10% Aluminium chloride (AlCl<sub>3</sub>)
- 5% Sodium nitrite (NaNO<sub>2</sub>)
- 4N Sodium hydroxide (NaOH)

5 g of sample was homogenized with 20 ml of methanol (80%) in a pestle and mortar 2-3 times. Pool the extracts and make up the volume to 50 ml. Take 1.0 ml of extract in tubes, add 0.3 ml of 5% NaNO<sub>2</sub>. Wait for 2 min and add 0.3 ml of 10% AlCl<sub>3</sub>. After another 2 min, add 3.4 ml of NaOH and allow to stand at room temperature for 10 minutes. The absorbance was read at 510 nm against blank. Catechin or quercetin was used as standard.

$$\text{Total flavonoid content (mg/100g)} = \frac{\text{OD}_{510\text{nm}} \times \text{Std. value (mg/OD)} \times \text{Total vol. of extract} \times 100}{\text{Assay volume} \times \text{Wt. of sample (g)}}$$

### 3.1.4 Statistical analysis of diversity studies on mango cultivars

Analysis of variance and components of genetic variability such as mean, range, genotypic and phenotypic coefficient of variance (GCV and PCV respectively), heritability and genetic advance as per cent mean (GAM) were estimated using standard procedures. Multivariate analysis (D<sup>2</sup> statistics), phenotypic and genotypic correlations and principal component analysis were performed on the data obtained, as detailed here under.

### 3.1.4.1 Analysis of variance

Analysis of variance for each character was carried out by using Randomized Block Design.

### 3.1.4.2 Coefficient of variation

Genotypic and phenotypic coefficients of variation were computed according to Burton (1952) based on the estimate of genotypic and phenotypic variance as follows:

$$GCV = \frac{\sqrt{GV}}{\bar{X}} \times 100 \qquad PCV = \frac{\sqrt{PV}}{\bar{X}} \times 100$$

Where, GCV = Genotypic coefficient of variation

PCV = Phenotypic coefficient of variation

GV = Genotypic variance

PV = Phenotypic variance

$\bar{X}$  = General mean of character

Further, the PCV and GCV were classified as suggested by Sivasubramanian and Madhavamenon (1973) :

0 – 10% : Low

10 – 20% : Moderate

20% and above : High

### 3.1.4.3 Heritability

Heritability in broad sense refers to the proportion of genetic variance to the total observed variance in the population. It has been estimated as per the formula given by Lush (1940).

$$h^2(b) = \frac{\text{Genotypic variance } (\sigma^2 g)}{\text{Phenotypic variance } (\sigma^2 p)} \times 100$$

Where,  $\sigma^2 g$  and  $\sigma^2 p$  are the genotypic and phenotypic variances. Further, the range of heritability in broad sense was classified as suggested by Johnson *et al.* (1955a).

Less than 30% : Low

30 – 60% : Moderate

More than 60% : High

#### 3.1.4.4 Genetic advance as per cent mean (GAM)

Genetic advance as per cent mean was worked out for each character adopting the formula given by Johnson *et al.* (1955b)

$$\text{GAM} = \frac{\text{GA}}{\bar{X}} \times 100$$

Where, Genetic advance (GA) =  $k \times \sigma^2_p \times h^2$

K : Selection differential which is equal to 2.06 at 5% intensity of selection

$\sigma^2_p$  : Phenotypic standard deviation

$h^2$  : Estimated heritability and

$\bar{X}$  : General mean

The range of genetic advance as per cent mean was classified according to Johnson *et al.* (1955b).

Low : Less than 10%

Moderate : 10 – 20%

High : More than 20%

#### 3.1.4.5 Correlation coefficient analysis

To determine the degree of association among the characters, the correlation coefficients were calculated. Both genotypic and phenotypic coefficients of correlation between two characters were determined by using the variance and covariance components as suggested by Al-Jibouri *et al.* (1958).

#### 3.1.4.6 Genetic divergence analysis

The genetic diversity in 156 cultivars for fifteen characters was estimated by using  $D^2$  analysis given by Mahalanobis (1936). Principal component analysis was carried out as per the procedure described by Banfield (1978) using WINDOWSTAT software package Version 8.1. It is defined as a method of data reduction to clarify the relationships between two or more characters and to divide the total variance of the original characters into limited number of uncorrelated new variables.

### 3.2 Characterization of the genotypes using molecular tools

#### 3.2.1 Isolation of DNA

DNA was extracted from 156 mango genotypes using fresh matured leaves. Standard protocol for the isolation and extraction of DNA by CTAB method was used.

### 3.2.2 Reagents

1. Extraction Buffer: prepare 20 mM Na EDTA and 100 mM Tris-HCl and mix, adjust the pH to 8.0, add 1.4 M NaCl and 2 per cent w/v CTAB (Cetyl trimethyl ammonium bromide); dissolve CTAB by heating to 60 °C and store at 37 °C. Autoclave and add 0.2 per cent  $\beta$ -mercaptoethanol just before use.
2. Chloroform: Isoamyl alcohol- 24:1 v/v.
3. 5 M Sodium chloride (Autoclave).
4. T.E Buffer: prepare 10 mM Tris-HCl and 1 mM EDTA and mix, adjust pH to 8.0 (Autoclave).
5. 7.5 M Ammonium acetate pH 7.7 (Autoclave).
6. Wash solution: 76 per cent v/v ethanol; chilled.
7. Alcohol 95 per cent stored at -20°C.
8. RNAase: (10 mg/ml) - Dissolve RNAase in 10 mM Tris-HCl, 15 mM NaCl; pH 7.5. Boil for 5 minutes and cool to room temperature.
9. PVPP- Polyvinyl polypyrrolidone.
10. TAE Buffer: Stock solution 50x- Dissolve in 1000 ml of water,  
242 g- Tris base  
57.1 ml- Glacial acetic acid  
100 ml- 0.5 M EDTA (pH 8.0)  
Sterilize by autoclaving  
Working solution 1x
11. Bromophenol blue: Working solution- 200 ml stock solution + 200 ml of 50 per cent glycerol.
12. Ethidium bromide- 10 mg/ml.

### 3.2.3 Protocol

DNA was extracted using CTAB method developed by Lodhi *et al.* (1994). The protocol followed is given below;

- 10 ml of the extraction buffer with 50  $\mu$ l of 0.5 per cent  $\beta$ -mercaptoethanol was preheated to 60 °C by keeping in water bath.

- Two grams of leaf tissue was grinded to fine powder with liquid nitrogen. 50 mg PVPP was added and mixed. The content was transferred into a centrifuge tube containing 10 ml CTAB buffer preheated to 60 °C and shaken gently.
- The tubes were incubated for one hr at 60°C, with intermittent shaking every 10 minutes and later cooled to room temperature.
- 10 ml of CHCl<sub>3</sub>: Isoamyl alcohol (24:1) was added and mixed gently by inverting tubes to form an emulsion.
- The tubes were centrifuged at 10000 rpm for 15 minutes. If cloudy, 6 ml of CHCl<sub>3</sub>: Isoamyl alcohol can be added and this step can be repeated.
- The clear aqueous phase was transferred to fresh centrifuge tubes. 2.5 ml of 5 M NaCl was added and mixed.
- 10 ml cold ethanol was added; gently mixed and refrigerated overnight at -20 °C.
- The tubes were centrifuged at 10000 rpm for 10 minutes at room temperature. The supernatant was poured off; pellets were washed with 200 µl of 70 per cent ice cold ethanol and centrifuged as above for two minutes.
- The washing was repeated twice or more.
- The supernatant was drained out; ethanol was removed without completely drying DNA by leaving the tubes uncovered at 37 °C for 20-30 minutes or vacuum dry at room temperature.
- The pellets were re-suspended in 50 µl TE buffer.
- 1 µl RNase (10 mg/ml) was added to the dissolved DNA. The tubes were incubated at 37 °C for 30 minutes and store at -20 °C.

### **3.2.4 Electrophoresis**

#### **3.2.4.1 Casting of the agarose gel**

- Agarose solution of 0.8 per cent was prepared in 1x TAE buffer for 30 ml [1.5% agarose solution for SSR]. It was heated to dissolve completely, cooled to 40 °C, and then ethidium bromide solution (0.5 g/ml) was added. It was then poured into the cast and the comb inserted.
- When the gel is set, the comb was removed and the tray was kept in the gel electrophoresis unit.

#### **3.2.4.2 Running the gel**

- The gel electrophoresis tray was filled with 0.5x TAE buffer; the gel was placed and the DNA solution was loaded.
- 75 Volts current was applied for 90 to 120 min.
- The slab was removed and if ethidium bromide is not added prior to casting pre-incubate in ethidium bromide solution (0.5 g/ml of distilled water) for 30-40 minutes and DNA was observed under UV light. A zigzag pattern of a single band indicates intact plant DNA.

#### 3.2.4.3 DNA Quantification

- Nano drop spectrophotometer was used for the quantification of DNA. The blank was made with TE buffer, 1µl of DNA sample was taken for the quantification.
- The absorbency of the solution was measured at 260 and 280 nm.
- The ratios A 260/A 280 were calculated.
- A good DNA preparation exhibited the following spectral properties.

$$A_{260}/A_{280} = 1.6-1.9 \text{ OD units}$$

- DNA concentration was calculated using the relationship for double stranded DNA:1 OD at 260 = 50 mg/ml.
- Total quantity of DNA (µg/ml)=  $\frac{\text{OD at 260nm} \times 50 \times \text{dilution factor}}{1000}$

$$\text{Dilution factor} = \frac{\text{Total volume of extract}}{\text{Volume of the aliquot}}$$

#### 3.2.4.4 Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR), developed in 1983 by Kary Mullis, is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular sequence. The PCR reaction were performed in a 25 µl reaction volume containing 10x incomplete buffer, 25 mM MgCl<sub>2</sub>, 1 mM dNTP's, 0.3 µl primers, 0.5 µl of Taq DNA polymerase (Genei, Bangalore) and 20 ng template DNA in Biometra thermal cycler. The SSR primers were obtained from Bioserve, Hyderabad (Table 3.2.1).

**Table 3.2.1 : SSR primer sequences and their expected size**

Sl. No.	Primer No.	Primers (5'-3') details	Allele Size (bp)
1.	MiIHR 23	F: TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC	107-156
2.	MiIHR 17	F: GCTTGCTTCCAAGTGGAGACC R: GCAAAATGCTCGGAGAAGAC	230-269
3.	MiIHR 18	F: TCTGACGTCACCTCCTTTCA R: ATACTCGTGCCTCGTCCTGT	148-193
4.	MiIHR 30	F: AGCTATCGCCACAGCAAATC R: GTCTTCTTCTGGCTGCCAAC	190-213
5.	MiIHR 31	F: TTCTGTTAGTGGCGGTGTTG R: CACCTCCTCCTCCTCCTCTT	207-260
6.	MiIHR 26	F: GCGAAAGAGGAGAGTGCAAG R: TCTATAAGTGCCCCCTCACG	127-171
7.	MiIHR 34	F: CTGAGTTTGGCAAGGGAGAG R: TTGATCCTTACCACCATCA	203-245
8.	MiIHR 36	F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAAGTAG	210-250
9.	MiKVR 965	F: GAAACCCCTAAGAGGGAAAA R: ACGCAACTTTGGTTTTGGAA	172-294
10.	MiMRD 273	F: ACTGGCTAGAAAGCAACACAA R: GGCAAGATTCAAAGCGAGAG	241-301
11.	MiKVR 642	F: TGCATGTGCCTATCCATCTC R: GCACGTGCAAAATTGTTATTG	158-221
12.	MiMRD 369	F: CCAGGTTATAGCAGCCAAGC R: TAAGGTTGCCAAACTGGACC	200-273

Optimisation of reaction condition should precede the actual PCR analysis so as to get repeatable results. Following optimization is essential:

1. Template DNA concentration
2. Taq DNA polymerase concentration
3. Primer concentration
4. Primer annealing temperature
5. Primer suitable for detection of polymorphic loci in the taxa to be analyzed

#### 3.2.4.5 Instruments utilized

The following instruments were utilized for the study:

DNA Thermo cycler, microfuge tubes, auto pipettes of range 2-20 µl, 20-200 µl and 200-1000 µl, electrophoresis unit with power supply, deep freezer of -20 °C, refrigerator, and laminar airflow.

### 3.2.4.6 Reagents

1. Reaction buffer (10x)
  - 750 mM Tris-HCL pH 8.8 at 25°C
  - 200 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>
  - 0.1 per cent Tween-20
  - 15 mM MgCl<sub>2</sub>
2. Primers – Stock 3 μm
3. Taq DNA polymerase –Stock 3 U/μl
4. Template DNA – Stock 20 ng/μl
5. dNTPs – Stock 1 mM
6. Bromophenol blue- Stock solution: 0.25 per cent in 50 per cent glycerol  
Working solution: 200 μl stock + 200 μl of 50 per cent glycerol.

### 3.2.5 Protocol

1. The Thermo cycler was switched on at least 15 minutes earlier.
2. The master mix was prepared for 48 samples of DNA and 20 μl of the reaction mixture each by adding the following components.
  - Reaction buffer (10 x) - 2.0 μl
  - Forward primer - 1.0 μl
  - Reverse primer - 1.0 μl
  - dNTPs (1 mM)- 1.0 μl
  - Taq DNA polymerase (3U/μl)- 0.3 μl
  - Template DNA (100 ng/μl)- 3.0 μl
  - Water - 11.7 μl
  - Total reaction mixture – 20 μl.
3. 20 μl of the master mix was transferred to sterile tubes.
4. 5 μl of different DNA samples was added to different tubes and centrifuged at top speed for 15 seconds.
5. The tubes were placed firmly in the wells of the Thermo cycler and the following temperature programme was set. 94 °C for 5 minutes, 94 °C for 2 minutes, 58 °C for 30 seconds, 72 °C for 1 minute, 72 °C for 4 minutes, hold at 4 °C (Table 3.2.2).

6. At the end of the run the tubes were taken out, 2.5 µl of diluted bromophenol blue was added and spin for 2-5 seconds at top speed in microfuge. The tubes were stored at 4 °C till electrophoresis.

**Table 3.2.2 PCR amplification programme for SSR**

Step 1	Initial denaturation	94° C for 5 minute
Step 2	Denaturation	94°C for 2 minute
Step 3	Annealing	58° C for 30 seconds
Step 4	Extension	72°C for 1 minutes
Step 5	Repeat step 2 to step 4 for 35 cycles	
Step 6	Final extension	72° C for 4 minutes

### **3.2.6 Electrophoresis and visualization of amplified products:**

10 µl of the amplified product was run along with 1 kb ladder on 1.5 per cent agarose gel and the bands were visualized under UV illuminator. The protocol followed here is the same as that used for visualizing DNA by agarose gel electrophoresis.

### **3.2.7 Analysis of molecular characters**

Amplified products were initially separated on 2 percent agarose gel for confirmation of the amplification. These samples were separated on the automatic capillary automated DNA sequencer.

The raw data generated was analyzed and compiled using Peak Scanner V1.0 software for detecting the alleles. This produces a size curve based on the known size standard fragments, with the help of which the unknown fragment sizes are determined. The results obtained were used for genetic analysis using Cervus 3.0 software (Kalinowski *et al.*, 2007) for determining the number of alleles, observed heterozygosity (Ho), expected heterozygosity (He) and Polymorphic Information Content (PIC).

### **3.3 Attempting marker assisted selections in the progeny population**

DNA was extracted from forty-two (21 Amrapali × Vanraj; 21 Amrapali × *M. odorata*) mango hybrids and their parents using fresh matured leaves. Standard protocol for the isolation and extraction of DNA by CTAB method, as described earlier, was used.

The raw data generated was analyzed and compiled using Peak Scanner V1.0 software for detecting the alleles. Using Cervus 3.0 software, the number of alleles, heterozygosity values and Polymorphic Information Content (PIC) were determined. With the use of the same software, parentage analysis of the hybrids was also worked out to determine the likelihood ratios of the parents used in hybridization programme.

### 3.4 Estimation of volatiles in certain progenies and their parents

Fresh mango leaf samples of various mango hybrids and their parents were obtained from the Field Gene Bank of IIHR, Bangalore for *HS-SPME* analysis. The volatile flavour constituents were analyzed by headspace-solid phase micro-extraction (HS-SPME) technique using capillary GC and GC–MS/MS. The mango hybrids along with their parents are given below in Table 3.4.1.

**Table 3.4.1 Mango hybrids and their parents analyzed for leaf volatiles**

Sl. No.	Hybrid	Parents
1.	Alfazli	Alphonso × Fazli
2.	Ambika	Amrapali × Janardhan Pasand
3.	Amrapali	Dashehari × Neelum
4.	Arka Anmol	Alphonso × Janardhan Pasand
5.	Arka Aruna	Banganapalli × Alphonso
6.	Arka Neelkiran	Alphonso × Neelum
7.	Arka Puneet	Alphonso × Banganapalli
8.	Arka Udaya	Amrapali × Arka Anmol
9.	Arunika	Amrapali × Vanraj
10.	Au Rumani	Rumani × Mulgoa
11.	H-151	Kalapadi × Neelum
12.	H-85	Kalapadi × Alampur Baneshan
13.	H-87	Kalapadi × Alampur Baneshan
14.	Konkan Ruchi	Neelum × Alphonso
15.	Mallika	Neelum × Dashehari
16.	Manjeera	Rumani × Neelum
17.	Neeleshan	Neelum × Baneshan
18.	Neeleshwari	Neelum × Dashehari
19.	Neelphonso	Neelum × Alphonso

20.	PKM-1	Suvarnarekha × Neelum
21.	PKM-2	Neelum × Mulgoa
22.	Prabha Shankar	Bombay Green × Kalapadi
23.	Ratna	Neelum × Alphonso
24.	Sindhu	Ratna × Alphonso
25.	Swarna Jehangir	Suvarnarekha × Jehangir

### 3.4.1 SPME extraction of volatiles

HS–SPME is now a well-established and very popular technique for head space (HS) sampling in several fields, including the study of the composition of the HS volatiles of medicinal and aromatic plants, flowers and fruits where it has assumed an ever-increasing importance. A manual SPME holder and three commercial SPME fibers were purchased from Supelco Inc., Bellefonte, PA, USA. The SPME fibers were conditioned in the GC injector as recommended by the manufacturer at some degrees below each fiber’s maximum tolerance temperature before they were used for volatile extraction. After optimizing the headspace sampling for the extraction of leaf volatiles for this study, the most suitable SPME fiber types DVB/CAR/PDMS, 50/30 μm, highly crossed linked, was used for the extraction of volatile compounds from mango leaf.

The selected fiber was first conditioned by inserting it into the GC injector port at 250 °C for 3 hours, then this conditioned fiber was inserted into the flask for extraction of flavour. Extraction process for head space volatiles of mango leaf was followed as described by Jirovetz *et al.*, (2002), Johnson *et al.*, (2004) and Rohloff (1999). In two separate 250 ml conical flasks, having screw caps with silicon rubber septum, 10 g of mango leaf were transferred. Immediately after closing the cap, sample was allowed to be at room temperature (25 ±1°C) for 10-15 min. to increase the transfer of the analytes and get equilibrated to the headspace. After the equilibration time was over, sampling was done by inserting the pre-conditioned SPME fiber into the head space of the vial for 5 hrs. at room temperature (25 ±1 °C).

### 3.4.2 Capillary Gas Chromatography and Mass Spectrometry (GC/MS)

#### 3.4.2.1 GC analysis :

Subsequently, the SPME device was introduced in the injector port for gas chromatographic analysis and was kept in the inlet for 10 min. to be desorbed. The GC-FID analysis was carried out using a Varian-3800 Gas Chromatograph, equipped with a FID detector. Nitrogen (1 ml/min) was used as the carrier gas. The components were separated on VF-5 (factor

Four) capillary column from Varian, USA, 30 m x 0.25 mm id, 0.25  $\mu\text{m}$  film thickness. The injector temperature was set at 260  $^{\circ}\text{C}$  and all injections were made in split mode (1:5). The detector temperature was kept at 270  $^{\circ}\text{C}$ , and the temperature programmes for column was as follows: 50  $^{\circ}\text{C}$  for 5 min. at an increment 4  $^{\circ}\text{C}/\text{min}$  to 170  $^{\circ}\text{C}$ , hold for 2 min., then 5  $^{\circ}\text{C}/\text{min}$  to 250  $^{\circ}\text{C}$  and maintaining the constant temperature for 7 min., total run time was 60 min.

#### **3.4.2.2 GC/MS analysis**

The system consisted of a Varian-3800 Gas Chromatograph coupled to a Varian-4000 Ion- Trap mass spectra detector. The ion trap, transfer line and ion source temperatures were maintained at 200  $^{\circ}\text{C}$ , 240  $^{\circ}\text{C}$  and 210  $^{\circ}\text{C}$  respectively. A fused-silica capillary column VF-5MS (Factor four) from Varian, USA, with 30 m x 0.25 mm id 0.25 mm film thickness was used for the analysis. Helium was used as carrier gas with the flow rate of 1 ml/min. The mass spectrometer was operated in the external electron ionization mode of 70 eV, with full mass scan-range 45–450 amu. Temperature programmes for column was the same as described for GC-FID analysis. The total volatile production was estimated by the sum of all GC-FID peak areas in the chromatogram and individual compounds was quantified as relative percent area. The individual volatile compounds were identified by comparing the retention index which was determined by using homologous series of n-alkanes ( $\text{C}_5$  to  $\text{C}_{32}$  procured from Sigma-Aldrich) as standard (Kovats, 1965) and comparing the spectra with the available two spectral libraries using Wiley and NIST-2007.

# *RESULTS*



The improvement of mango has been a challenge for many years. The success in mango improvement primarily depends on the nature and magnitude of variation present in the germplasm. Information on the genetic distance between the varieties is lacking for most of the germplasm collected from different diversity centres. The diversity studies would help in generating this information, which further would be useful in developing new recombinants with desirable traits by exploiting heterosis. Selection of parents for utilization in the hybridization programme would also become much easier.

The development of new varieties is mainly governed by the magnitude of genetic variability in base material and the extent of variability for desired characters. Germplasm with diverse genetic base is the major source for breeding programmes. Further, preservation of germplasm is a worldwide concern and conservation of specific gene pools will be useful to breeders.

Keeping this in view, the present investigation on “**Genetic diversity and characterization studies in mango**” was conducted during 2015-18 at the Division of Fruit Crops, Division of Biotechnology and Division of Physiology and Biochemistry, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru. The data collected were statistically analyzed and the results presented in this chapter.

In this study, the recorded data of 156 mango genotypes were analyzed for Mahalanobis  $D^2$  statistics to study the divergence, principal component analysis to identify the components contributing more towards diversity and also cluster analysis was done for morphological, biochemical as well as molecular data to know the relationship among the genotypes collected. The parentage analysis of Amrapali × Vanraj and Amrapali × *Mangifera odorata* hybrids was also worked out. The major volatiles were profiled from the leaves of mango hybrids and their parents. The results of all the parameters are presented under following headings:

#### **4.1 To study the diversity existing in *M. indica* varieties from different regions**

##### **4.1.1 Fruit morphological characters**

Considerable variation was observed with regard to the fruit morphological characteristics (Plate 4.1.1).

The data recorded on nine traits were subjected to statistical analysis.

#### **4.1.1.1 Fruit weight (g)**

Considerable variation was observed with regard to the fruit weight of mango cultivars and the data pertaining to it are presented in Table 4.1.1. The mean fruit weight recorded was 304.23 g, while the fruit weight ranged from 50.12 g to 1182.95 g. The maximum fruit weight was recorded in Safed Mulgoa (1182.95 g), followed by Sora (1102.5 g) and Khudadath (940.60 g), while, minimum fruit weight was recorded in Chandrakaran (50.12 g) which was at par with Elaichi (51.23 g) and Jeerige Neermavu (78.04 g), followed by Vhout (81.99 g) and Coorg Collection (84.75 g).

#### **4.1.1.2 Fruit length (cm)**

The fruit length revealed significant differences among the cultivars. The fruit length ranged from 4.70 cm to 17.50 cm with a mean fruit length of 9.81 cm (Table 4.1.1). Maximum fruit length was recorded in Sora (17.50 cm) which was at par with Safed Mulgoa (16.40 cm), followed by Totapuri (15.95 cm) and Thorappadi (14.75 cm), while, minimum fruit length was recorded in Elaichi (4.70 cm) which was at par with Haleema (5.30 cm), Chandrakaran (5.50 cm), Vhout (5.70 cm), Khas-ul-Khas (5.95 cm), Litchi (6.00 cm) and Coorg Collection (6.10 cm), followed by Jeerige Neermavu (6.35 cm).

#### **4.1.1.3 Fruit width (cm)**

The fruit width differed significantly with a mean of 7.61 cm, while it ranged from 4.15 cm to 12.90 cm among the cultivars studied (Table 4.1.1). Maximum fruit width was recorded in Sora (12.90 cm) which was on par with Safed Mulgoa (12.15 cm), followed by Thambva (11.35 cm) and Himayath Pasand (11.10 cm), while, minimum fruit width was recorded in Chandrakaran (4.15 cm), which was at par with Vellaikulamban (4.80 cm), Adderi Jeerige (4.90 cm), Jeerige Neermavu (4.95 cm) and Vhout (4.95 cm), followed by Hathizul (5.00 cm) and Elaichi (5.05 cm).

#### **4.1.1.4 Fruit thickness (cm)**

The fruit thickness of mango cultivars ranged from 2.05 cm to 10.50 cm with a mean value of 6.50 cm (Table 4.1.1). Minimum fruit thickness was noticed in Kalami Hindustani (2.05

**Table 4.1.1 Mean performance of 156 mango cultivars for fruit morphological characters**

Sl. No.	Germplasm	Fruit Weight (g)	Fruit Thickness (cm)	Fruit Length (cm)	Fruit Width (cm)	Skin Weight (g)	Skin Thickness (mm)	Stone Weight (g)	Pulp Weight (g)	Pulp Percent (%)
1	Adderi Jeerige	118.74	4.05	10.15	4.90	27.65	1.35	36.64	54.46	44.13
2	Agarbathi	396.96	7.70	9.85	8.10	112.38	2.90	48.19	236.39	59.54
3	Allampur Baneshan	366.99	8.25	13.25	7.95	97.79	1.50	50.90	218.30	59.51
4	Almas	257.77	6.25	8.50	7.25	61.56	2.15	35.85	160.36	62.22
5	Amini	467.06	8.20	11.35	9.25	119.22	2.90	59.22	288.63	61.82
6	Ananas	217.56	6.15	9.70	6.25	45.60	2.60	24.80	147.17	67.66
7	Apple Rumani	406.78	8.55	6.95	8.10	81.37	2.00	45.08	280.34	68.97
8	Aryavarthana Rasala	384.31	6.80	11.85	8.25	78.96	2.75	57.47	247.89	64.51
9	Asiquot	229.20	6.00	8.15	7.50	54.10	1.95	44.31	130.79	56.40
10	Atimadhuram	246.09	7.00	8.35	7.10	80.73	3.50	42.42	122.95	50.00
11	Balakondapari	589.44	8.50	14.10	8.85	142.79	2.90	74.66	371.99	63.12
12	Bangalore Sindhura	383.09	6.45	14.05	7.30	101.09	2.20	36.21	245.80	64.24
13	Banganapalli	329.37	6.95	12.30	8.65	93.78	3.25	56.55	179.05	54.36
14	Black Andrews	464.41	8.20	10.75	8.55	137.98	2.10	44.36	282.08	60.72
15	Blackseri	373.46	7.20	12.75	7.90	126.51	2.90	51.90	195.06	52.27
16	Bombay Peda	273.85	6.55	9.05	7.80	71.47	2.45	38.59	163.79	59.85
17	Chandanum	201.71	6.05	9.05	6.60	55.81	2.00	24.29	121.62	60.07
18	Chandrakaran	50.12	3.45	5.50	4.15	13.56	1.15	18.86	17.71	35.34
19	Chethalli	486.68	7.00	12.15	8.85	71.57	1.45	70.61	344.50	70.79
20	Coorg Collection	84.75	4.85	6.10	5.55	22.08	1.55	27.00	35.67	42.60
21	Creeping	107.45	5.10	6.45	6.00	21.69	1.80	17.69	68.07	63.23
22	Deorakhio	122.99	4.80	11.90	6.10	26.63	1.10	26.48	69.88	56.87
23	Dofasla	168.07	4.75	10.15	6.30	38.78	1.55	28.75	100.54	59.70
24	Dori	330.91	7.15	9.20	8.60	71.89	2.15	48.01	211.01	63.69
25	Dwarf Rumani	231.11	6.90	8.50	8.70	39.32	1.80	31.42	160.37	69.41
26	Ebatti Mavu	453.81	9.05	12.00	10.05	130.54	1.80	50.95	272.33	60.03
27	Elaichi	51.23	4.00	4.70	5.05	12.07	1.15	9.34	29.82	58.18
28	Gaddemar	572.55	8.25	13.15	9.75	95.40	2.00	57.58	419.58	73.29
29	Gidagana Mavu	365.43	7.00	10.10	8.60	119.01	4.00	33.29	213.14	58.37
30	Goa Kodur	437.33	8.55	11.00	10.50	72.22	2.95	50.12	315.00	72.09
31	Gulab Khas	207.12	6.25	9.20	6.30	44.59	3.00	41.72	120.82	58.40
32	Gulabi	426.57	8.05	11.55	8.15	99.57	2.75	49.59	277.42	65.04

33	H - 85	492.64	7.80	11.45	9.15	121.98	3.20	63.76	306.91	62.33
34	H -151	141.45	5.40	7.65	5.50	39.00	2.30	28.67	73.78	52.22
35	H-39	230.04	6.40	9.40	8.25	54.34	1.60	34.04	141.66	61.79
36	Hajeera	109.66	5.00	6.55	5.70	27.04	1.80	20.25	62.37	57.20
37	Haleema	92.87	5.15	5.30	6.40	18.34	1.75	18.94	55.59	60.76
38	Hamlet	530.71	8.20	12.65	9.10	57.40	1.85	27.66	445.66	83.99
39	Hamsa Mamidi	158.55	5.50	8.10	6.00	51.51	1.95	37.75	69.30	43.71
40	Hansraj	434.63	7.90	11.45	8.50	116.68	2.75	44.41	273.54	62.96
41	Harsha	520.59	8.65	10.05	9.10	104.05	2.95	43.58	372.96	71.70
42	Hathizul	89.44	4.50	6.85	5.00	21.04	1.85	19.92	48.49	54.64
43	Himam Pasand	347.15	5.90	12.65	7.05	99.10	1.30	45.86	202.19	58.15
44	Himayath Pasand	570.74	7.70	12.65	11.10	148.99	3.05	62.46	359.29	62.97
45	Hithalahalli Appe	140.45	4.65	9.20	5.40	25.57	2.00	33.07	81.82	58.08
46	Huli Appekai	182.84	5.85	8.00	7.00	28.71	1.15	37.65	116.49	63.70
47	Hydersaheb	228.09	6.05	9.90	7.60	49.73	1.70	40.28	138.08	60.73
48	Irugupalli Tokkukaya	218.71	6.35	8.40	7.25	50.75	2.15	30.64	137.33	62.85
49	Jawahar	183.64	5.22	10.10	7.00	35.68	2.10	35.60	112.37	61.12
50	Jeerige Neermavu	78.04	4.35	6.35	4.95	15.08	1.30	30.81	32.16	41.05
51	Jehangir	579.14	8.95	12.05	8.80	136.83	2.85	65.21	377.10	65.13
52	K-O-7	289.08	5.80	8.85	7.00	61.05	2.55	50.49	177.55	61.41
53	Kaju	188.24	5.55	8.85	6.50	43.69	1.45	52.31	92.24	49.04
54	Kalami Hindustani	440.66	2.05	9.90	9.45	69.30	2.05	42.66	328.70	74.67
55	Kalapadi	158.00	5.70	8.25	6.95	39.57	2.75	17.53	100.90	63.77
56	Kalapara	209.47	6.10	8.85	6.35	47.84	2.15	32.93	128.71	61.65
57	Kalgundi Koppa Appe	240.75	5.80	8.25	6.50	49.03	2.00	46.12	145.61	60.48
58	Kalkand	258.45	6.35	9.80	7.70	48.69	1.65	34.20	175.56	67.90
59	Kalneelum	342.09	7.30	9.65	8.15	104.03	2.25	49.77	188.29	54.75
60	Kalwa Gudda	239.78	6.40	9.15	6.65	52.89	1.75	29.83	157.07	65.53
61	Karanjio	175.26	5.95	7.50	6.90	46.50	1.45	24.33	104.43	59.46
62	Kari Ishad	297.63	7.40	9.10	9.65	86.06	4.05	39.04	172.54	58.02
63	Karigal Appe	248.39	5.95	7.85	6.70	54.11	1.70	40.40	153.88	61.77
64	Karkan Rumani	298.29	7.85	7.50	8.70	64.65	1.15	34.24	199.41	66.91
65	Kasturi Mamidi	302.92	6.75	10.85	9.10	50.09	1.85	61.18	191.65	63.14
66	Katta Gola	702.46	9.50	11.80	10.45	180.22	2.85	57.18	465.07	66.22
67	Kempikunde	111.78	4.95	7.20	5.95	22.20	1.50	28.45	61.13	54.66
68	Kerala Kalepad	188.16	5.90	7.45	6.40	46.03	2.05	41.16	100.97	53.67
69	Kerali Goa	428.85	7.20	11.10	8.30	91.40	2.05	48.77	288.68	67.31

70	Khas-ul-Khas	90.79	4.75	5.95	5.80	30.11	2.25	31.67	29.02	31.10
71	Khudadath	940.60	9.15	12.65	10.55	230.41	3.40	70.46	639.74	68.03
72	Khuddus	212.42	5.85	8.90	7.20	47.24	1.75	34.61	130.57	60.94
73	Kintalavenipeta	108.13	4.50	7.30	5.30	43.10	2.45	24.83	40.21	37.02
74	KMH-1	315.88	6.00	11.10	7.50	51.32	1.90	49.16	215.40	68.19
75	Kottur Konam	202.22	6.10	8.75	7.75	53.83	2.35	34.55	113.85	56.31
76	Krishna	266.50	7.05	7.85	7.45	69.71	2.70	32.96	163.84	61.44
77	Ku-8	596.18	8.25	12.90	9.15	188.71	2.80	72.77	334.71	56.16
78	Kutumba Appe	573.83	8.50	12.95	9.65	148.57	3.40	66.37	358.89	62.55
79	Lahara	613.60	7.80	13.25	8.60	103.29	2.00	66.05	444.26	72.40
80	Lal Muni	159.91	5.65	8.35	5.90	50.56	3.00	38.99	70.37	44.20
81	Lal Sundari	230.49	6.35	8.95	7.30	51.17	2.35	34.77	144.55	62.54
82	Lalpairi	257.38	6.33	10.70	8.75	78.13	3.15	34.44	144.82	56.33
83	Lemon	296.45	6.75	10.90	7.25	70.77	2.75	45.15	180.54	60.80
84	Litchi	116.78	5.30	6.00	5.90	34.39	2.35	31.89	50.51	43.26
85	Lord	457.28	6.05	13.35	7.55	71.06	3.05	37.25	348.97	76.33
86	Magemavu	434.37	8.53	10.75	9.35	103.47	4.05	38.54	292.37	67.34
87	Maharaja of Mysore	622.67	9.45	8.75	10.20	65.27	3.00	57.10	500.31	80.37
88	Makaram	661.48	8.65	12.20	10.90	162.36	3.10	53.15	445.97	67.40
89	Malai Misri	334.30	7.20	10.85	7.85	63.13	2.05	63.61	207.57	62.10
90	Manibhatta Appe	224.23	6.15	7.60	7.40	58.92	2.50	34.48	130.83	58.51
91	Mohammada Vikarabad	220.58	6.15	10.35	6.75	46.55	2.05	37.68	136.35	61.80
92	Mugalimelogra	331.53	7.25	9.65	7.50	81.33	2.10	45.90	204.30	61.59
93	Muvandan	198.70	5.60	8.75	7.50	47.49	2.25	37.34	113.87	57.33
94	Naagarappe	146.56	5.30	8.90	6.90	24.06	1.50	39.64	82.86	56.54
95	Nalla Mamidi	282.36	6.50	9.85	7.15	85.86	1.95	68.50	128.00	45.32
96	Narayana Sheni	126.28	5.90	7.00	6.15	25.99	1.70	27.21	73.08	57.92
97	Narela	180.08	6.20	8.30	7.00	40.47	2.20	29.14	110.48	61.07
98	Navneet	336.13	7.10	9.75	8.60	96.34	2.95	39.31	200.49	59.64
99	Neeleshan	250.20	6.00	10.35	7.80	82.75	2.05	44.77	122.69	49.04
100	Nekkare	124.43	4.90	7.65	6.20	39.16	3.15	31.21	54.07	43.30
101	Nuha	108.76	5.15	7.65	7.00	21.40	1.20	21.83	65.54	60.55
102	Olour	159.75	5.28	8.85	6.20	49.55	2.75	30.08	80.12	49.96
103	Pacharasi	177.79	6.15	9.40	7.10	56.81	2.95	30.11	90.88	51.03
104	Padari	259.49	6.50	10.35	7.70	65.52	1.90	46.52	147.45	56.92
105	Panakalu	191.88	6.05	8.65	6.90	51.28	2.25	36.74	103.86	54.10

106	Panchadara Kalasa	244.72	6.40	9.75	7.65	51.84	1.50	46.89	145.99	59.53
107	Panchavarnam	268.53	7.10	7.30	7.80	60.23	1.25	36.25	172.06	64.12
108	Papaya Khas	274.61	6.15	9.90	7.30	52.44	1.50	45.62	176.55	64.34
109	Papayaraju Goa	308.38	6.60	9.95	8.20	71.00	2.45	39.11	198.27	64.22
110	Peddarasam	516.99	7.20	12.65	8.40	112.27	2.25	68.32	336.41	65.08
111	Peter	207.79	6.40	8.10	7.20	52.62	2.85	35.73	119.45	57.59
112	PKM - 1	239.65	5.95	12.20	6.60	53.71	1.90	36.74	149.21	61.79
113	PKM - 2	307.49	6.23	11.90	10.00	59.35	2.35	48.44	199.71	65.03
114	Pulihora	175.68	5.70	8.30	6.95	38.37	1.70	29.11	108.20	61.59
115	Puttu	265.41	7.00	10.25	7.90	67.77	2.95	36.82	160.83	60.67
116	Putu	152.55	6.00	7.20	6.60	47.71	1.30	14.51	90.34	59.33
117	Ramphalya	265.28	6.90	8.55	7.25	87.75	3.00	40.71	136.82	51.48
118	Royal Special	179.32	6.25	7.35	7.50	49.29	2.70	22.30	107.74	59.53
119	Rumani x Neelum	339.59	7.95	8.05	8.45	85.45	2.85	38.09	216.05	63.67
120	Ruswani	415.79	7.05	12.10	8.15	101.38	2.40	59.62	254.79	61.24
121	Safed Mulgoa	1182.95	10.50	16.40	12.15	305.73	5.35	129.50	747.73	63.22
122	Safeda Lucknow	150.46	5.15	7.85	5.45	45.89	2.10	31.50	73.08	48.46
123	Sai Sugandh	361.21	6.35	12.15	7.80	83.03	2.75	46.43	231.75	64.17
124	Salem Bangalora	422.30	7.25	9.45	8.10	124.43	2.75	53.18	244.70	57.93
125	Sampar Totapuri	379.13	7.00	11.20	6.95	125.91	3.00	52.81	200.42	52.89
126	Santhoor Collection	245.48	6.00	9.30	7.40	66.85	2.95	40.57	138.07	56.10
127	Sardar	348.57	7.30	10.40	8.60	120.46	2.70	40.30	187.82	53.90
128	Saru	548.79	6.80	14.45	8.05	189.86	2.85	68.11	290.83	53.00
129	Shahjahan	507.71	8.30	11.00	9.75	136.75	2.70	49.90	321.07	63.24
130	Sharbathi Bagri	190.10	5.85	8.45	6.25	37.18	1.75	40.13	112.80	59.18
131	Shendriya	150.35	5.65	7.75	6.75	45.18	2.05	30.82	74.35	49.51
132	Shendryo	509.39	9.20	12.35	9.85	169.49	3.60	45.95	293.96	57.69
133	Siroli	92.44	4.85	6.40	5.35	27.20	1.95	15.74	49.51	53.63
134	Sora	1102.50	8.20	17.50	12.90	239.93	3.55	70.70	791.88	71.83
135	Surankudi	214.51	6.15	8.25	7.10	35.84	1.20	31.59	147.08	68.19
136	Sushanbhog	328.32	6.65	11.25	7.90	86.31	2.40	49.94	192.07	58.52
137	Suvarnarekha	223.15	6.00	10.65	6.90	61.16	1.95	30.99	131.00	58.56
138	Taimur pasand	232.50	6.25	10.75	7.90	54.63	2.50	44.19	133.68	57.75
139	Tatamdi	427.94	7.30	12.90	8.05	109.78	3.80	55.37	262.79	61.33
140	Tenkasi Banganapalli	517.83	7.70	14.35	10.60	91.01	3.00	52.68	374.15	72.29
141	Tenkasi Neelum	113.98	5.10	7.60	6.20	31.64	2.15	26.73	55.62	49.58
142	Tephala	237.94	6.15	11.45	6.55	53.41	1.65	43.36	141.17	59.42

143	Thali	114.97	5.20	6.70	5.55	27.43	1.90	29.37	58.17	50.57
144	Thambva	800.25	8.15	12.65	11.35	170.58	3.25	102.64	527.04	65.87
145	Thogarapalli	312.21	6.80	10.55	7.60	74.21	1.90	41.29	196.71	63.02
146	Thorappadi	332.58	6.30	14.75	8.05	76.21	3.10	51.23	205.15	61.57
147	Totapuri	271.77	6.00	15.95	7.70	79.08	3.00	49.05	143.64	52.95
148	Vattam	147.10	5.25	7.70	6.65	46.77	2.55	38.92	61.42	41.75
149	Vellaikulamban	127.90	4.15	8.60	4.80	27.86	1.45	31.33	68.71	53.72
150	Vhout	81.99	4.60	5.70	4.95	16.43	1.85	23.24	42.32	51.61
151	Vinayaka Hegde	146.56	5.25	7.45	5.80	35.36	2.00	28.87	82.34	56.26
152	Virudhanagar	196.05	5.75	9.00	8.10	41.84	1.95	49.50	104.71	53.35
153	Warale gidaga	366.80	7.70	10.00	8.90	120.83	2.45	34.35	211.63	57.73
154	Whiteseri	453.44	7.60	12.65	8.35	138.16	3.60	62.85	252.44	55.72
155	Willard	96.94	4.85	6.80	6.00	30.32	2.40	25.16	41.47	42.71
156	Yakutti	299.38	7.95	8.15	8.55	59.37	2.35	43.92	196.09	65.56
	<b>MINIMUM</b>	50.12	2.05	4.70	4.15	12.07	1.10	9.34	17.71	31.10
	<b>MAXIMUM</b>	1182.95	10.50	17.50	12.90	305.73	5.35	129.50	791.88	83.99
	<b>MEAN</b>	304.23	6.50	9.81	7.61	73.68	2.33	42.07	188.48	59.19
	<b>SE m ±</b>	10.807	0.206	0.51	0.301	5.278	0.183	3.801	9.745	3.2
	<b>CD (p=0.05)</b>	30.222	0.576	1.425	0.841	14.761	0.512	10.63	27.252	8.947
	<b>CV (%)</b>	5.024	4.477	7.35	5.589	10.132	11.114	12.779	7.312	7.645



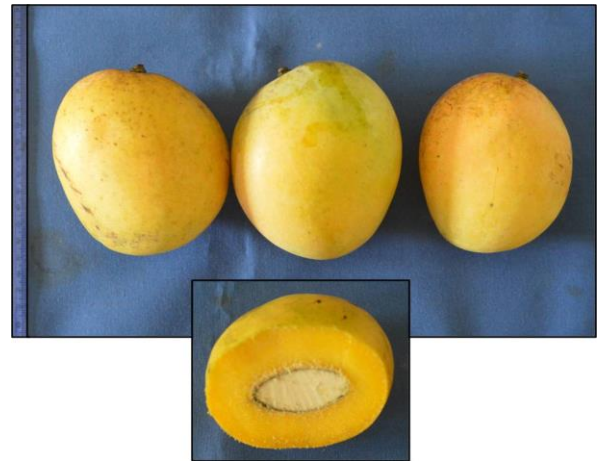
**1. Thorappadi**



**2. Jehangir**



**3. Lal Muni**



**4. Muvandan**



**5. Malai Misri**



**6. H-39**

**Plate 4.1.1 Variability in fruit morphology of mango cultivars**

cm), followed by Chandrakaran (3.45 cm) and Elaichi (4.00 cm), while, maximum was recorded in Safed Mulgoa (10.50 cm), followed by Katta Gola (9.50 cm) and Maharaja of Mysore (9.45 cm).

#### **4.1.1.5 Skin thickness (mm)**

The skin thickness varied significantly ranging from 1.10 mm to 5.35 mm among the cultivars studied with a mean of 2.33 mm (Table 4.1.1). Maximum skin thickness was recorded in Safed Mulgoa (5.35 mm), followed by Magemavu (4.05 mm) and Kari Ishad (4.05 mm), while, minimum was recorded in Deorakhio (1.10 mm), which was at par with Chandrakaran (1.15 mm), Huli Appekai (1.15 mm) and twenty-one other genotypes.

#### **4.1.1.6 Skin weight (g)**

Skin weight differed significantly among the cultivars studied with an average of 73.68 g and ranging from 12.07 g to 305.73 g (Table 4.1.1). Cultivar Elaichi recorded minimum skin weight of 12.07 g, which was at par with Chandrakaran (13.56 g), Jeerige Neermavu (15.08 g), and ten other genotypes. The cultivar Safed Mulgoa recorded maximum skin weight of 305.73 g, followed by Sora (239.93 g) and Khudadath (230.41 g).

#### **4.1.1.7 Stone weight (g)**

The mango genotypes revealed considerably higher range of variations for stone weight (Table 4.1.1). The stone weight of mango cultivars ranged from 9.34 g to 129.50 g with a mean value of 42.07 g. The least stone weight was noticed in Elaichi (9.34 g), which was at par with Putu (14.51 g), Siroli (15.74 g), Kalapadi (17.53 g), Creeping (17.69 g), Chandrakaran (18.86 g), Haleema (18.94 g) and Hathizul (19.92 g), followed by Hajeera (20.25 g) and Nuha (21.83 g). Highest stone weight was recorded in Safed Mulgoa (129.50 g), followed by Thambva (102.64 g) and Balakondapari (74.66 g).

#### **4.1.1.8 Pulp weight (g)**

The cultivar Chandrakaran recorded minimum pulp weight of 17.71 g, which was at par with Khas-ul-Khas (29.02 g), Elaichi (29.82 g), Jeerige Neermavu (32.16 g), Coorg Collection (35.67 g), Kintalavenipeta (40.21 g), Willard (41.47 g) and Vhout (42.32 g), followed by Hathizul (48.49 g) and Siroli (49.51 g). The cultivar Sora recorded maximum pulp weight of 791.88 g, followed by Safed Mulgoa (747.73 g) and Khudadath (639.74 g). The pulp weight ranged from 17.71 g to 791.88 g among the cultivars studied with a mean of 188.48 g (Table 4.1.1).

#### **4.1.1.9 Pulp content (%)**

The pulp content varied from 31.10 to 83.99% with an average of 59.19% (Table 4.1.1). The cultivar Hamlet recorded the highest pulp content (83.99%), which was at par with Maharaja of Mysore (80.37%) and Lord (76.33%), followed by Kalami Hindustani (74.67%) and Gaddemar (73.29%), while, the cultivar Khas-ul-Khas (31.10%) recorded the lowest pulp content, which was at par with Chandrakaran (35.34%) and Kintalavenipeta (37.02%), followed by Jeerige Neermavu (41.05%) and Vattam (41.75%).

#### **4.1.2 Fruit and leaf biochemical characters**

The data recorded on six biochemical characters were subjected to statistical analysis. Significant differences were observed among the cultivars for all the biochemical characters studied.

##### **4.1.2.1 TSS (°Brix)**

There were significant differences in the TSS content of mango cultivars studied (Table 4.1.2). TSS content ranged from 7.00 to 24.95 °Brix with a mean of 16.29 °Brix. TSS was observed highest in cultivar K-O-7 (24.95 °Brix), which was at par with Khas-ul-Khas (24.9 °Brix) and Chandrakaran (23.95 °Brix). Lowest TSS was observed in cultivar Huli Appekai (7 °Brix), which was at par with Adderi Jeerige (7.6 °Brix), Thorappadi (9.2 °Brix), Naagarappe (9.4 °Brix), Thambva (10.1 °Brix), Amini (10.25 °Brix), Pacharasi (10.6 °Brix) and Banganapalli (10.65 °Brix), followed by Sampar Totapuri (10.75 °Brix) and Gaddemar (10.8 °Brix).

##### **4.1.2.2 Titratable acidity (%)**

The range of titratable acidity (Table 4.1.2) among the mango cultivars analysed in the present investigation was 0.13 to 1.34% with a mean of 0.49%. The cultivar Adderi Jeerige recorded the highest (1.34%) titratable acidity, which was at par with Thambva (1.28%), Hithalahalli Appe (1.28%), Ebatti Mavu (1.28%), Naagarappe (1.28%), Jeerige Neermavu (1.28%), Huli Appekai (1.22%) and Kutumba Appe (1.22%), followed by Kintalavenipeta (1.09%) and Gaddemar (1.09%). The cultivar Neeleshan (0.13%) recorded the lowest titratable acidity, which was at par with Karanjio (0.13%), Banganapalli (0.13%) and forty-two other genotypes.

**Table 4.1.2 Mean performance of 156 mango cultivars for biochemical characters**

Sl. No.	Germplasm	TSS ( <sup>o</sup> Brix)	Titrateable acidity (%)	Total carotenoids (mg/100g)	Ascorbic acid (mg/100g)	Total phenols (mg/100g)	Total flavonoids (mg/100g)
1	Adderi Jeerige	7.60	1.34	1.10	78.50	9159.50	1087.80
2	Agarbathi	15.60	0.83	4.22	13.00	7314.98	823.20
3	Allampur Baneshan	12.05	0.42	0.97	22.00	7506.71	757.05
4	Almas	16.10	0.70	5.04	14.00	5742.29	518.18
5	Amini	10.25	0.96	1.68	97.00	5031.17	786.45
6	Ananas	15.75	0.26	6.75	11.50	1873.64	257.25
7	Apple Rumani	14.60	0.19	1.94	13.50	1919.76	367.50
8	Aryavarthana Rasala	14.25	0.70	1.82	5.50	7642.62	834.23
9	Asiquot	15.50	0.26	1.81	9.50	4863.71	742.35
10	Atimadhuram	19.50	0.32	1.25	27.50	5477.74	415.28
11	Balakondapari	14.55	0.45	1.37	23.00	4557.91	723.98
12	Bangalore Sindhura	12.90	0.64	1.56	9.00	3419.64	569.63
13	Banganapalli	10.65	0.13	1.56	13.50	5431.63	448.35
14	Black Andrews	16.75	0.26	2.29	72.00	5475.32	521.85
15	Blackseri	13.85	0.38	3.70	25.50	2854.16	466.73
16	Bombay Peda	15.10	0.45	2.30	12.00	6497.08	760.73
17	Chandanum	16.55	0.45	2.98	88.00	3407.51	510.83
18	Chandrakaran	23.95	0.83	6.59	90.00	6208.27	746.03
19	Chethalli	13.65	0.13	6.83	27.50	2594.47	463.05
20	Coorg Collection	22.50	0.77	3.58	107.00	6574.74	882.00
21	Creeping	15.90	1.09	3.47	34.50	8523.62	929.78
22	Deorakhio	15.25	0.19	3.83	79.00	4628.29	452.03
23	Dofasla	21.25	0.22	6.68	43.50	3150.25	385.88
24	Dori	18.45	0.38	4.16	36.50	6431.55	463.05
25	Dwarf Rumani	13.75	0.38	1.51	18.00	4642.85	646.80
26	Ebatti Mavu	13.55	1.28	2.86	6.00	3317.71	727.65
27	Elaichi	20.45	0.35	3.29	50.00	6300.50	496.13
28	Gaddemar	10.80	1.09	1.80	78.00	3737.58	455.70
29	Gidagana Mavu	19.45	0.42	3.22	38.00	4446.26	830.55
30	Goa Kodur	15.10	0.51	1.83	29.00	7016.46	867.30
31	Gulab Khas	17.10	0.13	4.97	61.50	1810.54	334.43
32	Gulabi	15.00	0.58	3.07	11.50	5356.39	621.08
33	H - 85	15.80	0.29	2.75	18.50	4652.56	764.40

34	H -151	17.75	0.13	2.49	103.00	5249.60	569.63
35	H-39	21.75	0.32	4.66	13.50	4946.23	782.78
36	Hajeera	12.15	0.38	4.10	11.00	4414.72	606.38
37	Haleema	13.45	0.45	3.72	43.50	4587.03	731.33
38	Hamlet	16.90	0.22	2.39	99.00	4215.70	712.95
39	Hamsa Mamidi	22.35	0.48	3.46	15.50	2295.94	422.63
40	Hansraj	19.85	0.29	1.29	52.00	6630.57	632.10
41	Harsha	18.85	0.45	6.41	16.00	5079.72	525.53
42	Hathizul	17.20	0.51	4.09	73.00	2849.30	294.00
43	Himam Pasand	16.30	0.45	0.91	27.00	6055.37	790.13
44	Himayath Pasand	18.70	0.29	1.07	21.00	7810.09	628.43
45	Hithalahalli Appe	11.40	1.28	1.87	113.00	9875.46	1006.95
46	Huli Appekai	7.00	1.22	2.52	107.00	8647.40	1036.35
47	Hydersaheb	18.45	0.38	4.29	32.50	7152.37	826.88
48	Irugupalli Tokkukaya	16.15	0.70	1.76	29.00	3909.90	396.90
49	Jawahar	22.70	0.32	5.70	14.00	6079.64	646.80
50	Jeerige Neermavu	12.65	1.28	1.41	73.50	6329.62	837.90
51	Jehangir	15.55	0.35	1.22	10.00	6552.90	624.75
52	K-O-7	24.95	0.45	0.87	20.50	5441.34	474.08
53	Kaju	22.80	0.29	2.82	93.00	4060.37	323.40
54	Kalami Hindustani	15.40	0.29	2.67	12.50	4164.73	746.03
55	Kalapadi	16.90	0.26	3.90	70.50	5688.89	569.63
56	Kalapara	17.95	0.45	5.05	8.50	3822.53	665.18
57	Kalgundi Koppa Appe	23.20	0.22	2.38	77.00	4533.64	576.98
58	Kalkand	16.25	0.19	2.40	10.50	6271.37	635.78
59	Kalneelum	17.85	0.35	2.57	33.50	6276.22	915.08
60	Kalwa Gudda	15.65	0.35	3.68	46.00	4693.82	433.65
61	Karanjio	18.35	0.13	3.59	28.50	5368.53	554.93
62	Kari Ishad	14.65	0.77	4.23	11.50	4669.55	808.50
63	Karigal Appe	17.80	0.58	2.45	39.00	4623.44	893.03
64	Karkan Rumani	15.50	0.22	1.69	22.00	5511.72	672.53
65	Kasturi Mamidi	14.00	0.90	0.98	10.00	4070.08	654.15
66	Katta Gola	13.50	0.58	0.92	8.50	5317.56	823.20
67	Kempikunde	19.10	0.51	1.16	6.50	2128.48	400.58
68	Kerala Kalepad	18.20	0.26	4.48	38.50	6805.31	808.50
69	Kerali Goa	22.25	0.22	1.23	26.50	5404.93	536.55
70	Khas-ul-Khas	24.90	0.45	5.23	112.00	6899.97	951.83

71	Khudadath	18.15	0.58	1.46	32.00	4332.20	463.05
72	Khuddus	20.05	0.22	1.33	38.00	3516.72	521.85
73	Kintalavenipeta	13.40	1.09	4.42	50.50	4356.47	654.15
74	KMH-1	18.00	0.22	2.43	34.50	4676.83	558.60
75	Kottur Konam	18.20	0.26	6.60	52.50	3016.76	279.30
76	Krishna	12.85	0.32	4.23	60.50	7404.78	867.30
77	Ku-8	17.95	0.38	3.05	31.00	2788.63	661.50
78	Kutumba Appe	10.90	1.22	1.31	49.00	2550.78	514.50
79	Lahara	17.05	0.51	3.48	37.50	4322.49	624.75
80	Lal Muni	17.10	0.83	1.58	41.00	3553.13	543.90
81	Lal Sundari	20.15	0.22	1.92	29.00	5708.31	720.30
82	Lalpairi	12.00	0.90	1.64	10.00	5159.81	815.85
83	Lemon	20.00	0.22	2.32	17.50	3735.16	485.10
84	Litchi	14.45	0.83	2.96	6.50	6123.32	716.63
85	Lord	14.70	0.45	1.44	17.50	2152.75	268.28
86	Magemavu	16.85	0.48	6.32	22.00	3645.36	536.55
87	Maharaja of Mysore	13.95	0.35	2.38	11.50	2757.07	459.38
88	Makaram	16.20	0.70	1.40	17.00	3873.49	882.00
89	Malai Misri	12.20	0.16	4.46	33.00	3271.60	429.98
90	Manibhatta Appe	16.25	0.35	8.21	32.00	4727.80	679.88
91	Mohammada Vikarabad	18.80	0.99	1.01	23.50	7800.38	591.68
92	Mugalimelogra	19.65	0.38	6.64	51.00	1691.62	389.55
93	Muvandan	15.15	0.48	1.93	64.50	8785.74	984.90
94	Naagarappe	9.40	1.28	1.63	84.00	7802.81	885.68
95	Nalla Mamidi	20.70	0.58	3.52	44.50	5053.02	874.65
96	Narayana Sheni	23.90	0.29	3.18	22.50	1577.55	371.18
97	Narela	13.15	0.77	0.90	18.50	4783.62	639.45
98	Navneet	20.10	0.45	2.65	8.50	6038.38	768.08
99	Neeleshan	14.95	0.13	2.74	36.50	5137.96	565.95
100	Nekkare	13.60	0.26	3.72	25.00	5664.62	705.60
101	Nuha	13.55	0.45	5.14	47.00	5089.42	521.85
102	Olour	14.50	0.26	4.93	34.50	5227.76	595.35
103	Pacharasi	10.60	0.38	4.04	12.50	4162.31	459.38
104	Padari	20.50	0.29	7.23	23.00	7215.47	753.38
105	Panakalu	19.05	0.32	2.81	26.50	7856.20	852.60
106	Panchadara Kalasa	18.95	0.32	6.04	14.50	6742.21	738.68

107	Panchavarnam	18.40	0.42	1.37	21.50	4123.47	705.60
108	Papaya Khas	21.65	0.26	4.89	58.00	5611.22	507.15
109	Papayaraju Goa	17.75	0.64	0.96	67.50	6790.75	716.63
110	Peddarasam	14.00	0.54	3.70	36.00	7065.00	742.35
111	Peter	12.95	1.02	3.06	22.00	6113.62	599.03
112	PKM - 1	14.05	0.58	2.02	10.50	3839.52	676.20
113	PKM - 2	12.05	0.29	1.67	26.00	5526.28	654.15
114	Pulihora	17.60	0.96	5.75	26.50	3463.33	668.85
115	Puttu	17.95	0.32	2.57	38.50	3914.75	771.75
116	Putu	14.15	0.35	4.88	37.50	5096.70	657.83
117	Ramphalya	23.75	0.32	2.75	76.00	1885.78	400.58
118	Royal Special	15.30	0.58	3.83	97.00	4878.27	668.85
119	Rumani x Neelum	14.30	0.35	1.12	19.50	6450.97	771.75
120	Ruswani	17.15	0.48	6.51	21.00	2240.12	569.63
121	Safed Mulgoa	15.40	0.45	1.27	51.50	2703.68	282.98
122	Safeda Lucknow	22.25	0.19	2.81	20.50	2062.95	657.83
123	Sai Sugandh	17.55	0.22	2.18	21.00	3528.86	341.78
124	Salem Bangalora	17.50	0.70	2.90	11.00	4921.96	804.83
125	Sampar Totapuri	10.75	0.38	3.15	14.00	3460.90	632.10
126	Santhoor Collection	15.25	0.70	6.81	19.00	3453.62	301.35
127	Sardar	13.70	0.90	1.32	26.50	6023.82	562.28
128	Saru	15.35	0.19	6.07	11.00	4749.64	823.20
129	Shahjahan	13.80	0.32	1.85	10.50	4436.56	797.48
130	Sharbathi Bagri	15.15	1.02	1.49	14.50	1815.40	305.03
131	Shendriya	18.55	0.48	1.37	16.50	3358.97	565.95
132	Shendryo	13.85	0.83	0.81	22.50	6001.97	665.18
133	Siroli	11.25	0.83	1.77	45.00	1694.05	441.00
134	Sora	11.95	0.58	1.15	18.50	5766.55	643.13
135	Surankudi	16.00	0.32	2.21	39.00	4244.82	529.20
136	Sushanbhog	20.35	0.29	4.31	11.50	2075.09	962.85
137	Suvarnarekha	15.70	0.19	2.61	10.50	6009.26	771.75
138	Taimur pasand	13.15	0.58	2.22	53.00	1985.29	878.33
139	Tatamdi	20.00	0.45	1.98	79.00	5708.30	782.78
140	Tenkasi Banganapalli	15.15	0.45	1.44	14.00	5077.29	878.33
141	Tenkasi Neelum	17.30	0.45	5.98	89.00	4465.68	477.75
142	Tephala	16.75	0.26	2.20	7.50	2980.36	485.10
143	Thali	16.95	0.45	4.63	55.00	3875.92	345.45

144	Thambva	10.10	1.28	1.85	109.00	2322.64	338.10
145	Thogarapalli	16.70	0.54	2.82	18.00	4016.69	257.25
146	Thorappadi	9.20	0.38	1.41	9.50	3642.93	338.10
147	Totapuri	10.95	0.45	1.54	13.00	4832.16	580.65
148	Vattam	13.15	0.22	2.61	26.00	6242.25	826.88
149	Vellaikulamban	13.65	0.32	1.14	22.00	4788.47	944.48
150	Vhout	16.40	0.90	2.10	23.50	4645.28	760.73
151	Vinayaka Hegde	19.90	0.45	5.86	29.50	5616.08	687.23
152	Virudhanagar	16.10	0.45	1.49	72.00	6186.43	757.05
153	Warale gidaga	21.65	0.26	3.43	93.00	3982.71	580.65
154	Whiteseri	12.65	0.58	2.29	30.00	3604.10	632.10
155	Willard	17.95	0.22	5.04	19.00	5722.87	720.30
156	Yakutti	19.65	0.32	2.78	24.00	6353.89	808.50
	<b>MINIMUM</b>	7.00	0.13	0.81	5.50	1577.55	257.25
	<b>MAXIMUM</b>	24.95	1.34	8.21	113.00	9875.46	1087.80
	<b>MEAN</b>	16.29	0.49	3.02	36.27	4856.80	633.54
	<b>SEm ±</b>	1.339	0.058	0.331	2.994	483.619	77.566
	<b>CD (p=0.05)</b>	3.746	0.163	0.926	8.373	1,352.39	216.905
	<b>CV (%)</b>	11.627	16.657	15.499	11.676	14.082	17.315

#### **4.1.2.3 Total carotenoids (mg/100g pulp)**

Total carotenoids ranged from 0.81 to 8.21 mg/100g with an average of 3.02 mg/100g (Table 4.1.2). Maximum total carotenoids content was found in Manibhatta Appe (8.21 mg/100g), followed by Padari (7.23 mg/100g) and Chethalli (6.83 mg/100g), while, minimum was found in Shendryo (0.81 mg/100g), which was at par with K-O-7 (0.87 mg/100g), Narela (0.9 mg/100g) and forty-one other genotypes, followed by Irugupalli Tokkukaya (1.76 mg/100g) and Siroli (1.77 mg/100g).

#### **4.1.2.4 Ascorbic acid (mg/100g pulp)**

Significant differences were observed in the ascorbic acid content of mango cultivars studied in the present investigation (Table 4.1.2). The ascorbic acid content ranged from 5.50 to 113.00 mg/100g with a mean of 36.27 mg/100g. The highest ascorbic acid content was found in Hithalahalli Appe (113 mg/100g), which was at par with Khas-ul-Khas (112.00 mg/100g), Thambva (109.00 mg/100g), Coorg Collection (107.00 mg/100g) and Huli Appekai (107.00 mg/100g). On the other hand, the lowest ascorbic acid content was found in Aryavarthana Rasala (5.50 mg/100g), which was at par with Ebatti Mavu (6.00 mg/100g), Kempikunde (6.50 mg/100g) and thirty-one other genotypes, followed by Almas (14.00 mg/100g) and Jawahar (14.00 mg/100g).

#### **4.1.2.5 Total phenols (mg/100g pulp)**

The highest total phenol content was found in Hithalahalli Appe (9875.46 mg/100g), which was at par with Adderi Jeerige (9159.5 mg/100g), Muvandan (8785.74 mg/100g) and Huli Appekai (8647.40 mg/100g), followed by Creeping (8523.62 mg/100g) and Panakalu (7856.20 mg/100g). Lowest total phenols was found in Narayana Shen (1577.55 mg/100g), which was at par with Mugalimelogra (1691.62 mg/100g), Siroli (1694.05 mg/100g) and twenty other genotypes, followed by Tephala (2980.36 mg/100g) and Kottur Konam (3016.76 mg/100g). The mean value of total phenol content was 4856.80 mg/100g, while it ranged from 1577.55 to 9875.46 mg/100g (Table 4.1.2).

#### **4.1.2.6 Total flavonoids (mg/100g pulp)**

Significant differences were observed in total flavonoids content of mango cultivars studied in the present investigation (Table 4.1.2). The total flavonoids content ranged from 257.25 to 1087.80 mg/100g with an average of 633.54 mg/100g. The highest total flavonoids

content was found in Adderi Jeerige (1087.80 mg/100g), which was at par with Huli Appekai (1036.35 mg/100g) and fourteen other genotypes, while the lowest content was found in Thogarapalli (257.25 mg/100g), which was at par with Ananas (257.25 mg/100g), Lord (268.28 mg/100g) and thirty-three other cultivars.

#### **4.1.2 Genetic variability studies**

The genetic parameters, *viz.*, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense and genetic advance as per cent mean were estimated for all the characters under study and the results are presented in Table 4.1.3. Further, the variability studied in different characters is presented character wise under the following heads:

##### **4.1.2.1 Fruit weight (g)**

All the variability parameters studied for the trait (Table 4.1.3), namely phenotypic coefficient of variation (61.26%), genotypic coefficient of variation (61.05%), genetic advance as per cent mean (125.34%) and heritability (99.33%) were found to be high for this parameter. The high PCV and GCV of this character indicated the presence of large amount of variation.

##### **4.1.2.2 Fruit thickness (cm)**

High estimates of phenotypic coefficient of variation (20.70%), genotypic coefficient of variation (20.21%), genetic advance as per cent mean (40.66%) and high estimates of heritability (95.32%) were observed for this character indicating the presence of additive gene action in the inheritance of this trait (Table 4.1.3).

##### **4.1.2.3 Fruit length (cm)**

Phenotypic coefficient of variation (24.53%), genotypic coefficient of variation (23.41%), genetic advance as per cent mean (46.00%) and heritability (91.02%), observed to be high for this character, indicated the presence of additive gene action in the inheritance of this trait and simple selection would be highly rewarding for improving this character (Table 4.1.3).

##### **4.1.2.4 Fruit width (cm)**

The variability parameters studied for the trait (Table 4.1.3), namely phenotypic coefficient of variation (20.42%), genetic advance as per cent mean (38.93%) and heritability

**Table 4.1.3 Estimates of variability and genetic parameters for different quantitative characters**

Character	Range	Mean $\pm$ S.E.m.	Coefficient of variation		Heritability (%)	Genetic advance	Genetic advance (% of mean)
			Genotypic	Phenotypic			
<b>Fruit weight (g)</b>	50.12-1182.95	304.23 $\pm$ 10.807	61.05	61.26	99.33	381.33	125.34
<b>Fruit thickness (cm)</b>	2.05-10.50	6.5 $\pm$ 0.206	20.21	20.70	95.32	2.64	40.66
<b>Fruit length (cm)</b>	4.70-17.50	9.81 $\pm$ 0.51	23.41	24.53	91.02	4.51	46.00
<b>Fruit width (cm)</b>	4.15-12.90	7.61 $\pm$ 0.301	19.65	20.42	92.52	2.96	38.93
<b>Skin weight (g)</b>	12.07-305.73	73.68 $\pm$ 5.278	63.23	64.04	97.5	94.76	128.61
<b>Skin thickness (mm)</b>	1.10-5.35	2.33 $\pm$ 0.183	29.37	31.40	87.47	1.32	56.59
<b>Stone weight (g)</b>	9.34-129.50	42.07 $\pm$ 3.801	36.73	38.89	89.2	30.07	71.47
<b>Pulp weight</b>	17.71-791.88	188.48 $\pm$ 9.745	69.58	69.96	98.91	268.67	142.54
<b>Pulp %</b>	31.10-83.99	59.19 $\pm$ 3.2	12.73	14.85	73.49	13.31	22.48
<b>TSS (<math>^{\circ}</math>Brix)</b>	7.00-24.95	16.29 $\pm$ 1.34	20.20	23.31	75.12	5.88	36.07
<b>Titrateable acidity (%)</b>	0.13-1.35	0.49 $\pm$ 0.06	57.77	60.12	92.32	0.56	114.34
<b>Total carotenoids (mg/100g)</b>	0.81-8.21	3.02 $\pm$ 0.33	55.44	57.57	92.75	3.32	110.00
<b>Ascorbic acid (mg/100g)</b>	5.50-113.00	36.27 $\pm$ 2.99	75.83	76.73	97.68	55.99	154.40
<b>Total phenols(mg/100g)</b>	1577.55-9875.46	4856.80 $\pm$ 483.59	33.95	36.75	85.32	3137.41	64.60
<b>Total flavonoids(mg/100g)</b>	257.25-1087.80	633.54 $\pm$ 77.57	26.62	31.76	70.27	291.27	45.98

PCV, GCV and Genetic advance as per cent mean: Low = 0-10%; Medium = 10-20%; High = 20% above  
Heritability: Low = Less than 30%; Medium = 30-60%; High = More than 60%

(92.52%) were found to be high, whereas moderate genotypic coefficient of variation (19.65%), observed for this trait, indicated the presence of both additive and non-additive gene action.

#### **4.1.2.5 Skin weight (g)**

All the variability parameters (Table 4.1.3), namely phenotypic coefficient of variation (64.04%), genotypic coefficient of variation (63.23%), genetic advance as per cent mean (128.61%) and heritability (97.50%) were found to be high for peel weight which indicated the presence of additive gene action.

#### **4.1.2.6 Skin thickness (mm)**

The variability parameters studied for this trait (Table 4.1.3), namely, phenotypic coefficient of variation (31.40%), genotypic coefficient of variation (29.37%), genetic advance as per cent mean (56.59%) and heritability (87.47%) were found to be high.

#### **4.1.2.7 Stone weight (g)**

Phenotypic coefficient of variation (38.89%), genotypic coefficient of variation (36.73%), heritability (89.20%) and genetic advance as per cent mean (71.47%) were found to be high indicating presence of additive gene action.

#### **4.1.2.8 Pulp weight (g)**

High estimates of phenotypic coefficient of variation (69.96%), genotypic coefficient of variation (69.58%), genetic advance as per cent mean (142.54%) and heritability (98.91%), observed for this trait indicated additive gene action and improvement could be done for this trait by simple direct selection (Table 4.1.3).

#### **4.1.2.9 Pulp %**

Moderate estimates of phenotypic coefficient of variation (14.85%) and genotypic coefficient of variation (12.73%), while high estimates of heritability (73.49%) and genetic advance as per cent mean (22.48%) observed for this trait revealed the predominance of both additive and non-additive gene action (Table 4.1.3).

#### **4.1.2.10 TSS (<sup>o</sup>B)**

The estimates of phenotypic coefficient of variation (23.31%), genotypic coefficient of variation (20.20%), heritability (75.12%) and genetic advance as per cent mean (36.07%) were observed to be high for this character (Table 4.1.3).

#### **4.1.2.11 Titratable acidity (%)**

High estimates of phenotypic coefficient of variation (60.12%) and genotypic coefficient of variation (57.77%) and high estimates of genetic advance as per cent mean (114.34%) and heritability (92.32%) were observed for this character (Table 4.1.3).

#### **4.1.2.12 Total carotenoids (mg/100g)**

Phenotypic coefficient of variation (57.57%), genotypic coefficient of variation (55.44%), genetic advance as per cent mean (110%) and heritability (92.75 %) were observed to be high for this character showing that the character was governed by additive genes and selection will be rewarding for improvement (Table 4.1.3).

#### **4.1.2.13 Ascorbic acid (mg/100g)**

High estimates of phenotypic coefficient of variation (76.73%), genotypic coefficient of variation (75.83%), genetic advance as per cent mean (154.40%) and heritability (97.68%) were observed for this character.

#### **4.1.2.14 Total phenols (mg/100g)**

Phenotypic coefficient of variation (36.75%), genotypic coefficient of variation (33.95%), genetic advance as per cent mean (64.60%) and heritability (85.32%) were observed to be high for this character indicated that this character was governed by additive gene action (Table 4.1.3).

#### **4.1.2.15 Total flavonoids (mg/100g)**

All the variability parameters (Table 4.1.3), namely phenotypic coefficient of variation (31.76%), genotypic coefficient of variation (26.62%), genetic advance as per cent mean (45.98%) and heritability (70.27%) were found to be high, indicating that selection will be highly rewarding for improvement of this character.

### 4.1.3 Correlation studies

To determine the nature and magnitude of association, phenotypic and genotypic correlation coefficients between pairs of traits were estimated and presented in Table 4.1.4. The results on correlation coefficients have been described as under.

#### 4.1.3.1 Fruit weight

Fruit weight had significant positive correlation (Table 4.1.4) with pulp weight ( $r_p=0.985$ ,  $r_g=0.988$ ), skin weight ( $r_p=0.915$ ,  $r_g=0.924$ ), fruit width ( $r_p=0.852$ ,  $r_g=0.895$ ), stone weight ( $r_p=0.811$ ,  $r_g=0.849$ ), fruit thickness ( $r_p=0.799$ ,  $r_g=0.817$ ), fruit length ( $r_p=0.752$ ,  $r_g=0.792$ ), skin thickness ( $r_p=0.562$ ,  $r_g=0.598$ ) and pulp per cent ( $r_p=0.518$ ,  $r_g=0.600$ ). It was negatively correlated with total carotenoids ( $r_p= -0.259$ ,  $r_g= -0.271$ ), TSS ( $r_p= -0.163$ ,  $r_g= -0.190$ ), ascorbic acid ( $r_p= -0.158$ ,  $r_g= -0.160$ ) and total phenols ( $r_p= -0.129$ ,  $r_g= -0.140$ ). Non-significant negative correlation was observed for the rest of characters.

#### 4.1.3.2 Fruit thickness

At both phenotypic and genotypic levels (Table 4.1.4), fruit thickness recorded significant positive correlation with fruit width ( $r_p=0.795$ ,  $r_g=0.843$ ), pulp weight ( $r_p= 0.775$ ,  $r_g=0.795$ ), skin weight ( $r_p=0.769$ ,  $r_g=0.793$ ), stone weight ( $r_p=0.641$ ,  $r_g=0.681$ ), fruit length ( $r_p=0.574$ ,  $r_g=0.603$ ), skin thickness ( $r_p=0.525$ ,  $r_g=0.567$ ) and pulp per cent ( $r_p= 0.485$ ,  $r_g=0.584$ ). Negative correlation was significant with respect to total carotenoids ( $r_p= -0.224$ ,  $r_g= -0.245$ ), ascorbic acid ( $r_p= -0.201$ ,  $r_g= -0.210$ ), TSS ( $r_p= -0.114$ ,  $r_g= -0.164$ ) and total phenols ( $r_p= -0.104$ ,  $r_g= -0.119$ ). Non-significant negative correlation was observed for acidity and total flavonoids.

#### 4.1.3.3 Fruit length

At both phenotypic and genotypic levels (Table 4.1.4), fruit length recorded significant positive correlation with pulp weight ( $r_p=0.723$ ,  $r_g=0.760$ ), skin weight ( $r_p=0.711$ ,  $r_g=0.762$ ), stone weight ( $r_p=0.686$ ,  $r_g=0.775$ ), fruit width ( $r_p=0.685$ ,  $r_g=0.691$ ), skin thickness ( $r_p=0.446$ ,  $r_g=0.491$ ) and pulp per cent ( $r_p=0.407$ ,  $r_g=0.480$ ). It was significantly negatively correlated with TSS ( $r_p= -0.257$ ,  $r_g= -0.313$ ), total carotenoids ( $r_p= -0.252$ ,  $r_g= -0.280$ ), ascorbic acid ( $r_p= -0.196$ ,  $r_g= -0.213$ ) and total phenols ( $r_p= -0.100$ ,  $r_g= -0.114$ ). Titratable acidity and total flavonoids exhibited non-significant negative correlation.

#### 4.1.3.4 Fruit width

Significant positive correlation (Table 4.1.4) at both phenotypic and genotypic levels was observed for fruit width with pulp weight ( $r_p=0.848$ ,  $r_g=0.890$ ), skin weight ( $r_p=0.765$ ,  $r_g=0.814$ ), stone weight ( $r_p=0.668$ ,  $r_g=0.754$ ), pulp per cent ( $r_p=0.560$ ,  $r_g=0.675$ ) and skin thickness ( $r_p=0.528$ ,  $r_g=0.584$ ). Negative correlation was significant for total carotenoids ( $r_p= -0.254$ ,  $r_g= -0.278$ ), ascorbic acid ( $r_p= -0.211$ ,  $r_g= -0.223$ ) and TSS ( $r_p= -0.194$ ,  $r_g= -0.229$ ). Correlation was non-significant for the rest of characters *viz.*, titratable acidity, total phenols and total flavonoids.

#### 4.1.3.5 Skin weight

Skin weight recorded significant positive correlation with pulp weight ( $r_p=0.839$ ,  $r_g=0.859$ ), stone weight ( $r_p=0.775$ ,  $r_g=0.806$ ), skin thickness ( $r_p=0.651$ ,  $r_g=0.679$ ) and pulp per cent ( $r_p=0.248$ ,  $r_g=0.350$ ). Skin weight was negatively correlated with total carotenoids ( $r_p= -0.232$ ,  $r_g= -0.244$ ), ascorbic acid ( $r_p= -0.174$ ,  $r_g= -0.177$ ), TSS ( $r_p= -0.133$ ,  $r_g= -0.156$ ) and total phenols ( $r_p= -0.114$ ,  $r_g= -0.118$ ).

#### 4.1.3.6 Skin thickness

Significant positive correlation at both phenotypic and genotypic levels (Table 4.1.4) was observed for fruit skin thickness with pulp weight ( $r_p=0.502$ ,  $r_g=0.545$ ) and stone weight ( $r_p=0.482$ ,  $r_g=0.514$ ). Ascorbic acid ( $r_p= -0.159$ ,  $r_g= -0.172$ ), TSS ( $r_p= -0.127$ ,  $r_g= -0.159$ ), total phenols ( $r_p= -0.127$ ,  $r_g= -0.140$ ) and total carotenoids ( $r_p= -0.122$ ,  $r_g= -0.137$ ) were observed to be negatively correlated. Non-significant correlation was observed for the rest of characters *viz.*, titratable acidity, pulp per cent and total flavonoids.

#### 4.1.3.7 Stone weight

Significant positive correlation at both phenotypic and genotypic levels (Table 4.1.4) was observed for stone weight with pulp weight ( $r_p=0.745$ ,  $r_g=0.798$ ) and pulp per cent ( $r_p= 0.208$ ,  $r_g=0.372$ ). Stone weight was found to be significantly correlated negatively with total carotenoids ( $r_p= -0.224$ ,  $r_g= -0.248$ ), TSS ( $r_p= -0.146$ ,  $r_g= -0.179$ ), ascorbic acid ( $r_p= -0.107$ ,  $r_g= -0.112$ ) and total phenols ( $r_p=-0.100$ ,  $r_g= -0.128$ ). Non-significant correlation was observed for the rest of the characters.

**Table 4.1.4 Genotypic (rg) and phenotypic (rp) correlation coefficients of various quantitative characters in 156 mango cultivars**

Character		Fruit Weight	Fruit Thickness	Fruit Length	Fruit Width	Skin Weight	Skin Thickness	Stone Weight	Pulp Weight	Pulp %	TSS	Acidity	Carotenoids	Ascorbic Acid	Phenols	Flavonoids
Fruit Weight	rp rg	1.000														
Fruit Thickness	rp rg	0.799** 0.817**	1.000													
Fruit Length	rp rg	0.752** 0.792**	0.574** 0.604**	1.000												
Fruit Width	rp rg	0.852** 0.895**	0.795** 0.843**	0.685** 0.691**	1.000											
Skin Weight	rp rg	0.915** 0.924**	0.769** 0.793**	0.711** 0.762**	0.765** 0.814**	1.000										
Skin Thickness	rp rg	0.562** 0.598**	0.525** 0.567**	0.446** 0.491**	0.528** 0.584**	0.651** 0.679**	1.000									
Stone Weight	rp rg	0.811** 0.849**	0.641** 0.681**	0.686** 0.775**	0.668** 0.754**	0.775** 0.806**	0.482** 0.514**	1.000								
Pulp Weight	rp rg	0.985** 0.988**	0.775** 0.795**	0.723** 0.760**	0.848** 0.890**	0.839** 0.859**	0.502** 0.545**	0.745** 0.798**	1.000							
Pulp %	rp rg	0.518** 0.600	0.485** 0.584**	0.407** 0.480**	0.560** 0.675**	0.248** 0.350**	0.061 0.147	0.208** 0.372**	0.618** 0.681**	1.000						
TSS	rp rg	-0.163** -0.190**	-0.114* -0.164*	-0.257** -0.313**	-0.194** 0.229**	-0.133* -0.156*	-0.127* -0.159*	-0.146** -0.179**	-0.165** -0.193**	-0.128* -0.170*	1.000					
Acidity	rp rg	0.005 0.005	-0.036 -0.027	-0.038 -0.031	-0.021 -0.016	0.011 0.009	0.011 -0.002	0.035 0.038	-0.001 0.000	-0.121* -0.147*	-0.403** -0.431**	1.000				
Carotenoids	rp rg	-0.259** -0.271**	-0.224** -0.245**	-0.252** -0.280**	-0.254** -0.278**	-0.232** -0.244**	-0.122* -0.137*	-0.224** -0.248**	-0.255** -0.269**	-0.143* -0.188*	0.256** 0.293**	-0.216** -0.225**	1.000			
Ascorbic Acid	rp rg	-0.158** -0.160**	-0.201** -0.210**	-0.196** -0.213**	-0.211** -0.223**	-0.174** -0.177**	-0.159** -0.172**	-0.107 -0.112	-0.148** -0.150**	-0.188** -0.240**	0.058 0.074	0.204** 0.211**	0.054 0.057	1.000		
Phenols	rp rg	-0.129* -0.140*	-0.104 -0.119	-0.100 -0.114	-0.045 -0.048	-0.114* -0.118*	-0.127* -0.140*	-0.100 -0.128	-0.129* -0.141*	-0.093 -0.129	-0.075 -0.099	0.173** 0.197**	-0.116* -0.145*	0.147** 0.165**	1.000	
Flavonoids	rp rg	-0.074 -0.093	-0.065 -0.074	-0.064 -0.082	0.009 0.017	-0.035 -0.044	-0.093 -0.076	-0.055 -0.061	-0.085 -0.108	-0.121* -0.196	-0.099 -0.146	0.213** 0.272**	-0.129* -0.146*	0.055 0.061	0.549** 0.747**	1.000

\* and \*\* indicates significance of values at P=0.05 and P=0.01, respectively

#### **4.1.3.8 Pulp weight**

Pulp weight (Table 4.1.4) recorded significant positive correlation with pulp per cent ( $r_p = 0.618$ ,  $r_g = 0.681$ ). Significant negative correlation was observed with respect to total carotenoids ( $r_p = -0.255$ ,  $r_g = -0.269$ ), TSS ( $r_p = -0.1647$ ,  $r_g = -0.1931$ ), ascorbic acid ( $r_p = -0.148$ ,  $r_g = -0.150$ ) and total phenols ( $r_p = -0.129$ ,  $r_g = -0.141$ ). Acidity and total flavonoids showed non significant correlation.

#### **4.1.3.9 Pulp per cent**

Pulp per cent was observed to be negatively correlated with ascorbic acid ( $r_p = -0.188$ ,  $r_g = -0.240$ ), total carotenoids ( $r_p = -0.143$ ,  $r_g = -0.188$ ), TSS ( $r_p = -0.128$ ,  $r_g = -0.170$ ), acidity ( $r_p = -0.121$ ,  $r_g = -0.147$ ) and total flavonoids ( $r_p = -0.121$ ,  $r_g = -0.196$ ). Total phenols was found to be non significantly correlated with pulp per cent.

#### **4.1.3.10 TSS**

Total Soluble Solids exhibited significant positive correlation (Table 4.1.4) with total carotenoids ( $r_p = 0.256$ ,  $r_g = 0.293$ ) while it was observed to have significant negative correlation with acidity ( $r_p = -0.403$ ,  $r_g = -0.431$ ). It exhibited non significant correlation with other characters *viz.*, ascorbic acid, total phenols and total flavonoids.

#### **4.1.3.11 Acidity**

Significant positive correlation at both phenotypic and genotypic levels (Table 4.1.4) was observed for acidity with total flavonoids ( $r_p = 0.213$ ,  $r_g = 0.272$ ), ascorbic acid ( $r_p = 0.204$ ,  $r_g = 0.211$ ) and total phenols ( $r_p = 0.173$ ,  $r_g = 0.197$ ). Negative correlation was significant with respect to total carotenoids ( $r_p = -0.216$ ,  $r_g = -0.225$ ).

#### **4.1.3.12 Total carotenoids**

Total carotenoids showed significant negative correlation with total flavonoids ( $r_p = -0.129$ ,  $r_g = -0.146$ ) and total phenols ( $r_p = -0.116$ ,  $r_g = -0.145$ ) while it showed non significant correlation with ascorbic acid.

#### **4.1.3.13 Ascorbic acid**

Ascorbic acid showed significant positive correlation with total phenols ( $r_p = 0.147$ ,  $r_g = 0.165$ ) and titratable acidity ( $r_p = 0.204$ ,  $r_g = 0.211$ ). Significant negative correlation was observed with respect to fruit weight and size, skin weight and thickness and pulp characteristics.

#### **4.1.3.14 Total phenols**

Significant positive correlation was observed in case of total flavonoids (rp=0.549, rg=0.747). Negative correlation was significant with respect to fruit weight (rp= -0.129, rg= -0.140), skin weight (rp= -0.114, rg= -0.118), skin thickness (rp= -0.127, rg= -0.140), pulp weight (rp= -0.129, rg= -0.141), titratable acidity (rp= -0.173, rg= -0.197) and total carotenoids (rp= -0.116, rg= -0.145).

#### **4.1.3.15 Total flavonoids**

Total flavonoids exhibited significant positive correlation with total phenols (rp=0.549, rg=0.747) and titratable acidity (rp=0.213, rg=0.272). Significant negative correlation was observed with total carotenoids (rp= -0.129, rg= -0.146) and pulp per cent (rp= -0.121, rg= -0.196). The rest of the characters showed non-significant correlation with total flavonoids.

#### **4.1.4 Genetic divergence studies**

The genetic divergence among 156 cultivars of mango was quantitatively assessed by adopting Mahalanobis D<sup>2</sup> statistics and principal component analysis, while the divergence at molecular level was studied by using SSR markers.

##### **4.1.4.1 Relative contribution of different characters towards divergence**

The relative ranking of various character components to divergence was furnished in Table 4.1.5 and Fig. 4.1.1. The result of character wise contribution towards total genetic divergence showed that fruit weight (47.07%) had the greatest contribution by ranking first in 5691 times out of 12090 combinations, followed by ascorbic acid (24.85%), which ranked first 3004 times. The characters *viz.*, carotenoids, acidity and fruit width, contributed 8.06, 5.37 and 5.06 per cent towards the diversity by ranking 974, 649 and 612 times, respectively. Total phenols, fruit length, skin thickness, skin weight, fruit thickness, stone weight, TSS, total flavonoids, pulp per cent and pulp weight contributed 1.91, 1.69, 1.51, 1.36, 1.08, 0.86, 0.55, 0.41, 0.22 and 0.01 per cent respectively to the genetic divergence in decreasing order.

##### **4.1.4.2 Mean intra and inter cluster distances**

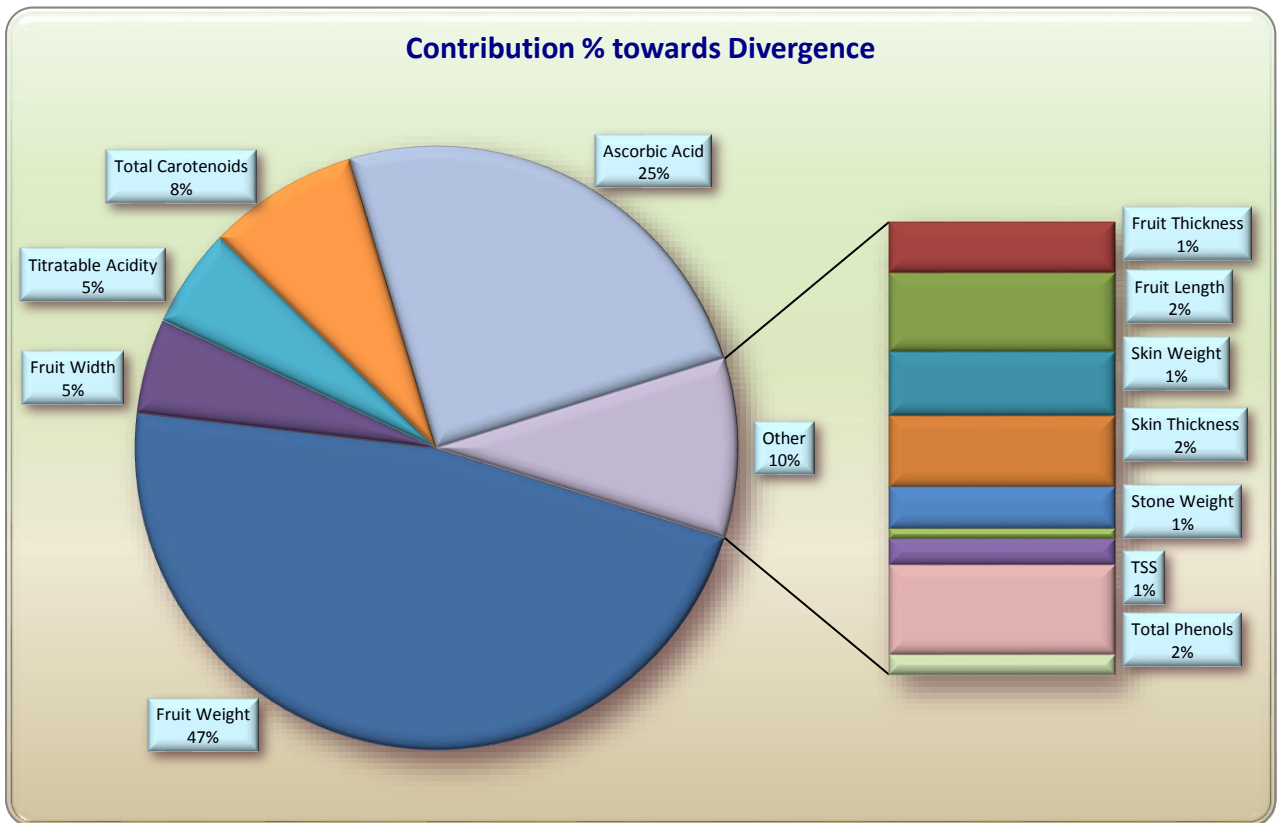
The information regarding proximity and divergent clusters from each cluster is given in Table 4.1.6, which revealed that cluster I was closely related to cluster II (408.903) and distantly related to cluster XIII (6572.160).

**Table 4.1.5 Relative contribution of different characters to genetic divergence**

Sl. No.	Character	Per cent contribution	Number of times ranked 1st
1.	Fruit weight	47.07	5691
2.	Fruit thickness	1.08	131
3.	Fruit length	1.69	204
4.	Fruit width	5.06	612
5.	Skin weight	1.36	164
6.	Skin thickness	1.51	182
7.	Stone weight	0.86	104
8.	Pulp weight	0.01	0
9.	Pulp %	0.22	27
10.	TSS	0.55	67
11.	Titrateable acidity	5.37	649
12.	Total carotenoids	8.06	974
13.	Ascorbic acid	24.85	3004
14.	Total phenols	1.91	231
15.	Total flavonoids	0.41	50

**Table 4.1.6 Proximity and divergent clusters from each other based on D<sup>2</sup> values**

Cluster	Proximity clusters with D <sup>2</sup> values	Divergent cluster with D <sup>2</sup> values
I	II (408.903)	XIII (6572.160)
II	IV (236.627)	XIII (5687.560)
III	IV (172.472)	XIII (5618.797)
IV	III (172.472)	XIII (5172.170)
V	IV (184.199)	XIII (4886.274)
VI	IV (208.634)	XIII (6441.035)
VII	V (240.960)	XIII (3913.113)
VIII	VII (349.159)	XIII (3571.356)
IX	VII (382.715)	XIII (2512.650)
X	VII (405.798)	XIII (3197.877)
XI	XII (680.848)	VI (2325.925)
XII	IX (497.283)	I (2786.493)
XIII	XII (1540.243)	I (6572.160)



**Fig. 4.1.1 Relative contribution of different characters towards divergence**

Clusters II (177.064), III (172.472), V (184.199) and VI (208.634) were closely related to cluster IV, while all four were furthest from cluster XIII (5687.560, 5618.797, 4886.274 and 6441.035, respectively).

Clusters IV (172.472) showed close proximity with cluster III while exhibiting wide diversity with cluster XIII (5172.170). Cluster VII was observed to have close proximity with cluster V (240.960) while being widely divergent from cluster XIII (3913.113).

Clusters VIII (349.159), IX (382.715) and X (405.798) exhibited intimate relation with cluster VII and wide diversity with cluster XIII (3571.356, 2512.650 and 3197.877, respectively).

Clusters XI (680.848) and XIII (1540.243) exhibited close proximity with cluster XII while being widely divergent from cluster VI (2323.925) and cluster I (6572.160), respectively. Cluster XII (497.283) was closely related to cluster IX and showed wide diversity with cluster I (2876.493).

The mean inter and intra cluster distances among thirteen clusters were presented in Table 4.1.7. The inter cluster  $D^2$  values ranged from 172.472 to 6572.160 indicating presence of substantial amount of genetic diversity in the genetic material. The highest inter cluster  $D^2$  value was recorded between clusters I and XIII (6572.160), while the lowest was recorded between clusters III and IV (172.472).

The highest intra cluster  $D^2$  value was observed in cluster XI (491.651) while the lowest (93.041) intra cluster  $D^2$  value was observed in cluster IV.

#### **4.1.4.3 Mean performance of characters in clusters**

Cluster means indicated the average performance of all cultivars clubbed into a cluster. The clusters mean values for all the 15 characters are presented in Table 4.1.8.

Fruit weight was observed maximum in cluster XIII (1075.35), followed by cluster XII (622.20) and was found minimum in cluster I (111.53), followed by cluster VI (120.98). Cluster XIII (9.28) followed by cluster XII (9.20) had maximum fruit thickness, while cluster I (4.66) followed by cluster VI (5.06) had minimum fruit thickness.

The maximum fruit length was found in cluster XIII (15.52), followed by cluster XI (12.92), while minimum was reported in cluster VI (7.14), followed by cluster I (7.52) and cluster

II (8.51). Maximum fruit width was observed in cluster XIII (11.87), followed by cluster XII (10.52), while minimum fruit width were reported in clusters I (5.58), VI (5.897) and II (6.65).

With respect to the skin weight, cluster XIII (258.69) exhibited the maximum, followed by cluster XII (135.95), while clusters I (23.35) and VI (33.25) showed minimum skin weight. Maximum skin thickness was observed in cluster XIII (4.10) and minimum in cluster I (1.53).

Highest stone weight was recorded in cluster XIII (90.21), while, lowest was observed in cluster VI (27.19). Maximum pulp weight (726.44) was recorded in cluster XIII, followed by cluster XII (470.44) and minimum in clusters I (56.27) and VI (60.55). The maximum pulp per cent was observed in cluster XI (73.89) and minimum in cluster I (46.57).

With respect to the biochemical characters, maximum acidity (1.06), ascorbic acid (95.63), total phenols (7687.22) and total flavonoids (929.32) were observed in cluster I, while, minimum was observed in cluster IV (99.00), cluster XII (12.33), cluster VIII (2990.60) and cluster XIII (463.05), respectively.

Maximum TSS (17.86) was exhibited by clusters II and IV, followed by cluster X (17.71), and minimum by clusters XI (13.71) and XII (14.55). Cluster III had maximum mean (5.78) for total carotenoids, followed by cluster VIII (5.07) and cluster II (3.86), while, minimum mean for total carotenoids were reported in cluster XII (1.57), preceded by cluster XIII (1.29).

#### **4.1.4.4 Grouping of cultivars into different clusters (D<sup>2</sup> analysis)**

The entire germplasm was grouped into thirteen distinct clusters using the Ward's method (Table 4.1.9) with the criterion that the average intra-cluster D<sup>2</sup> values should be less than the average inter-cluster D<sup>2</sup> values. The distribution of 156 cultivars into thirteen clusters is illustrated in Fig. 4.1.2.

Cluster V was the largest cluster consisting of 23 cultivars (Asiquot, Kalkand, Bombay Peda, Suvarnakha, Banganapalli, PKM-2, Lemon, Sai Sugandh, KMH-1, Thogarapalli, K-O-7, PKM-1, Tephala, Thorappadi, Totapuri, Irugupalli Tokkukaya, Narela, Peter, Mohammada Vikarabad, Sharbathi Bagri, Kari Ishad, Lalpairi and Kasturi Mamidi), followed by cluster VII with 21 cultivars (Agarbathi, Salem Bangalora, Gulabi, Navneet, Sardar, Aryavarthana Rasala, Dori, Kalneelum, Gidagana Mavu, Bangalore Sindhura, Himam Pasand, Allampur Baneshan, Blackseri, Sampar Totapuri, Whiteseri, Dwarf Rumani, Panchavarnam, Karkan Rumani, Yakutti, Rumani x Neelum and Apple Rumani).

Table 4.1.7 Intra &amp; inter cluster distance of 156 mango cultivars

Cluster	1	2	3	4	5	6	7	8	9	10	11	12	13
1	<b>242.49</b>	408.903	780.479	591.305	895.622	564.993	1076.677	1278.589	1742.326	956.192	2138.539	2786.493	6572.160
2		<b>186.719</b>	380.679	236.627	465.207	318.176	589.491	717.708	1157.247	598.460	1754.025	2096.159	5687.560
3			<b>111.254</b>	172.472	230.202	211.243	368.960	501.467	932.136	803.073	1988.788	1846.234	5618.797
4				<b>93.041</b>	184.199	208.634	273.914	458.872	768.263	508.700	1635.806	1627.959	5172.170
5					<b>161.711</b>	327.341	240.960	440.968	658.427	614.387	1652.522	1455.213	4886.274
6						<b>141.211</b>	565.133	781.639	1291.973	955.861	2325.925	2332.443	6441.035
7							<b>174.611</b>	349.159	382.715	405.798	1234.074	975.658	3913.113
8								<b>346.588</b>	464.878	519.865	1063.551	932.251	3571.356
9									<b>247.018</b>	437.646	792.722	497.283	2512.650
10										<b>171.489</b>	713.465	895.973	3197.877
11											<b>491.651</b>	680.848	1842.523
12												<b>341.264</b>	1540.243
13													<b>409.990</b>

Intra (**bold**) and inter cluster  $D^2$  values for 15 quantitative characters

**Table 4.1.8 Cluster means of 156 mango cultivars for morpho-biochemical characters**

Cluster	Fruit Weight	Fruit Thickness	Fruit Length	Fruit Width	Skin Weight	Skin Thickness	Stone Weight	Pulp Weight	Pulp %	TSS	Titrateable Acidity	Total Carotenoids	Ascorbic Acid	Total Phenols	Total Flavonoids
<b>1</b>	111.53	4.66	7.52	5.58	23.35	1.53	31.91	56.27	46.57	14.93	1.06	2.99	95.63	7687.22	929.32
<b>2</b>	179.69	5.69	8.51	6.65	45.12	2.18	32.89	101.67	56.16	17.86	0.34	3.86	73.82	4463.40	517.53
<b>3</b>	211.29	6.00	8.98	7.08	50.86	2.16	34.26	126.17	59.61	17.13	0.45	5.78	16.67	4720.31	589.84
<b>4</b>	227.04	6.22	8.95	7.16	56.66	2.00	38.16	132.22	58.33	17.86	0.33	3.05	38.28	5140.39	675.74
<b>5</b>	267.54	6.33	10.61	7.68	62.31	2.40	42.34	162.89	60.67	15.23	0.52	1.97	16.22	4765.89	566.11
<b>6</b>	120.98	5.06	7.14	5.89	33.25	2.06	27.19	60.55	50.72	16.74	0.52	3.19	28.95	4477.94	634.67
<b>7</b>	357.15	7.35	10.23	8.14	95.52	2.43	45.17	216.46	60.90	15.55	0.48	2.33	20.48	5255.03	687.75
<b>8</b>	416.61	6.71	11.12	8.41	83.51	2.50	50.19	282.91	67.14	16.53	0.35	5.07	23.56	2990.60	543.49
<b>9</b>	523.05	8.12	12.50	9.44	134.38	2.81	60.00	328.66	63.07	15.46	0.52	2.18	22.00	5141.60	711.11
<b>10</b>	411.53	7.65	11.07	8.57	112.58	2.74	46.13	252.82	61.46	17.71	0.48	1.94	76.75	5603.13	670.08
<b>11</b>	629.28	8.10	12.92	9.70	106.66	2.28	63.48	459.13	73.89	13.71	0.77	2.38	80.88	3649.60	532.88
<b>12</b>	662.20	9.20	10.92	10.52	135.95	2.98	55.81	470.44	71.33	14.55	0.54	1.57	12.33	3982.71	721.52
<b>13</b>	1075.35	9.28	15.52	11.87	258.69	4.10	90.21	726.44	67.70	15.17	0.53	1.29	34.00	4267.47	463.05

**Table 4.1.9 Distribution of mango cultivars in different clusters (Ward's method)**

Cluster	No. of cultivars	Name of the cultivars
I	8	Adderi Jeerige, Jeerige Neermavu, Huli Appekai, Naagarappe, Hithalahalli Appe, Coorg Collection, Khas-ul-Khas, Chandrakaran
II	17	Chandanum, Royal Special, Kalapadi, H-151, Kaju, Kalgundi Koppa Appe, Ramphalya, Muvandan, Virudhanagar, Krishna, Gulab Khas, Kottur Konam, Dofasla, Hathizul, Thali, Tenkasi Neelum, Deorakhio.
III	12	Almas, H-39, Padari, Panchadara Kalasa, Manibhatta Appe, Santhoor Collection, Pulihora, Hajeera, Pacharasi, Jawahar, Kalapara, Ananas
IV	16	Atimadhuram, Puttu, Kalwa Gudda, Karigal Appe, Khuddus, Surankudi, Neeleshan, Taimur Pasand, Nalla Mamidi, Papaya Khas, Hydersaheb, Kerala Kalepad, Lal Sundari, Panakalu, Karanjio, Putu
V	23	Asiquot, Kalkand, Bombay Peda, Suvarnarekha, Banganapalli, PKM-2, Lemon, Sai Sugandh, Kmh-1, Thogarapalli, K-O-7, PKM-1, Tephala, Thorappadi, Totapuri, Irugupalli Tokkukaya, Narela, Peter, Mohammada Vikarabad, Sharbathi Bagri, Kari Ishad, Lalpairi, Kasturi Mamidi
VI	20	Haleema, Nuha, Elaichi, Nekkare, Vattam, Olour, Vinayaka Hegde, Willard, Lal Muni, Siroli, Kintalavenipeta, Litchi, Vhout, Creeping, Hamsa Mamidi, Safeda Lucknow, Narayana Sheni, Kempikunde, Shendriya, Vellaikulamban
VII	21	Agarbathi, Salem Bangalora, Gulabi, Navneet, Sardar, Aryavarthana Rasala, Dori, Kalneelum, Gidagana Mavu, Bangalore Sindhura, Himam Pasand, Allampur Baneshan, Blackseri, Sampar Totapuri, Whiteseri, Dwarf Rumani, Panchavarnam, Karkan Rumani, Yakutti, Rumani x Neelum, Apple Rumani
VIII	9	Chethalli, Lord, Harsha, Magemavu, Malai Misri, Mugalimelogra, Ruswani, Sushanbhog, Kalam Hindustani
IX	14	Balakondapari, Jehangir, H-85, Shahjahan, Himayath Pasand, Goa Kodur, Tenkasi Banganapalli, Kerali Goa, Peddarasam, Ku-8, Saru, Kutumba Appe, Shendryo, Ebatti Mavu
X	6	Black Andrews, Hansraj, Warale Gidaga, Amini, Papayaraju Goa, Tatamdi
XI	4	Gaddemar, Lahara, Hamlet, Thambva
XII	3	Katta Gola, Makaram, Maharaja of Mysore
XIII	3	Khudadath, Sora, Safed Mulgoa

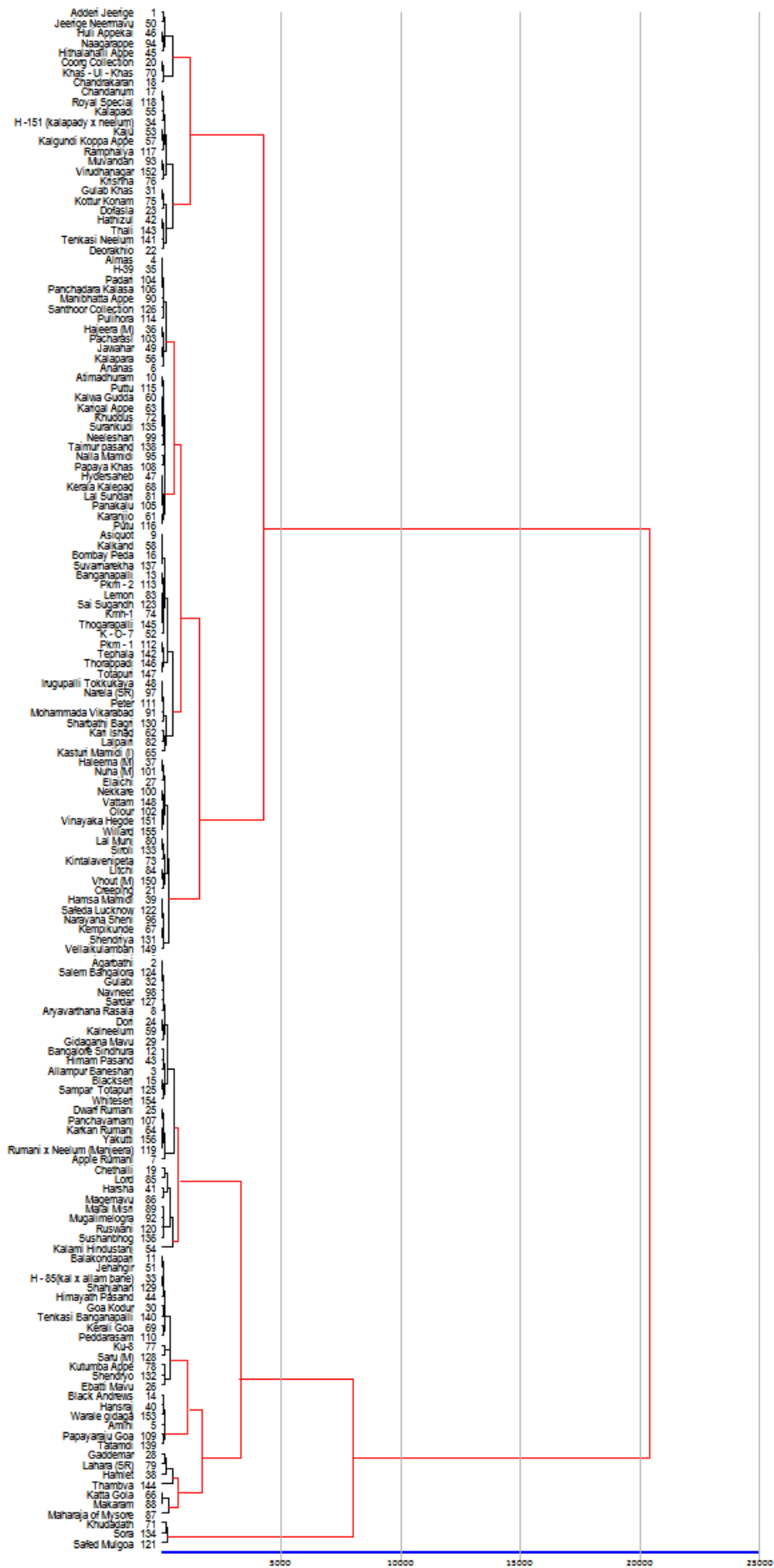


Fig. 4.1.2 Ward's minimum variance dendrogram for 156 mango cultivars

Cluster VI consisted of 20 cultivars including Haleema, Nuha, Elaichi, Nekkare, Vattam, Olour, Vinayaka Hegde, Willard, Lal Muni, Siroli, Kintalavenipeta, Litchi, Vhout, Creeping, Hamsa Mamidi, Safeda Lucknow, Narayana Sheni, Kempikunde, Shendriya and Vellaikulamban), whereas cluster II consisted of 17 cultivars (Chandanum, Royal Special, Kalapadi, H-151, Kaju, Kalgundi Koppa Appe, Ramphalya, Muvandan, Virudhanagar, Krishna, Gulab Khas, Kottur Konam, Dofasla, Hathizul, Thali, Tenkasi Neelum and Deorakhio).

Cluster IV consisted of 16 cultivars (Atimadhuram, Puttu, Kalwa Gudda, Karigal Appe, Khuddus, Surankudi, Neeleshan, Taimur Pasand, Nalla Mamidi, Papaya Khas, Hydersaheb, Kerala Kalepad, Lal Sundari, Panakalu, Karanjio and Putu), followed by cluster IX (Balakondapari, Jehangir, H-85, Shahjahan, Himayath Pasand, Goa Kodur, Tenkasi Banganapalli, Kerali Goa, Peddarasam, Ku-8, Saru, Kutumba Appe, Shendryo and Ebatti Mavu) with 14 cultivars.

Clusters III (Almas, H-39, Padari, Panchadara Kalasa, Manibhatta Appe, Santhoor Collection, Pulihora, Hajeera, Pacharasi, Jawahar, Kalapara, Ananas), VIII (Chethalli, Lord, Harsha, Magemavu, Malai Misri, Mugalimelogra, Ruswani, Sushanbhog, Kalami Hindustani) and I (Adderi Jeerige, Jeerige Neermavu, Huli Appekai, Naagarappe, Hithalahalli Appe, Coorg Collection, Khas-ul-Khas, Chandrakaran) consisted of 12, 9 and 8 cultivars, respectively.

Clusters X (Black Andrews, Hansraj, Warale Gidaga, Amini, Papayaraju Goa, Tatamdi) and XI (Gaddemar, Lahara, Hamlet, Thambva) consisted of 6 and 4 cultivars each, followed by clusters XII (Katta Gola, Makaram, Maharaja of Mysore) and XIII (Khudadath, Sora, Safed Mulgoa) with 3 cultivars in each group.

#### **4.1.4.5 Principal component analysis**

The principal components, eigen values, per cent variability, cumulative per cent of variability and factor loading of different morphological and biochemical characters studied are furnished in Table 4.1.10. The principal components with eigen values less than one were considered as non-significant as per the procedure. In the present investigation, the first four principal components with eigen values more than one contributed 70.77 per cent of cumulative variability among the 156 cultivars of mango evaluated for 15 morphological and bio-chemical characters.

The first principal component (PC I) contributed highest variability (42.36%). The character loading values for principal components represented the weights defining the

contribution of different characters for the respective principal components. Characters like fruit weight (0.385), fruit width (0.347), fruit length (0.326), skin weight (0.206), total phenols (0.167), fruit thickness (0.105), titratable acidity (0.054) and ascorbic acid (0.026) coupled with negative loadings for pulp weight (-0.351), stone weight (-0.348), pulp per cent (-0.303), total carotenoids (-0.239), skin thickness (-0.238), total flavonoids (-0.230) and TSS (-0.191), in decreasing order of the elements, explained about variability in the first principal component (Table 4.1.11).

The second principal component, contributing 11.09 per cent of variability, showed high positive loadings for fruit thickness (0.268), pulp per cent (0.220), skin weight (0.175), fruit length (0.069), total carotenoids (0.057), TSS (0.045), fruit weight (0.020), skin thickness (0.017) and fruit width (0.002) were noted to explain the maximum variability, while the characters, ascorbic acid (-0.552), total phenols (-0.511), acidity (-0.451), total flavonoids (-0.207), pulp weight (-0.159) and stone weight (-0.052) contributed negatively towards divergence.

The third principal component explained 10.27 per cent variability and showed high positive correlation for TSS (0.435), ascorbic acid (0.260), total carotenoids (0.190), fruit length (0.147), fruit weight (0.085), pulp weight (0.063) and stone weight (0.016), while the characters, fruit thickness (-0.520), total flavonoids (-0.446), acidity (-0.272), pulp per cent (-0.230), total phenols (-0.210), skin thickness (-0.147), skin weight (-0.103) and fruit width (-0.037) contributed negatively towards divergence.

The fourth principal component (7.05% variability) showed high positive loadings for skin thickness (0.283), fruit thickness (0.243), fruit width (0.191), pulp weight (0.100), ascorbic acid (0.094), fruit weight (0.058) and acidity (0.024). The characters which contributed negatively towards divergence were skin weight (-0.656), TSS (-0.316), total phenols (-0.315), pulp per cent (-0.295), total flavonoids (-0.246), fruit length (-0.154), stone weight (-0.028) and total carotenoids (-0.026).

The fifth principal component (6.26% variability) had total phenols (0.317) contribute positively to the diversity followed by skin thickness (0.313), fruit thickness (0.289), ascorbic acid (0.288), TSS (0.287), total flavonoids (0.195), total carotenoids (0.182), fruit width (0.178), skin weight (0.029) and fruit weight (0.014). The characters, acidity (-0.555), fruit length (-0.236), stone weight (-0.236), pulp weight (-0.146) and pulp per cent (-0.091) contributed negatively towards divergence. A three dimensional plot was illustrated in Fig. 4.1.3 depicting the spatial distribution of 156 mango cultivars by using principal component analysis (PCA)

**Table 4.1.10 Eigen values, per cent variability and cumulative variability for principal components of morphological and bio-chemical characters in mango**

<b>Principal component</b>	<b>Eigene Value (Root)</b>	<b>Per cent variation extracted.</b>	<b>Cumulative variation explained</b>
1 Vector	6.35	42.36	42.36
2 Vector	1.66	11.09	53.45
3 Vector	1.54	10.27	63.72
4 Vector	1.06	7.05	70.77
5 Vector	0.94	6.26	77.03

**Table 4.1.11 Character loading of principal components for morphological and bio-chemical characters in mango**

	<b>1 Vector</b>	<b>2 Vector</b>	<b>3 Vector</b>	<b>4 Vector</b>	<b>5 Vector</b>
Fruit weight	0.385	0.020	0.085	0.058	0.014
Fruit thickness	0.105	0.268	-0.520	0.243	0.289
Fruit length	0.326	0.069	0.147	-0.154	-0.236
Fruit width	0.347	0.002	-0.037	0.191	0.178
Skin weight	0.206	0.175	-0.103	-0.656	0.029
Skin thickness	-0.238	0.017	-0.147	0.283	0.313
Stone weight	-0.348	-0.052	0.016	-0.028	-0.236
Pulp weight	-0.351	-0.159	0.063	0.100	-0.146
Pulp %	-0.303	0.220	-0.230	-0.295	-0.091
TSS	-0.191	0.045	0.435	-0.316	0.287
Titrateable acidity	0.054	-0.451	-0.272	0.024	-0.555
Total carotenoids	-0.239	0.057	0.190	-0.026	0.182
Ascorbic acid	0.026	-0.552	0.260	0.094	0.288
Total phenols	0.167	-0.511	-0.210	-0.315	0.317
Total flavonoids	-0.230	-0.207	-0.446	-0.246	0.195

### 3D Plot

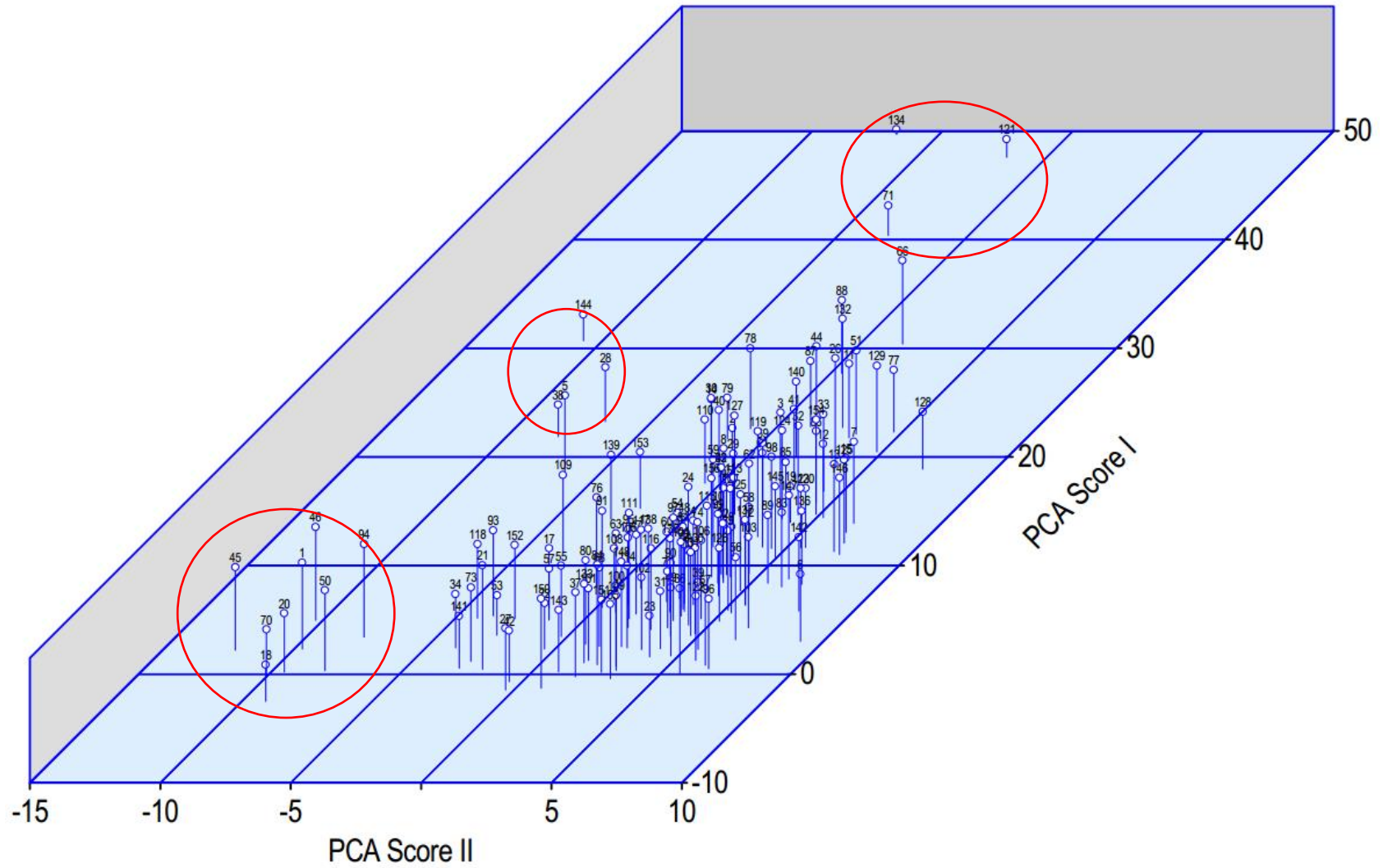


Fig. 4.1.3 Relative position of mango cultivars based on PCA scores of morphological and bio-chemical characters (3-D)

score. The wider distribution of cultivars indicated the presence of considerable diversity in the cultivars.

## **4.2 Characterization of the genotypes using molecular tools**

Molecular diversity among 156 mango genotypes was studied using SSR markers. The DNA isolated from young healthy leaves using the modified CTAB method yielded good amount of DNA of relatively high purity, suitable for PCR amplification. The twelve selected SSR primers amplified alleles across the 156 genotypes showing varied levels of polymorphism. High level of polymorphism was observed with MiKVR 965 primer. The SSR primers were labelled with four different dyes, namely, FAM, PET, NED and VIC at their 5' ends and used as probes.

### **4.2.1 Polymorphic information content (PIC) of SSR marker analysis**

The analysis of 12 SSR markers revealed that the PCR product size (bp) ranged from 107 (Mi IIHR-23) to 301 (MiMRD-273) in 156 mango genotypes. It resulted in the detection of alleles, with an average of alleles/SSRs, ranging from 23 alleles in MiIIHR-30 to 105 alleles/SSRs in MiKVR-965. The allele size ranged from 107-156 bp in Mi IIHR-23 and from 241–301 bp in MiMRD-273. The expected heterozygosity ranged from 0.885 in MiIIHR-18 to 0.990 in MiKVR-965. The observed heterozygosity ranged from 0.545 in Mi IIHR-18 to 0.795 in MiKVR-642, indicating high polymorphism. The polymorphic information content (PIC) value was maximum (0.987) in MiKVR-965 and minimum (0.877) in MiIIHR-18 (Table 4.2.1).

### **4.2.2 Diversity analysis**

Genetic diversity tree was constructed by Neighbour joining method using Darwin software (Fig. 4.2.1). The genotypes were grouped into three major clusters; cluster I consisting of 67 number of genotypes. Further, cluster I is sub-divided into four sub-clusters (Ia, Ib, Ic and Id) consisting of 21, 22, 14 and 10 progenies, respectively. Cluster Ia consisted of 21 genotypes (Magemavu, Kalneelum, Coorg Collection, Sharbathi Bagri, Jeerige Neermavu, Dofasla, Tenkasi Neelum, Maharaja of Mysore, Surankudi, Apple Rumani, Kutumba Appe, Naagarappe, Kaju, Shahjahan, Karigal Appe, Tephala, Sushanbhog, Thorappadi, Kalami Hindustani, Whiteseri and Vinayaka Hegde), whereas, cluster Ib consisted of 22 genotypes (Virudhanagar, Safeda Lucknow, Ruswani, Hathizul, H-39, Mugalimelogra, Blackseri, Kerala Kalepad, Gaddemar, Salem Bangalora, Himam Pasand, Yakutti, Almas, Sai Sugandh, Nuha, Warale Gidaga, Thali, Santhoor Collection, Safed Mulgoa, Ramphalya, Ku-8 and Hajeera).

Clusters Ic and Id comprised of 14 (PKM-2, Goa Kodur, Thambva, Lal Muni, Tenkasi Banganapalli, Makaram, Nalla Mamidi, Balakondapari, Katta Gola, H-85, Panchavarnam, H-151, Harsha and Hamsa Mamidi) and 10 (Willard, Gidagana Mavu, Krishna, Lahara, Kalgundi Koppa Appe, Papaya Khas, Lord, Kottur Konam, KMH-1 and Deorakhio) genotypes, respectively.

Cluster II consisted of 7 genotypes which were further sub-divided into two clusters (IIa and IIb) consisting of 3 (Thogarapalli, Karkan Rumani and Taimur Pasand) and 4 (Royal Special, Padari, Kempikunde and Kalkand) genotypes, respectively.

Cluster III comprised of the remaining 84 genotypes, sub-clustered into 7 groups. Cluster IIIa consisted of 6 genotypes (Lemon, Vhout, Gulab\_Khas, Hamlet, Kintalavenipeta and Lal Sundari), cluster IIIb of 15 genotypes (Suvarnarekha, Papayaraju Goa, Lalpairi, Peter, Totapuri, Bangalore Sindhura, Sampar Totapuri, Jawahar, Puttu, Putu, Aryavarthana Rasala, Kalapara, Chethalli, Ananas and Mohammada Vikarabad), cluster IIIc of 11 genotypes (Asiquot, Haleema, Tatamdi, Kerali Goa, Allampur Baneshan, Himayath Pasand, Irugupalli Tokkukaya, Manibhatta Appe, Rumani, Atimadhuram and K-O-7), while cluster IIId comprised of 11 genotypes (Agarbathi, Elaichi, Dori, Pulihora, Saru, Siroli, Malai Misri, Khuddus, Licthi, Khudadath and Navneet).

The sub-clusters IIIe, IIIf and IIIg consisted of 23 (Chandrakaran, Vellaikulamban, Muvandan, Olour, Nekkare, Vattam, Narayana Sheni, Kalapadi, Panakalu, Karanjio, Sardar, Shendriya, Black Andrews, Hansraj, Bombaypeda, PKM-1, Jehangir, Panchadara Kalasa, Chandanum, Ebatti Mavu, Pacharasi, Kalwa Gudda and Shendryo), 4 (Adderi Jeerige, Huli Appikai, Hithalahalli Appe and Narela) and 12 (Creeping, Gulabi, Dwarf Rumani, Neeleshan, Kasturi Mamidi, Hydersaheb, Kari Ishad, Khas-UI-Khas, Sora, Peddarasam, Amini and Banganapalli) genotypes, respectively.

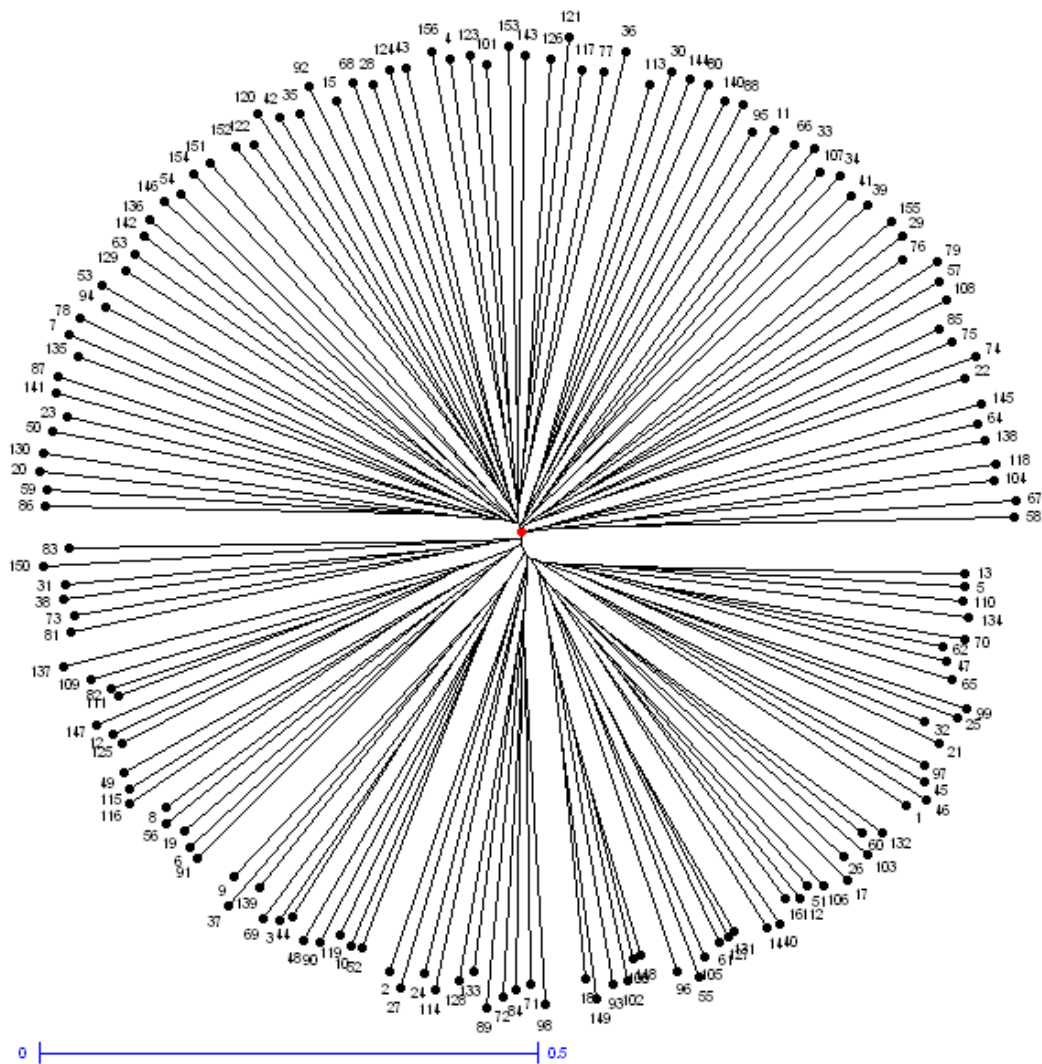
#### **4.3 Attempting marker assisted selections in the progeny population**

Molecular characterization for forty-two mango hybrids and their parents was carried out using eight SSR markers *viz.*, MiIIHR 17, MiIIHR 18, MiIIHR 23, MiIIHR 26, MiIIHR 30, MiIIHR 31, MiIIHR 34 and MiIIHR 36 developed by Ravishankar *et al.* (2011). A glimpse of the results (Table 4.3.1) reveal that the observed heterozygosity ( $H_0$ ) ranged from 0.898 to 0.980, while, expected heterozygosity ( $H_e$ ) ranged from 0.874 to 0.906 in mango hybrids and parents. All the 8 SSR markers were found to be informative primers as their calculated polymorphic information content (PIC) values varied from 0.850 to 0.888. The maximum PIC value was noted

**Table 4.2.1 List of SSR primers and their characteristics based on genetic analysis of 156 mango cultivars**

Sl.No.	Primer No.	Primers (5'-3') details	Allele Size (bp)	k	Ho	He	PIC
1.	MiIHR 23	F: TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC	107-156	43	0.622	0.955	0.950
2.	MiIHR 17	F: GCTTGCTTCCAAGTGGAGACC R: GCAAAATGCTCGGAGAAGAC	230-269	35	0.705	0.937	0.930
3.	MiIHR 18	F: TCTGACGTCACCTCCTTTCA R: ATACTCGTGCCTCGTCCTGT	148-193	42	0.545	0.885	0.877
4.	MiIHR 30	F: AGCTATCGCCACAGCAAATC R: GTCTTCTTCTGGCTGCCAAC	190-213	23	0.763	0.936	0.929
5.	MiIHR 31	F: TTCTGTTAGTGGCGGTGTTG R: CACCTCCTCCTCCTCCTCTT	207-260	44	0.731	0.913	0.906
6.	MiIHR 26	F: GCGAAAGAGGAGAGTGCAAG R: TCTATAAGTGCCCCCTCACG	127-171	40	0.686	0.962	0.957
7.	MiIHR 34	F: CTGAGTTTGGCAAGGGAGAG R: TTGATCCTTACCACCATCA	203-245	38	0.679	0.924	0.917
8.	MiIHR 36	F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAAGTAG	210-250	36	0.699	0.929	0.923
9.	MiKVR 965	F: GAAACCCCTAAGAGGGAAAA R: ACGCAACTTTGGTTTTGGAA	172-294	105	0.641	0.990	0.987
10.	MiMRD 273	F: ACTGGCTAGAAAGCAACACAA R: GGCAAGATTCAAAGCGAGAG	241-301	61	0.673	0.981	0.977
11.	MiKVR 642	F: TGCATGTGCCTATCCATCTC R: GCACGTGCAAAATTGTTATTG	158-221	62	0.795	0.983	0.979
12.	MiMRD 369	F: CCAGGTTATAGCAGCCAAGC R: TAAGGTTGCCAAACTGGACC	200-273	71	0.692	0.983	0.979
	Mean			50.00	0.6859	0.9482	0.9426
	Range			23-105	0.545-0.795	0.885-0.990	0.877-0.987

k= Number of alleles per locus, Ho= Observed heterozygosity, He= Expected heterozygosity, PIC= Polymorphic information content



**Fig. 4.2.1 Dendrogram of mango cultivars based on SSR markers**

- |                   |                  |                     |                   |                      |                     |
|-------------------|------------------|---------------------|-------------------|----------------------|---------------------|
| 1. A. Jeerige     | 27. Elaichi      | 53. K. Hindustani   | 79. Lahara        | 105. Panakalu        | 131. Shendriya      |
| 2. Agarbathi      | 28. Gaddemar     | 54. Kalapadi        | 80. Lal Muni      | 106. P. Kalasa       | 132. Shendryo       |
| 3. A. Baneshan    | 29. G. Mavu      | 55. Kalapara        | 81. Lal Sundari   | 107. Panchavarnam    | 133. Siroli         |
| 4. Almas          | 30. Goa Kodur    | 56. K. K. Appe      | 82. Lalpairi      | 108. Papaya Khas     | 134. Sora           |
| 5. Amini          | 31. Gulabi       | 57. Kalkand         | 83. Lemon         | 109. P. Goa          | 135. Surankudi      |
| 6. Ananas         | 32. Gulab Khas   | 58. Kalneelum       | 84. Litchi        | 110. Peddarasam      | 136. Sushanbhog     |
| 7. A. Rumani      | 33. Hajeera      | 59. Kalwa Gudda     | 85. Lord          | 111. Peter           | 137. Suvarnarekha   |
| 8. A. Rasala      | 34. Haleema      | 60. Karanjio        | 86. Magemavu      | 112. Pkm -1          | 138. T. Pasand      |
| 9. Asiquot        | 35. Hamlet       | 61. Karigal Appe    | 87. M. Mysore     | 113. Pkm -2          | 139. Tatamdi        |
| 10. Atimadhuram   | 36. H. Mamidi    | 62. Kari Ishad      | 88. Makaram       | 114. Pulihora        | 140. Tenkasi B.     |
| 11. Balakondapari | 37. Hansraj      | 63. Karkan Rumani   | 89. Malai Misri   | 115. Puttu           | 141. T. Neelum      |
| 12. B. Sindhura   | 38. Harsha       | 64. Kasturi Mamidi  | 90. M. Appe       | 116. Putu            | 142. Tephala        |
| 13. Banganapalli  | 39. Hathizul     | 65. Katta Gola      | 91. M. Vikarabad  | 117. Ramphalya       | 143. Thali          |
| 14. B. Andrews    | 40. H. Pasand    | 66. Kempikunde      | 92. Mugalimelogra | 118. Royal Special   | 144. Thambva        |
| 15. Blackseri     | 41. H. Pasand    | 67. Kerala Kalepad  | 93. Muvandan      | 119. Rumani x Neelum | 145. Thorapalli     |
| 16. Bombaypeda    | 42. H. Appe      | 68. Kerali Goa      | 94. Naagarappe    | 120. Ruswani         | 146. Thorappadi     |
| 17. Chandanum     | 43. H. Appekai   | 69. Khas-Ul-Khas    | 95. Nalla Mamidi  | 121. Safed Mulgoa    | 147. Totapuri       |
| 18. Chandrakaran  | 44. Hydersaheb   | 70. Khudadath       | 96. N. Sheni      | 122. Safeda Lucknow  | 148. Vattam         |
| 19. Chethalli     | 45. H-85         | 71. Khuddus         | 97. Narela        | 123. Sai Sugandh     | 149. Vellaikulamban |
| 20. C. Collection | 46. H-151        | 72. Kintalavenipeta | 98. Navneet       | 124. Salem Bangalora | 150. Vhout          |
| 21. Creeping      | 47. H-39         | 73. Kmh 1           | 99. Neeleshan     | 125. Sampar Totapuri | 151. V. Hegde       |
| 22. Deorakhio     | 48. I. Tokkukaya | 74. K-O-7           | 100. Nekkare      | 126. S. Collection   | 152. Virudhanagar   |
| 23. Dofasla       | 49. Jawahar      | 75. Kottur Konam    | 101. Nuha         | 127. Sardar          | 153. Warale gidaga  |
| 24. Dori          | 50. J. Neermavu  | 76. Krishna         | 102. Olour        | 128. Saru            | 154. Whiteseri      |
| 25. D. Rumani     | 51. Jehangir     | 77. Ku-8            | 103. Pacharasi    | 129. Shahjahan       | 155. Willard        |
| 26. E. Mavu       | 52. Kaju         | 78. Kutumba Appe    | 104. Padari       | 130. Sharbathi Bagri | 156. Yakutti        |

**Table 4.3.1 List of SSR primers and their characteristics based on genetic analysis of mango hybrids and parents**

Sl.No.	Primer No.	Primers (5'-3') details	Allele Size (bp)	k	Ho	He	PIC
1.	MiIHR 23	F: TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC	107-156	21	0.898	0.875	0.853
2.	MiIHR 17	F: GCTTGCTTCCAAGTGGAGACC R: GCAAATGCTCGGAGAAGAC	230-269	24	0.959	0.899	0.881
3.	MiIHR 18	F: TCTGACGTCACCTCCTTTCA R: AACTCGTGCCTCGTCCTGT	148-193	20	0.898	0.882	0.860
4.	MiIHR 30	F: AGCTATCGCCACAGCAAATC R: GTCTTCTTCTGGCTGCCAAC	190-213	17	0.980	0.888	0.868
5.	MiIHR 31	F: TTCTGTTAGTGGCGGTGTTG R: CACCTCCTCCTCCTCCTCTT	207-260	21	0.980	0.874	0.850
6.	MiIHR 26	F: GCGAAAGAGGAGAGTGCAAG R: TCTATAAGTGCCCCCTCACG	127-171	21	0.978	0.882	0.860
7.	MiIHR 34	F: CTGAGTTTGGCAAGGGAGAG R: TTGATCCTTACCACCATCA	203-245	22	0.959	0.879	0.856
8.	MiIHR 36	F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAGTAG	210-250	24	0.980	0.906	0.888
	Mean			21.25	0.954	0.885	0.864
	Range			17-24	0.898-0.980	0.874-0.906	0.850-0.888

k= Number of alleles per locus, Ho= Observed heterozygosity, He= Expected heterozygosity, PIC= Polymorphic information content

in MiIHR 36 (0.888) and the minimum in MiIHR 31 (0.850). The number of alleles per locus ranged from 17 (MiIHR 30) to 24 (MiIHR 17 and MiIHR 36), with an average of 21.25.

Table 4.3.2 shows the results of paternity analysis of the 42 mango hybrids using Cervus 3.0. Simulation of parentage analysis was done for likelihoods using the corrected likelihood equations of Kalinowski *et al.* (2007). LOD score was calculated for candidate parent, and out of 21 hybrids involving crosses between Amrapali × Vanraj, it was possible to assign parentage for 12 hybrids with strict confidence (95%) and 5 with relaxed confidence (80%). Five of the hybrids were unassigned as the candidate parent is not most likely its parent.

Among the hybrids developed from crosses between Amrapali × *M. odorata*, 6 of the hybrids were assigned parentage with strict confidence (95%), while 7 were done so with relaxed confidence (80%). Four of the hybrids were unassigned as the candidate parent is not most likely its parent.

The most likely candidate parent is the one with the highest (most positive) LOD score. A negative LOD score means that the candidate parent is less likely to be the true parent. Negative LOD scores can occur when the candidate parent and offspring share very common alleles at every locus. More commonly, negative LOD scores indicate that a candidate parent mismatches at one or more loci.

For a most likely candidate parent, the confidence of parentage assignment is shown: \* for strict confidence (95%) or + for relaxed confidence (80%). - is shown for most likely candidate parent, but not assigned confidence. If the candidate parent is not most likely, this column will be blank. In this study nine hybrids were left unassigned. One possible reason for this may be that the true parents were not sampled for these hybrids or otherwise there may be need of additional loci for assigning parentage with strict confidence.

#### **4.4 Estimation of volatiles in certain mango progenies and their parents**

Mango leaf volatile fragments were assessed through GC-MS/MS in some hybrids and their parents. Principal component analysis and cluster analysis were also carried out to see the components which were mainly contributing to specific aroma of the cultivar and their relative grouping.

#### 4.4.1 Leaf volatile aroma composition

The leaf aroma components of 39 mango hybrids and their parents were determined independently with HS-SPME-GC-MS/MS technique (Plate 4.4.1). The chemical diversity of the leaves of 25 mango hybrids and their parents were analysed, wherein 77 different volatile constituents were detected (Table 4.4.1), which consists of different chemical classes, namely, hydrocarbons, alcohols, aldehydes and ketones, monoterpenoids and sesquiterpenoids. Four compounds, namely, 'Benzene, 1-ethyl-4-methoxy', 'Hexane, 1-(hexyloxy)-5-methyl', 'Caryophyllene oxide' and '1,5,5,8-Tetramethyl-12-oxabicyclo [9.1.0] dodeca-3,7-diene' were placed under 'miscellaneous' category in this analysis. Within this set of cultivars, H-85 contained the highest number of volatiles (67), followed by Ratna, Rumani and H-151, each containing 66 number of volatiles. Sindhu contained the least (52) number of volatiles followed by Manjeera, Swarna Jehangir and Suvarnarekha with 54 each.

#### 4.4.2 Genotypic variability in leaf volatile composition

The results revealed that, mango leaves were rich in volatile components and the genotype Jehangir (125.88 ng/g) had the highest concentration of leaf volatiles followed by Arka Anmol and Rumani with 123.41 and 106.20 ng/g, respectively. In contrast, Neelam had the lowest (17.88 ng/g) volatile components followed by Suvarnarekha (18.27 ng/g) and Kalapadi (18.80 ng/g).

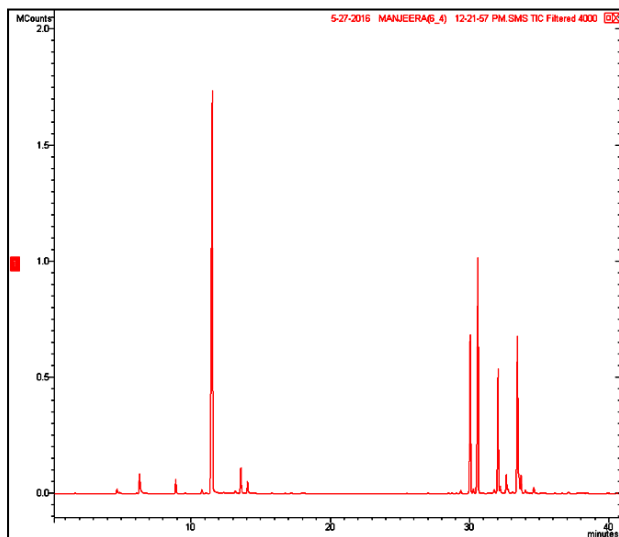
Significant differences in the composition and proportion of leaf volatile components were observed among the 39 mango hybrids and their parents. In all the 39 cultivars, monoterpenoids and sesquiterpenoids dominated the compound profiles, being represented in all the cultivars (Table 4.4.1). The genotype Fazli had the highest per cent monoterpenoids proportion (65.87%) followed by Alphonso (59.56%) and Vanraj (57.39%). The least monoterpenoids proportion was observed in genotype Rumani (4.10%), followed by Amrapali and Mulgoa with 5.85 and 6.13%, respectively.

Sesquiterpenoids, the other major group of compounds showed significant differences among the 39 genotypes. The highest per cent of sesquiterpenoids composition was observed in genotype Rumani (91.48%), followed by H-151 (90.17%). The least content was noticed in genotype Dashehari (26.22%), followed by Alphonso (34.16%) and Vanraj (34.40%)

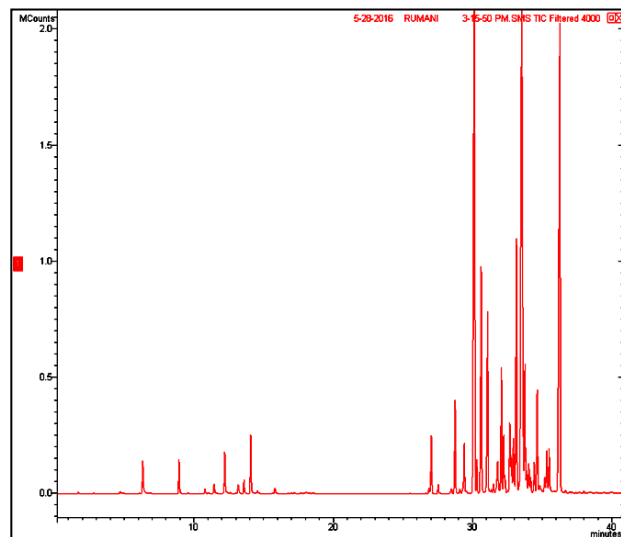
Among the monoterpenoids, cis-ocimene (7.22%), 3-carene (5.33%) and dl-limonene (4.52%) contributed higher proportion to leaf volatiles (Table 4.4.2), whereas, in case of sesqui

**Table 4.3.2 Summary of parentage analysis of 42 mango hybrids obtained with Cervus 3.0**

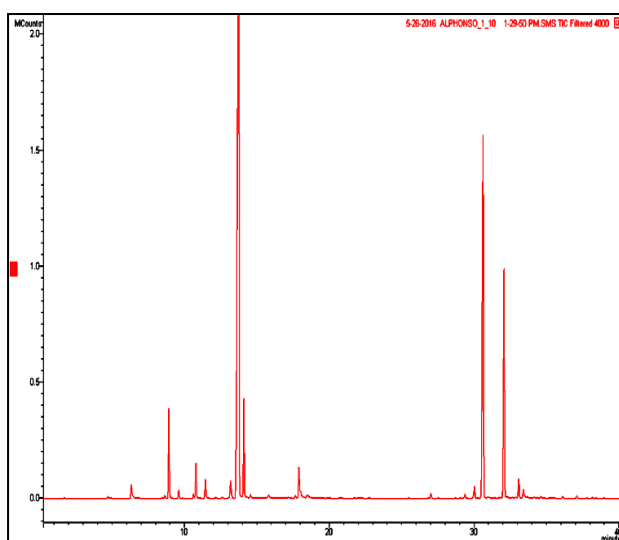
Offspring ID	Candidate father ID	Loci typed	Pair loci compared	Pair loci mismatching	Pair LOD score	Pair confidence
<b>Amrapalli × Vanraj</b>						
AV 1-14	Vanraj	8	8	1	1.89E+00	-
AV 1-16	-	8	8	2	-3.47E+00	
AV 2-65	Vanraj	8	8	0	6.06E+00	+
AV 2-67	Vanraj	8	8	0	6.28E+00	+
AV 2-68	Vanraj	8	8	0	5.26E+00	+
AV 2-69	Vanraj	8	8	0	6.80E+00	*
AV 2-70	-	8	8	6	-2.29E+01	
AV 2-71	Vanraj	8	8	0	7.39E+00	*
AV 2-72	Vanraj	8	8	0	6.77E+00	*
AV 2-73	Vanraj	8	8	0	7.37E+00	*
AV 2-74	Vanraj	8	8	0	6.80E+00	*
AV 2-75	Vanraj	8	8	0	6.81E+00	*
AV 2-76	-	8	8	2	-2.72E+00	
AV 3-1	Vanraj	8	8	0	6.95E+00	*
AV 3-2	Vanraj	8	8	0	6.70E+00	*
AV 3-3	Vanraj	8	8	0	6.09E+00	+
AV 3-4	-	8	8	4	-1.20E+01	
AV 3-5	Vanraj	8	8	1	3.12E+00	-
AV 3-6	Vanraj	8	8	0	6.03E+00	+
AV 3-7	-	8	8	4	-1.40E+01	
AV 3-8	Vanraj	8	8	1	2.54E+00	-
<b>Amrapalli × <i>M. odorata</i></b>						
AO 1-4	<i>M. odorata</i>	8	8	0	6.99E+00	*
AO 1-5	<i>M. odorata</i>	8	8	0	5.79E+00	+
AO 1-6	-	8	8	4	-1.38E+01	
AO 1-57	<i>M. odorata</i>	8	8	0	7.38E+00	*
AO 1-60	<i>M. odorata</i>	8	8	0	5.08E+00	+
AO 1-61	<i>M. odorata</i>	8	8	0	4.99E+00	+
AO 1-62	-	8	8	2	-2.76E+00	
AO 1-63	<i>M. odorata</i>	8	8	0	4.96E+00	+
AO 1-64	<i>M. odorata</i>	8	8	0	7.47E+00	*
AO 1-70	<i>M. odorata</i>	8	8	0	7.91E+00	*
AO 1-71	<i>M. odorata</i>	8	8	1	2.24E+00	-
AO 1-72	-	8	8	2	-5.41E+00	
AO 1-73	<i>M. odorata</i>	8	8	0	7.62E+00	*
AO 1-74	<i>M. odorata</i>	8	8	0	6.96E+00	*
AO 1-75	<i>M. odorata</i>	8	8	0	6.33E+00	+
AO 1-76	<i>M. odorata</i>	8	8	1	1.71E+00	-
AO 2-2	<i>M. odorata</i>	8	8	0	5.86E+00	+
AO 2-6	<i>M. odorata</i>	8	8	1	1.52E+00	-
AO 2-7	<i>M. odorata</i>	8	8	0	4.93E+00	+
AO 2-8	-	8	8	2	-3.25E+00	
AO 7-5	<i>M. odorata</i>	8	8	1	1.82E+00	-



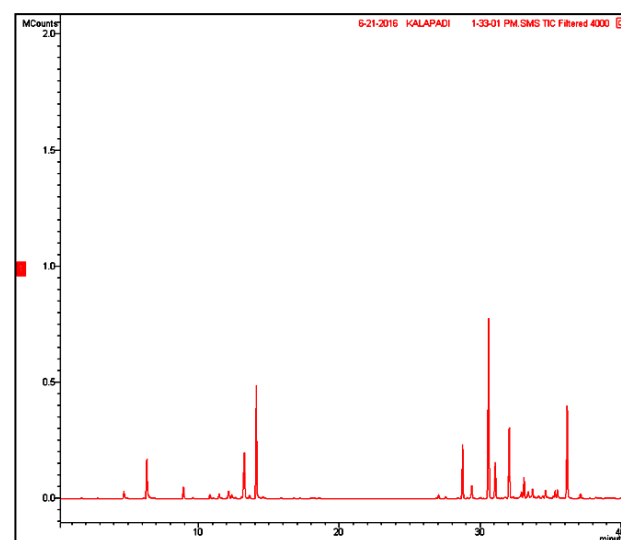
**1. Manjeera**



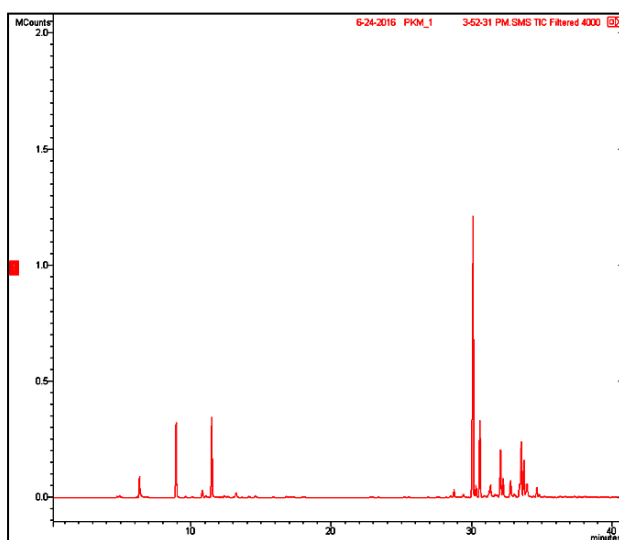
**2. Rumani**



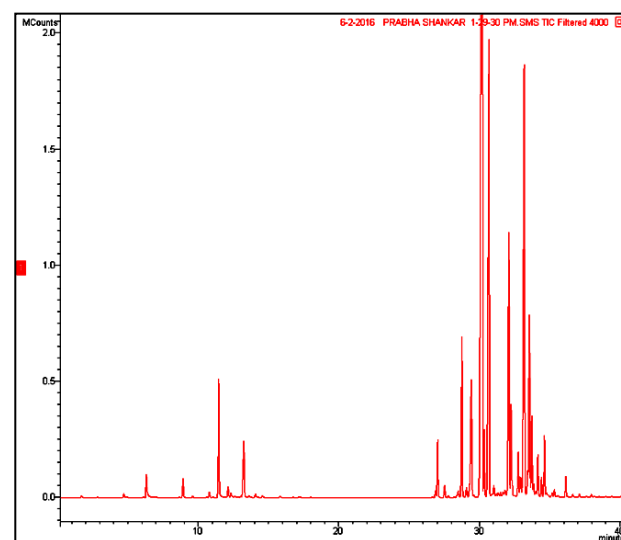
**3. Alphonso**



**4. Kalapadi**



**5. PKM-1**



**6. Prabha Shankar**

**Plate 4.4.1 Leaf volatile chromatogram of mango hybrids and parents**

**Table 4.4.1 Volatile compounds (%) in mango hybrids and their parents**

RT		Alampur Baneshan	Alfazli	Alphonso	Ambika	Amrapalli	Arka Anmol	Arka Aruna	Arka Neelkiran	Arka Puneet	Arka Udaya	Arunika	Au Rumani	Banganapalli
	<b>HYDROCARBONS</b>													
1.710	Cyclopentene	0.011	0.026	0.044	0.016	N.D	0.014	0.009	0.013	0.023	0.056	0.013	0.039	0.007
2.826	Furan, 2-Ethyl	0.005	0.004	0.015	0.007	0.031	0.003	0.001	0.008	N.D	0.044	0.005	N.D	0.007
3.965	Toluene	N.D	0.005	0.008	0.001	N.D	0.002	0.005	0.003	0.016	0.009	N.D	0.024	0.001
8.472	3-Methyl-apopinene	0.002	0.017	0.039	0.007	0.019	0.008	0.010	0.005	0.002	0.024	0.018	0.002	0.001
17.657	1,2-Dimethyl-3-vinyl-1,4-cyclohexadiene	0.002	N.D	0.117	N.D	0.031	0.012	0.013	0.092	0.090	N.D	N.D	0.123	0.016
17.187	2-methyl-2-bornene	0.006	0.033	0.051	0.030	0.040	0.017	0.014	0.007	0.036	0.008	0.011	0.003	0.026
12.390	Cyclopentene, 1-pentyl-	0.011	N.D	0.003	0.121	0.014	0.002	0.027	N.D	0.028	0.059	0.019	0.052	0.040
16.288	Benzene, 1-ethenyl-4-ethyl-	0.002	0.034	0.020	0.043	0.007	N.D	N.D	N.D	0.031	0.005	0.008	0.065	N.D
13.079	Benzene, 2-ethyl-1,3-dimethyl-	N.D	N.D	0.008	0.031	N.D	0.005	0.022	0.008	N.D	0.046	N.D	N.D	0.170
35.162	Naphthalene, 1,2,3-	0.026	0.015	0.004	0.009	0.050	N.D	0.009	N.D	0.016	N.D	0.027	0.015	0.013
35.620	Bicyclo[3.3.1]nonane, 1-phenyl-	N.D	0.008	N.D	N.D	0.066	N.D	N.D	N.D	0.023	N.D	0.008	0.004	N.D
		<b>0.065</b>	<b>0.143</b>	<b>0.309</b>	<b>0.264</b>	<b>0.258</b>	<b>0.063</b>	<b>0.112</b>	<b>0.135</b>	<b>0.266</b>	<b>0.251</b>	<b>0.109</b>	<b>0.327</b>	<b>0.281</b>
	<b>ALCOHOLS</b>													
6.142	3-Hexen-1-ol	0.008	0.010	0.013	0.009	0.006	0.006	0.007	0.008	0.010	0.038	0.009	0.011	0.010
16.989	3-Nonen-1-ol, (Z)-	N.D	N.D	0.002	N.D	N.D	0.001	0.005	0.022	0.007	0.007	0.002	0.011	0.003
37.029	Spathulenol	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
37.285	Viridiflorol	0.031	0.001	N.D	N.D	0.051	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.005
37.616	Agarupiol	0.044	0.008	0.002	0.002	0.166	0.008	0.004	0.018	0.009	N.D	N.D	N.D	0.019
37.966	Ledol	0.190	0.027	0.002	N.D	0.118	0.023	N.D	0.040	0.032	0.017	N.D	0.074	0.075
37.780	Santalol	N.D	N.D	0.005	0.005	N.D	0.003	0.010	0.003	N.D	0.009	0.005	N.D	N.D
		<b>0.273</b>	<b>0.046</b>	<b>0.020</b>	<b>0.016</b>	<b>0.340</b>	<b>0.041</b>	<b>0.026</b>	<b>0.091</b>	<b>0.058</b>	<b>0.062</b>	<b>0.011</b>	<b>0.097</b>	<b>0.113</b>
	<b>ALDEHYDES &amp; KETONES</b>													
4.731	cis-3-Hexenal	0.055	0.028	0.075	0.036	0.021	0.035	0.031	0.050	0.025	0.153	0.054	0.039	0.095
6.327	2-Hexenal	0.481	1.253	0.820	0.885	0.523	0.301	0.695	0.417	0.913	2.614	0.638	1.020	0.371
		<b>0.536</b>	<b>1.280</b>	<b>0.895</b>	<b>0.921</b>	<b>0.544</b>	<b>0.336</b>	<b>0.726</b>	<b>0.467</b>	<b>0.938</b>	<b>2.767</b>	<b>0.692</b>	<b>1.059</b>	<b>0.467</b>
	<b>MONO-TERPENOIDS</b>													
8.656	$\alpha$ -Thujene	N.D	0.061	0.133	0.021	0.020	0.027	0.053	0.016	0.011	0.083	0.064	0.003	0.006
8.943	$\alpha$ -Pinene	0.259	1.991	3.647	0.636	1.637	2.608	6.652	2.318	0.244	5.308	6.255	0.280	1.727
9.611	Camphene	0.025	0.187	0.352	0.086	0.039	0.090	0.123	0.064	0.022	0.247	0.185	0.011	0.021
10.646	Sabinene	N.D	0.031	0.164	0.008	0.020	0.168	0.028	0.168	0.013	0.057	0.038	0.004	0.020
10.799	$\beta$ -Pinene	0.112	0.667	1.383	0.340	0.243	0.499	0.640	0.419	0.105	1.205	0.830	0.065	0.128
12.134	1-Phellandrene	N.D	3.035	0.049	0.497	0.072	0.027	0.292	0.050	0.029	0.022	0.082	0.044	0.026
15.643	2-Carene	N.D	0.330	N.D	0.007	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
11.453	3-Carene	20.625	3.254	0.845	1.561	0.712	0.427	0.654	0.407	0.271	15.002	0.994	0.347	0.100
15.812	$\alpha$ -Terpinene	0.018	3.588	0.139	13.927	0.048	0.050	0.135	0.040	0.008	0.149	0.206	N.D	0.023
14.095	$\beta$ -Ocimene	0.461	0.299	3.054	0.180	0.077	0.783	5.318	1.280	0.645	0.575	0.991	1.893	12.531
13.198	dl-Limonene	0.835	2.354	0.870	0.560	1.277	0.534	0.961	0.530	0.143	0.965	11.638	0.116	N.D
13.621	Cis-Ocimene	0.123	0.142	48.488	0.138	1.563	9.723	2.971	15.576	11.205	1.873	0.289	28.750	0.315
14.577	$\gamma$ -Terpinene	0.018	0.197	0.139	0.118	0.081	0.118	0.166	0.135	0.015	0.312	0.158	0.014	0.044
12.615	$\alpha$ -Terpinolene	0.009	0.314	0.075	0.443	0.060	0.052	0.070	0.059	0.006	0.161	0.068	0.004	0.014
18.500	Allo-Ocimene	N.D	N.D	0.226	0.005	N.D	0.056	0.040	0.100	0.060	N.D	0.007	0.123	0.158
		<b>22.485</b>	<b>16.450</b>	<b>59.565</b>	<b>18.523</b>	<b>5.851</b>	<b>15.162</b>	<b>18.063</b>	<b>21.162</b>	<b>12.777</b>	<b>25.959</b>	<b>21.804</b>	<b>31.653</b>	<b>14.953</b>

<b>SESQUI-TERPENOIDS</b>														
26.870	$\alpha$ -Ylangene	0.018	0.023	0.014	0.025	0.005	0.049	0.013	0.032	0.076	0.014	0.023	0.075	0.035
26.658	(+)-Sativene	N.D	0.003	N.D	N.D	0.006	N.D	N.D	N.D	0.007	N.D	0.004	N.D	N.D
27.525	$\alpha$ -Cubebene	0.035	0.047	0.014	0.016	0.067	0.034	0.024	0.039	0.107	0.019	0.095	0.048	0.044
27.029	$\delta$ -Elemene	0.062	0.295	0.223	0.387	0.032	0.662	0.206	0.185	0.977	0.189	0.362	1.070	0.295
26.444	$\alpha$ -Longipinene	0.001	N.D	N.D	N.D	0.009	0.001	N.D	0.002	N.D	N.D	N.D	N.D	N.D
27.804	Longifolene	N.D	N.D	N.D	N.D	N.D	N.D	0.004	N.D	N.D	N.D	0.021	N.D	N.D
28.729	$\alpha$ -Copaene	0.129	0.063	0.014	0.057	0.714	0.043	0.084	0.108	0.202	0.099	0.665	0.076	0.076
29.550	$\beta$ -Cubebene	N.D	0.009	N.D	0.005	0.014	N.D	0.007	N.D	0.025	N.D	0.018	0.014	N.D
28.160	Clovene	0.001	0.007	0.003	0.003	0.071	0.003	0.005	0.005	0.003	N.D	0.004	0.002	0.001
28.456	$\beta$ -Gurjunene	0.162	0.047	0.005	0.006	0.098	0.054	0.014	0.118	0.157	0.052	0.018	0.064	0.123
29.372	$\beta$ -Elemene	0.603	0.714	0.198	0.391	0.121	0.757	0.133	1.347	1.273	0.341	0.280	0.664	1.257
30.086	$\alpha$ -Gurjunene	37.136	12.329	0.589	0.033	27.490	11.316	0.082	25.411	26.528	13.939	0.059	14.134	26.451
31.020	Junipene	N.D	0.025	0.025	0.063	N.D	0.074	0.037	0.011	0.210	0.023	0.124	0.223	0.035
32.644	$\alpha$ -Guaiene	0.456	0.063	0.034	0.040	0.039	0.432	0.023	0.088	1.129	0.026	0.068	0.065	1.215
31.206	$\alpha$ -Himachalene	N.D	0.025	0.006	0.019	N.D	N.D	0.006	N.D	0.032	0.010	0.032	0.058	0.004
30.275	Cadinene	1.341	0.409	0.021	0.007	0.801	0.364	0.003	1.019	1.000	0.379	0.016	0.455	0.856
30.624	Caryophyllene	10.338	37.101	20.295	41.654	26.062	36.412	46.648	23.081	18.560	32.219	39.532	21.025	14.763
31.478	$\delta$ -Gurjunene	0.062	0.023	0.010	0.002	0.049	0.026	0.009	0.046	0.205	0.016	0.005	0.049	0.072
31.316	Aromadendrene	0.164	0.062	N.D	N.D	0.216	0.011	N.D	0.066	0.036	0.015	N.D	0.011	0.068
32.084	$\alpha$ -Humulene	6.016	20.384	11.067	N.D	15.838	19.499	27.141	13.935	10.015	16.564	23.666	12.938	7.898
30.901	Allo aromadendrene	0.052	0.032	0.009	N.D	0.131	0.003	0.014	0.042	N.D	N.D	N.D	N.D	0.017
33.088	Germacone-D	0.362	1.292	0.849	2.515	0.129	2.889	0.881	0.730	5.654	0.684	5.513	4.445	1.717
32.743	$\alpha$ -Amorphene	1.036	0.227	0.006	N.D	1.443	0.136	N.D	0.913	0.297	0.381	0.012	0.431	0.304
32.316	(+)-Epi-bicyclosquiphellandrene	N.D	N.D	0.031	0.054	N.D	N.D	0.043	N.D	0.045	N.D	0.154	0.086	N.D
31.808	$\beta$ -Selinene	0.241	0.096	0.029	0.012	0.268	0.126	0.046	0.043	0.641	0.022	0.025	0.053	0.405
33.994	Patchoulene	0.194	N.D	0.007	0.012	N.D	0.114	0.015	0.011	0.300	N.D	0.020	0.018	0.312
32.885	$\gamma$ -Muuroleone	N.D	0.058	0.038	0.109	0.093	0.067	0.133	0.016	0.232	0.021	0.194	0.194	0.065
33.412	Aciphyllene	5.762	1.620	0.302	0.118	0.920	3.843	0.265	0.170	9.725	N.D	0.066	0.084	N.D
33.526	Leden	2.320	0.379	N.D	0.009	2.924	N.D	0.000	3.090	N.D	0.992	N.D	1.290	20.021
33.844	$\alpha$ -Muuroleone	0.058	N.D	0.009	0.012	0.099	0.019	0.019	0.040	0.045	0.011	0.046	0.049	0.027
33.692	$\alpha$ -Selinene	2.459	1.079	0.061	0.192	1.692	0.705	0.165	1.027	1.537	0.301	0.138	0.592	1.997
32.255	Valencene	2.124	0.314	N.D	29.815	1.313	0.202	N.D	1.216	1.020	0.367	N.D	0.544	1.280
34.818	Calamenene	N.D	N.D	N.D	0.010	N.D	0.020	N.D	N.D	N.D	N.D	N.D	N.D	N.D
34.610	$\delta$ -Cadinene	1.078	0.377	0.074	0.162	1.170	0.384	0.170	0.358	0.964	0.121	0.543	0.364	0.927
36.131	Eremophilene	0.047	0.172	0.103	0.203	0.086	0.301	0.123	0.084	0.407	0.105	0.184	0.558	0.138
35.332	Selina-3,7(11)-diene	0.027	0.047	0.017	0.028	0.148	0.034	0.023	0.020	0.095	0.010	0.069	0.051	0.039
34.140	$\delta$ -Selinene	0.139	0.217	0.062	0.071	5.423	0.214	0.071	0.347	0.202	0.108	0.065	0.147	0.278
34.402	Gamma Cadinene	0.122	0.117	0.042	0.077	0.203	0.093	0.075	0.064	0.275	0.035	0.193	0.154	0.113
		<b>72.546</b>	<b>77.657</b>	<b>34.156</b>	<b>76.109</b>	<b>87.676</b>	<b>78.885</b>	<b>76.480</b>	<b>73.664</b>	<b>81.981</b>	<b>67.063</b>	<b>72.212</b>	<b>60.032</b>	<b>80.834</b>
<b>OTHERS</b>														
17.915	Benzene, 1-ethyl-4-methoxy-	0.010	0.034	1.877	0.012	0.084	0.363	0.051	0.313	0.294	0.115	0.011	0.680	0.150
26.148	Hexane, 1-(hexyloxy)-5-	N.D	N.D	0.008	0.003	0.025	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
37.103	Caryophyllene Oxide	0.070	0.077	0.120	0.104	0.091	0.111	0.172	0.121	0.122	0.219	0.114	0.186	0.035
38.174	1,5,5,8-Tetramethyl-12-	0.015	0.030	0.045	0.041	0.017	0.038	0.071	0.047	0.049	0.075	0.041	0.076	0.009
		<b>0.095</b>	<b>0.141</b>	<b>2.051</b>	<b>0.161</b>	<b>0.216</b>	<b>0.513</b>	<b>0.293</b>	<b>0.480</b>	<b>0.465</b>	<b>0.409</b>	<b>0.166</b>	<b>0.943</b>	<b>0.194</b>
	<b>TOTAL</b>	<b>96.000</b>	<b>95.717</b>	<b>97.000</b>	<b>96.000</b>	<b>94.885</b>	<b>95.000</b>	<b>95.740</b>	<b>96.000</b>	<b>96.486</b>	<b>96.519</b>	<b>95.000</b>	<b>94.111</b>	<b>97.000</b>



26.870	$\alpha$ -Ylangene	0.029	0.008	N.D	0.412	0.041	0.024	0.023	0.022	0.033	0.021	0.013	0.003	0.062
26.658	(+)-Sativene	0.017	N.D	N.D	N.D	0.002	N.D	0.004	N.D	N.D	N.D	N.D	N.D	0.029
27.525	$\alpha$ -Cubebene	0.444	0.150	0.012	0.367	0.055	0.111	0.095	0.028	0.275	0.011	0.032	0.008	0.142
27.029	$\delta$ -Elemene	0.614	0.153	0.009	0.021	0.477	0.186	0.362	0.163	0.542	0.023	0.086	0.028	0.826
26.444	$\alpha$ -Longipinene	N.D	N.D	N.D	0.031	0.002	0.002	N.D	0.001	N.D	0.004	N.D	N.D	0.001
27.804	Longifolene	0.086	N.D	0.018	0.017	0.001	N.D	0.021	N.D	0.010	N.D	N.D	N.D	N.D
28.729	$\alpha$ -Copaene	1.762	0.645	0.090	10.408	0.151	3.422	0.665	0.080	6.052	0.124	0.067	0.039	0.140
29.550	$\beta$ -Cubebene	0.061	N.D	0.017	N.D	N.D	N.D	0.018	N.D	N.D	N.D	N.D	N.D	N.D
28.160	Clovene	0.005	0.013	0.083	0.002	0.001	N.D	0.004	0.003	0.004	0.002	N.D	0.002	0.008
28.456	$\beta$ -Gurjunene	0.171	0.046	0.024	0.152	0.078	0.193	0.018	0.094	0.143	0.055	0.101	0.038	0.168
29.372	$\beta$ -Elemene	0.599	0.197	0.022	1.468	1.130	1.075	0.280	0.741	1.540	0.269	0.371	0.224	0.927
30.086	$\alpha$ -Gurjunene	17.606	0.069	0.105	37.666	28.077	31.609	0.059	21.501	0.108	3.058	26.541	10.691	27.977
31.020	Junipene	0.243	N.D	N.D	0.030	0.060	0.109	0.124	N.D	4.112	0.007	N.D	N.D	0.149
32.644	$\alpha$ -Guaiene	0.078	0.053	0.025	0.082	1.060	0.654	0.068	0.042	0.107	0.219	0.024	0.987	0.079
31.206	$\alpha$ -Himachalene	0.038	0.011	N.D	0.141	0.008	0.007	0.032	N.D	N.D	3.113	N.D	N.D	N.D
30.275	Cadinene	0.384	0.016	0.019	1.536	1.070	1.011	0.016	0.818	0.032	0.085	0.631	0.307	0.880
30.624	Caryophyllene	12.132	22.557	15.975	9.217	19.020	11.936	39.532	25.122	23.097	9.306	15.967	17.520	23.004
31.478	$\delta$ -Gurjunene	0.022	N.D	N.D	0.050	0.126	0.102	0.005	0.033	0.041	0.091	0.026	0.008	0.065
31.316	Aromadendrene	0.089	0.032	0.045	1.089	0.051	0.068	N.D	0.066	N.D	0.193	0.037	0.029	0.245
32.084	$\alpha$ -Humulene	5.159	14.862	8.666	4.686	11.206	5.448	23.666	14.277	9.033	6.433	7.341	8.216	15.157
30.901	Allo aromadendrene	N.D	0.027	0.216	0.118	0.015	0.009	N.D	0.022	N.D	0.028	N.D	0.027	0.022
33.088	Germacone-D	8.055	2.830	0.137	0.842	2.460	1.294	5.513	0.781	2.293	0.245	0.392	0.093	3.576
32.743	$\alpha$ -Amorphene	0.443	0.030	0.019	1.392	0.357	0.386	0.012	0.778	N.D	0.075	0.617	0.099	1.395
32.316	(+)-Epi-bicyclosesquiphellandrene	N.D	0.129	N.D	N.D	N.D	N.D	0.154	N.D	0.150	N.D	N.D	N.D	N.D
31.808	$\beta$ -Selinene	0.086	0.100	0.164	0.192	0.536	0.402	0.025	0.035	0.138	0.102	N.D	0.248	0.108
33.994	Patchoulene	N.D	N.D	N.D	0.382	0.417	0.203	0.020	0.010	N.D	0.477	0.023	0.199	0.082
32.885	$\gamma$ -Muuroleone	0.839	0.453	0.073	0.052	0.095	0.147	0.194	N.D	0.672	0.010	N.D	N.D	0.562
33.412	Aciphyllene	0.223	N.D	0.070	0.539	N.D	6.606	0.066	0.232	0.851	2.940	N.D	9.878	0.625
33.526	Leden	0.804	0.077	N.D	3.894	10.116	N.D	N.D	3.094	0.044	N.D	1.509	N.D	4.497
33.844	$\alpha$ -Muuroleone	0.028	0.077	N.D	N.D	N.D	0.053	0.046	0.049	0.109	N.D	0.012	N.D	0.143
33.692	$\alpha$ -Selinene	0.272	0.127	0.078	11.524	2.191	1.966	0.138	1.051	1.009	1.368	0.439	0.996	1.490
32.255	Valencene	0.472	0.009	N.D	2.141	1.312	1.193	N.D	0.806	0.014	0.174	0.747	0.312	1.471
34.818	Calamenene	N.D	0.065	0.023	0.091	0.009	N.D	N.D	N.D	0.111	N.D	N.D	N.D	N.D
34.610	$\delta$ -Cadinene	2.147	1.024	0.192	1.044	1.224	1.068	0.543	0.433	0.967	0.332	0.165	0.423	1.342
36.131	Eremophilene	0.233	0.083	0.010	0.006	0.224	0.127	0.184	0.118	11.612	0.028	0.064	0.011	0.599
35.332	Selina-3,7(11)-diene	0.293	0.149	0.033	0.030	0.052	0.034	0.069	0.022	0.832	0.017	0.012	0.007	0.220
34.140	$\delta$ -Selinene	0.230	0.053	0.009	0.419	0.337	0.220	0.065	0.259	0.247	N.D	0.080	0.029	0.256
34.402	Gamma Cadinene	1.002	0.473	0.083	0.130	0.171	0.159	0.193	0.082	0.320	27.916	0.035	0.014	0.587
		<b>54.666</b>	<b>44.519</b>	<b>26.218</b>	<b>90.168</b>	<b>82.133</b>	<b>69.825</b>	<b>72.212</b>	<b>70.764</b>	<b>64.496</b>	<b>56.725</b>	<b>55.331</b>	<b>50.438</b>	<b>86.836</b>
	<b>Others</b>													
17.915	Benzene, 1-ethyl-4-methoxy-	0.018	0.030	0.070	0.051	0.039	0.046	0.011	0.450	N.D	0.071	0.031	0.065	0.732
26.148	Hexane, 1-(hexyloxy)-5-	N.D	N.D	0.009	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.272
37.103	Caryophyllene Oxide	0.011	0.147	0.021	0.012	0.108	0.107	0.114	0.125	0.500	0.027	0.271	0.120	0.070
38.174	1,5,5,8-Tetramethyl-12-	0.004	N.D	N.D	N.D	0.035	0.022	0.041	0.040	0.156	0.008	0.075	0.040	0.030
		<b>0.033</b>	<b>0.177</b>	<b>0.101</b>	<b>0.063</b>	<b>0.181</b>	<b>0.175</b>	<b>0.166</b>	<b>0.616</b>	<b>0.656</b>	<b>0.106</b>	<b>0.377</b>	<b>0.225</b>	<b>1.104</b>
	TOTAL	<b>96.657</b>	<b>94.782</b>	<b>97.000</b>	<b>96.000</b>	<b>97.000</b>	<b>94.639</b>	<b>95.000</b>	<b>95.000</b>	<b>95.000</b>	<b>96.000</b>	<b>96.990</b>	<b>95.000</b>	<b>94.978</b>



26.870	$\alpha$ -Ylangene	0.048	0.154	0.002	0.022	0.048	0.147	0.133	0.017	0.092	0.011	0.006	0.004	0.015
26.658	(+)-Sativene	N.D	0.018	N.D	N.D	N.D	N.D	0.013	N.D	0.006	N.D	N.D	N.D	N.D
27.525	$\alpha$ -Cubebene	0.046	0.224	0.005	0.028	0.062	0.048	0.337	0.021	0.200	0.002	0.024	0.003	0.067
27.029	$\delta$ -Elemene	0.114	0.058	0.030	0.163	0.005	0.004	1.522	0.022	1.270	0.100	0.072	0.062	0.123
26.444	$\alpha$ -Longipinene	0.005	0.013	N.D	0.001	0.007	0.011	0.005	0.002	0.001	0.016	N.D	N.D	N.D
27.804	Longifolene	N.D	0.015	N.D	N.D	N.D	N.D	0.058	N.D	N.D	N.D	N.D	0.014	0.019
28.729	$\alpha$ -Copaene	0.351	0.841	0.027	0.080	0.963	0.514	4.564	0.058	2.130	0.008	0.049	0.028	0.460
29.550	$\beta$ -Cubebene	N.D	0.734	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.010	N.D	0.013
28.160	Clovene	N.D	0.005	0.006	0.003	0.027	0.009	N.D	0.003	N.D	N.D	0.016	N.D	0.014
28.456	$\beta$ -Gurjunene	0.067	0.301	0.001	0.094	0.233	0.184	0.155	0.098	0.171	N.D	N.D	0.004	0.065
29.372	$\beta$ -Elemene	0.555	N.D	0.249	0.741	0.437	0.610	3.227	0.848	1.095	0.146	0.114	0.118	0.478
30.086	$\alpha$ -Gurjunene	15.538	33.444	0.065	21.501	37.089	24.626	4.636	21.779	19.911	0.027	0.082	0.487	15.970
31.020	Junipene	0.052	N.D	N.D	N.D	N.D	N.D	0.296	N.D	4.628	N.D	N.D	N.D	0.096
32.644	$\alpha$ -Guaiene	1.075	0.061	1.735	0.042	0.012	0.023	0.292	0.051	1.173	0.011	0.020	0.013	0.052
31.206	$\alpha$ -Himachalene	0.252	N.D	0.005	N.D	0.232	N.D	0.053	0.006	0.011	0.011	N.D	N.D	0.049
30.275	Cadinene	0.551	1.101	0.001	0.818	1.340	0.993	15.283	0.809	0.662	N.D	0.012	0.018	0.476
30.624	Caryophyllene	7.149	7.542	15.136	25.122	9.294	18.761	18.261	21.538	5.843	31.985	35.071	37.641	6.217
31.478	$\delta$ -Gurjunene	0.050	N.D	0.008	0.033	0.047	0.031	0.100	0.030	0.179	N.D	N.D	N.D	0.019
31.316	Aromadendrene	0.034	2.147	N.D	0.066	1.025	1.551	0.071	0.074	0.047	N.D	0.047	N.D	0.017
32.084	$\alpha$ -Humulene	3.577	4.111	9.561	14.277	5.618	11.947	8.557	12.574	2.869	18.308	23.778	22.295	3.184
30.901	Allo aromadendrene	0.014	0.174	0.020	0.022	0.163	0.257	N.D	0.047	N.D	N.D	0.172	N.D	N.D
33.088	Germacrene-D	0.792	2.506	0.120	0.781	0.473	N.D	15.963	0.091	6.559	0.376	0.260	0.249	3.341
32.743	$\alpha$ -Amorphene	0.219	1.467	N.D	0.778	2.043	1.120	1.217	0.709	0.279	N.D	N.D	0.016	0.369
32.316	(+)-Epi-bicyclosesquiphellandrene	N.D	N.D	0.020	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.056
31.808	$\beta$ -Selinene	0.438	0.116	0.328	0.035	0.207	0.133	0.145	0.031	N.D	0.013	0.146	N.D	0.031
33.994	Patchoulene	0.417	N.D	0.377	0.010	1.009	0.304	N.D	0.004	0.535	N.D	N.D	0.007	0.010
32.885	$\gamma$ -Muurolene	0.025	0.348	0.102	N.D	N.D	N.D	0.435	N.D	1.093	0.014	0.140	N.D	0.082
33.412	Aciphyllene	12.742	0.624	17.821	0.232	N.D	0.390	0.167	0.113	N.D	0.036	0.243	0.387	N.D
33.526	Leden	N.D	4.753	0.000	3.094	5.561	4.109	4.600	2.320	19.730	N.D	N.D	N.D	1.333
33.844	$\alpha$ -Muurolene	N.D	N.D	N.D	0.049	N.D	N.D	0.172	0.027	0.203	N.D	N.D	0.005	0.033
33.692	$\alpha$ -Selinene	2.761	5.925	1.396	1.051	3.933	4.965	1.894	0.892	2.248	0.048	0.197	0.226	0.468
32.255	Valencene	0.726	2.056	N.D	0.806	1.821	1.520	1.912	0.928	0.986	0.041	N.D	N.D	0.555
34.818	Calamenene	0.069	0.125	N.D	N.D	0.257	0.083	N.D	N.D	N.D	N.D	N.D	N.D	N.D
34.610	$\delta$ -Cadinene	0.952	1.747	0.618	0.433	1.138	0.602	1.669	0.258	2.314	0.023	0.266	0.068	0.423
36.131	Eremophilene	0.069	0.039	0.018	0.118	0.020	0.012	0.606	0.015	15.552	0.047	0.056	0.052	0.057
35.332	Selina-3,7(11)-diene	0.022	0.182	0.017	0.022	0.052	0.017	0.207	0.007	0.785	0.005	0.062	0.005	0.039
34.140	$\delta$ -Selinene	0.097	0.135	0.027	0.259	0.040	0.107	1.110	0.197	0.223	0.073	N.D	0.081	0.139
34.402	Gamma Cadinene	0.107	0.620	0.016	0.082	0.177	0.072	0.567	0.033	0.681	0.011	0.132	0.015	0.129
		<b>48.915</b>	<b>71.587</b>	<b>47.711</b>	<b>70.764</b>	<b>73.331</b>	<b>73.151</b>	<b>88.226</b>	<b>63.602</b>	<b>91.477</b>	<b>51.312</b>	<b>60.975</b>	<b>61.796</b>	<b>34.397</b>
	<b>Others</b>													
17.915	Benzene, 1-ethyl-4-methoxy-	0.063	0.282	0.016	0.450	0.032	0.055	0.009	1.009	0.042	N.D	0.175	0.206	1.239
26.148	Hexane, 1-(hexyloxy)-5-	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.003	N.D	N.D	N.D	N.D	N.D
37.103	Caryophyllene Oxide	0.077	0.011	0.045	0.125	0.031	0.067	0.078	0.079	0.026	0.149	0.212	0.370	0.065
38.174	1,5,5,8-Tetramethyl-12-	0.018	N.D	0.018	0.040	0.057	0.047	0.023	0.026	0.010	0.056	0.106	0.156	0.025
		<b>0.158</b>	<b>0.293</b>	<b>0.078</b>	<b>0.616</b>	<b>0.119</b>	<b>0.168</b>	<b>0.109</b>	<b>1.118</b>	<b>0.077</b>	<b>0.205</b>	<b>0.494</b>	<b>0.732</b>	<b>1.328</b>
	<b>TOTAL</b>	<b>94.496</b>	<b>95.746</b>	<b>95.000</b>	<b>95.000</b>	<b>96.000</b>	<b>95.000</b>	<b>96.000</b>	<b>96.000</b>	<b>97.000</b>	<b>95.543</b>	<b>96.923</b>	<b>95.716</b>	<b>96.298</b>

**Table 4.4.2 Major monoterpenoids present in mango hybrids and parents**

Compound (%)	$\alpha$ -Pinene	I-Phellandrene	3-Carene	$\beta$ -Ocimene	dl-Limonene	Cis-Ocimene
Alampur Baneshan	0.26	N.D	20.63	0.46	0.84	0.12
Alfazli	1.99	3.03	3.25	0.30	2.35	0.14
Alphonso	3.65	0.05	0.85	3.05	0.87	48.49
Ambika	0.64	0.50	1.56	0.18	0.56	0.14
Amrapalli	1.64	0.07	0.71	0.08	1.28	1.56
Arka Anmol	2.61	0.03	0.43	0.78	0.53	9.72
Arka Aruna	6.65	0.29	0.65	5.32	0.96	2.97
Arka Neelkiran	2.32	0.05	0.41	1.28	0.53	15.58
Arka Puneet	0.24	0.03	0.27	0.64	0.14	11.21
Arka Udaya	5.31	0.02	15.00	0.57	0.96	1.87
Arunika	6.25	0.08	0.99	0.99	11.64	0.29
Au Rumani	0.28	0.04	0.35	1.89	0.12	28.75
Banganapalli	1.73	0.03	0.10	12.53	N.D	0.32
Bombay Green	2.71	1.49	0.76	0.68	1.52	0.07
Dashehari	0.89	0.11	0.59	1.66	41.98	0.08
Fazli	2.61	N.D	0.71	0.42	21.77	0.05
H-151	1.93	0.04	0.60	0.13	N.D	1.34
H-85	0.63	0.00	11.25	0.43	0.24	1.01
H-87	7.60	1.44	0.81	0.08	9.75	0.16
Jan. Pasand	2.78	0.01	7.12	0.23	0.49	0.47
Jehangir	0.70	0.01	0.29	1.37	0.11	19.90
Kalapadi	1.42	0.80	0.60	12.37	7.26	0.46
Konkan Ruchi	1.31	21.25	0.88	0.35	11.11	2.41
Mallika	5.85	0.02	2.98	0.13	23.54	1.34
Manjeera	0.88	N.D	38.28	0.91	0.21	1.62
Mulgoa	1.05	0.01	3.43	0.12	0.20	0.86
Neeleshan	6.45	0.03	31.27	1.89	1.48	0.92
Neeleshwari	3.69	0.10	0.36	15.11	0.74	0.35
Neelphonso	1.33	26.36	1.37	0.56	15.24	0.20
Neelum	12.44	0.16	18.62	0.40	1.42	0.20
Pkm-1	8.12	0.02	9.00	0.16	0.67	0.02
Pkm-2	3.37	0.02	0.33	1.85	0.42	12.63
Prabha Shankar	0.52	0.29	3.17	0.10	1.87	0.04
Ratna	3.46	0.03	0.40	1.68	0.51	23.37
Rumani	0.75	0.87	0.22	1.34	0.23	0.32
Sindhu	2.31	0.02	1.30	2.45	0.64	34.34
Suvarnarekha	5.36	0.12	14.38	0.61	1.52	2.33
Swn. Jehangir	0.25	0.00	18.05	1.02	0.10	12.11
Vanraj	1.11	0.03	0.54	3.35	0.58	50.71
<b>Mean</b>	2.86	1.57	5.33	2.25	4.52	7.22

terpenoids, caryophyllene (20.87%),  $\alpha$ -gurjunene (16.11%) and  $\alpha$ -humulene (11.47%) contributed maximum to the leaf volatiles in the genotypes studied (Table 4.4.3).

Hydrocarbons and alcohols were also represented in the entire set; however, their quantities were low in most of the cultivars. Relative concentrations of hydrocarbons ranged between 0.05% in Mulgoa to 2.17% in Fazli, while it ranged between 0.01% (Bombay Green) and 0.45% (PKM-1) in case of alcohols. Aldehydes and ketones were also detected in the present study, ranging from 0.30% in Konkan Ruchi to 6.23% in Suvarnarekha.

#### **4.4.3 Cluster analysis based on leaf volatile components**

Cluster analysis was performed based on the leaf volatile composition and proportion for major monoterpenoids and sesquiterpenoids content among 39 mango hybrids and their parents (Fig. 4.4.1), and the results revealed that cluster I consisted of genotypes Alfazli, Arka Anmol, Amrapalli, Arka Udaya, Suvarnarekha, Swn. Jehangir, Arka Aruna and Arunika, being rich in caryophyllene and  $\alpha$ -humulene. Cluster II (Fazli, Mallika and Dashehari) and cluster III (Konkan Ruchi and Neelphonso) consisted of genotypes rich in dl-limonene and l-phellandrene, respectively (Table 4.4.4).

Cluster IV (Alphonso, Sindhu, Vanraj, Arka Neelkiran, Jehangir, PKM-2, Ratna, Au Rumani, Ambika, Arka Puneet, Mulgoa, H-85, Jan. Pasand, H-1 51, Bombay Green and Rumani) and cluster V (Banganapalli, Kalapadi and Neeleshwari) consisted of cultivars rich in  $\alpha$ -gurjunene and caryophyllene. Cluster IV also showed relatively higher proportion of cis-ocimene, whereas cluster V was also rich in  $\beta$ -ocimene.

Cluster VI consisted of a single cultivar, Prabha Shankar, rich in caryophyllene and germacrene-D. The cultivars, H-87, PKM-1, Neelum, Manjeera, Neeleshan and Alampur Baneshan were grouped into a single cluster (cluster VII) with diverse proportion of  $\alpha$ -gurjunene, 3-carene and caryophyllene as major composition.

#### **4.4.4 Principal component analysis (PCA) for leaf volatiles**

The principal components, eigen values, per cent variability, cumulative per cent of variability and component loading of different leaf volatile components studied are furnished in Table 4.4.5 The principal components with eigen values less than one were considered as non-significant as per the procedure. In the present investigation, the first five principal components with eigen values more than one contributed to 74.98 per cent of cumulative variability among

the 39 cultivars of mango evaluated for leaf volatile components. Factor 1 is contributing more towards total variability having the highest eigen value of 2.437 and 22.15 per cent variability. The factors 2, 3, 4 and 5 also showed higher eigen values (1.897, 1.599, 1.283 and 1.033, respectively) and contributed positively towards total variability (17.241, 14.536, 11.665 and 9.387, respectively). The projections of the PC loadings defined by first two principal components shows 39.39% of variability and allowed us to visualize the position of different mango genotypes as shown in plot (Fig 4.4.2).

The correlation matrix for monoterpenoids and sesquiterpenoids showed the variables that mostly define six principal components (Table 4.4.6): F1 was strongly correlated with caryophyllene (0.528) and  $\alpha$ -humulene (0.519), F2 with  $\alpha$ -pinene (0.442) and 3-carene (0.407), F3 with caryophyllene (0.248) and  $\alpha$ -humulene (0.241), F4 with 3-carene (0.330) and germacrene-D (0.302), F5 with  $\beta$ -ocimene (0.641) and F6 with  $\alpha$ -pinene (0.381). The results of PCA analysis and dendrogram interpreted that the leaf caryophyllene,  $\alpha$ -humulene,  $\alpha$ -pinene, 3-carene and cis-ocimene were important compounds which clearly distinguish the variability among mango genotypes.

#### 4.4.5 Correlation between mango hybrids and parents

Based on the volatile composition, correlation analysis was carried out between the various mango hybrids and their parents. Amongst the 25 mango hybrids, 14 of them showed higher correlation with the female parent. The hybrids Alfazli, Ambika, Amrapalli, Arka Anmol, Arka Neelkiran, Au Rumani, H-85, H-87, Mallika, Manjeera, Neeleshwari, PKM-1, Sindhu, Swarna Jehangir showed greater correlation with the female parent, *viz.*, Alphonso (0.4744\*\*), Amrapali (0.2498), Dashehari (0.4179\*\*), Alphonso (0.8701\*\*), Alphonso (0.9413\*\*), Rumani (0.5606), Kalapadi (0.6243), Kalapadi (0.7825), Neelum (0.9049), Rumani (0.4881), Neelum (0.1941), Chinnasuvarnakha (0.1399), Ratna (0.3115), and Suvarnakha (0.7089), respectively (Table 4.4.7).

Eleven of the hybrids, namely, Arka Aruna, Arka Puneet, Arka Udaya, Arunika, H-151, Konkan Ruchi, Neeleshan, Neelphonso, PKM-2, Prabha Shankar and Ratna showed greater correlation with the male parent *viz.*, Alphonso (0.9620\*\*), Banganapalli (0.8871), Arka Anmol (0.8847), Vanraj (0.9098), Neelum (0.2885), Alphonso (0.7257), Baneshan (0.6524), Alphonso (0.3996), Mulgoa (0.4613), Kalapadi (0.9585) and Alphonso (0.6387), respectively.

**Table 4.4.3 Major sesquiterpenoids present in mango hybrids and parents**

Compound (%)	$\delta$ -elemene	$\alpha$ -copaene	$\beta$ -elemene	$\alpha$ -gurjunene	Caryophyllene	$\alpha$ -humulene	Germacrene-d	Aciphyllene	Leden	$\alpha$ -selinene	Valencene	$\delta$ -cadinene
Al. Baneshan	0.06	0.13	0.60	37.14	10.34	6.02	0.36	5.76	2.32	2.46	2.12	1.08
Alfazli	0.29	0.06	0.71	12.33	37.10	20.38	1.29	1.62	0.38	1.08	0.31	0.38
Alphonso	0.22	0.01	0.20	0.59	20.30	11.07	0.85	0.30	N.D	0.06	N.D	0.07
Ambika	0.39	0.06	0.39	0.03	41.65	N.D	2.51	0.12	0.01	0.19	29.82	0.16
Amrapalli	0.03	0.71	0.12	27.49	26.06	15.84	0.13	0.92	2.92	1.69	1.31	1.17
Arka Anmol	0.66	0.04	0.76	11.32	36.41	19.50	2.89	3.84	N.D	0.70	0.20	0.38
Arka Aruna	0.21	0.08	0.13	0.08	46.65	27.14	0.88	0.26	0.00	0.16	N.D	0.17
Arka Neelkiran	0.19	0.11	1.35	25.41	23.08	13.94	0.73	0.17	3.09	1.03	1.22	0.36
Arka Puneet	0.98	0.20	1.27	26.53	18.56	10.02	5.65	9.73	N.D	1.54	1.02	0.96
Arka Udaya	0.19	0.10	0.34	13.94	32.22	16.56	0.68	N.D	0.99	0.30	0.37	0.12
Arunika	0.36	0.67	0.28	0.06	39.53	23.67	5.51	0.07	N.D	0.14	N.D	0.54
Au Rumani	1.07	0.08	0.66	14.13	21.03	12.94	4.44	0.08	1.29	0.59	0.54	0.36
Banganapalli	0.30	0.08	1.26	26.45	14.76	7.90	1.72	N.D	20.02	2.00	1.28	0.93
Bombay Green	0.61	1.76	0.60	17.61	12.13	5.16	8.05	0.22	0.80	0.27	0.47	2.15
Dashehari	0.15	0.65	0.20	0.07	22.56	14.86	2.83	N.D	0.08	0.13	0.01	1.02
Fazli	0.01	0.09	0.02	0.10	15.98	8.67	0.14	0.07	N.D	0.08	N.D	0.19
H-151	0.02	10.41	1.47	37.67	9.22	4.69	0.84	0.54	3.89	11.52	2.14	1.04
H-85	0.48	0.15	1.13	28.08	19.02	11.21	2.46	N.D	10.12	2.19	1.31	1.22
H-87	0.19	3.42	1.08	31.61	11.94	5.45	1.29	6.61	N.D	1.97	1.19	1.07
Jan. Pasand	0.25	0.12	1.38	23.64	17.79	9.58	1.03	N.D	20.03	2.09	1.16	0.90
Jehangir	0.16	0.08	0.74	21.50	25.12	14.28	0.78	0.23	3.09	1.05	0.81	0.43
Kalapadi	0.54	6.05	1.54	0.11	23.10	9.03	2.29	0.85	0.04	1.01	0.01	0.97
Konkan Ruchi	0.02	0.12	0.27	3.06	9.31	6.43	0.25	2.94	N.D	1.37	0.17	0.33
Mallika	0.09	0.07	0.37	26.54	15.97	7.34	0.39	N.D	1.51	0.44	0.75	0.17
Manjeera	0.03	0.04	0.22	10.69	17.52	8.22	0.09	9.88	N.D	1.00	0.31	0.42
Mulgoa	0.83	0.14	0.93	27.98	23.00	15.16	3.58	0.62	4.50	1.49	1.47	1.34
Neeleshan	0.11	0.35	0.56	15.54	7.15	3.58	0.79	12.74	N.D	2.76	0.73	0.95
Neeleshwari	0.06	0.84	N.D	33.44	7.54	4.11	2.51	0.62	4.75	5.92	2.06	1.75
Neelphonso	0.03	0.03	0.25	0.06	15.14	9.56	0.12	17.82	0.00	1.40	N.D	0.62
Neelum	0.01	0.25	0.59	30.37	9.27	4.32	0.12	N.D	1.96	4.51	0.98	0.21
Pkm-1	0.00	0.96	0.44	37.09	9.29	5.62	0.47	N.D	5.56	3.93	1.82	1.14
Pkm-2	0.00	0.51	0.61	24.63	18.76	11.95	N.D	0.39	4.11	4.97	1.52	0.60
Prabha Shankar	1.52	4.56	3.23	4.64	18.26	8.56	15.96	0.17	4.60	1.89	1.91	1.67
Ratna	0.02	0.06	0.85	21.78	21.54	12.57	0.09	0.11	2.32	0.89	0.93	0.26
Rumani	1.27	2.13	1.10	19.91	5.84	2.87	6.56	N.D	19.73	2.25	0.99	2.31
Sindhu	0.10	0.01	0.15	0.03	31.98	18.31	0.38	0.04	N.D	0.05	0.04	0.02
Suvarnarekha	0.07	0.05	0.11	0.08	35.07	23.78	0.26	0.24	N.D	0.20	N.D	0.27
Swn. Jehangir	0.06	0.03	0.12	0.49	37.64	22.30	0.25	0.39	N.D	0.23	N.D	0.07
Vanraj	0.12	0.46	0.48	15.97	6.22	3.18	3.34	N.D	1.33	0.47	0.55	0.42
<b>Mean</b>	<b>0.30</b>	<b>0.91</b>	<b>0.70</b>	<b>16.11</b>	<b>20.87</b>	<b>11.47</b>	<b>2.17</b>	<b>2.67</b>	<b>4.42</b>	<b>1.69</b>	<b>1.86</b>	<b>0.72</b>

**Table 4.4.4 Cluster means of mango genotypes based on major monoterpenoids and sesquiterpenoids**

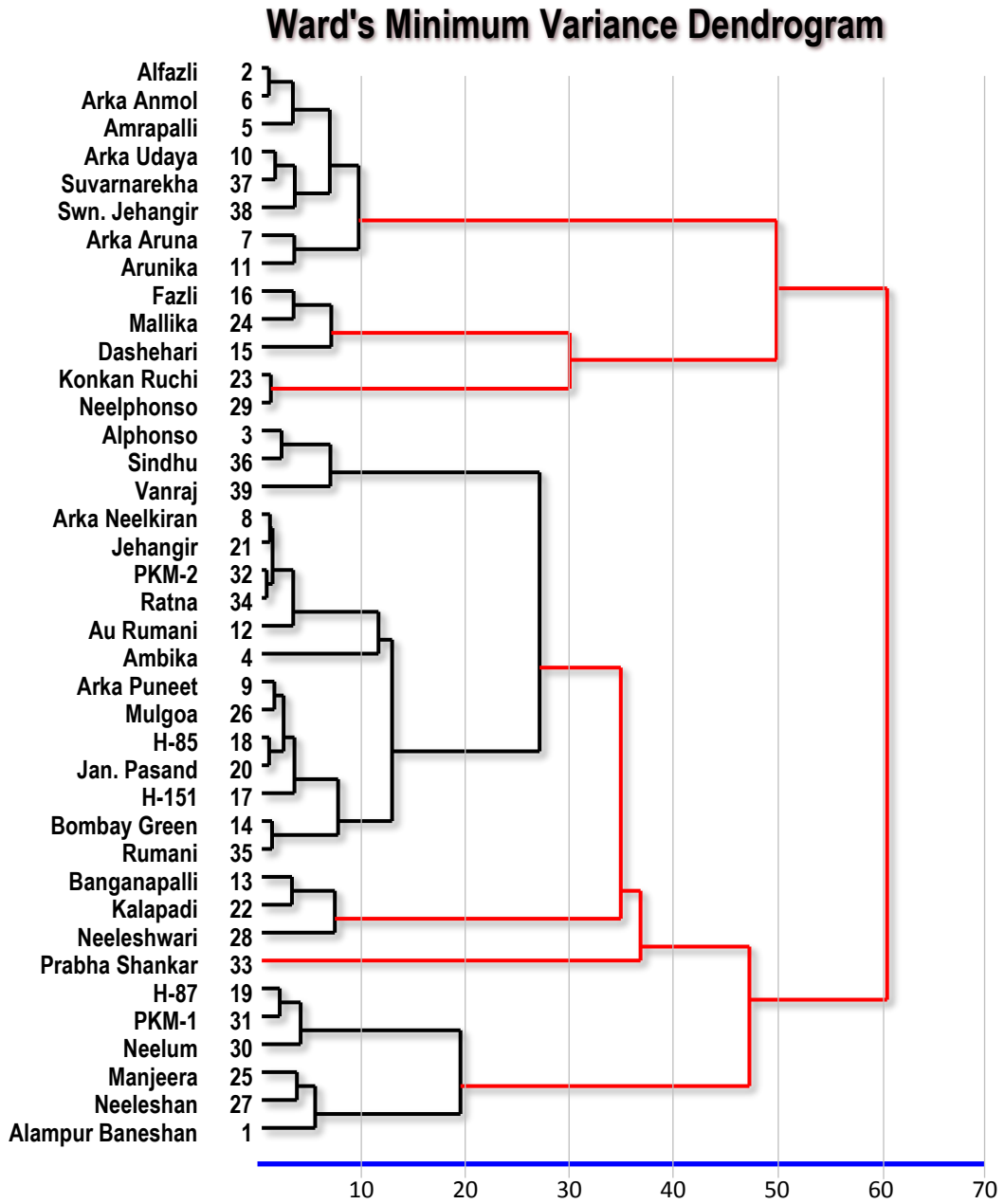
Cluster	$\alpha$ -pinene	l-phellandrene	3-carene	$\beta$ -ocimene	dl-limonene	Cis-ocimene	$\beta$ -elemene	$\alpha$ -gurjunene	caryophyllene	$\alpha$ -humulene	Germacrene-D
1	3.757	0.457	6.684	1.208	2.418	3.876	0.322	8.223	36.336	21.146	1.487
2	3.120	0.045	1.425	0.738	29.096	0.491	0.197	8.905	18.166	10.290	1.120
3	1.322	23.801	1.123	0.456	13.171	1.304	0.259	1.562	12.221	7.997	0.183
4	1.745	0.201	1.855	1.292	0.446	15.574	0.831	19.092	19.703	9.806	2.581
5	2.279	0.307	0.353	13.338	2.667	0.375	0.932	20.001	15.134	7.014	2.172
6	0.516	0.285	3.168	0.100	1.870	0.044	3.227	4.636	18.261	8.557	15.963
7	5.960	0.277	19.767	0.649	2.393	0.507	0.581	27.073	10.917	5.532	0.522

**Table 4.4.5 Eigen values, per cent variability and cumulative variability for principal components of volatile compounds in mango**

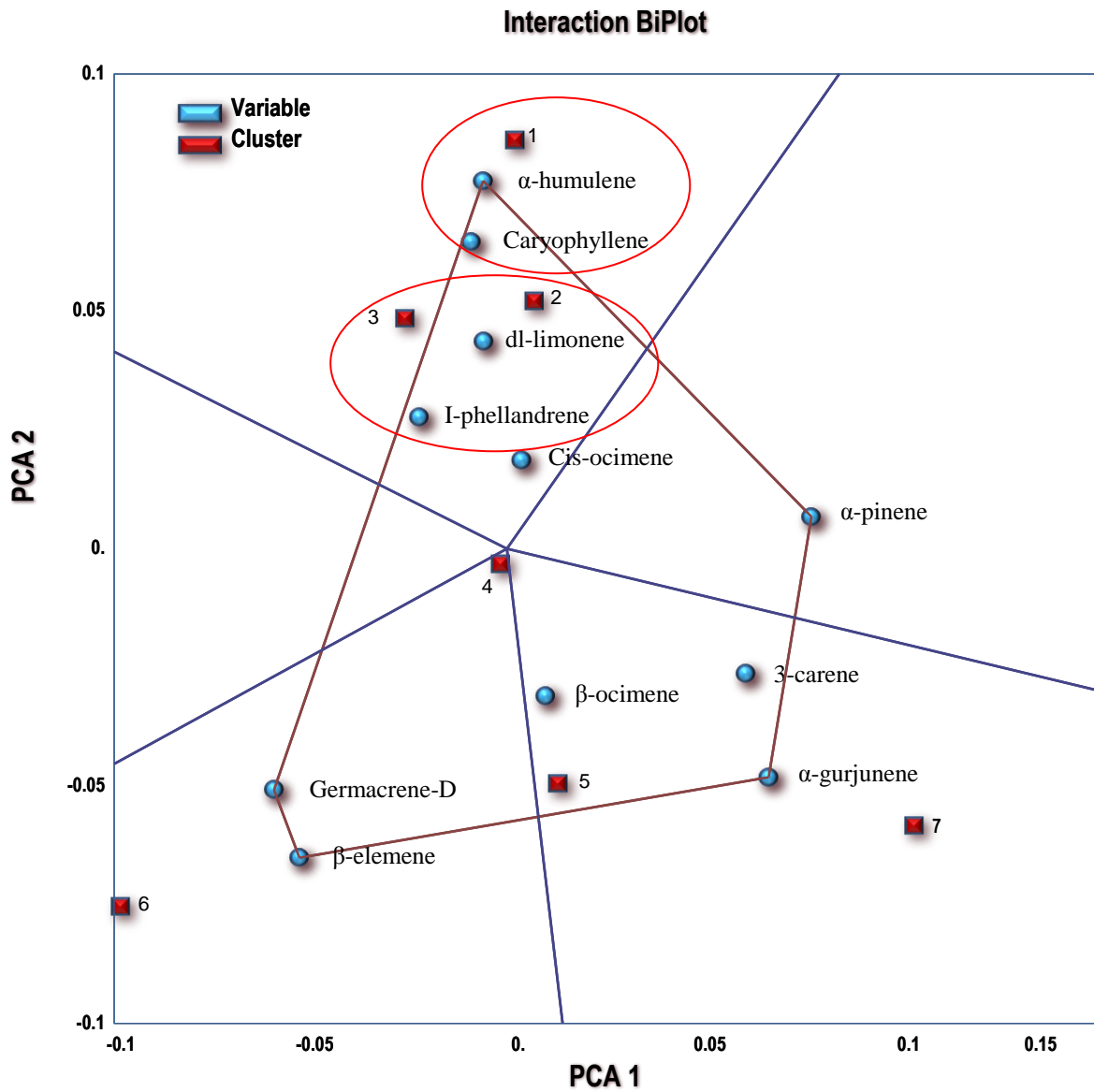
<b>Principal component</b>	<b>Eigene Value (Root)</b>	<b>Per cent variation extracted</b>	<b>Cumulative variation explained</b>
1 Vector	2.437	22.152	22.152
2 Vector	1.897	17.241	39.393
3 Vector	1.599	14.536	53.929
4 Vector	1.283	11.665	65.594
5 Vector	1.033	9.387	74.981
6 Vector	0.832	7.559	82.541

**Table 4.4.6 Character loading of principal components for monoterpenoids and sesquiterpenoids in mango**

	<b>1 Vector</b>	<b>2 Vector</b>	<b>3 Vector</b>	<b>4 Vector</b>	<b>5 Vector</b>	<b>6 Vector</b>
$\alpha$ -pinene	0.015	0.442	0.164	0.162	0.387	0.381
I-phellandrene	0.072	0.066	-0.609	-0.055	-0.262	-0.208
3-carene	-0.033	0.407	0.223	0.330	-0.330	-0.504
$\beta$ -ocimene	-0.074	-0.079	0.044	-0.470	0.641	-0.546
dl-limonene	0.161	0.075	-0.572	0.129	0.289	0.298
Cis-ocimene	0.107	-0.166	0.198	-0.628	-0.387	0.321
$\beta$ -elemene	-0.378	-0.435	0.082	0.281	0.027	0.004
$\alpha$ -gurjunene	-0.461	0.229	0.238	-0.040	0.075	0.245
Caryophyllene	0.528	-0.200	0.248	0.190	0.094	-0.058
$\alpha$ -humulene	0.519	-0.135	0.241	0.148	0.097	0.046
Germacrene-D	-0.218	-0.543	-0.036	0.302	0.051	0.040



**Fig. 4.4.1 Dendrogram based on major monoterpenoids and sesquiterpenoids of 39 mango cultivars**



**Fig. 4.4.2** Relative position of mango cultivars based on PCA scores along with major volatile components

**Table 4.4.7 Correlation coefficients among different mango genotypes based on volatile components**

	Alampur Baneshan	Alfazli	Alphonso	Ambika	Amrapalli	Arka Anmol	Arka Aruna	Arka Neelkiran	Arka Puneet	Arka Udaya	Arunika	Au Rumani	Banganapalli
Alfazli		1.0000	0.4744**	0.0604	0.1540	0.7366**	0.4242**	0.2111	0.6808**	0.7540**	0.6367**	0.2014	0.3988**
Alphonso			1.0000	0.3694*	0.6545**	0.8701**	0.9620**	0.9413**	0.7912**	0.7144**	0.9368**	0.9223**	0.6404**
Ambika				1.0000	0.2498	0.2993*	0.5735**	0.4498**	0.6126**	0.4756**	0.3778**	0.3854**	0.8949**
Amrapalli					1.0000	0.4645**	0.6222**	0.6480**	0.4163**	0.3516*	0.5854**	0.6249**	0.3652*
Arka Anmol						1.0000	0.8486**	0.7073**	0.9185**	0.8847**	0.8467**	0.6835**	0.6661**
Arka Aruna							1.0000	0.9389**	0.8573**	0.7812**	0.9009**	0.9011**	0.7953**
Arka Puneet									1.0000	0.9321**	0.7889**	0.6158**	0.8871**

	Bombay Green	Dashehari	Fazli	H-151	H-85	H-87	Jan. Pasand	Jehangir	Kalapadi	Konkan Ruchi	Mallika	Manjeera	Mulgoa
Alampur Baneshan	-0.0855	-0.0697	-0.0096	-0.0297	-0.0711	-0.0842	-0.0618	-0.0812	-0.0720	-0.0368	-0.0524	-0.0478	-0.0726
Alfazli	0.6721**	0.4172**	0.0921	0.0344	0.8170**	0.8911**	0.8235**	0.7541**	0.5893**	0.1223	0.1484	0.5353**	0.7007**
Alphonso	0.5512**	0.4662**	0.5195**	0.4058**	0.4319**	0.7349**	0.5552**	0.6665**	0.7908**	0.7257**	0.4475**	0.4700**	0.4931**
Ambika	0.1402	0.0803	0.1944	0.1154	0.0688	0.2087	0.1022	0.1803	0.7195**	0.3056*	0.1969	0.1228	0.1703
Amrapalli	0.2469	0.4179**	0.3180*	0.4380**	0.1281	0.3541*	0.2050	0.3322*	0.4428**	0.5341**	0.2145	0.2029	0.2756
Arka Anmol	0.7687**	0.4949**	0.3680*	0.2275	0.7675**	0.9079**	0.8199**	0.8289**	0.8666**	0.5157**	0.3209*	0.5968**	0.4321**
Au Rumani	0.3589*	0.2976*	0.7042**	0.4448**	0.1685	0.5091**	0.3669*	0.4763**	0.6431**	0.7942**	0.4491**	0.4171**	0.3737*
Dashehari		1.0000	0.1500	0.7604**	0.4452**	0.4768**	0.4795**	0.4161**	0.4100**	0.2018	0.1380	0.3228*	0.1850
H-151				1.0000	0.0068	0.1391	0.1895	0.1310	0.1969	0.3363*	0.2845	0.3147*	0.1040
H-85					1.0000	0.8043**	0.8769**	0.7260**	0.6243**	0.1404	0.1195	0.5290**	0.2641
H-87						1.0000	0.8019**	0.9433**	0.7825**	0.3669*	0.2341	0.5711**	0.6164**
Jan. Pasand							1.0000	0.7069**	0.6559**	0.2702	0.3221*	0.7033**	0.3440*

	Neeleshan	Neeleshwari	Neelphonso	Neelum	PKM-1	PKM-2	Prabha Shankar	Ratna	Rumani	Sindhu	Suvarnarekha	Swn. Jehangir	Vanraj
Alphonso	0.8423**	0.3150*	0.3996**	0.4566**	0.4556**	0.4752**	0.7506**	0.6387**	0.7050**	0.2714	0.6720**	0.9108**	0.8688**
Amrapalli	0.4303**	0.1017	0.1084	0.2455	0.1420	0.1383	0.3719*	0.4631**	0.3755*	0.0654	0.4618**	0.5939**	0.5847**
Arka Neelkiran	0.6599**	0.1679	0.2009	0.4131**	0.2368	0.2244	0.6061**	0.6088**	0.6183**	0.1096	0.7499**	0.9417**	0.8962**
Arunika	0.8453**	0.5739**	0.4391**	0.3389*	0.6520**	0.5889**	0.7557**	0.6040**	0.7135**	0.2903	0.6437**	0.9292**	0.9098**
Au Rumani	0.6414**	0.1642	0.1623	0.4799**	0.2334	0.2150	0.5577**	0.6513**	0.5606**	0.1111	0.6871**	0.9182**	0.8538**
Banganapalli	0.6524**	0.2010	0.4022**	0.1883	0.3653*	0.4199**	0.8397**	0.4279**	0.9593**	0.2798	0.9186**	0.5676**	0.6973**
Bombay Green	0.8061**	0.2990*	0.8559**	0.0881	0.6321**	0.7766**	0.7725**	0.3947**	0.6246**	0.7668**	0.2619	0.3406*	0.3300*
Dashehari	0.5162**	0.1913	0.4304**	0.0918	0.4030**	0.4618**	0.4520**	0.3081*	0.3838**	0.2982*	0.1743	0.2715	0.2385
H-151	0.1936	0.0252	0.0081	0.2885	0.0537	0.0358	0.1588	0.1772	0.1680	-0.0393	0.2315	0.3484*	0.2948*
Jehangir	0.8715**	0.4617**	0.7054**	0.1510	0.7309**	0.8148**	0.8028**	0.4828**	0.6651**	0.7557**	0.3421*	0.5192**	0.5072**
Kalapadi	0.8525**	0.2955*	0.5892**	0.2353	0.5486**	0.6318**	0.9585**	0.4876**	0.9876**	0.4179**	0.8258**	0.6508**	0.7292**
Konkan Ruchi	0.4765**	0.0977	0.2412	0.3822**	0.1241	0.1143	0.4193**	0.5066**	0.4242**	0.2627	0.5495**	0.7061**	0.6736**
Mallika	0.2908*	0.1087	0.1066	0.9049**	0.1419	0.1378	0.2825	0.1993	0.2877	0.0333	0.3037*	0.3610*	0.3423*
Manjeera	0.5914**	0.2998*	0.5157**	0.2702	0.5422**	0.5842**	0.5607**	0.2465	0.4881**	0.3137*	0.2254	0.3144*	0.2859
Mulgoa	0.4818**	0.9360**	0.2430	0.2434	0.6652**	0.4613**	0.3776**	0.3133*	0.3514*	0.1224	0.3006*	0.6380**	0.6779**
Neeleshan	1.0000	0.4395**	0.7599**	0.2363	0.7465**	0.8414**	0.9171**	0.5705**	0.7948**	0.5829**	0.4728**	0.6455**	0.6261**
Neeleshwari		1.0000	0.3757*	0.1941	0.7787**	0.5849**	0.3601*	0.1777	0.2957*	0.1795	0.1122	0.4152**	0.4415**
Neelphonso			1.0000	0.0118	0.7553**	0.8793**	0.7586**	0.2403	0.5865**	0.6080**	0.1069	0.1437	0.1367
Neelum				1.0000	0.0521	0.0335	0.1915	0.2058	0.1941	-0.0350	0.2721	0.3949**	0.3604*
PKM-1					1.0000	0.9394**	0.6998**	0.2511	0.5586**	0.4776**	0.1399	0.3704*	0.3546*
Ratna								1.0000	0.4093**	0.3115*	0.4088**	0.5858**	0.5654**
Suvarnarekha											1.0000	0.7089**	0.8183**

\* and \*\* indicates significance of values at P=0.05 and P=0.01, respectively

# *DISCUSSION*



Mango (*Mangifera indica* L.) is an important tropical as well as subtropical fruit crop of India. By virtue of its excellent flavour, delicious taste, attractive colour and nutritive value, mango ranks first among fruits. All edible commercial cultivars of mango belonging to *Mangifera indica* L. have been developed through chance seedlings from the naturally occurring open pollinated seedlings. Mango is highly cross-pollinated, heterozygous and huge variability exists in the diversity centres. Almost all the commercial mango varieties have some demerits which needs improvement. An ideal mango variety should be dwarf, precocity and regularity in bearing, attractive and good quality fruits, high productivity and resistant to major diseases and pests along with good transport and processing qualities.

In any breeding programme, inclusion of genetically diverse parents is essential in order to produce high heterosis effects and to create new reservoirs of genetic variability. The development of new varieties is mainly governed by the magnitude of genetic variability in base material and the extent of variability for desired characters. Genetic variability and divergence is of greatest interest to the plant breeder as it plays a vital role in framing a successful breeding programme. The multivariate analysis ( $D^2$ ) is a powerful tool to measure the genetic divergence and to choose appropriate parents for hybridization programme.

However, molecular markers, being environmentally neutral as opposed to morphological characters, focus directly on the variations controlled by genes or on the differences in DNA sequences, yield a more reliable estimate of the inherent diversity. The range of molecular markers that can be relatively used on most plant germplasm is quite extensive. The higher resolution of molecular markers makes them a valuable tool for a variety of purposes including fingerprinting, genotyping and facilitating appropriate choice of parents for breeding programmes and assessment of genetic diversity.

Germplasm with diverse genetic base is the major source for breeding programmes. Further, preservation of germplasm is a worldwide concern and conservation of specific diverse gene pools will be useful to breeders to ensure the effectiveness of their mango improvement programmes. Morphological markers combined with molecular characterisation are essential for better understanding of genetic diversity in mango (Singh *et al.*, 2009; Begum *et al.*, 2012). Current knowledge on genetic variability and divergence in mango cultivars is meagre. Keeping

this in view, the present investigation on “**Genetic diversity and characterization studies in mango**” was conducted during 2015-18 at the Division of Fruit Crops, Division of Biotechnology and Division of Physiology and Biochemistry, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru. The data collected was statistically analyzed and the results obtained were discussed under the following sub-heads.

## **5.1 To study the diversity existing in *M. indica* varieties from different regions**

### **5.1.1 Fruit morphological characters**

Morphological characterization is the simplest and formal standardized method for evaluating crop genetic diversity which could be the first step and need to follow first step before more profound studies like biochemical or molecular analysis based on the visual attributes (Hoogendijk and Williams, 2001).

The data recorded on nine traits were subjected to statistical analysis. Analysis of variance was found significant among the cultivars for all the characters studied. Wide variation was recorded among the 156 genotypes and these genotypes differed significantly for all the characters studied. Mango genotypes exhibit wide range of diversity with respect to fruit size, fruit weight, pulp content, TSS content, acidity, total carotenoids content, total phenols content and total flavonoids content which can be utilized for various purposes. These differences among genotypes provide variation for making selection in the crop improvement programme.

Variability in fruit shape was the most important and stable character for discriminating varieties from each other. Other fruit characters also have a degree of varying importance for the purpose of identification. Fruits have been exclusively used as the major descriptors for identification of different varieties of fruit crops (Toili *et al.*, 2013). Several procedures for the identification and characterization of mango genotypes have been developed based on outstanding morphological characters of fruits. Morphological variations of mango varieties have been studied extensively (Watson and Winston, 1984; Uddin *et al.*, 2006 and 2007; Mhamed and Ahmed, 2015).

The genotypes recorded significant variation for fruit weight ranging from 50.12 g to 1182.95 g with a mean value of 304.23 g. Safed Mulgoa (1182.95 g) registered highest fruit weight, whereas, lowest was observed in Chandrakaran (50.12 g). The results were similar to those reported by Bhuyan and Islam (1986) and Dinesh (2004) who recorded fruit weight in range of 202.8 g to 1014.4 g. Himabindu *et al.* (2017) also recorded fruit weight in the range of 131.43

g to 1395.45 g with a mean fruit weight of 400.74 g. However, the difference in fruit weight might be due to differences in climatic conditions and also due to genetic behaviour of the genotype.

The fruit length revealed significant differences among the cultivars. The fruit length of mango cultivars studied in the present investigation ranged from 4.70 cm to 17.50 cm with a mean fruit length of 9.81 cm. In the present study, significantly maximum fruit length was recorded in Sora (17.50 cm), while, minimum fruit length was recorded in Elaichi (4.70 cm). Himabindu *et al.* (2017) also recorded maximum fruit length in Sora Mamidi (17.56 cm), while minimum fruit length was recorded in Nuzividu Tiyya Mamadi (5.93 cm). Similar range of fruit length (4.4 cm to 18.1 cm) was reported by Simi (2006) in mango. Begum *et al.* (2014) assessed 30 accessions of 'Cherukurasam' and reported that fruit length ranged from 7.60 to 14.00 cm with an average of 11.20 cm.

The fruit width differed significantly with a mean of 7.61 cm, ranging from 4.15 cm to 12.90 cm among the cultivars studied. The findings are in accordance with those reported by Himabindu *et al.* (2017) who observed fruit width in the range of 4.47 to 12.20 cm. Simi (2006), in their investigation, also recorded fruit width in the range of 3.90 cm to 12.00 cm. Radha and Nair (1998) observed fruit width to be ranging from 2.48 cm to 11.10 cm. The variation in length and width of fruit in mango was also observed by Kher and Sharma (2002) and Abirami *et al.* (2004). The variations in the fruit size depends upon genetic makeup of an individual variety and are highly influenced by environmental factors.

With respect to fruit thickness, significant differences were observed among the cultivars. The fruit thickness of mango cultivars ranged from 2.05 cm to 10.50 cm with a mean value of 6.50 cm. Majumder *et al.* (2012b) reported fruit thickness in the range of 5.10 to 9.71 cm, while Rajan *et al.* (2009) observed it to be ranging from 4.30 to 8.40 cm.

The skin thickness differed significantly ranging from 1.10 mm to 5.35 mm with a mean of 2.33 mm. Simi (2006) reported fruit skin thickness in the range of 0.60 mm to 2.00 mm in mango, while, Himabindu *et al.* (2017) noted it to be in the range of 0.37 mm to 2.05 mm. Mannan *et al.* (2003) reported that the range of peel thickness varied from 1.48 mm to 2.72 mm in different mango varieties. Peel thickness provides protection against fruit fly and help to reduce post harvest losses; however, this fact could increase the difficulty of removing peel before processing.

Among the cultivars studied, skin weight ranged from 12.07 g to 305.73 g with an average of 73.68 g. Cultivar Elaichi recorded minimum skin weight of 12.07 g, while, the cultivar Safed Mulgoa recorded maximum skin weight of 305.73 g. Similar results were reported by Dinesh (2004) in mango, whereas, Himabindu *et al.* (2017) noted skin weight to be in the range of 10.89 g to 117.12 g. Mitra and Mitra (2001) evaluated 19 cultivars and reported significant difference in peel weight in various cultivars.

The stone weight of mango cultivars ranged from 9.34 g to 129.50 g with a mean value of 42.07 g. Similar results were reported by Himabindu *et al.* (2017) who observed stone weight in the range of 21.05 to 96.50 g with a mean of 45.11 g. Majumdar *et al.* (2011) found stone weight to be ranging from 21.33 to 111.61 g. Jilani *et al.* (2010) and Anila and Radha (2003), observed that stone weight ranged from 22.55 g to 48.67 g.

Pulp weight differed significantly with a mean of 188.48 g and ranging from 17.71 g to 791.88 g. Cultivar Chandrakaran recorded minimum pulp weight of 17.71 g, while, the cultivar Sora recorded maximum pulp weight of 791.88 g. Similar results were reported by Dinesh (2004) in mango. In their investigations of different mango cultivars, Himabindu *et al.* (2017) recorded mean pulp weight of 303.39 g, while it ranged from 89.12 g to 1178.2 g.

Considerable variation was observed with respect to pulp content among the mango cultivars studied in the present investigation. It varied from 31.10 to 83.99% with a mean of 59.19%. Similar findings were reported by Majumdar *et al.* (2011) who observed that per cent edible portion pulp varied significantly from 45.22 to 79.83% in different cultivars of mangos. Palaniswamy *et al.* (1974) found that pulp content varied from 53 to 83 per cent.

### **5.1.2 Fruit and leaf biochemical characters**

There were significant differences in the TSS content of mango cultivars studied. TSS content ranged from 7.00 °Brix to 24.95 °Brix with a mean of 16.29 °Brix. TSS was observed highest in cultivar K-O-7 (24.95 °Brix) and lowest in cultivar Huli Appekai (7 °Brix). Similar trend in TSS content of mango cultivars was earlier reported by Kaur *et al.* (2014), who observed a high range of variability for TSS content (11.35-28.95 °Brix) in evaluated mango germplasm. Kulkarni and Rameshwar (1981) observed that TSS ranged from 13.1% to 27%. Satyavati *et al.* (1972) reported that TSS of ripe fruits of local varieties of Kerala ranged between 10 and 24 °Brix, while, Passam (1982) observed Total soluble solids to be varying between 10 to 23 °Brix.

The range of titratable acidity among the mango cultivars studied in the present investigation was 0.13 to 1.34% with a mean of 0.49%. Pradeepkumar *et al.* (2006) reported similar acidity values ranging from 0.13 to 1.65%. Similarly low titratable acidity content of 0.14% in mango cultivars were reported by Mitra *et al.* (2000) and Singh (2005).

The differences observed in total carotenoids values were found to be significant. Total carotenoids ranged from 0.81 to 8.21 mg/100g with an average of 3.02 mg/100g. Maximum total carotenoids content was found in Manibhatta Appe (8.21 mg/100g), while, minimum content was found in Shendryo (0.81mg/100g). Bora *et al.* (2017) and Hada and Singh (2018) reported similar findings with total carotenoids content ranging from 1.53 mg/100g to 8.78 mg/100 g. Rajwana *et al.* (2010) noted it to be in the range of 1.77 to 6.99 mg/100 g. Total carotenoids provide an expression of natural appearance to the fruit product and their higher content in fruits offer distinct advantages, particularly in international trade where addition of artificial colour is discouraged.

In the present investigation, ascorbic acid content ranged from 5.50 to 113.00 mg/100g with a mean of 36.27 mg/100g. Teaotia and Singh (1971) recorded ascorbic acid ranging from 8 mg/100 g to 105.2 mg/100 g. Kumar (1998) observed fruit vitamin C to be as high as 150 mg/100g and as low as 6.2 mg/100g. Mitra *et al.* (2000) reported the highest ascorbic acid content (123.33 mg/100 g) in fruits of Kashir Langra. Vitamin-C content in different cultivars ranged from 12 to 108.0 mg/100 g (Rajwana *et al.*, 2010). The results were in agreement with Palaniswamy *et al.* (1974) and Singh (2005) who stated that smaller sized mango fruits recorded higher ascorbic acid content than larger sized fruits.

Total phenols are being used as a biochemical index for screening mango progenies (Sharma *et al.*, 2000). The mean value of total phenol content in leaves was 4856.80 mg/100g, while it ranged from 1577.55 to 9875.46 mg/100g. The highest total phenol content was found in Hithalahalli Appe (9875.46 mg/100g), while, lowest was found in Narayana Sheni (1577.55 mg/100g). Begane *et al.* (2018) reported total phenolic content in the leaf extracts of mango hybrids and parents to be ranging from 2977.60 to 7627.43 mg/100 g.

Significant differences were observed in total flavonoids content of mango cultivars studied in the present investigation. The total flavonoids content ranged from 257.25 to 1087.80 mg/100g with a mean of 633.54 mg/100g. Maximum content was found in Adderi Jeerige (1087.80 mg/100g) and minimum in Thogarapalli (257.25 mg/100g). Begane *et al.* (2018) found

total flavonoids in mango leaves to be ranging from 100.74 to 292.95 mg/100g. In their investigation on Arumanis leaves (*Mangifera indica* L. var. Arumanis), Marjoni *et al.* (2018) found total flavonoid contents to be 1240.1 mg, expressed in terms of quercetin equivalent.

### **5.1.3 Genetic variability studies of morphological and bio-chemical characters**

Success of any plant breeding programme largely depends upon the knowledge of genetic variability present in a given crop species for the characters under improvement. The understanding of such variability provides many avenues for genetic amelioration of a crop. The genotypic coefficient of variation measures the range of variability available in a crop and also enables to compare the amount of variability present in different characters. The phenotypic expression of the character is the result of interaction between genotype and environment. Higher phenotypic coefficient of variation over genotypic coefficient of variation indicated the influence of environment over the character.

Besides genetic variability, knowledge on heritability and genetic advance measures the relative degree to which a character is transmitted from one generation to next. High heritability indicated that a major part of phenotypic variability in any character is controlled by additive gene effect, which can be improved by simple selection procedures (Lush, 1940). Heritability also provides information to predict genetic gain obtained by selection. Therefore, for successful improvement of any crop, it is necessary to have a thorough knowledge on the above genetic parameters. The characters with high heritability coupled with high genetic advance further indicated the possibility of making selections in earlier generations.

The genetic variability together with the high heritability would give a better idea on the amount of genetic advance expected out of selection. The magnitude of heritable variability is the most important aspect of genetic contribution of the breeding material, which has close relationship on its response to selection.

The genetic parameters, *viz.*, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense and genetic advance as per cent mean were estimated for all the characters under study and the results are presented in Table 4.1.3.

#### **5.1.3.1 Phenotypic and genotypic coefficient of variation**

Among the fruit morphological characters, the phenotypic coefficient of variation (PCV) ranged from 14.85 to 69.96, while, it ranged from 23.31 to 76.73 among the bio-chemical

characters. On the other hand, genotypic coefficient of variation (GCV) ranged from 12.73 to 69.58 among the fruit morphological characters and from 20.20 to 75.83 among the bio-chemical characters. Similarly, Himabindhu *et al.* (2016a) found PCV and GCV to be ranging from 6.36-75.81 and 6.24-75.66, respectively.

In the present investigation, phenotypic coefficient of variation was greater than the corresponding genotypic coefficient of variation for all the characters indicating the importance of environment in expression of characters. It also indicates that phenotypic variability is a reliable measure of genetic variability. The range of PCV and GCV were classified as suggested by Sivasubramanian and Madhavamenon (1973), *i.e.*, 0-10% as low, 10-20% as moderate and more than 20% as high. High values of PCV with corresponding high values of GCV were recorded for fruit weight, fruit thickness, fruit length, fruit width, skin weight, fruit skin thickness, stone weight, pulp weight, TSS, titratable acidity, total carotenoids, ascorbic acid, total phenols and total flavonoids, which indicates that there exists high genetic variability and better scope for improvement of these characters through selection. Similar to the present investigation, Rai *et al.* (2001) found high range of variation for peel, pulp and stone weight in mango.

The results are in consonance with the findings of Yadav *et al.* (1995), Attri *et al.* (1999), Simi (2006), Rajan *et al.* (2009) and Himabindu *et al.* (2016a) who found high phenotypic coefficient of variation compared to corresponding genotypic coefficient of variation (GCV) for most of the traits studied and indicated the presence of additive gene action in the inheritance of these traits. From the foregoing discussions, it is clear that these characters offer good scope for selection in mango.

### **5.1.3.2 Heritability**

The genotypic coefficient of variation does not offer full scope to estimate the variation that is heritable and therefore, estimation of heritability becomes necessary. The variability existing in a population is the sum total of heritable and non-heritable components. A high value of heritability indicates that the phenotype of that trait strongly reflects its genotype. Swarup and Chougule (1962) suggested that the estimates of genotypic coefficient of variation alone was not sufficient to quantify the amount of variation which is heritable and Burton (1952) inferred that genotypic coefficient of variation effects together with heritability estimates would furnish more reliable information.

Heritability values are useful in predicting the expected progress to be achieved through the process of selection. Heritability values ranged from 73.49 to 99.33 among the fruit morphological characters, while, it ranged from 70.27 to 97.68 among the biochemical characters. Further, the range of heritability in broad sense was classified as suggested by Johnson *et al.* (1955a) *i.e.*, less than 30% as low, 30-60% as moderate and more than 60% as high. In their study of 34 mango cultivars, Himabindhu *et al.* (2016a) found heritability values to be ranging from 71.90 to 99.61 for the fruit morphological and biochemical characters. Further, similar to the present results, high heritability for pulp per cent, total sugars and  $\beta$ -carotene was reported by Attri *et al.* (1999), Singh (2002) and Simi (2006) in mango. Heritability estimates were high for all the characters under study indicating that selection would be the best step for selecting genotypes having traits with high heritability. This is because there would be a close correspondence between the cultivars and the phenotype due to relative small contribution of the environment to the total variability. Higher values of heritability indicates that either these were simply inherited characters governed by a few major genes or additive gene effects, even if they were under polygenic control and therefore, selection of these characters would be more effective for improvement (Johnson *et al.*, 1955a; Panse, 1957).

### **5.1.3.3 Genetic advance**

Estimates of heritability are important to find out the heritable portion of variability and genetic gain that can be likely achieved in the next generation. Although estimates of heritability are useful to plant breeders as they provide basis for selection on phenotypic performance, however, for more reliable and maximum genetic information, heritability estimates coupled with genetic advance should be considered. Genetic advance is the improvement over the base population that can potentially be made from the selection of a character.

The genetic advance as per cent mean ranged from 22.48 to 142.54 among the fruit morphological characters, while, it ranged from 36.07 to 154.40 among the bio-chemical characters. High values of genetic advance as percentage of mean (> 20 %) were obtained in the present study for all the characters studied. Similarly, Himabindhu *et al.* (2016a) observed the genetic advance to be ranging from 12.6 to 155.55 for the characters under study.

The result pertaining to genetic advance coupled with heritability clearly depicts that environmental effects might not be contributing much to the total phenotypic variation for fruit weight and pulp weight and could have moderate to high influence on fruit and pulp weight. According to Johnson *et al.* (1955a), an estimated heritability associated with genetic advance is

more reliable than heritability alone for prognosticating the impact of selection. High heritability accompanied with high genetic advance is mainly referred to the action of additive genes (Panse, 1957). A low genetic advance implies that heritability of a particular trait in a specific environment was mainly due to non-additive gene action, whereas, if the heritability was due to additive gene effect, it would be associated with high genetic advance (Shadakshari *et al.*, 1995). So, it can be concluded that different characters specially fruit weight and pulp weight under study can be improved through phenotypic selection.

The phenotypic coefficient of variation ranged from 14.85 (pulp per cent) to 76.73 (ascorbic acid) among the morphological and bio-chemical characters, while the genotypic coefficient of variation ranged from 12.73 (pulp per cent) to 75.83 (ascorbic acid). Further, the heritability values ranged from 70.27 (total flavonoids) to 99.33 (fruit weight), while, the genetic advance as per cent mean ranged from 22.48 (pulp per cent) to 154.40 (ascorbic acid).

From the results presented in the aforesaid paragraphs, it was evident that the characters, namely, fruit weight, skin weight, titratable acidity, total carotenoids and ascorbic acid had recorded higher estimates for phenotypic coefficient of variation, genotypic coefficient of variation, genetic advance as per cent mean and heritability indicating the presence of additive gene action in the inheritance of these traits and simple selection would be highly rewarding for improving these characters. High heritability coupled with high genetic advance were observed for all characters studied indicating these characters are governed by additive gene action and phenotypic selection may be more fruitful. Hence, direct selection may be followed for the improvement of mango for these characters.

#### **5.1.4 Correlation studies**

The aim of correlation studies is primarily to know the suitability of various characters for indirect selection, because the selection of any particular trait may bring about undesirable changes in other associated characters. Phenotypic correlation is the association between two variables which can be directly observed. It includes both genotypic and environmental effects and, therefore, it differs under different environmental conditions. Genotypic correlation is the inherent or heritable association between two variables. This type of correlation may be either due to pleiotropic action of genes or due to linkage or more likely both. This type of correlation is more stable and is of paramount importance for a plant breeder to bring about genetic improvement in one character by selecting the other character of a pair that is genetically correlated.

The correlation studies between different morphological and bio-chemical characters would certainly provide an idea, which might be utilized for selection of desirable parameters for future breeding programmes. The highly significant positive correlation between desirable characters is favourable to a plant breeder, because it might help in simultaneous improvement of those characters. On the other hand, the negative correlation would hinder the synchronized expression of those characters. In such a situation, it would require to make some compromise including economic ones. The genotypic (G) and phenotypic (P) correlation coefficients were worked out for fifteen characters in 156 mango cultivars. In general genotypic correlation coefficients were higher than phenotypic correlation coefficient for almost all the characters.

#### **5.1.4.1 Correlation coefficient analysis**

To determine the nature and magnitude of association, phenotypic and genotypic correlation coefficients between pairs of traits were estimated and presented in Table 4.1.4. The results on correlation coefficients are discussed below.

In the present investigation genotypic coefficients of correlation ( $r_g$ ) revealed higher magnitude than their corresponding phenotypic coefficients ( $r_p$ ) in most of the cases, indicating that there is an inherent association among the characters studied. In few cases the phenotypic correlation coefficients are same or higher than the genotypic correlation coefficients which indicated that both environmental and genotypic correlations in these cases acted in the same direction and finally maximised their expression at phenotypic level.

Fruit weight recorded significant positive correlation with pulp weight, skin weight, fruit width, stone weight, fruit thickness, fruit length, skin thickness and pulp per cent. It was negatively correlated with total carotenoids, TSS, ascorbic acid and total phenols. The selection indices based on fruit size (length, width and thickness) with high peel, pulp and stone content were the most important factors for identifying high fruit weight types. Yadav *et al.* (2003) showed significant positive association of fruit weight with stone weight, peel weight and pulp weight, which is in accordance with the results of present study. Yadav *et al.* (1995) reported positive association of fruit weight with pulp weight and negative association of fruit weight with TSS, which is akin to the results reported here. As in the present study, Gill and Navprem (2015) observed fruit weight to be significantly correlated with fruit length, fruit width, skin weight, stone weight and pulp weight. The results are in agreement with earlier findings of Suman *et al.* (1985), Singh *et al.* (1990), Balakrishnan *et al.* (1998), Attri *et al.* (1999), Jha *et al.* (2003), Singh (2005) and Simi (2006) in mango. None of the chemical characteristics, however, exhibited

significant positive correlation with fruit weight. This was supported by the findings of Pradeepkumar *et al.* (2006) who observed similar results.

Fruit length and width recorded significant positive correlation with pulp weight, skin weight, stone weight, fruit width, skin thickness and pulp per cent. Fruit length was positively correlated with pulp weight. Therefore, cultivars having more fruit length might have more pulp and less stone. The results are in agreement with the reports of Yadav *et al.* (1995), Gupta *et al.* (1996), Attri *et al.* (1999) and Kumar (2000) suggesting significant positive association of fruit length with fruit weight. Positive correlation of fruit length with fruit weight and width was reported by Suman *et al.* (1985) and Simi (2006). Yadav *et al.* (2003) showed positive association of fruit length with pulp weight and peel weight, which support the results of present study.

The physical characteristics had significant positive correlations with each other, but had negative correlations with the biochemical characteristics. Similar result of physical characters being negatively correlated with biochemical characters was reported in acid lime by Prasad and Rao (1989).

### **5.1.5 Genetic divergence studies**

For a successful breeding programme, the diversity of parents is of utmost importance, since the crosses made between the parents with maximum genetic divergence are more likely to yield desirable recombinants in the progenies. Hence, it is desirable to select suitable and genetically divergent parents, based on information about the genetic variability and genetic diversity present in the available germplasm (Singh, 1998). The genetic divergence among 156 cultivars of mango was quantitatively assessed by adopting Mahalanobis  $D^2$  statistics and principal component analysis, while the divergence at molecular level was studied by using SSR markers.

#### **5.1.5.1 Mahalanobis $D^2$ statistic**

The  $D^2$  statistic, proposed by Mahalanobis (1936) is one of the potent techniques of measuring genetic divergence based on multiple characters. This technique measures the forces of differentiation at two levels *viz.*, intracluster and intercluster levels and thus helps in the selection of genetically divergent parents for exploitation in hybridization programmes. The multivariate analysis ( $D^2$ ) is a powerful tool for determining the degree of divergence between populations and relative contribution of different components to total divergence in isolation of suitable parents. The  $D^2$  value between any two cultivars was calculated as the sum of squares of the differences

between the mean values of all the fifteen characters and is used for the final grouping of the cultivars.

### **5.1.5.2 Relative contribution of different characters towards divergence**

The result on character wise contribution towards total genetic divergence showed that fruit weight (47.07%) had the greatest contribution by ranking first in 5691 times out of 12090 combinations, followed by ascorbic acid (24.85%) which ranked first 3004 times. The characters *viz.*, carotenoids, acidity and fruit width, contributed 8.06%, 5.37% and 5.06% towards the diversity by ranking 974, 649 and 612 times, respectively. Total phenols, fruit length, skin thickness, skin weight, fruit thickness, stone weight, TSS, total flavonoids, pulp per cent and pulp weight contributed 1.91, 1.69, 1.51, 1.36, 1.08, 0.86, 0.55, 0.41, 0.22 and 0.01 per cent respectively to the genetic divergence in decreasing order.

Fruit weight, fruit width, ascorbic acid, carotenoids and acidity contributed maximum towards diversity; the characters with maximum contribution towards diversity should be given due consideration for mango crop improvement. Similar trend of findings with regard to contribution of fruit weight towards divergence were reported by Majumder *et al.* (2013) and Barholia and Sangeetha (2014) in mango. The character which contributed maximum to the divergence should be given greater emphasis for selection (Jagadev *et al.*, 1991).

### **5.1.5.3 Mean intra and inter cluster distances**

Choice of particular cluster and selection of particular cultivar from selected cluster are the two important points to be considered before initiating the crossing programme. The hybrids between cultivars of different clusters will express high heterosis and throw more useful segregants.

The information regarding proximity and divergent clusters from each cluster was given in Table 4.1.6 which revealed that Clusters II (177.064), III (172.472), V (184.199) and VI (208.634) were closely related to cluster IV, while all four were furthest from cluster XIII (5687.560, 5618.797, 4886.274 and 6441.035, respectively). Clusters VIII (349.159), IX (382.715) and X (405.798) exhibited intimate relation with cluster VII and wide diversity with cluster XIII (3571.356, 2512.650 and 3197.877, respectively).

Cultivars grouped into the same cluster presumably differ little from one another as the aggregate of characters measured. Therefore, it would be desirable to attempt crosses between

cultivars belonging to most distant clusters for getting highly heterotic crosses which are likely to yield wide range of segregants on which selection can be practiced. More diverse, the greater chances of obtaining high heterotic  $F_1$ s and broad spectrum of variability in segregating generations (Arunachalam, 1981).

The mean inter and intra cluster distances among thirteen clusters were presented in Table 4.1.7. The inter cluster  $D^2$  values ranged from 172.472 to 6572.160 indicating presence of substantial amount of genetic diversity in the genetic material. The highest (6572.160) inter cluster  $D^2$  value was recorded between clusters I and XIII, which suggested wide genetic diversity between these clusters. The lowest (172.472) was recorded between clusters III and IV indicating close relationship and similarity for most traits in the cultivars.

The highest intra cluster  $D^2$  value was observed in cluster XI (491.651) indicating the presence of wide genetic diversity among the cultivar present within the cluster while the lowest (93.041) intra cluster  $D^2$  value was observed in cluster IV indicating less genetic diversity among the cultivars present within the group. The cluster distance ranged from 93.041 to 491.651 within clusters and 172.472 to 6572.160 between clusters. This indicated that the clusters were homogenous within themselves and heterogeneous between themselves.

Genetic diversity is the most important tool to select prospective parents for crop improvement programme. The cultivars belonging to the clusters separated by high estimated distance could be utilized in hybridization programme for obtaining wide variation among segregants. Based on these studies, crosses may be made between the genotypes from clusters that are far apart genetically to obtain new recombinants in mango since the magnitude of heterosis depends largely on the degree of genetic diversity of parents. Therefore, it is proposed to evolve the hybrids involving cultivars from the clusters with high inter cluster distance to isolate superior segregants in advanced generations with high yield potential and desirable characters (Himabindu *et al.*, 2017).

#### **5.1.5.4 Mean performance of characters in clusters**

Cluster means indicated the average performance of all cultivars clubbed into a cluster. The clusters mean values for all the 15 characters are presented in Table 4.1.8. Cluster XIII comprised of genotypes having the highest average fruit weight, fruit length, fruit width, skin weight, skin thickness, stone weight and pulp weight. The mean values of genotypes present in

cluster I were the lowest among all the clusters for fruit weight, fruit width skin thickness, pulp weight and pulp per cent.

With respect to the biochemical characters, maximum acidity, ascorbic acid, total phenols and total flavonoids were observed in cluster I while minimum were observed in cluster IV, cluster XII, cluster VIII and cluster XIII, respectively. Maximum TSS was exhibited by clusters II and IV and minimum by cluster XI. Although cluster V (23) and cluster VII (21) had the maximum number of genotypes, no remarkable feature was noticed in these two clusters for different characters. Similar results were also noticed by Ismail (2008) in case of lemon.

#### **5.1.5.5 Grouping of cultivars into different clusters**

The entire germplasm was grouped into thirteen distinct clusters using the Ward's method (Table 4.1.9). The distribution of 156 cultivars into thirteen clusters is illustrated in Fig. 4.1.2. The clustering of genotypes is based on the basis of the trait in which they share similarity. The genotypes were grouped based on the degree to which they were similar in the characters studied. However these traits may vary from time to time and region to region, as the performance and quality of fruit is dependent upon the favourable environmental conditions, and expression of the genes responsible for the desired traits.

Ward's minimum variance dendrogram (cluster analysis) created subgroup within a cluster, so relative position of the cultivars within the clusters could be examined by seeing the dendrogram distance.

Cluster V was the largest cluster consisting of 23 cultivars followed by cluster VII with 21 cultivars. Cluster VI consisted of 20 cultivars whereas cluster II consisted of 17 cultivars. Clusters III, VIII and I consisted of 12, 9 and 8 cultivars, respectively. Clusters X and XI consisted of 6 and 4 cultivars each. Cluster XII (Katta Gola, Makaram and Maharaja of Mysore) and cluster XIII (Khudadath, Sora and Safed Mulgoa) consisted of genotypes with large sized fruits. The polyembryonic varieties *viz.*, Vattam, Nekkare, Olour and Vellaikulamban were grouped in a single cluster (cluster VI) except for Chandrakaran and Muvandan which were grouped in cluster I and cluster II, respectively.

Cultivars with high fruit weight can be utilized in crossing programme to realize the broad spectrum of genetic variability in segregating generations to effect the selection for fruit weight improvement. Similar results in relation to large sized table fruit cultivars grouping into a cluster were reported by Pradeepkumar *et al.* (2006) and Rathod (2007) in mango.

Genetic diversity was the outcome of several factors along with geographical diversity. This genetic diversity among the cultivars could be due to factors like heterogeneity, genetic architecture of the populations and developmental traits as described by Murty and Arunachalam (1966). Hence, the selection for hybridization should be more based on genetic diversity rather than geographic diversity. This result suggested that the factors other than geographical separation are responsible for divergence and the genotypes that have originated from the same place may have different genetic architecture or vice-versa. These findings are in agreement with those of Ismail (2008) and Rahman *et al.* (2006). Lack of true relationship between geographical and genetic diversity was also explained by Murthy and Arunachalam (1966) that genetic drift and natural selection in different environment can cause high diversity among the genotypes than geographical isolation.

#### **5.1.5.6 Principal component analysis**

Principal component analysis or canonical (vector) analysis is a sort of multivariate analysis where canonical vectors or roots representing different axes of differentiation and amount of variation accounted by each of such axes, respectively, are derived (Rao, 1952).

Principal component analysis confirms the group constellations obtained by  $D^2$  analysis. The advantage of PCA over  $D^2$  analysis is that it reduces the dimensionality of the data set by creating significant principal components that contributed maximum towards variability of the cultivars.

In principal component analysis, the number of variables were reduced to a linear expression called the principal components and these account for most of the variation produced. Principal component analysis (PCA) for morphological and bio-chemical characters was performed by adopting correlation matrix method. The character loading values for principal components represented the weights defining the contribution of different characters for the respective principal components. Further, the loading signs (+/-) are indicative of the direction of contribution, similar to that of regression coefficients.

The principal component analysis sorted only significant principal components out of the 15 attributes studied. The contribution of the main characters for variance was easily identified by the characters loaded on the PC-1 with high loading values. PCA facilitates the in-depth analysis for genetic diversity.

The pattern grouping obtained by cluster analysis was confirmed by PCA by dividing it into five important principal components contributing more towards variability. The PCA biplot can be used to classify mango genotypes and visualize the relationship among the varieties. The distribution of mango accessions in the PCA based on the PC-1 and PC-2 showed the variations among the accessions.

The analysis thus identified the maximum contributing variables *viz.*, fruit weight, fruit width, fruit length, skin weight and fruit thickness significantly loaded in vector-1 (PC-1) and vector 2 (PC-2), contributing more towards variability. The above characters were positive across the two axes indicating their importance as components of genetic divergence among the studied characters. Negative values for both the vectors for stone weight, pulp weight and total flavonoids indicated the lowest contribution towards total divergence of mango. Majumder *et al.* (2013), in their study of 60 mango genotypes found that weight of harvested fruits per plant, number of fruits per plant, percent of flowering shoot, percent perfect flower and fruit weight were some of the important characters responsible for genetic divergence in the characters studied. Krishnapillai and Wijeratnam (2016) reported that PC-1 explained 34.2% variations, the components being fruit length, breadth, thickness and weight, whereas, PC-2 showed 21.8% variations with the parameters of leaf length, leaf breadth and inflorescence length. Saifullah *et al.* (1999) reported that fruit per primary branch, % TSS, seed diameter, spine density and yield per plant played major role in both axes for determining genetic divergence of jackfruit.

#### **5.1.5.7 Comparative study of D<sup>2</sup> analysis and Principal component analysis**

Mahalanobis' D<sup>2</sup> statistic and Jackson's principal component analysis were used for analyzing multivariate data. In D<sup>2</sup> analysis, the intra and inter-cluster distances were low compared to the principal component analysis. This is same with the utilization of correlation matrix in principal component analysis derived from covariance matrix. The standardization made the principal component analysis to support the clustering analysis.

PCA confirms the group constellations obtained by D<sup>2</sup> analysis. It determines the effective number of axes for differentiation of primary and secondary or based on number of canonical vectors. The advantage of PCA over D<sup>2</sup> analysis is that it reduces the dimensionality of the data set by creating significant principal components that contributed maximum towards variability of the cultivars. In PCA, standardization of data made attributes to contribute equally towards the divergence studies irrespective of the units taken.

The principal component analysis sorted only significant principal components out of 15 attributes studied. The contribution of the main characters for variance was easily identified by the characters loaded on the PC I with high loading values. PCA facilitate the in-depth analysis for genetic diversity.

In D<sup>2</sup> analysis, fruit weight followed by ascorbic acid, carotenoids, acidity and fruit width contributed maximum towards diversity, while in PCA the characters *viz.*, fruit weight, fruit width, fruit length, skin weight and total phenols contributed more towards variability. It could be concluded that the characters which contributed more towards divergence in D<sup>2</sup> analysis were loaded in PC-1 except total carotenoids which showed negative loadings in PC-1.

## **5.2 Characterization of the genotypes using molecular tools**

Although morphological traits are very useful, they have several disadvantages. They are often limited in number and suffer from lack of decisiveness. 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 based on morphological traits needs complementation with molecular markers.

The information obtained by using molecular markers like SSR offer many benefits for identifying variation and for establishing diversity among the cultivars. Molecular markers enable the assessment of genetic similarity among cultivars in the early stages of development and are advantageous for diversity studies (Nicolosi *et al.*, 2000). Understanding the genetic diversity among the varieties is important in mango production, improvement, and breeding; knowledge on this field can supply useful information for further scientific progress in developing new accessions (Ravishankar *et al.* 2000; Rajwana *et al.* 2011). In the present study, apart from using the morphological and bio-chemical traits, SSR markers were also used to estimate the extent of molecular diversity among mango cultivars. All the 156 mango cultivars were studied for their genetic diversity at molecular level by using 12 Simple Sequence Repeat (SSR) markers. The results are discussed below.

### **5.2.1 Polymorphic information content (PIC) of SSR marker analysis**

The SSR primers used in this study showed relatively higher values of heterozygosity and PIC. The genetic analysis was done using Cervus 3.0 (Kalinowski *et*

*al.*, 2007). The expected heterozygosity ( $H_e$ ) values ranged from 0.885 to 0.990, observed heterozygosity from 0.545 to 0.795 and polymorphic information content from 0.877 to 0.9987 with a mean of 0.943. These results indicated that, the markers selected in the present study were highly informative, efficient and highly reproducible genetic markers although the development of microsatellites was considered to be laborious and expensive (Eiadthong *et al.*, 1999). SSRs are widely used as a versatile tool in plant breeding programmes as well as in evolutionary studies because of their ability for showing diversity among cultivars (Adato *et al.*, 1995). Therefore in the present investigation twelve selected SSR primers were used to analyse inter and intra cultivar diversity among 156 mango genotypes. High level of polymorphism was observed with MiKVR 965 primer. The higher efficiency of SSR markers in molecular characterization of mango compared to morphological markers is because of the higher values of heterozygosity and PIC values indicating their usefulness for fingerprinting mango genotypes (Ravishankar *et al.*, 2011). It was comparable with the results generated by polymorphic bands ranging from 90 bp to 370 bp (Begum *et al.*, 2012), 100 bp to 480 bp (Anshuman *et al.*, 2012), 130 bp to 245 bp (Begum *et al.*, 2013) in mango.

Understanding the diversity and relatedness of accessions can assist breeders to better select parents with the potential to contribute desired genes to progeny and for developing new commercial cultivars. Genetic diversity within a breeding program is highly desirable to enable new cultivars to be produced with novel productivity and fruit quality traits necessary for sustainable productivity and market competitiveness. (Dillon *et al.*, 2014a). The high levels of polymorphism obtained with twelve SSRs in the present study was consistent with their known characteristics that they are more variable and revealed greater diversity than RFLPs or RAPDs (Powell *et al.*, 1996). The high polymorphism associated with SSR was to be expected because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Tautz and Renz, 1984; Tautz *et al.*, 1986) rather than by single nucleotide mutations and insertions or deletions.

### **5.2.2 Diversity analysis**

The dendrogram obtained through Neighbour joining method using Darwin 5 software resulted in the formation of three major clusters which divided into thirteen important sub clusters, as that of morphological cluster analysis, depending upon their genetic relatedness/ distance.

Cluster I consists of 67 number of progenies. Further, cluster I is sub-divided into four sub-clusters (1a, 1b, 1c and 1d) consisting of 21, 22, 14 and 10 progenies, respectively.

Cluster II consisted of 7 genotypes which were further divided into two clusters (2a and 2b) consisting of 3 and 4 genotypes, respectively.

Cluster III comprised of the remaining 84 genotypes sub-clustered into 7 groups. The sub-clusters 3a, 3b, 3c, and 3d consisted of 6, 15, 11 and 11 genotypes, respectively, while, sub-clusters 3e, 3f and 3g were found to comprise of 23, 4 and 12 genotypes, respectively.

All the polyembryonic varieties *viz.*, Olour, Muvandan, Vattam, Nekkare, Vellaikulamban and Chandrakaran are grouped into one cluster. Similarly the Goan varieties *viz.*, Karanjio, Sardar and Shendriya are grouped in one cluster indicating their common genetic base. The appemidi types *viz.*, Huli Appekai, Adderi Jeerige and HittalhalliAppe, that are unique to Uttar Kannada region are also clustered together. These types are unique in the way that they are highly acidic, fibrous and rich in characteristic raw mango flavour, medium to high latex flow and firm pulp with good keeping quality. They are gaining importance in the export market because of their suitability for pickling as whole fruit (tender mangoes). The results of the present study are in line with the observations made by Ravishankar *et al.* (2000) who showed high genetic relatedness among cultivars from the same geographical region using RAPD markers. They found northern and eastern regions formed one zone and western and southern regions formed another zone of mango diversity in India. Region-based grouping of Indian cultivars was earlier reported by Lopez *et al.* (1997). Karibasappa *et al.* (1999) also carried out diversity studies in which cluster analysis showed eleven clusters in sixty-nine genotypes of mango.

India being the primary centre of origin for mango, greatest genetic diversity has been reported. Further, the crop exhibits rich genetic diversity for various horticultural traits and has a great scope for improvement. The tendency of cultivars grouping in clusters was irrespective of geographic boundaries, demonstrated that geographical isolation was not the only factor for causing genetic diversity. This suggested that there are forces other than geographical separation such as natural or artificial selection, exchange of breeding material, genetic drift and environmental variation. The existence of duplicates might be due to multiple donations of the same seed source but from different collections. Therefore, diverse geographic origins among cultivars might not always be a reliable indicator for sampling of genetically diverse materials. From the conservation point of view, identification and elimination of duplicates in a collection could save time and resources, both financial and human, in germplasm maintenance due to reduced number of cultivars.

The present study of use of microsatellite markers for characterization of mango cultivars revealed that 12 polymorphic microsatellite markers were found efficient to distinguish cultivars and would certainly be useful for purposes such as the certification of varieties, for identification of pest and disease resistant lines and for development of superior plants from the crosses of these mango cultivars.

The information obtained from diversity analysis can be utilized in making the crosses and selection of divergent parents to maximize heterosis in future breeding programmes. SSR analysis shows great potential for mango improvement and can be performed for variety identification and estimation of genetic variation in existing populations (Brettell *et al.*, 2002). Knowledge about the extent of genetic diversity is the major component in designing future breeding strategies for sustainability in mango production. It is important to couple phenotypic analysis with genetic diversity for germplasm conservation in gene bank collections.

### **5.3 Attempting marker assisted selections in the progeny population**

Mukherjee *et al.* (1961) were the first to describe inter-varietal hybridization in mango with 1.45 per cent success. Mango breeding in India is in tune with the modern mango breeding programmes in other countries which have focused on cultivars with higher production, red peel colour, distinct TSS: acid ratio, disease resistance and extended shelf-life. These criteria are justified by the identified needs of the growers and of the world-wide mango market (Campbell and Zill, 2009). 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.

Of late, hybrid Amrapali has emerged as the premium choice of farmers in the country, particularly in the eastern states. In spite of its desirable characters, variety Amrapali suffers from few demerits such as high susceptibility to mango malformation, uneven fruit size, unattractive fruit appearance and decline in productivity after few years. To overcome this, Amrapali was crossed with Vanraj and *M. odorata*. The inclusion of coloured variety Vanraj as male parent in hybridization programme was to impart the desirable red peel characteristic into the newly developed hybrids whereas *M. odorata* was used to incorporate its resistance to fruit fly.

Molecular characterization for forty-two mango hybrids and their parents was carried out using eight SSR markers which revealed that the observed heterozygosity ( $H_0$ ) ranged from 0.898 to 0.980, while expected heterozygosity ( $H_e$ ) ranged from 0.874-to 0.906 in mango hybrids and parents. All the 8 SSR markers were found to be informative primers as their calculated

polymorphic information content (PIC) values varied from 0.850 to 0.888. The maximum PIC value was noted in MiIHR 36 (0.888) and the minimum in MiIHR 31 (0.850). The number of alleles per locus ranged from 17 (MiIHR 30) to 24 (MiIHR 17 and MiIHR 36), with an average of 21.25.

The SSR markers that have been developed for mango are easily used to verify parentage using a software package like CERVUS (Marshall *et al.*, 1998), which, in this case, was used for paternity analyses of 42 mango hybrids. Simulation of parentage analysis was done for likelihoods using the corrected likelihood equations of Kalinowski *et al.* (2007), which is superior because they have higher power to assign parentage, and more accurately take account of typing errors.

LOD score was calculated for candidate parent, and out of 21 hybrids involving crosses between Amrapali  $\times$  Vanraj, it was possible to assign parentage for 12 hybrids with strict confidence (95%) and 5 with relaxed confidence (80%). 5 of the hybrids were unassigned as the candidate parent is not most likely its parent. Among the hybrids developed from crosses between Amrapali  $\times$  *M. odorata*, 6 of the hybrids were assigned parentage with strict confidence (95%), while 7 were done so with relaxed confidence (80%). In this study, out of the forty-two hybrids, nine hybrids were left unassigned. One possible reason for this may be that the true parents were not sampled for these hybrids or otherwise there may be need of additional loci for assigning parentage with strict confidence.

As in the present study, Begane *et al.* (2018) carried out the molecular characterization of thirty-eight hybrids with eight SSRs (Simple Sequence Repeats), and observed paternal allele inheritance for the hybrids, Arka Udaya, Konkan Ruchi, Manjeera and AU-Rumani to the extent of 50, 50, 54.54 and 50%, respectively. Among the 64 Florida cultivars evaluated in the parentage analysis by Schnell *et al.* (2006), 31 cultivars were found to have 'Haden' as one of the most likely parents. Likewise, the other important early Florida selection 'Brooks' is the parent of seven cultivars. Singh *et al.* (2012) analyzed forty eight mango hybrids using 17 simple sequence repeat (SSR) markers. SSRs gave moderate values of Polymorphic Information Content (PIC) and heterozygosity. The tendency of clustering among mango hybrids revealed that they had stronger affinity towards female parent Amrapali.

Despite the drawbacks ailing mango breeding, like, high heterozygosity and single seed per fruit, breeding can be successful because of a number of positive attributes *viz.*, wide range of available genetic variation and the ease with which a selected hybrid can be vegetatively

propagated (Iyer and Schnell, 2009). The presence of delicate flowers, complex floral biology, poor fruit set and absence of pre-selection indices have made validation a necessity for determining the parentage of a hybrid. Of all the markers ‘Simple Sequence Repeats (SSR)’ show great potential for mango improvement and can be performed for variety identification and validation of parentage (Singh *et al.*, 2012). Analysis of hybrids and their parents is essential to know the contribution of each parent to their progenies, which will help in further analysis of hybridization programmes (Vasanthaiah, 2009).

#### **5.4 Estimation of volatiles in certain mango progenies and their parents**

Mango varieties differ in the amount and type of flavour compounds present, which is dependent on their place of origin; major and minor volatile compounds play a key role in their aroma (Kanjana *et al.*, 2005). There is vast literature on chemical analysis of the aroma of several mango cultivars around the world (Macleod and Troconis, 1982) with a wide range of compounds having been identified as esters, lactones, mono- and sesqui-terpenes. The different proportions of volatile components and the presence or absence of trace components often determine aroma properties (Zavala *et al.*, 2004).

Although, morphological and molecular diversity analyses have helped significantly in cultivar identification (Kumar *et al.*, 2001; Karihaloo *et al.*, 2003; Pandit *et al.*, 2007), use of biochemical features, which are the actual desired traits, is necessary to supplement this task and confer a functional dimension. Leaf volatile blend presents a good experimental system for such an endeavour as it includes array of chemicals from various classes such as hydrocarbon, alcohol, aldehyde, ketone and terpenes.

Therefore, in this work, 25 mango hybrids and their parents have been analysed for volatile profiles in the leaves. An attempt has also been made to assess the diversity and the relationships amongst these cultivars, based on presence of such compounds. In addition, this study has enabled us to have a glimpse into the aroma-bank of mango hybrids and also to highlight the discrepancies amongst the cultivars based on this attribute.

The aroma components of 39 mango hybrids and their parents were determined independently with HS-SPME-GC-MS/MS technique, the major proportion being contributed by the monoterpenoids and sesuiterpenoids composition. Similar results was observed by Wetungu *et al.* (2015) who studied the chemical profile of six mango varieties and reported that the mango

leaves were rich in monoterpenes (46.98%) and sesquiterpenes (38.17%) with minor quantities of their analogues (10.67%).

The chemical diversity of the leaves of 39 mango cultivars (Table 4.4.1) were analysed, wherein 77 different volatile constituents were detected. This total of 77 volatile compounds was contributed by different chemical classes, namely, hydrocarbons, alcohols, aldehydes and ketones, monoterpenoids and sesquiterpenoids. Four compounds were placed under 'miscellaneous' category in this analysis. Within this set of cultivars, H-85 contained the highest number of volatiles (67), followed by Ratna, Rumani and H-151, each containing 66 number of volatiles. Sindhu contained the least (52) volatiles followed by Manjeera, Swarna Jehangir and Suvarnarekha with 54 each.

#### 5.4.1 Genotypic variability in leaf volatile composition

Significant differences in the composition and proportion of leaf volatile components were observed among the mango genotypes studied. The genotype Fazli had the highest per cent monoterpenoids (65.87%), followed by Alphonso (59.56%) and Vanraj (57.39%). Among the monoterpenoids, cis-ocimene (7.22%), 3-carene (5.33%) and dl-limonene (4.52%) contributed higher proportion to leaf volatiles.

Sesquiterpene hydrocarbons form the second largest group of aroma volatiles in mango (Pandit *et al.*, 2009). Sesquiterpenoids are major active molecules present in mango leaves and reported an anticancer activity in the leaf oil for the first time by Simionatto *et al.* (2010). Significant differences in composition of total sesquiterpenoids were recorded among genotypes studied. The highest per cent of sesquiterpenoids composition was observed in genotype Rumani (91.48%) followed by H-151 (90.17%), while, the least content was noticed in genotype Dashehari (26.22%). In case of sesqui terpenoids, caryophyllene (20.87%),  $\alpha$ -gurjunene (16.11%) and  $\alpha$ -humulene (11.47%) contributed maximum to the leaf volatiles in the genotypes studied. This was also supported by the investigation carried out by Gebara *et al.* (2011) in *M. indica* var. coquinho. They reported that cyperene, E-caryophyllene,  $\alpha$ -humulene and terpinolene were the main compounds found in immature leaves, whereas, in mature leaves, cyperene,  $\alpha$ -gurjunene, E-caryophyllene, cedrene and  $\alpha$ -humulene were the main substances detected. Dzbreveamic *et al.* (2010) observed that the leaves of *M. indica* were rich in sesquiterpenes (70.3%), the dominant compounds in mango leaf oil being  $\delta$ -3-carene (20.5%),  $\alpha$ -gurjunene (19.2%),  $\beta$ -selinene (13.9%) and  $\beta$ -caryophyllene (13.7%).

Earlier findings of Pino *et al.* (2005) indicated that terpenes are the primary aroma constituents present in most of the Indian mango genotypes and classified the mango cultivars into terpinolene cultivars, 3-carene cultivars, and myrcene cultivars, based on their different constituents among terpenes. The present study also found similar results and indicated that, terpenes were the main aroma constituents among the genotypes which include monoterpenes like  $\alpha$ -pinene,  $\beta$ -phellandrene, limonene, cis-ocimene and sesquiterpene like  $\alpha$ -humulene,  $\alpha$ -selinene, caryophyllene and terpinolene. Similar results were found by Wetungu *et al.* (2015) who reported that seven compounds, namely,  $\alpha$ -pinene, camphene,  $\beta$ -pinene,  $\alpha$ -copaene,  $\beta$ -elemene,  $\alpha$ -gurjunene and  $\alpha$ -humulene were present in significant but varying amounts (0.3-21.9%) among the mango cultivars studied. However, the large differences in the terpenes compositions and proportions in the present study might be attributed due to differences in genetic composition of cultivars.

Aldehyde occurs at a low level, but plays a key role in mango flavour. This observation was supported by the earlier finding by Macleod and Troconis (1982) that the aldehyde content of mangoes was 0.03%-14.36%. In the present study, only few cultivars contained aldehydes as they are more of fruit specific volatiles. Pino *et al.* (2005) considered that the aldehydes in Cuban mango cultivars were associated with the sweet herbal flavour of their fruit.

Aldehydes are easily broken down to alcohols and lactones (Matsui, 2006). Alcohols occur in small amounts in mango fruit and contribute slightly to their aroma and flavor (Engel and Tressl, 1983; Quijano *et al.*, 2007). However, in the present study on leaves, cultivars contained less per cent of alcohols ranging between 0.01% (Bombay Green) and 0.45% (PKM-1). Cis-3-hexanal and 2-hexanal were the only aldehyde and ketone detected among the total flavor volatile compounds in the present study, with its highest concentration in Suvarnarekha (6.23%).

Principal component analysis (PCA) for major leaf monoterpenoids and sesquiterpenoids composition was performed by adopting correlation matrix method. The factor loading values for principal components represented the weights defining the contribution of different characters for the respective principal components. The analysis thus identified the maximum contributing variables *viz.*,  $\alpha$ -pinene, 1-phellandrene and dl-limonene significantly loaded in vector-1 (PC-1) and vector 2 (PC-2), contributing more towards variability. The above characters were positive across the two axes indicating their importance as components of genetic divergence among the studied characters. Negative values for both the vectors for  $\beta$ -ocimene,  $\beta$ -elemene and germacrene-D indicated the lowest contribution towards total divergence of mango.

The results differ qualitatively and quantitatively with similar reported essential oil chemical compositions of mango varieties from other countries. This suggests that variations in climate and soils can influence the chemical composition of mango cultivars. The variations in the essential oil compositions of the mango varieties could account for the differences in the natural aroma of the mango varieties.

In summary, mango cultivars differ in the total concentration of volatiles, both in terms of qualitative and quantitative composition of these volatiles and in their principle volatile components. Monoterpenoids and sesquiterpenoids are the major constituents of mango cultivars and these cultivars can be classified into mono- or sesqui-terpene dominating groups. On the quantitative basis,  $\alpha$ -gurjunene, caryophyllene,  $\alpha$ -humulene, cis-ocimene and 3-carene are the major compounds in mango leaves. This work can be extended to determine the sensory impact of the detected volatiles and the possibility of utilization of the leaf oils to improve the aroma quality of processed mangoes can be given further thought. The volatiles can serve as a biochemical marker for screening hybrid progenies in the breeding programme as well.

The present investigation entitled “**Genetic diversity and characterization studies in mango**” was conducted at Division of Fruit Crops, Division of Biotechnology and Division of Physiology and Biochemistry, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru during 2015-18 with the following objectives:

- Study the diversity existing in *M. indica* varieties from different regions
- Characterization of the genotypes using molecular tools
- Attempting marker assisted selections in the progeny population
- Estimation of volatiles in certain progenies and their parents

The study was carried out with one hundred and fifty six mango (*Mangifera indica* L.) cultivars to document information on fifteen morphological and bio-chemical characters and to determine the relationship between different characters through correlation analysis. The study also aimed to assess the extent of genetic diversity by D<sup>2</sup>, PCA analysis and at molecular level using SSR markers. Parentage analysis was also carried out in Amrapali × Vanraj and Amrapali × *M. odorata* hybrids using eight SSR primers. The volatile profile of leaves of 39 mango hybrids and their parents was also analysed by headspace-solid phase micro-extraction (HS-SPME) technique using capillary GC and GC–MS/MS. The salient features of the experimental findings are summarized below under following heads.

## **6.1 Study the diversity existing in *M. indica* varieties from different regions**

### **6.1.1 Fruit morphological characters**

Various fruit morphological characterizations were carried out systematically for the entire 156 mango genotypes using standard descriptor for mango by IPGRI (2006). The analysis of variance for the nine fruit morphological traits revealed significant differences for all the characters studied thus indicating wide variation among the cultivars.

The cultivars, Safed Mulgoa, Sora and Khudadath appeared to be promising donors for fruit yield showing maximum fruit weight and pulp weight. The cultivars, Safed Mulgoa and Magemavu reported maximum fruit skin thickness which can be used as a source for high shelf

## *SUMMARY & CONCLUSION*



life of mango fruits. The cultivars Elaichi, Putu and Siroli recorded minimum stone weight, while the cultivars, Hamlet, Maharaja of Mysore and Lord recorded maximum pulp per cent.

### **6.1.2 Bio-chemical characters**

The cultivars Adderi jeerige and Hithalahalli Appe recorded highest titratable acidity, total phenols and total flavonoids content and also exhibited high values with respect to ascorbic acid, while, the cultivars K-O-7 and Khas-ul-Khas recorded maximum TSS indicating that these cultivars could be used as a source for quality improvement. The cultivars Manibhatta Appe, Padari and Chethalli recorded maximum total carotenoids content implying their usefulness as a source for vitamin A.

### **6.1.3 Correlation studies**

The association of fruit length, fruit width, fruit thickness, skin weight, skin thickness, stone weight, pulp weight and pulp per cent with fruit weight was positive and highly significant and these traits can be used for the genetic improvement of fruit weight. The genetic improvement of fruit quality thus can be obtained by direct selection of these components. The morphological characteristics had significant positive correlations with each other, but had negative correlations with the biochemical characteristics. Genotypic coefficients of correlation ( $r_g$ ) revealed higher magnitude than their corresponding phenotypic coefficients ( $r_p$ ) in most of the cases, indicating that there is an inherent association among the characters studied.

### **6.1.4 Genetic variability studies**

Genetic variability studies revealed that the range of PCV was 14.85 to 76.73 while the range of GCV was 12.73 to 75.83 for morphological and bio-chemical characters. The higher PCV and GCV values for fruit weight, skin weight, pulp weight, titratable acidity, total carotenoids and ascorbic acid indicated high variability among the different cultivars of mango for these characters.

The estimates for heritability ranged from 70.27 to 99.33 and genetic advance as per cent mean ranged from 22.48 to 154.40 for morphological and bio-chemical characters.

High heritability coupled with high genetic advance was recorded for fruit weight, skin weight, pulp weight, ascorbic acid, total phenols and total flavonoids indicating the role of additive gene action governing the inheritance of these traits and these traits can be improved through simple selection.

### **6.1.5 Genetic divergence (Mahalanobis D<sup>2</sup> statistic)**

The multivariate analysis indicated the presence of considerable genetic divergence among the mango cultivars. The 156 mango cultivars were grouped into thirteen clusters in D<sup>2</sup> analysis. The result of character wise contribution towards total genetic divergence showed that fruit weight (47.07%) had the greatest contribution by ranking first in 5691 times out of 12090 combinations followed by ascorbic acid (24.85%) which ranked first 3004 times. The characters *viz.*, carotenoids, acidity and fruit width, contributed 8.06, 5.37 and 5.06 per cent towards the diversity by ranking first 974, 649 and 612 times, respectively. The characters with maximum contribution towards diversity should also be given due consideration for mango crop improvement.

The highest inter cluster D<sup>2</sup> value was recorded between clusters I and XIII (6572.160) indicating that the cultivars of these clusters are highly divergent. Based on these studies crosses may be made between the genotypes from clusters that are far apart genetically to obtain new recombinants in mango since the magnitude of heterosis depends largely on the degree of genetic diversity of parents. On the other hand, the minimum inter cluster D<sup>2</sup> value recorded between cluster III and IV (172.472) indicated almost parallel diversity among the cultivars included in these clusters.

The highest intra cluster D<sup>2</sup> value observed in cluster XI (491.651) indicated the presence of wide genetic diversity among the genotypes present in cluster, while, the lowest (93.041) intra cluster D<sup>2</sup> value was observed in cluster IV indicating less genetic diversity among the cultivars present within the group.

Cluster means indicated the average performance of all cultivars clubbed into a cluster. Cluster XIII comprised of genotypes having the highest average fruit weight, fruit length, fruit width, skin weight, skin thickness, stone weight and pulp weight, whereas, cluster I exhibited maximum acidity, ascorbic acid, total phenols and total flavonoids which can be used for creating variability.

### **6.1.6 Principal component analysis**

Principal component analysis (PCA) recognized four principal components (PCs) with eigen values more than one which contributed 70.77 per cent of cumulative variance. The analysis identified the maximum contributing variables *viz.*, fruit weight, fruit width, fruit length, skin weight and fruit thickness significantly loaded in vector-1 (PC-1) and vector 2 (PC-2),

contributing more towards variability. The above characters were positive across the two axes indicating their importance as components of genetic divergence among the studied characters. Negative values for both the vectors for stone weight, pulp weight and total flavonoids indicated the lowest contribution towards total divergence of mango.

## **6.2 Characterization of the genotypes using molecular tools**

Molecular diversity among 156 mango genotypes was studied using twelve SSR primers. The analysis of 12 SSR markers resulted in the detection of alleles, with an average of alleles/SSRs, ranging from 23 alleles in MiIHR-30 to 105 alleles/SSRs in MiKVR-965. The expected heterozygosity ranged from 0.885 in MiIHR-18 to 0.990 in MiKVR-965. The observed heterozygosity ranged from 0.545 in MiIHR-18 to 0.795 in MiKVR-642, indicating high polymorphism. The polymorphic information content (PIC) value was maximum (0.987) in MiKVR-965 and minimum (0.877) in Mi IHR-18.

Genetic diversity tree constructed by Neighbour joining method using Darwin software grouped the genotypes into three major clusters; cluster I consisting of 67 number of genotypes, cluster II comprised of 7 genotypes, while the remaining 84 genotypes were grouped in cluster III, sub-clustered into 7 groups.

## **6.3 Attempting marker assisted selections in the progeny population**

LOD score was calculated for candidate parent, and out of 21 hybrids involving crosses between Amrapali × Vanraj, it was possible to assign parentage for 12 hybrids with strict confidence (95%) and 5 with relaxed confidence (80%). 5 of the hybrids were unassigned as the candidate parent is not most likely its parent. Among the hybrids developed from crosses between Amrapali × *M. odorata*, 6 of the hybrids were assigned parentage with strict confidence (95%), while 7 were done so with relaxed confidence (80%). In the present study, out of the forty-two hybrids, nine hybrids were left unassigned.

## **6.4 Estimation of volatiles in certain progenies and their parents**

The chemical diversity of the leaves of 39 mango cultivars were determined independently with HS-SPME-GC-MS/MS technique, the major proportion being contributed by the monoterpenoids and sesquiterpenoids composition. The seventy seven different volatile constituents detected were composed of various chemical classes, namely, hydrocarbons, alcohols, aldehydes and ketones, monoterpenoids and sesquiterpenoids.

The genotype Fazli had the highest (65.87%) monoterpenoids proportion while the highest per cent of sesquiterpenoids composition was observed in genotype Rumani (91.48%). On the quantitative basis,  $\alpha$ -gurjunene, caryophyllene,  $\alpha$ -humulene, cis-ocimene and 3-carene are the major compounds in mango leaves.

#### **6.4.1 Principal component analysis**

Principal component analysis (PCA) for major leaf monoterpenoids and sesquiterpenoids composition was performed. The analysis identified the maximum contributing variables *viz.*,  $\alpha$ -pinene, l-phellandrene and dl-limonene significantly loaded in vector-1 (PC-1) and vector 2 (PC-2), contributing more towards variability. The above characters were positive across the two axes indicating their importance as components of divergence among the studied characters. Negative values for both the vectors for  $\beta$ -ocimene,  $\beta$ -elemene and germacrene-D indicated lowest contribution towards total divergence.

#### **6.5 Conclusion**

1. The cultivars with high desirable characters from this study can be used as parents for hybridization programme.
2. For the protection of valuable germplasm, molecular markers can be employed for characterization, conservation of elite genotypes and establishment of geographical indications.
3. The high degree of divergence observed among mango hybrids could be attributed to the highly heterozygous and cross pollinated nature of the mango crop, different parental combinations of hybrids and high discriminatory power of the SSR markers.
4. The results indicated that SSR markers are useful not only for varietal identification and detection of duplicate entries, but also for the use in future mango breeding programmes to design crosses that maximize genetic variability with the objective of developing superior mango hybrids suited to emerging consumer demands.
5. The molecular fingerprinting of mango hybrids using SSR markers assumes significance in the face of demand for exacting quality in international trade, need for ensuring fidelity in planting stocks and solving the disputes related to intellectual property rights on indigenous biodiversity.

# *ABSTRACT*



## GENETIC DIVERSITY AND CHARACTERIZATION STUDIES IN MANGO

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### ABSTRACT

Knowledge about the extent of genetic diversity/relatedness in mango germplasm is vital for developing coherent strategies for future gains in productivity and quality. The present investigation was conducted at ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru during 2015-18 by studying the morphological and biochemical characters to assess the extent of variability and the genetic diversity by using SSR markers. Wide variation was recorded among the 156 genotypes and these genotypes differed significantly for all the morphological and bio-chemical characters studied. Genetic variability studies revealed that the range of PCV was 14.85 to 76.73, while, the range of GCV was 12.73 to 75.83. The estimates for heritability ranged from 70.27 to 99.33 and genetic advance as per cent mean ranged from 22.48 to 154.40. The 156 mango cultivars were grouped into thirteen clusters in D<sup>2</sup> analysis. The analysis of 12 SSR markers resulted in the detection of alleles, with an average of alleles/SSRs, ranging from 23 alleles in Mi IIHR-30 to 105 alleles/SSRs in MiKVR-965. The polymorphic information content (PIC) value was maximum (0.987) in MiKVR-965 and minimum (0.877) in Mi IIHR-18. Parentage analysis was also carried out in mango hybrids using eight SSR primers. LOD score was calculated for candidate parents, and out of 42 hybrids involving crosses between Amrapali, Vanraj and *M. odorata*, it was possible to assign parentage for 14 hybrids with strict confidence (95%) and 10 hybrids with relaxed confidence (80%). The chemical diversity of the leaves of 39 mango cultivars were determined independently with HS-SPME-GC-MS technique, the major proportion being contributed by the monoterpenoids and sesquiterpenoids composition. The seventy seven different volatile constituents detected were composed of various chemical classes, namely, hydrocarbons, alcohols, aldehydes and ketones, monoterpenoids and sesquiterpenoids.

Keywords: mango; diversity; morphological; bio-chemical; volatiles

# आम की आनुवंशिक विविधता और लक्षणीकरण का अध्ययन

## सारांश

आम के जननद्रव्य में आनुवंशिक विविधता/संबद्धता के विस्तार का ज्ञान उत्पादकता और गुणवत्ता के क्षेत्र में भविष्य में लाभ पाने के लिए सुसंगत कार्य-पद्धति के विकास में महत्वपूर्ण है। यह अध्ययन भा.कृ.अनु.प.-भारतीय बागवानी अनुसंधान संस्थान, बेंगलूरु में 2015-18 के दौरान एसएसआर मार्करों का इस्तेमाल करते हुए परिवर्तिता की व्याप्ति और आनुवंशिक विविधता के विश्लेषण के लिए आकारकीय और जैवरासायनिक लक्षणों के अध्ययन के द्वारा किया गया। एक सौ छप्पन जीनप्ररूपों में व्यापक परिवर्तन दर्ज किया गया और ये जीनप्ररूप अध्ययन किए गए सभी आकारकीय और जैवरासायनिक लक्षणों के संबंध में काफी भिन्न थे। आनुवंशिक परिवर्तिता अध्ययन से पता चला कि पीसीवी 14.85 से 76.73 के बीच था, जबकि जीसीवी 12.73 से 75.83 के बीच था। आनुवंशिकता का अनुमान 70.27 से 99.33 के बीच और प्रतिशत औसत के अनुसार आनुवंशिक प्रगति 22.48 से 154.40 के बीच थी। आम की 156 प्रजातियों को डी2 विश्लेषण में 13 समूहों में बाँट दिया गया। बारह एसएसआर मार्करों के विश्लेषण के परिणामस्वरूप औसतन एमआई आईआईएचआर-30 में 23 एलीलों से लेकर एमआईकेवीआर-965 में 105 एलील/ एसएसआर तक एलीलों की पहचान की जा सकी। बहुरूपता सूचना मात्रा (पीआईसी) मूल्य एमआईकेवीआर-965 में अधिकतम (0.987) और एमआई आईआईएचआर-18 में न्यूनतम (.0.877) था। आठ एसएसआर प्राइमरों के उपयोग से आम का व्युत्पत्ति-विश्लेषण भी किया गया। प्रत्याशी जनकों का एलओडी स्कोर की गणना की गई, और 42 संकरों में से, जिनमें आमपाली, वनराज और एम. ओडोराटा का संकरण शामिल है, पूरे आत्मविश्वास (95%) के साथ 14 संकरों के लिए और शिथिल आत्मविश्वास (80%) के साथ 14 संकरों के लिए जनक निर्धारित करना संभव था। एचएस-एसपीएमई-जीसी-एमएस तकनीक, जिसमें मुख्य अनुपात मोनोटर्पेनोइडों और सेस्क्विटर्पेनोइडों के संयोजन से बनता है, से आम की 39 प्रजातियों के पत्तों की रासायनिक विविधता स्वतंत्र रूप से निर्धारित की गई। पहचान किए गए 77 विभिन्न वाष्पशील संघटक कई रासायनिक वर्गों, जैसे हाइड्रोकार्बन, एल्कोहल, आल्डीहाइल और कीटोन, मोनोटर्पेनोइड और सेस्क्विटर्पेनोइड से बने हैं।

मुख्य शब्द : आम; विविधता; आकारकीय; जैव-रासायनिक; वाष्पशील

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# *APPENDICES*



**Meteorological observations during October, 2015 to February, 2018 at ICAR-IIHR, Bengaluru**

Month	Temperature (°C)		Relative Humidity (%)		USWB Class 'A' Pan Evaporation (mm)	Mean wind speed (km/h)	Rainfall (mm)	Average Day length (hrs)
	Max.	Min.	0.7.30 hrs.	14.00 hrs.				
October, 2015	30.35	19.97	78.77	52.61	3.41	2.52	4.60	11:50:35
November, 2015	28.17	17.97	84.23	53.13	1.43	2.77	5.77	11:32:14
December, 2015	28.00	17.81	79.74	52.84	1.68	2.54	0.00	11:22:49
January, 2016	27.77	15.35	77.68	45.61	3.16	2.34	0.02	11:27:24
February, 2016	32.31	19.03	75.38	48.34	4.74	3.24	0.00	11:43:06
March, 2016	35.55	21.55	69.39	43.32	5.95	2.99	0.00	12:03:52
April, 2016	37.6	24	71.67	43.6	6.16	3.65	0	12:25:31
May, 2016	35.77	22.42	72.23	37.81	5.36	4.14	3.03	12:43:39
June, 2016	30.83	20.00	80.53	40.00	3.14	5.74	4.28	12:52:36
July, 2016	29.23	20.55	78.10	49.97	3.38	6.36	5.62	12:48:01
August, 2016	27.55	21.19	78.84	57.84	3.57	5.23	0.53	12:32:11
September, 2016	25.33	20.97	79.63	55.73	2.70	3.91	1.93	12:11:28
October, 2016	26.84	20.35	65.81	43.16	4.68	2.28	0.49	11:50:05
November, 2016	26.57	19.10	63.70	34.07	4.33	2.02	0.30	11:31:52
December, 2016	27.32	17.39	74.81	43.03	3.44	2.78	2.00	11:22:45

Month	Temperature (°C)		Relative Humidity (%)		USWB Class 'A' Pan Evaporation (mm)	Mean wind speed (km/h)	Rainfall (mm)	Average Day length (hrs)
	Max.	Min.	0.7.30 hrs.	14.00 hrs.				
January, 2017	28.61	16.32	72.71	36.16	3.80	2.21	0.00	11:27:41
February, 2017	27.30	14.95	54.60	24.65	5.76	2.50	0.00	11:43:15
March, 2017	31.97	17.55	54.97	25.48	6.24	0.78	0.85	12:03:41
April, 2017	34.93	20.28	70.37	30.00	6.50	3.71	1.13	12:25:21
May, 2017	33.01	21.18	75.55	48.71	4.05	2.69	9.49	12:43:32
June, 2017	28.38	20.92	77.10	64.40	4.45	5.14	1.97	12:52:34
July, 2017	28.85	20.18	75.10	60.39	4.46	6.09	1.34	12:48:07
August, 2017	28.49	20.96	82.90	64.23	3.41	4.82	7.89	12:32:20
September, 2017	28.52	20.40	85.87	65.37	2.64	3.86	11.97	12:11:38
October, 2017	28.66	19.53	82.52	61.87	2.75	2.64	7.80	11:50:15
November, 2017	27.58	17.51	77.67	56.37	3.37	3.00	0.39	11:31:59
December, 2017	27.20	14.17	79.74	45.32	3.57	3.92	0.18	11:22:46
January, 2018	28.36	12.66	78.45	36.35	4.25	2.70	0.00	11:27:36
February, 2018	30.20	12.74	67.54	27.79	5.59	3.37	0.02	11:43:06