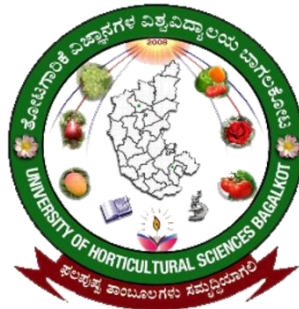


STUDIES ON DIVERSITY OF INDIGENOUS MANGO
(*Mangifera indica* L.) GENOTYPES

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BAGALKOT, KARNATAKA, INDIA

2018



*Affectionately Dedicated
to
My respected Guide,
Beloved Amma, Appa,
Atthe, Belli, Vini
And*

*My Best half- Rajath
&
To the farming community*



UNIVERSITY OF HORTICULTURAL SCIENCES, BAGALKOT

**COLLEGE OF HORTICULTURE, BENGALURU
DEPARTMENT OF HORTICULTURE**

CERTIFICATE

This is to certify that the thesis entitled “**STUDIES ON DIVERSITY OF INDIGENOUS MANGO (*Mangifera indica* L.) GENOTYPES**” submitted by **Mrs. VEENA, G.L., UHS12PGD53** for the degree of **DOCTOR OF PHILOSOPHY in HORTICULTURE** to the University of Horticultural Sciences, Bagalkot, is a record of *bona-fide* research work done by her during the period of her study at University of Horticultural Sciences, Bagalkot under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bengaluru
July, 2018

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BENGALURU

(VEENA, G.L.)

STUDIES ON DIVERSITY OF INDIGENOUS MANGO (*Mangifera indica* L.) GENOTYPES

ABSTRACT

Western Ghats region of Karnataka is one of the diversity centres of indigenous pickling mango variety ‘Appemidi’. Keeping this in view, the present investigation entitled “Studies on diversity of indigenous mango genotypes” was conducted at ICAR-IIHR, Bengaluru, during 2013-2014 and 2016-18. An attempt was made to study the diversity of indigenous mango genotypes with special reference to Appemidi from Chikmagalur district, by conducting a survey. Morphological characterization of 40 genotypes was described systematically as per the IPGRI mango descriptor, which consisted of leaf, inflorescence, fruit and other parameters. Among the characters studied, fruit diameter contributed most to the total diversity followed by fruit weight and inflorescence diameter. Among the pollen characters, colpi per cent showed the maximum contribution towards total divergence followed by equatorial diameter and colpi length. 14 SSR primers were also used to analyse the inter and intra cultivar diversity. The morphological, palynological and molecular diversity analysis showed grouping of genotypes into different clusters irrespective of their place of collection. Appemidi leaf volatiles were profiled and compared with their fruit sap aroma constituents using GCMS/MS. A total of 80+ volatiles were observed in all the genotypes belonging to mono and sesquiterpenoids groups. The highest significant positive correlation was observed between major monoterpenoids of leaf and fruit sap, particularly for β -Phellandrene, which proved that leaf volatiles were directly correlated with fruit sap in appemidi, which can be used as a pre-selection index. Water soluble and fat soluble vitamins, as well as important organic acids were profiled in fifteen genotypes at three different forms. Indigenous traditional knowledge associated was also documented. Conservation of important genotypes were carried out by grafting in addition pollen samples were cryopreserved.

VEENA, G. L.
(Student)

M. R. DINESH
(Major Advisor)

ಸ್ಥಳೀಯ ಮಾವಿನ (ಮ್ಯಾಂಜಿಫೆರಾ ಇಂಡಿಕಾ ಎಲ್.) ತಳಿಗಳ ಜೀವವೈವಿಧ್ಯತೆಯ ಅಧ್ಯಯನ

ಪ್ರಬಂಧದ ಸಾರಾಂಶ

ಕರ್ನಾಟಕದ ಪಶ್ಚಿಮಘಟ್ಟ ಪ್ರದೇಶವು ಅಪ್ಪೆಮಿಡಿಗಳ ಜೀವವೈವಿಧ್ಯತೆಯ ಕೇಂದ್ರವಾಗಿದೆ. ಈ ಅಂಶವನ್ನು ಗಮನದಲ್ಲಿಟ್ಟುಕೊಂಡು 2013-14 ಮತ್ತು 2016-18 ನೇ ಸಾಲಿನಲ್ಲಿ ಬೆಂಗಳೂರಿನ ಭಾ.ಕೃ.ಅನು.ಪ-ಭಾರತೀಯ ತೋಟಗಾರಿಕಾ ಸಂಶೋಧನಾ ಸಂಸ್ಥೆಯಲ್ಲಿ ಸ್ಥಳೀಯ ಮಾವಿನ ತಳಿಗಳ ಜೀವ ವೈವಿಧ್ಯತೆಯ ಅಧ್ಯಯನವನ್ನು ಕೈಗೊಳ್ಳಲಾಯಿತು. ಸ್ಥಳೀಯ ಮಾವಿನ ತಳಿಗಳ ಜೀವವೈವಿಧ್ಯತೆಯನ್ನು ಅಧ್ಯಯನ ಮಾಡಲು ಚಿಕ್ಕಮಗಳೂರು ಜಿಲ್ಲೆಯಲ್ಲಿ ವಿಶೇಷ ಸಮೀಕ್ಷೆಯನ್ನು ನಡೆಸಲಾಯಿತು. ಈ ಸಮೀಕ್ಷೆಯಲ್ಲಿ ಸುಮಾರು 40 ವಿವಿಧ ತಳಿಗಳ ರೂಪ ವಿಜ್ಞಾನದ ಗುಣಲಕ್ಷಣಗಳನ್ನು ಐಪಿಜಿಆರ್‌ಐ ವಿವರಣಾಕಾರದ ಪ್ರಕಾರ ವ್ಯವಸ್ಥಿತ ರೂಪದಲ್ಲಿ ವಿವರಿಸಲಾಗಿದೆ. ಇದರಲ್ಲಿ ಎಲೆ, ಹೂಗೊಂಚಲು, ಹಣ್ಣು ಮತ್ತು ಇನ್ನಿತರ ನಿಯತಾಂಕಗಳನ್ನು ಸೇರಿಸಿಕೊಳ್ಳಲಾಗಿದೆ. ಈ ಅಧ್ಯಯನದ ಪ್ರಕಾರ ಎಲ್ಲಾ ಗುಣಗಳಿಗಿಂತ ಹಣ್ಣಿನ ವ್ಯಾಸವು ಒಟ್ಟು ಜೀವವೈವಿಧ್ಯತೆಗೆ ಹೆಚ್ಚಿನ ಕೊಡುಗೆ ನೀಡಿದೆ. ತದನಂತರ ಹಣ್ಣಿನ ತೂಕ ಮತ್ತು ಹೂಗೊಂಚಲಿನ ವ್ಯಾಸವು ಹೆಚ್ಚಿನ ಕೊಡುಗೆಯನ್ನು ಕೊಟ್ಟಿವೆ. ಪರಾಗರೇಣುಗಳ ಪೈಕಿ ಶೇಕಡ ಕೋಲ್ಟ್ರೈ ಒಟ್ಟು ಜೀವವೈವಿಧ್ಯತೆಗೆ ಗರಿಷ್ಠ ಪ್ರಮಾಣದ ಕೊಡುಗೆ ನೀಡಿದೆ. ತದನಂತರ ಸಮಭಾಜಕ ವ್ಯಾಸವು ಮತ್ತು ಕೋಲ್ಟ್ರೈ ಉದ್ದವು ಸಹ ಜೀವವೈವಿಧ್ಯತೆಗೆ ಗಮನಾರ್ಹ ಕೊಡುಗೆ ನೀಡಿದೆ. ಅಷ್ಟೇ ಅಲ್ಲದೆ 14 ಎಸ್‌ಎಸ್‌ಆರ್ ಅಣ್ವಿಕಗಳನ್ನು ಅಪ್ಪೆಮಿಡಿ ತಳಿಗಳ ನಡುವಿನ ಹಾಗೂ ಅವುಗಳ ಆಂತರಿಕ ವೈವಿಧ್ಯತೆಯನ್ನು ವಿಶ್ಲೇಷಿಸಲು ಬಳಸಲಾಯಿತು. ಒಟ್ಟಾರೆ ರೂಪ ವಿಜ್ಞಾನದ ಗುಣಲಕ್ಷಣ, ಜೀವರಸಾಯನ ಹಾಗೂ ಆಣ್ವಿಕ ಅಧ್ಯಯನದ ಪ್ರಕಾರ ಅಪ್ಪೆಮಿಡಿ*o*Ai ಜೀವವೈವಿಧ್ಯತೆಯ ವಿಶೇಷತೆಗಳು ಅವುಗಳನ್ನು ಸಂಗ್ರಹಿಸಿದ ಸ್ಥಳಗಳನ್ನು ಹೊರತುಪಡಿಸಿ ತನ್ನದೇ ಆದ ವರ್ಗಗಳಾಗಿ ವಿಂಗಡಣೆಗೊಂಡಿದೆ. ಅಪ್ಪೆಮಿಡಿ ಎಲೆಯಲ್ಲಿರುವ ಪರಿಮಳ ಸುಗಂಧ ರಸಯನಗಳನ್ನು ಜಿಸಿಎಂಎಸ್/ಎಮ್‌ಎಸ್‌ನಿಂದ ಅಧ್ಯಯಿಸಿ ತದನಂತರ ಅವುಗಳನ್ನು ಅಪ್ಪೆಮಿಡಿ*o*Ai ಸೊನೆಯಲ್ಲಿರುವ ಪರಿಮಳಘಟಕಗಳೊಂದಿಗೆ ಹೋಲಿಸಲಾಗಿದೆ. ಈ ಅಧ್ಯಯನದ ಪ್ರಕಾರ ಅಪ್ಪೆಮಿಡಿಯಲ್ಲಿರುವ ಸುಗಂಧ ದ್ರವ್ಯಗಳನ್ನು ಪ್ರಮುಖವಾಗಿ ಮೋನೋ ಮತ್ತು ಸೆಸ್‌ಕ್ಯೂ ಟರ್ಟೀನಾಯ್ಡ್‌ಗಳ ಗುಂಪುಗಳನ್ನಾಗಿ ವಿಂಗಡಿಸಲಾಗಿದೆ. ಒಟ್ಟು 80ಕ್ಕೂ ಹೆಚ್ಚು ಸುಗಂಧ ಭಾಷ್ಮಶೀಲಗಳು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಕಂಡುಬಂದವು. ಎಲೆ ಹಾಗೂ ಮಿಡಿಯ ಸೊನೆಯ ಪ್ರಮುಖವಾದ ಮೋನೋ ಟರ್ಟೀನಾಯ್ಡ್‌ಗಳ ನಡುವೆ ನಿರ್ದಿಷ್ಟವಾಗಿ ಬೀಟಾ-ಫಿಲ್ಯಾಂಡ್ರಿನ್ ಎಂಬ ಸುಗಂಧಕಣವು ಧನಾತ್ಮಕವಾದ ಪರಸ್ಪರ ಸಂಬಂಧವನ್ನು ಹೊಂದಿರುವುದು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಕಾಣಬಹುದಾಗಿದೆ. ಈ ಫಲಿತಾಂಶದ ಪ್ರಕಾರ ಎಲೆಯಲ್ಲಿರುವ ಪರಿಮಳಘಟಕಗಳು ನೇರವಾಗಿ ಅಪ್ಪೆಮಿಡಿ*o*Ai ಸೊನೆಯ ಸುಗಂಧಕಣಗಳೊಂದಿಗೆ ಧನಾತ್ಮಕ ಸಂಬಂಧವನ್ನು ಹೊಂದಿದೆಯೆಂದು ಸಾಬೀತು ಪಡಿಸಲಾಗಿದೆ. ಆದುದರಿಂದ ಈ ಅಂಶವನ್ನು ತಳಿಗಳ ಪೂರ್ವ ಆಯ್ಕೆಯ ಸೂಚ್ಯಂಕವಾಗಿ ಬಳಸಬಹುದಾಗಿದೆ. ಅಷ್ಟೇ ಅಲ್ಲದೆ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಅಪ್ಪೆಮಿಡಿ*o*Aiಲ್ಲಿರುವ ನೀರಿನಲ್ಲಿ ಕರಗಬಲ್ಲ ಮತ್ತು ಕೊಬ್ಬಿನಲ್ಲಿ ಕರಗಬಲ್ಲ ಜೀವಸತ್ವಗಳು ಹಾಗೂ ಸಾವಯವ ಆಮ್ಲಗಳನ್ನು 15 ತಳಿಗಳಲ್ಲಿ 3 ವಿವಿಧ ಆಯಾಮಗಳಲ್ಲಿ ಅಧ್ಯಯನ ನಡೆಸಿ ಪ್ರಮುಖವಾದವುಗಳನ್ನು ಹೆಸರಿಸಲಾಗಿದೆ. ಜೀವವೈವಿಧ್ಯತೆಯ ಸಂರಕ್ಷಣೆಯ ಒಂದು ಭಾಗವಾಗಿ ಸಮೀಕ್ಷೆಯಲ್ಲಿ ದೊರೆತ ತಳಿಗಳನ್ನು ಕಸಿ ಮಾಡಲಾಯಿತು. ಜೊತೆಗೆ ಪರಾಗರೇಣುಗಳನ್ನೂ ಸಹ ಶೀತಸಂರಕ್ಷಕದಲ್ಲಿ ಸಂಗ್ರಹಿಸಿಡಲಾಗಿದೆ.

ವೀಣಾ, ಜಿ. ಎಲ್.

(ವಿದ್ಯಾರ್ಥಿ)

ಎಂ. ಆರ್. ದಿನೇಶ್

(ಪ್ರಧಾನ ಮಾರ್ಗದರ್ಶಕರು)

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I. INTRODUCTION

Mango (*Mangifera indica* L.) is an important member of the family Anacardiaceae in the order Sapindales. It is one of the most important fruit crops in India, having a great cultural, socio-economic and religious significance since ancient times. Its long period of domestication is well evident from its mention in ancient scriptures. It has originated from north-eastern India, Indo Myanmar border region and Bangladesh, where it is still found as a wild tree with very small fruits. Mukherjee (1953) opined that mango has been under cultivation for at least 4000 years and over 1000 varieties are in cultivation. Geographical distribution, polygenic traits, pollen morphology and breeding behaviour indicate the highest diversity of *Mangifera* species which is found in the Malayan peninsula followed by Sudan Islands and Eastern peninsula comprising Burma, Thailand and Indo-China. Such variability is because of the long period of domestication, high genetic variability, selection and future multiplication by vegetative means.

By virtue of its excellent flavor, delicious taste, attractive color along with various vitamins and minerals as well as availability and accessibility to common man, mango has been regarded as the 'King of fruits' in tropical world and the 'National Fruit of India'. Mughal emperor Babar called it as the choicest fruits of Hindustan and nurtured mango by evolving grafting techniques and making huge collection of its diversity.

A very large unexploited varietal diversity in mango can be seen especially in the Western Ghats region of Karnataka. This region is known for the pickling types of mango, 'Appemidi' (midi means tender mango in Kannada), which enjoys geographical indication (GI) tag. The appemidi is not just an ordinary mango, its fragrance is so strong that adding just a few midis to an ordinary pickle can change its taste and aroma. "Appemidi" is the king of all tender mangoes as far as its use in pickle industry is concerned. The local populace has conserved few of them in their backyard after evaluating them and they have a special place in Malnad cuisine and its culture. The fruits, at all the stages of development are used for culinary purpose. The tender and the slightly older ones are used for pickling. The fully matured ones consisting of the stone or the developing seed are boiled and preserved for longer durations in salt solution. They are used as an alternative for tomato or tamarind. Appemidis are also used in preparation of gojju (a kind of chutney), sasuve (a different preparation), appehuli, chutney and thambuli which are good digestives.

The turnover of Appemidi pickle business runs into millions of rupees. This "Appemidi" (tender mango) market is valued around Rs.12 crore (on the lower side) per annum in Karnataka alone. Ripponpet market in Shimoga sees a turnover of several lakhs of rupees during the months of March and April. A good quality tender mango costs Rs.1-1.50; the price for a quintal could go up to almost Rs 6,000/- depending upon the demand and availability. Since the supply of appemidi meets only one-tenth of the demand in the market, they are often mixed with ordinary tender mangoes (Dinesh *et al.*, 2014).

Appemidi products have high potential for marketing. The Appemidi's unique size and taste make the pickle as one of the best cuisines in India. People from distant places like Chennai and Hyderabad come here to purchase tender mangoes. Some of the characteristics which are attributed to Appemidi types are, generally these types can be seen on the banks of the rivers and streams. The trees observed in their original habitat in most cases are more than 100 years old. The fruits are characterized by bunch bearing habit and the yield in most of the trees is anywhere between 2000 to 6000 fruits (Dinesh *et al.*, 2014).

The shape of the fruit varies from round to oblong to ovoid. Fruits in most of the varieties are small sized and have strong aroma. The large variability observed in 'Appemidi' is because of the multiplication through seeds. These are available in an array of strong aroma ranging from jeera (cumin) to camphor, with three basic categories *viz.*, Jeerige (cumin seeds flavoured), Karpooora Appe (camphor flavoured), Kancha Appe (sour orange flavoured) and Saada Appe (mango flavoured). They are highly acidic having thick stalk, small seed, thin skin, thick pulp and generally long fruit and most of them

cannot be used as fresh fruit. The shelf life of pickles prepared from these varieties varies between 2 to 5 years.

About 300 known varieties of Appemidi have been found in Uttara Kannada district and 700 varieties in the Malnad region. They differ in shape, aroma, skin color, flesh color, stalk length, taste, as well as shelf life. Each one of them is unique in nature. Uttara Kannada district is said to be the “Cradle of Appemidi”. About 46 per cent of all mango varieties found in the home-gardens and farmlands of Uttara Kannada district are of ‘Appemidi’ types (Vasudeva *et al.*, 2015).

Most of these trees are found on the river banks in these regions, which is also one of the reasons for its diversity. Since, there is a large unexplored diversity present in these areas, several Appemidi types have not yet been evaluated and conserved. At present ICAR-IIHR has conserved and characterized 188 Appemidi varieties. Knowledge about the extent of genetic diversity is the major component in designing future breeding strategies for sustainability in mango production (Ravishankar *et al.*, 2000).

Morphological features were generally used to characterize fruit tree cultivars, rootstocks and landraces, a time consuming process that is prone to environmental effects. DNA-based markers are useful tools for characterizing and studying genetic similarities among land races, varieties and cultivars (Duneman, 1994). Various DNA markers, including restriction fragment length polymorphism (RFLP) (Ravishankar *et al.*, 2004), random amplified polymorphic DNA (RAPD) (Karihaloo *et al.*, 2003; Ravishankar *et al.*, 2004), amplified fragment length polymorphism (AFLP) (Yamanaka *et al.*, 2006) and simple sequence repeats (SSRs) (Viruel *et al.*, 2005; Schnell *et al.*, 2006) have been utilized to determine taxonomic identity (Schnell *et al.*, 2006), to estimate genetic diversity (Viruel *et al.*, 2005) and draw evolutionary histories of mango (Yamanaka *et al.*, 2006).

In addition to the assessment of diversity using morphological and molecular tools, studying the differences in the pollen morphological features (palynology) such as shape, aperture sculpture, exine ornamentation and other traits can be used as an alternative tool to distinguish species and cultivars as well as taxonomic evaluation of plants (Perveen and Qaiser, 2010; Bera *et al.*, 2007; Keshavarzi *et al.*, 2012 and Bhattacharya *et al.*, 2012). Hence, detailed palynological study in the selected Appemidi population could be a multivariate approach to facilitate characterization and conservation of the germplasm as well as to provide clue to the aspects of breeding systems and hybridization (Ferguson, 1985).

The present study was taken up with the objective of collecting, evaluating and conserving some of the wildy grown Appemidi types in the Western Ghat regions of Chikmagalur district. Morphological and pollen characterization coupled with the chemical profiling of the fruit sap and leaves as well as assessment of genetic diversity and genetic relationships among the indigenous mango with special reference to Appemidi types were attempted. In this background, the present investigation was carried out to find out the genetic diversity through morphological, biochemical and molecular means among the indigenous Appemidi populations with the following objectives.

1. To study the variability in various morphological characters of indigenous mango.
2. To study the diversity within the population through molecular characterization.
3. To study the chemical profile of fruit sap and leaf.
4. To document the traditional knowledge on south Indian mango.

II. REVIEW OF LITERATURE

India is the home of mango diversity, where more than thousands of varieties are widely distributed in different agro ecological zones. The perennial nature coupled with high heterozygosity in mango leads to delay in the crop improvement. However in the recent past even work on mango genomics has started. Nevertheless, Knowledge about the extent of genetic diversity is the major component in designing future breeding strategies for sustainability in mango production. Hence, the study was initiated in the Western Ghats region of Karnataka which is a home to the diversity of pickling varieties. Appemidi in particular and the previous research works carried out by different workers relevant to the present study have been reviewed in this chapter under the following heads

Studies on;

1. Genetic Variability
2. Genetic Diversity
3. Palynology
4. Biochemical aspects
5. Molecular diversity
6. Indigenous traditional documentation

2.1 Genetic variability studies

Crop improvement mainly depends upon the extent, nature and magnitude of genetic variability existing in the material and the extent to which it is heritable. Evaluation of genetic variability among the available germplasm helps to identify superior parents to be included for developing new genotype (Rajan *et al.*, 2005). If the parents are diverse for the characters, then there is a great chance of getting wide spectrum of recombinants (Vasugi *et al.*, 2013). The nature and magnitude of variability among the genetic stock of a crop is of prime importance. Evaluation of variability is important, to know the source of gene for a particular trait within the available germplasm (Tomooka, 1991). The information on genetic divergence of fruit crops including mango were mainly based on morphological data.

2.1.1 Morphological characteristics

The first description of mango varieties including those from India was attempted by Maries (1901). Special importance was given to fruit characters. Particularly fruit shape was given importance in classification of mango grown in Florida (USA) by Ralphs (1915). Burns and Prayag (1920) classified eighty nine mango cultivars from Erstwhile Bombay Presidency based on fruit characters. According to Naik and Gangolly (1950), no other single structure in mango gives so many morphologically important characters as that of fruit. The form of beak was a primary trait for variety identification. Nature of venation on stone was considered as tertiary character. Fruit characters alone were used by Sturrock (1951) for development of artificial key for identification of mango varieties grown in Florida (USA).

Fruits were described by its shape, depth of fruit stalk cavity, slope of fruit ventral shoulder, sinus, apex, beak, skin surface texture, skin color, pulp color, quantity of fibre, pattern of stone venation, seed shape *etc.*, (IPGRI, 2006).

2.1.1.1 Fruit shape

The shape of fruit including presence and absence of beak was considered as the most important character of mango fruits (Gangolly and Ranjith, 1957). Tripathi (2001) evaluated 93

accessions of mango germplasm under Tarai conditions and found roundish, ovate oblong and oblong fruit shapes in different cultivars.

Dinesh (2004) evaluated 130 mango varieties for fruit characteristics and reported oblong, elliptic and roundish fruit shapes. Whereas, Singh *et al.*, (2011) observed, fruit shape was ovate/ oblong/ obliquely oval/ ovate oblong/ roundish oblique and roundish in 30 varieties of mango. Fruit shapes of nine mango cultivars grown under a Mediterranean subtropical climate in Spain were reported to be oval to oblique (Rodriguez *et al.*, 2012). Hammedunnisa Begum *et al.* (2013c) screened 31 accessions of 'Beneshan' (BN Acc-I to BN Acc-31) and observed various shape of fruits *i.e.*, obliquely oval, ovate reniform, oblong oval and ovate oblong.

Vasugi *et al.* (2013) investigated the mango germplasm to identify duplicates in the germplasm using morphological methods and reported that fruit shape was elliptic (2), round (8) and oblong.

Wang *et al.* (2013) studied 16 varieties of mango introduced from different parts of the world includes, Australia, USA, India, Thailand and Vietnam origin and reported by South Subtropical Crops Institute in China that all the varieties exhibited rich diversity for fruit shape with S-shape, oval-shape, long oval shape, flat circular and spherical shape under China conditions.

Hammedunnisa Begum *et al.* (2014) assessed 30 accessions of 'Cherukuramam' (CKR Acc-I to CKR Acc-30) and reported that fruit shape was oblong in all the 30 accessions collected from three eco-geological regions of Andhra Pradesh. Sinus was present with rounded apex and no beak. Ventral shoulders were equal and higher than dorsal side and slope of shoulders was slightly rising and then rounded in ventral side and ending in a long curve in dorsal side.

2.1.1.2 Fruit size

Variation in fruit characters of polyembryonic varieties was studied by Prasad and Prasad (1972) who reported that fruit length varied from 6.20 to 10.34 cm, breadth from 3.74 to 6.82 cm, thickness from 4.10 to 5.22 cm, weight from 85.43 to 111.32 g. Satyawati *et al.* (1972) found that, the average weight of mango fruits ranged from 156 g (Sundari) to 301 g (Cripe) at Trichur (Kerala). Eight mango varieties grown in the orchards of IIHR, Hesaraghatta were studied by Lodh *et al.* (1974) with regard to their fruit weight, shape and size.

Wide variations were noticed in the physical characters of the ripe fruits of twenty two important mango cultivars at Sangareddy, Andhra Pradesh by Kulkarni and Rameshwar, (1981) and they observed Alampur Baneshan was the heaviest (400 g/fruit), while Alphonso and Pairi exhibited the minimum fruit weight of 180 g/fruit.

Passam (1982) compared some local mango cultivars with the introduced ones and observed that the fruits of local cultivars weighed approximately from 100 g/fruit (Doodooth) to 500 g/fruit (Graham). In contrast, the introduced cultivars (Haden, Sensation and Zill) produced medium to heavy fruits with 250-500 g of weight.

A comprehensive survey was conducted to assess the potentiality of some comparatively less known mango cultivars grown in the district of Murshidabad, West Bengal. The largest fruit was found in Mocha, each weighing over 500 g. However, maximum length and diameter was found in Burya Fazri (13.9 × 9.2 cm) which was followed by Rumali and Hapus (Sadhu and Bose, 1982).

In an evaluation of ten varieties of mango in West Bengal, Ghosh *et al.* (1985) illustrated that the variety Jangale was superior with respect to fruit size. Bombay Green, Bombay Yellow and Sarikhas were also better with respect to fruit weight, while the fruit of Piarafully, Amriti and Meghalantan were smaller in size.

Iyer *et al.* (1986) stated that fruits of cultivar Padiri had more fruit weight and volume compared to Alphonso. Six mango cultivars were evaluated by Minhas *et al.* (1991) showed that the fruit weight of Langra was maximum followed by Dashehari and Amrapali under Patiala, Punjab conditions. However, the lowest fruit weight was recorded in local seedling. The maximum fruit length was found in Dashehari followed by Langra and Late Bhaduran however, Langra had the highest fruit breadth.

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

Significant variations in length and width of fruit (cm) were noted in 30 mango varieties evaluated by Singh *et al.* (2011). The higher length of fruit was noted in Mallika, Fazli and Kakaria followed by Banglora, Dilsad, Baneshan, 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, followed by Banglora and Krishnabhog, whereas minimum width of fruits was obtained from Dashehari Chhotee, Safeda and Kelam.

Rodriguez *et al.* (2012) assessed the physico-chemical characteristics of 9 mango cultivars grown under a Mediterranean subtropical climate in Spain and reported that Osteen fruits had maximum weight (697 ± 95 g).

Hammedunnisa Begum *et al.* (2014) assessed 30 accessions of 'Cherukurasam' (CKR Acc-I 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. The fruit weight ranged from 130 to 380 g with an average of 266.55 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/fruit (Vanraj).

Wang *et al.* (2013) studied 16 varieties of mango in China all introduced from Australia by South Subtropical Crops Institute, among them 4 were of Australian origin, 7 were of U.S. origin, 3 were of Indian origin, and 2 others were respectively of Thailand and Vietnam origin. 'Valencia' had the highest fruit shape index of 1.68, while 'Magovar' had the lowest index of 0.86. 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' was the smallest fruit size of 207 g.

Kaur *et al.* (2014) characterized 14 mango genotypes and reported maximum fruit weight in Chausa. Eight mango cultivars were evaluated by Nazi *et al.* (2014) and reported 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.

2.1.1.3 Fruit skin characters

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 and stone content were lowest in Suvarnarekha.

Kumar (1998) reported the minimum peel percentage was observed in Langra in an experiment with 101 mango varieties at Sabour in Bihar. Fruit of Mussarat (Bardez and Salcette types), Fernandin and Bemcorado selection had attractive skin colour of crimson to dark red blushed on shoulders was observed by Desai and Dhandar (2000).

Vasquez *et al.* (2002) studied nine Thai mango cultivars and reported that peel content varied from 15 to 17.5 per cent and peel thickness ranged from 0.63 to 1.23 mm.

Anila and Radha (2003) studied 6 varieties of mango and reported that the skin color was yellow in Alphonso, Ratna and H-151 and green yellow in Prior, Muvandan and Neelum. In all the varieties except prior, the skin was smooth and in Prior the skin was rough.

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

Dinesh (2004) evaluated 130 mango varieties for fruit characteristics and observed that mature skin color ranged from yellow to greenish yellow to yellow with red blush. The skin texture was classified into two categories *viz.*, smooth and rough. Out of 130 varieties, 66 varieties had smooth textured skin while others are rough.

Morphological characterization of 12 mango (*Mangifera indica* L.) varieties cultivated in Mauritius, indicated that, skin color of ripe fruit were green (1), yellow (5), orange (1) and red (Anusha and Sanmukhiya, 2011).

The peel per cent was highest in Dashehari followed by Banglora and lowest in Baneshan and still lower in Shukul, Mallika and Fazli (Singh *et al.* 2011). Fruit skin color was differed significantly among the mango varieties and observed yellowish near top and greenish at bottom, light yellow, light chrome, dark green, deep yellow, light green, yellow, pale yellow, golden yellow, light cadmium, greenish and reddish yellow in the 30 varieties studied.

Rodriguez *et al.* (2012) assessed the morphological characteristics of 9 mango cultivars grown under a Mediterranean subtropical climate in Spain and reported that skin color was greenish yellow to red and purple.

Hammedunnisa Begum *et al.* (2013b) assessed 16 trees of 'Panchadarakalasa' (PK Acc-I to PK Acc-16) spread over the three eco-geographical regions (Coastal Andhra, Rayalaseema and Telangana) and skin color of matured fruit was observed to be green/yellow/yellowish green/ dark yellow with smooth skin texture.

Hammedunnisa Begum *et al.* (2013c) screened 31 accessions of 'Beneshan' (BN Acc-I to BN Acc-31) and reported that the peel content ranged from 8.80 to 25.80 per cent. Skin color of mature fruit varied from different tones of yellow and red blush over shoulders with smooth skin surface.

Hammedunnisa Begum *et al.* (2014) assessed 30 accessions of 'Cherukuram' (CKR Acc-I to CKR Acc-30) and reported that peel content ranged from 14.70 to 23.10 per cent and skin color was yellowish green/ greenish yellow with smooth skin texture.

Vasugi *et al.* (2013) reported that pulp and skin color was with shades of yellow and orange in various cultivars and the same trait can be utilised to identify the duplicates among the different mango germplasms. Wang *et al.* (2013) studied 16 varieties of mango in China all introduced from Australia by South Subtropical Crops Institute, among them 4 were of Australian origin, 7 were of U.S. origin, 3 were of Indian origin, and 2 others were respectively of Thailand and Vietnam origin and reported that mature fruit peel color were mostly yellow or red-yellow.

Kaur *et al.* (2014) characterized 14 mango genotypes and reported that mango genotypes attained yellowish green color at maturity. However, different color tones of yellowish green were evidenced in different genotypes.

2.1.1.4 Fruit pulp characters

Kumar (1998) noted a great variation in pulp content in an experiment with 101 mango varieties at Sabour in Bihar. A range of 56 per cent (Safeda Malihabadi) to 85 per cent pulp (Puttu) was recorded. Three varieties viz., Dalma, Mandhappa and Puttu contained more than 80 per cent pulp, while pulp percentage of 24 varieties was in the range of 70 to 79.9. Therefore found that only 27 out of 101 varieties had high pulp.

Pulp content of thirteen mango varieties investigated by Desai and Dhandar (2000), varied from 67.56 per cent (Bemcorado) to 83.21 per cent in RC-MS-1 (Bemcorado selection). Fruit pulp was prominently orange in Mankurad and Furtado, RC-MS-1 (Bemcorado Selection) and also in Bardez Mussarat which indicated a source for higher carotenoid contents. 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 was varied from 304.75 g (Surya Pasand) to 456.25 g (Talabi). The high pulp to stone ratio in Rugh Sheeradhar and Surya Pasand indicated their suitability for fruit processing.

Dinesh (2004) evaluated 130 mango varieties for fruit characteristics and reported that pulp content was more in variety Tenneru (81%). The highest pulp percentage was noted in Baneshan, followed by Aswaniya, Mallika, Langra and then Chausa, while the lowest pulp percentage was obtained in Safeda followed by Bombay green. The pulp color of fruit showed variations like light yellow, deep yellow, light cadmium, white, yellow, lemon yellow, radish yellow and orange (Singh *et al.*, 2011).

A study was conducted to assess the physico-chemical quality characteristics of 9 mango cultivars grown under a Mediterranean subtropical climate in Spain and reported that Osteen fruits recorded with high pulp: seed ratio (20:2) significantly higher than those in the other cultivars tested. The lowest pulp: seed ratio was recorded for the cultivars Kensington (6.3) and Sensation (7.6). The fruits with the highest percentages of pulp belonged to cultivars Gleen, Palmer, and Osteen, each averaging 85%. Pulp color was varied from yellow to orange with low to medium amount of fibre (Rodriguez *et al.*, 2012).

Shamili *et al.* (2012) analyzed 41 mango genotypes in Iran and reported that the yellow pulp colour and presence of fiber in 36 genotypes and orange pulp colour in 4 genotypes.

Hammedunnisa Begum *et al.* (2013b) assessed 16 trees of 'Panchadarakalasa' (PK Acc-I to PK Acc-16) spread over the three eco-geographical regions (Coastal Andhra, Rayalaseema and Telangana) and reported yellow or golden yellow pulp colour with abundant quantity of fiber in pulp.

Hammedunnisa Begum *et al.* (2013c) screened 31 accessions of 'Beneshan' (BN Acc-I to BN Acc-31) and reported that the pulp content ranged from 53.50 to 79.40 per cent and pulp color varied from yellow to dark yellow.

Hammedunnisa Begum *et al.* (2014) assessed 30 accessions of 'Cherukuram' (CKR Acc-I to CKR Acc-30) and reported that pulp content was ranged from 54.30 to 67.40 per cent and pulp color was yellow to dark yellow with abundant quantity of fibre in pulp.

Kaur *et al.* (2014) characterized 14 mango genotypes and reported that maximum pulp percentage and pulp stone ratio in Chausa and Langra Baramasi.

2.1.1.5 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).

Fruits of seven mango cultivars were analyzed (Jagmohan *et al.*, 1989) for physico-chemical characters over two seasons (1983 and 1984) in Himachal Pradesh and reported maximum stone per cent in Dashehari (16.8 and 17.0%).

Stone weight was found to be significantly higher in cultivar S.B. Rampur than Dashehari by Badiyala and Awasthi (1990) at Kangra valley in Himachal Pradesh. They also observed the minimum stone weight in S.B. Chausa.

Ali *et al.* (1992) evaluated the fruits of 32 varieties of mango grown at Shujabad and found smallest stone in Pohilot (7.6%). Maximum stone percentage was observed in Safdar Pasand, while the minimum in Fazli (Kundu and Ghosh, 1992)

Kumar (1998) reported the minimum stone percentage was observed in Puttu in an experiment with 101 mango varieties at Sabour in Bihar. 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 was varied from 12.8 to 29%.

Dinesh (2004) evaluated 130 mango varieties for stone characteristics and reported the 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. The veins on the surface of stones were found to be depressed in 16 varieties where as it was elevated and in level with the surface in 48 and 66 varieties, respectively. The pattern of venation was found to be parallel and forked in 23 and 103 varieties, respectively.

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.

Rajwana *et al.* (2011) analyzed 17 indigenous mango germplasm available in Punjab and found that stone shape was oblong (14), elliptical (2) and ovate (1). The percentage weight of stone was highest in the variety Banglora followed by Taimuriya and then Safeda and lowest in Baneshan and then in Fazli (Singh *et al.*, 2011).

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 seed-weight of the fruits of cv. Osteen proved the lowest (4.2%) among all the cultivars, confirming the most desirable relationship with the pulp (Rodriguez *et al.*, 2012).

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

2.1.2 Biochemical properties of fruit

2.1.2.1 Ripe fruits biochemical properties

According to Bhutiani (1946) the vitamin A content of different mango varieties of India ranged from 121.9 to 422.6 mg/100g pulp. Among all the varieties tested Dr. King and Langra proved to be the richest sources of vitamin A.

Cheema *et al.* (1954) also reported that the vitamin A content of Indian varieties of mango was about 4800 IU/100g. Among the South Indian mango varieties, Neelum recorded the highest value of total carotenoids (Das *et al.*, 1955).

Soule and Hatton (1955) found that the total soluble solids of mature fruits of Irwin, Haden, Sensation, Zill, Keitt and Kent mangoes ranged from 7.2 to 10.4 per cent.

After a detailed analysis of the fruit quality of 60 mango varieties of Punjab, 24 varieties had a TSS of 17.5°Brix and (or) more were classified as high quality mangoes and a further 21 varieties having TSS between 15 -17.5°Brix were classified as medium quality (Bakshi and Bajwa, 1959).

Under Basti (Uttar Pradesh) conditions, Teatota and Srivastava (1961) and Teatota and Singh (1963) reported maximum TSS in Gilas, Yakuti Barabanki and Yusuf Pasand and maximum sugar in Gaurjit followed by Yakuti Barabanki while Marke-Ara contained the minimum sugar content.

While working with certain off-season mango varieties of Tamilnadu, Srinivasan and Shanmugavalu (1971) obtained maximum TSS in cultivars Kalepad and Alphonso and minimum in Athimathuram and Mundappa. The varieties Alphonso, Mulgoa, Khudadad and Neelum showed a high reducing sugar content, while Mundappa showed the least. Further, titratable acidity was also low in Alphonso, high in Khudadad and Mundappa and moderate in other varieties.

Significant differences in the values of reducing and non-reducing sugars, total acidity and vitamin C content were reported in Ketiki Bihar and Langra Dudhia by Teatota and Singh (1971). Ketiki Bihar with 6.7 per cent reducing sugar, 8.7 per cent non-reducing sugar, 0.36 per cent total acidity and 8 mg/100 g ascorbic acid was significantly superior in quality than Langra Dudhia which showed the respective values of 2.9 per cent, 13.6 per cent, 0.37 per cent and 105.2 mg/100 g.

Variation in fruit characters of polyembryonic varieties was studied by Prasad and Prasad (1972) and reported that TSS varied from 15.60 to 17.20°Brix. Qualitative characters of local varieties of mango in Kerala were analyzed and reported by Satyawati *et al.* (1972). Total soluble solids were varied from 10.0 to 20.4°Brix, acidity from 0.2 to 0.48 per cent and ascorbic acid from 19.84 to 54.72 mg/100g.

Roy *et al.* (1972) found that Bombay Green had the maximum total carotenoids among the North Indian varieties of mango.

The bio-chemical nature of Neelum, Bride of Russia, Anupam, Langra, Kesar and Nisar Pasand has been studied by Teatota *et al.* (1972). Neelum had the highest vitamin C content, whereas Nisar Pasand had the highest TSS and sugar content. The earliest ripening variety, Bride of Russia having an attractive reddish blush on its shoulder, exhibited minimum acidity (0.24%).

Twelve popular late mango varieties of Northern India were studied for their performance at Eastern Uttar Pradesh. Acidity was high in Sukul and very low in Chausa. Variety Bathule, Darbar-I-Kalan, Ketiki Bihar and M.M. Gorakhpur were poor in ascorbic acid content while the rest contained an optimum amount of vitamin C. TSS and sugar contents of ripe fruits, however, varied from 15 to 18 per cent and 1.91 to 15.65 per cent, respectively. On the whole, Chausa was the richest in both while M.M. Gorakhpur, the poorest (Moti and Gangwar, 1973).

Sharma *et al.* (1999) reported that Dashehari had the highest total soluble solids (20.04%), reducing sugar content (3.87%), TSS to acid ratio (75.42) and sugar: acid ratio (14.33) among four cultivars evaluated under arid-irrigated conditions of Punjab.

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%), whereas Jhumko Rani showed the highest sugar content (14.83%). The lowest titratable acidity (0.14%) and the highest ascorbic acid content (123.33 mg/100 g) were observed in fruits of Jahanara and Kashir Langra, respectively.

Reddy *et al.* (2000) reported the highest TSS (24.20°Brix) and total sugars (18.67%) 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.

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

Vasquez *et al.* (2002) studied nine Thai mango cultivars and reported that total phenol index varied from 35 to 117.1 mg/ 100g and β - carotene was ranged from 0.66 to 15.72 mg/ kg fresh weight. TSS varied from 15 to 18.7°Brix and TSS/ TA ratio ranged from 47.6 to 114.1. Titratable acidity was 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.* (2003b) recorded the highest TSS, sugars and ascorbic acid content for Dashehari among the 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. The phenol content ranged from 48.40 mg/100g in cv. Haden to 208.70 mg/100 g of edible portion in cv. Uba (Riberio *et al.* 2007).

Rodriguez *et al.*, (2012A) assessed 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 (TSS). The TSS: TA ratios proved the highest for cvs. Kent (382) and Lippens (333), which might be indicative of the effect on their flavour. All the mango cultivars tested in this subtropical marginal area, especially cvs. Osteen and Tommy Atkins, met the standard parameters for high quality fruits, and can be recommended for their performance and sustainable yield.

Hammedunnisa Begum *et al.* (2013c) screened 31 accessions of 'Beneshan' (BN Acc-I to BN Acc-31) and reported that the TSS ranged from 15 to 22°Brix with an average of 16.75°Brix.

Fruit weight had highly significant and positive correlation with fruit length, fruit diameter, fruit volume and seed stone length in twelve genetically diverse strains of mango. Path coefficient analysis revealed that the fruit length, seed stone length and seed stone diameter had positive direct effects on fruit weight (Kumar, 2000).

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 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 among fruit characters of 69 types of mango cv. Langra and indicated a strong genetic correlation of characters than the phenotypic level, In addition, selection indices based on fruit size (length and diameter) with high pulp content were the most important factors for identifying high fruit weight types. Stone weight showed a strong and positive correlation with the peel weight. Fruit length was negatively and significantly correlated with total soluble solids and total sugars, but was positively correlated with fruit acidity, pulp weight and peel weight.

Kumar *et al.* (2006) studied variability in physico-chemical characteristics of 31 mango genotypes in Northern Kerala. They observed the highest significant correlation between fruit length and pulp weight while, ascorbic acid exhibited negative correlation with fruit length and peel weight.

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

2.1.2.2 Immature fruits in pickle preparations

Pickling is done in high concentration salt solution. The lactic acid bacteria can tolerate a high salt concentration, which give them an advantage over other less salt-tolerant species and allows them to grow and produce acid that inhibits the growth of undesirable microorganisms. The main purpose of addition of salt is to create a saline environment within the fruit pieces that ensures a perfect growing and multiplication environment for the beneficial microbes and thus preventing other detrimental microorganisms.

Geeta and Prakash (2006) examined the quality characteristics of lime pickles prepared using different salts and found that the ones prepared with free flow, iodized and healthy salts were found acceptable with sensory scores of 7.91, 7.51 and 8.11 respectively. Whereas, pickles made with rock salt and black salt obtained lower scores (6.72 and 5.09 respectively).

Bhagwati *et al.*, (2004) conducted an experiment in which ten bamboo species were screened to standardize the recipe for pickle preparation. The fermented product of *Bholuka (Bambusa balcooa)* secured the highest sensory score of eight among all the species. Among the recipes tried grating then blanching and treating with two per cent salt concentration, spicing and allowing for fermentation was found to be the best as it recorded the highest sensory score (8.00).

Based on the sensory evaluation of the whole immature green mango pickle prepared by standard fermentation and curing method among 19 unique mango accessions revealed that, the accessions viz., Kashimidi, Isagoor Appe, Malange, Appemidi, Dantimamidi and Jeerige were most suitable for preparation of tender mango pickles (Vasugi *et al.*, 2008).

Pruthi and Bedekar (1963) studied the physio-chemical characteristics and suitability of different varieties of pickling mangoes for pickle namely *Beerj*, *Amllet* and *Beenamini* varieties and found that *Amllet* variety scored highest fruit weight (466 g) and the recovery of mango slices. The ascorbic acid, dehydro-ascorbic acid, ascorbigen content ranged from 69.6 to 86.2, 0 to 61 and 1.4 to 2.1 mg/100g respectively and all the three varieties were found to be good in terms of pickling.

2.1.3 Genetic heritability

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. Genetic variability was determined with the help of certain genetic parameters such as coefficient of variation, heritability and genetic advance. Heritability was the heritable portion of total phenotypic variance and it was a good index of transmission of a character from parents to their offspring (Falconer, 1981).

The knowledge of heritability helps the plant breeder in selection for a particular character. Heritability was the ratio of genotypic variance to phenotypic variance. Its estimation was important, because it determines the additive effect of genes. If the heritability of a character was high, the phenotypic performance for predicting the effect of selection, heritability estimates along with genetic advance are more useful than the heritability estimates alone (Johnson *et al.*, 1955a).

Johnson *et al.* (1955b) and Lerner (1958) were first to study the heritability along with genetic advance. Assessment of genetic variability in the population was the first step in crop improvement because the progress of any breeding programme depends largely on the extent of variability present in population. A brief review of the research work done so far in mango with respect of coefficient of variation, heritability and genetic advance was presented in this part of the chapter.

In a highly divergent collection of 40 varieties, high genotypic coefficient of variation was found for fruit weight, fruit volume, reducing sugar and ascorbic acid, which revealed least influence of environment. Significant negative correlation was noted between total sugar and acidity (Gangwar and Tripathi, 1973).

Jintanawongse *et al.* (1987) characterized eighty local and eight introduced mango cultivars in Thailand based on leaf and fruit shape and reported that both the characters were heritable, easy to see and expressed in all environments.

In a study carried out using half-sib analysis, the fruit characters *viz.*, 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 was low (Dinesh, 2003).

Fruit weight, fruit shape, ground surface color, fruit width and pulp depth have high heritability and can therefore, facilitate the selection of the progeny (Brettell *et al.*, 2004). The blush colour and fruit flavour have a strong genetic component. A high frequency of hybrids with red peel or burgundy blush can be recovered from crosses where one of the parents has an intense red blush color.

Singh and Singh (2004) evaluated five mango hybrids and observed variability in fruit weight, pulp percentage, TSS, Vitamin-C and total carotenoids. Singh (2005) reported that, high phenotypic coefficient of variation along with high genotypic coefficient of variation for peel color, fruit weight and ascorbic acid content was observed among the 20 genotypes studied. High heritability along with high expected genetic advance was noticed for fruit weight and ascorbic acid content.

Kumar *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.

Rajan *et al.* (2009) reported that, high genotypic coefficient of variation was observed for weight of pulp, fruit, stone, and peel, length of fruit and stone ratio among the 42 cultivars studies and indicated heritability accompanied with greater genetic advance for these traits plays major role in selection of parents for hybridization programme. Using group constellation, cultivars were grouped into three distinct clusters. Pulp weight contributed maximum towards the genetic divergence (34.03 %) followed by peel weight, TSS, stone weight and stone width.

2.2 Genetic diversity studies

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 (Mahalanobi's, 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. The genetic diversity in an available germplasm is one of the most important criteria for the optimal design of a breeding programme, which helps in the choice of desired parents for establishing new breeding population. Better knowledge on genetic diversity or genetic similarity can help in sustaining long term selection gain (Majumder *et al.*, 2011).

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 for North Indian, South Indian and Exotic Cultivars.

Rathod (2007) assessed genetic diversity by Mahalanobi'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.

Silva *et al.* (2012) studied the genetic diversity of fifteen mango cultivars, eight from Brazil and seven from Florida (USA), produced in the Zona da Mata Mineira region. 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. There was correlation between the color of the pulp, hue angle, and total soluble sugar content and between the color of the peel, shape index and percentage of peel and pulp.

Vasugi *et al.* (2012) considering the difficulty in traditional divergence study microsatellite markers were successfully used for genetic diversity analysis of indigenous Appemidi types and further reported that the major compounds contribute to the unique aroma of these types were due to totally different combination of monoterpenes.

Vasugi *et al.* (2013) studied on diversity of Appemidi. An enormous diversity was seen in its flavour, taste and fruit form unique to particular regions of India. On evaluation of tender fruit accessions Chansi Appe, Dodderi Jeerige, Manibhatta Appe, Gorana Appe, Isgoor Appe, Malange, Gurusurthy Appe and Kashmiridi were found with good attributes for tender whole mango pickle.

Majumder *et al.* (2013) assessed the genetic diversity in 60 mango genotypes through D^2 Statistics and principal component analysis. The genotypes were grouped into eight clusters and diversity was influenced by the morphological characteristics not by the geographical distribution. Depending upon the magnitude of genetic distance, contribution of different characters towards the total divergence, magnitude of cluster means for different characters and the *per se* performances the genotypes of cluster I, VII and VII could be considered as parents in hybridization programme.

Dinesh *et al.* (2015) studied the genetic diversity in certain indigenous mango varieties of Chittoor area of Andhra Pradesh. Most of the indigenous varieties from one region are grouped in the same cluster. It was interesting to note that the morphological and molecular characterization follow almost the same pattern suggesting the genetic control of these fruit that being grown in that region.

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. Mango varieties Langra, Amrapali, Dashehari, Chausa, Neelum, Fazli, Alphonso, Totapari, Mallika, SBM 01-10, SBM 01-13, SBM 01- 14, SBM 01-38 and SBM 01-19 appeared most divergent and showed real genetic diversity. 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 (*Mangifera indica* L.).

Thirty-eight local mango trees in Eastern and Central Kenya were sampled and characterized by 75 selected qualitative (44) and quantitative (31) morphological descriptors selected from the descriptor list developed for mango by Bioversity International (former IPGRI). The cluster analysis conducted with all 56 finally 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 egg-shaped fruits, which mostly had a completely yellow-orange skin when ripe. Most samples in cluster 1 had a shallow stalk depth in contrast to other clusters. 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. Their weight was almost 3 times as much as those of cluster 1. Four samples with long fruits having curved fruit shapes and a typical deep sinus were grouped in cluster 5. Fruits from this mango type had the highest length to width ratio and were of intermediate weight. Unique was the acute shape of the fruit apex. Cluster 6 grouped five samples characterized by oblong, almost quadratic shaped fruits without sinus. The fruit apex in this cluster was obtuse and the skin color was green without any flush (Sennhenn *et al.*, 2014).

2.3 Palynological studies

The pollen morphology is an expression that comes from genome and like any other characters is critical to understand differences of the genotypes. The macro-morphological properties may be useful in taxonomic studies of some species or plant groups. In many species, valuable information can be derived from a full pollen study leading to understand both the morphology and where possible, the functional role of the characters of the pollen grain. The pollen grain may be studied not only for comparative morphological data alone but also as clue to the unexpected aspects of breeding systems and hybridization (Ferguson, 1985).

Prunus is one of the most problematic genera in the family of Rosaceae and its taxonomy is very complicated because of the polymorphism and the wide ecological tolerance of the species, as well as the presence of an enormous number of cultivars. For the identification of plant material, morphological characteristics such as plant vigour and leaf, flower, and fruit parameters could be utilized. In addition to these features, differences in the size and the surface ultrastructure of pollen grain are a useful tool for distinguishing species and cultivars of fruit trees. Using pollen morphology in identification has become more important since the advent of the scanning electronic microscope (SEM). The cluster analysis shows a great morphological affinity among nine cultivars and three rootstocks, while the two cultivars of *P. avium* (C1 and C2) were different due to the medium-sized pollen grains (45.74–48.20 $\mu\text{m} \times 23.55$ –24.78 μm) and the very small diameter of perforations (0.26–0.28 μm , respectively), which were sparsely distributed (Anna Geraci *et al.*, 2012).

The efficacy of crosses, both between varieties and cultivars of the same species and between different species, depends directly on the pollen grain viability. *In vitro* pollen grain germination rates were related to the highest percentage of fruits and number of seeds obtained in *in vivo* conditions (Souza *et al.*, 2015).

Plant pollen has excellent genetic conservation and is not easily affected by environmental factors. Palynological studies have been widely used in many fruit trees and ornamental plants. A systematic study on pollen morphology of 55 representative's pomegranate cultivars from different eco-geographical populations in China. The results may provide a Palynological basis for the taxonomy and evolution of *Punica* plants and facilitate the identification of fossil pollens. Pollen grains were 3-lobed monads, rounded or triangle in polar view, and prolate or perprolate spheroid in equatorial view. Three types of exine ornamentation, psilate with verrucate, micromesh verrucate and scabrate verrucate were observed. The evolutionary trend was from psilate with verrucae to micromesh verrucae to scabrate verrucae. Palynological analysis was discordant with the morphological classification and irrelevant with geographical locations (Shangshang *et al.*, 2015).

2.3.1 Pollen viability

Pollen viability and germination are very important in choosing pollinating varieties needed to ensure high and quality yields. In this study, the viability and germination of pollen from ten apple cultivars. The viability, tube growth and morphological homogeneity related to pollen quality are the most important properties in fruit trees. These properties are useful for plant breeders, geneticists, and growers. Viability of pollen was highest in the cultivar Mahali Sheik and the highest germination (71.00%) was obtained in 15% sucrose along with highest pollen tube growth (180.72) (Fariba Moshtagh *et al.*, 2015).

An attempt was made to study pollen–pistil interactions in four mango cultivars such as 'Amrapali', 'Mallika', 'Pusa Arunima' and 'Pusa Surya', developed at the Indian Agricultural Research Institute (IARI), New Delhi, India. The pollen adhesion was highest (20.01 pollens) in 'Pusa Surya' followed by the 'Amrapali' (18.67 pollens) after 6 h of self-pollination. The minimum pollen retention was observed in 'Mallika' (13.02 pollens) followed by 'Pusa Arunima' (15.67 pollens) after 6 h of self-pollination. The adhesion of pollen on stigma is critical step for success of reproduction 'Amrapali' and 'Mallika' showed significantly low fruit-set as a result of self-pollination than open-pollination at different intervals of fruit growth. Fruitlets and ovules growth of selfed and open-pollinated flowers clearly indicated that selfed fruitlets of 'Amrapali' and 'Mallika' were smaller than fruitlets obtained from open-pollination. The findings of present investigation bring out clearly that self-incompatibility in mango is post-zygotic and mango cultivar 'Amrapali' and 'Mallika' are post-zygotic self-incompatible. (Dutta *et al.*, 2013).

2.4 Biochemical studies

It is well documented that mango fruit is an important source of vitamins and other phytochemicals. Moreover mango fruit provides energy, dietary fibre, carbohydrates, proteins, fats and phenolic compounds (Tharanathan, Yashoda and Prabha, 2006). Each part of mango tree is having one or the other use which shows the nutritional and functional importance of mango by products.

Aroma volatiles of mango determine consumer acceptability and influence selection whilst breeding. To assess their variety, composition and possible impact on cultivar relationship volatile blends of 22 Indian and 5 non-Indian cultivars were used. Mango cultivars differ in the total concentration of volatiles that they produce in the qualitative and quantitative composition of these volatiles and their principal volatile components. Mono and sesqui terpenes hydrocarbons are the major constituents of Indian as well as Non-Indian cultivars these cultivars can be classified as mono and sesqui dominating groups (Sagar *et al.*, 2008).

Flavonoids aid in cultivar identification and assessing phylogenetic kinship among some cultivars of pomegranate (*Punica granatum* L.) Secondary metabolites, foliar flavonoids have gained reputation as being excellent phyto-chemical markers for cultivar identification. These markers come handy at places where the morphological parameters become limiting and hence recourse to such phyto-chemical markers is taken to delimit the taxon. Nine cultivars of pomegranate to develop phyto-chemical markers for them are using foliar flavonoid. The data thus generated have shown that distribution of flavonoid spots or spot combination is specific for each cultivar which can be used not only for varietal identification but also for assessing the phylogenetic kinship among pomegranate cultivars (Bhargava *et al.*, 2009).

Vasugi *et al.* (2012) reported that the major compounds that contribute to the unique aroma of were estimated. Analysis of sap volatiles was done using GCMS fitted with a DB-5 MS column using helium as the carrier gas. 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. The aroma of pickle type of mangoes is due to totally different type of terpenes as well as a completely different combination of monoterpenes. The volatile compounds separated from the water fraction of the sap indicated that there is a clear distinction between the mango types in volatile profiles.

The sap constituted mainly terpenoid compounds in all the varieties. Total concentration of aroma compounds was maximum in Anantha Bhatta Appe (14.743 mg/ml), followed by Adderi Jeerige (12.39 mg/ml), Isagoor Appe (10.93 mg/ml) and Totapuri (10.91 mg/ml). Aroma concentration was minimum in Kana Appe (6.11 mg/ml) and Amrapali (0.75 mg/ml). Total aroma concentration indicates that Anantha Bhatta Appe has a very strong odour when compared to the other genotypes. Amrapali had a very weak odour. Aromatic profile of compounds showed that the relative percentage of β -phellandrene, limonene and α -terpinene was about 81–89% of the total volatiles in Anantha Bhatta Appe, Adderi Jeerige, Isagoor Appe and Kana Appe. However, in Totapuri δ -3-carene, (–)- β -pinene, cis-ocimene and allo-ocimene were the major compounds amounting to 83% of the total volatiles; in Amrapali, δ -3-carene, (–)- β -pinene, cis-ocimene, caryophyllene and humulene amounted to 85% of the volatiles. This indicates that the aroma of pickle type of mangoes is due to totally different type of terpenes compared to the other two fruit-type mangoes (Vasugi *et al.*, 2012).

Dalbir singh *et al.* (2013), conducted a study on total phenolics, antioxidant activity and functional properties of Tommy Atkins mango peel and kernel as affected by drying methods. Freeze dried powders had the lowest bulk density values among different techniques tried. The cabinet dried waste powders can be potentially used in food products to enhance their nutritional and antioxidant properties.

Jean *et al.* (2014), determined the qualitative composition and distribution of phytochemicals in peel and flesh of fruits of four different mangos using mass spectrometry profiling using fractionation of methanol extracts by HPLC. This work provided additional information regarding mango fruit phytochemical composition and its potential contribution to human health and nutrition. The presence of secondary metabolites across different fruit parts and varieties, it is important to recognize compounds that are specifically present in one particular variety or fruit part. The uniqueness of a specific compound reflects the genetic basis of a variety to biosynthesis such a compound.

Mango fruit is consumed as dietary supplements with health benefits; it is widely used in food industry. Bruno *et al.* (2016), studied the chemical profile of Uba mango at four distinct maturation stages using negative-ion mode electrospray ionisation Fourier transform ion cyclotron resonance mass spectrometry (ESI(-)FT-ICR MS) and physicochemical characterisation analysis (total titratable acidity (TA), total soluble solids (TSS), TSS/ TA ratio, and total polyphenolic content). Primary (organic acids and sugars) and secondary metabolites (polyphenolic compounds) were mostly identified in the third maturation stage, thus indicating the best stage for harvesting and consuming the fruit and also concluded that fruit polyphenols have the potential for cancer chemoprevention because of the secondary metabolites content.

2.4.1 Pre selection indices in fruit crops

Most of the perennial fruit trees remain juvenile for longer period if they are propagated sexually through seed. Since seed is the outcome of the breeding programme, sexual propagation becomes indispensable. The juvenile phase of the seedlings thus determines the number of years the trees have to be grown before the results can be assessed.

Some of the successful pre-selection indices developed in mango breeding are:

- ✓ Leaf flavour was reported to be directly correlated with fruit flavour (Majumder *et al.* 1972)
- ✓ Emergence of new growth flushes simultaneously with fruiting or immediately after harvest is indicative of regular bearing (Sharma *et al.*, 1981)
- ✓ Higher phenolics in the apical bud have also been shown to be associated with dwarfing (Iyer, 1991)
- ✓ Lower stomata density is an indication of dwarfness Majumder *et al.* (1981)

2.5 Molecular studies

Mango cultivars were identified based on morphological characters, which lack high degree of accuracy, as these are often modified by environment. Biotechnological methods have a definite role in understanding the genetics, crop improvement, germplasm characterization and removing confusion in the nomenclature of cultivars.

2.5.1 RAPD markers

RAPD analysis can be used to identify several useful polymorphisms quickly and efficiently, and has tremendous potential in cultivar/hybrid identification.

Schnell and Knight (1993) were the first to apply RAPD technique for molecular characterization of mango. Nine *Mangifera* species were analyzed and the resultant grouping compared to the traditional taxonomic classification based on morphological characters. One hundred and nine bands were resolved by using ten primers. The UPGMA cluster analysis based on the band distribution grouped subgenus *Mangifera* in conformity with the current taxonomy based on flower morphology.

The genetic diversity of Kensington mangoes was investigated using 10 RAPD markers. However, there was very little evidence of significant genetic variation within Kensington Pride selections. Fifteen of the selections were identical in all 107 markers. Only 2 selections, WEAN2 and ML2N1, differed by more than 5%. These plants provided the best options for use in genetic improvement of the Kensington Pride mango (Bally *et al.*, 1996).

Random Amplified Polymorphic DNA analysis was carried out in 29 Indian mango cultivars comprising popular landraces and some advanced cultivars. PCR amplification with 24 primers generated 314 bands, 91.4% of which were polymorphic. Jaccard's similarity between pairs of cultivar ranged between 0.318 and 0.75 with a mean of 0.565. A UPGMA dendrogram showed the majority of the cultivars from Northern and Eastern regions of India clustering together and separate from Southern and Western cultivars. Analysis of molecular variance revealed that 94.7% of the genetic diversity in mango existed within regions. However, differences among regions were significant; Northern and Eastern regions formed one zone and Southern and Western regions formed another zone of mango diversity in India (Karihaloo *et al.*, 2003).

Samal *et al.* (2004) assessed the genetic relationship of twenty varieties of cashew by using RAPD markers. Cluster analysis clearly showed that 20 varieties of cashew grouped into 2 major

clusters based on similarity indices. The variety Vengurla-2 has very close similarity (85%) with variety VRI-3.

Thirty eight mango cultivars and one wild mango species (*Mangifera persiciforma*) were examined using Random Amplified Polymorphic DNA (RAPD) markers. PCR [Polymerase Chain Reaction] amplification with 19 primers generated 223 bands, 87.89% of which were polymorphic. The results suggested the presence of high genetic diversity among the cultivars. Based on the UPGMA [Unweighted Pair-Group Method with Arithmetic Averages] dendrogram, the 38 cultivars were classified into 3 groups (similarity coefficient of 75.5 per cent), the classification of which was significantly correlated with embryo type. One marker associated with skin color, and several markers associated with embryo type in mango were identified (Xie JiangHui *et al.*, 2005).

Simi (2006) characterized 50 mango varieties in Kerala with 157 RAPDs. Out of which, 4 decamer primers were amplified showing 96.18 per cent polymorphism. The pair wise similar coefficient values ranged from 0.217 to 0.833. The coefficients were utilized to construct a dendrogram using the Unweighted Pair Group of Arithmetic Means (UPGMA) algorithm segregated the mango genotypes clustering into 7 clusters.

Rahman *et al.* (2007) analyzed 28 mango germplasm using RAPD markers and revealed that out of 20 primers screened, four were selected, which gave 50 clear and bright fragments, out of which 48 fragments were considered polymorphic. The proportion of polymorphic loci and gene diversity values across all loci were 96 per cent and 0.29, respectively. The UPGMA dendrogram based on genetic distance segregated the 28 mango germplasm into two main clusters with the lowest intervarietal similarity index (14.29%) and highest genetic distance (0.87). The results of the present study indicated that the RAPD analysis could be utilized by breeders for further improvement of mango varieties.

Bajpai *et al.* (2008) screened 46 mango cultivars using 30 RAPD markers. Out of which 9 primers gave 110 discrete fragments ranging from 300 to 3500 bp showing 87.3% polymorphism. UPGMA tree constructed on RAPD data on the basis of Jaccard's coefficient clustered the accessions into 3 groups, one comprising majority of North Indian varieties and other having Eastern Indian and third cluster comprising accessions from both the regions.

Damodaran *et al.* (2009) examined 18 mango clones for genetic diversity using 60 RAPD primers. Of the 60 primers screened, 20 primers gave polymorphic DNA amplification patterns. The number of bands generated ranged from 5-8 per primer. Banding patterns of the primers used to distinguish between clones indicated that PCR by using RAPD primers was an efficient method for clonal identification. Similarity coefficients were calculated based on 57 selected bands and UPGMA clustering analysis was performed. Six local clones were found to be related closely with four clones from South India. The remaining eight clones were classified into four groups based on the dendrogram. This analysis separated the differentially flowering polyembryonic local clones with single flowering monoembryonic ones.

The genetic diversity and relationship in mango genotypes (*Mangifera indica* L.) of Malda district was investigated using Random Amplified Polymorphic DNA (RAPD) markers by (Roy and Abhishek, 2011). A total of 15 primers were screened, which produced 173 alleles, out of which 160 were polymorphic. The range of polymorphism was 83 to 100 per cent depending upon the primer. The UPGMA dendrogram based on Jaccard's similarity coefficient grouped the 15 mango genotypes into three main clusters. Four genotypes namely Laxmanbhog, Baramasia, Fazli and Kohitur were formed one cluster, second cluster comprised of four genotypes namely Michrikand, Golapkhash, Himsagar, Sinduri and third cluster included Mulayomjam, Gopalbhog, Langra, Aswina, Kachamitha and Malagyobhog. Two genotypes Sinduri and Himsagar were grouped together at highest similarity index 0.94. The Principal Coordinate (PCO) analysis showed that there was sufficient genetic variability among the mango genotypes studied.

Hassan *et al.* (2014) assessed 10 mango genotypes grown in Suez Canal and Sina region of Egypt using 30 arbitrary ten-mer primers. Of these, 11 primers were selected which gave 92 clear and bright fragments. A total of 72 polymorphic RAPD bands were detected out of 92 bands, generating 78 per cent polymorphism. A dendrogram based on Nei's genetic distance coefficient implied a moderate degree of genetic diversity among the cultivars. Cluster analysis clearly showed two main groups, the first consisting of indigenous to the Delta of Egypt cultivars and the second consisting of indigenous to the Suez Canal and Sinai region.

2.5.2 SSR markers

Recently, micro-satellite markers have been characterized in mango and used for analysis of cultivars collected mainly in Thailand (Honsho *et al.*, 2005). Six micro-satellite loci revealed 2 to 6 putative alleles. Among the 36 mango cultivars tested in this study, 29 showed unique micro-satellite patterns, whereas seven cultivars could not be identified because of genotype similarities.

Sagar *et al.* (2000), with 33 polymorphic inter simple sequence repeat markers analysed the genetic diversity among 70 mango cultivars and a distant outgroup. No separation was observed for North Indian and South Indian cultivars. Twelve different cultivar specific bands were detected for six cultivars,

Singh and Bhat (2009) analyzed a set of 241 mango landraces and commercial cultivars from 15 different regions of India for micro satellite variability. 18 primer pairs selected for high polymorphism were used for the analysis. A total of 103 alleles with an average of 5.78 alleles per primer pair were scored in the cultivars analyzed. The pair wise similarity coefficients for the cultivars ranged from 0.024 to 0.808 with an average of 0.258 indicated presence of high genetic diversity among the cultivars analyzed. Partitioning of total molecular variance among germplasm from 15 regions representing 8 groups based on geographic contiguity in the Indian sub-continent showed that among groups difference accounted for 1.91 per cent, among populations within groups for 3.80%, among cultivars within population 57.87 per cent and differences within individual cultivars accounted for 36.42 per cent of the total variations. The First value derived from these estimates was 0.019 indicated that the cultivars in the 8 major geographic groups were not strongly differentiated except Manipur of the North Eastern region. The validity of synonyms and homonyms for cultivar names were investigated by analysing cultivars bearing the same names but under cultivation in different regions. Results indicated presence of differences in molecular profiles for most cultivars analyzed. Analysis of molecular variance, low Fst value, cluster analysis and high values for estimates of Nm indicated presence of substantial gene flow between the cultivar from different regions, possibly due to cultivar exchange followed by gene introgression by crossing and selection. These processes might have homogenized the population structure of mango cultivars in the country.

Ravishankar *et al.* (2011) assessed the genetic diversity among mango (*Mangifera indica*) cultivars to test their amplification in closely related species. Thirty six microsatellite (Simple Sequence Repeats; SSR) 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. Polymorphic information content values ranged from 0.185 to 0.920 with a mean of 0.687. The total value for the probability of identity was 2.42×10^{-31} . 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.

Anshuman *et al.* (2012) analyzed 48 mango hybrids using 17 Simple Sequence Repeat (SSR) markers which detected 59 scorable loci, of which 45 were polymorphic. The sizes of the alleles detected ranged from 100 to 480 bp. SSR markers were highly polymorphic with an average of 3.47 alleles per primer. SSRs gave moderate values of Polymorphic Information Content (PIC) and heterozygosity. 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. Two unique fingerprints were identified in hybrid H-9-6. The size of unique fingerprints ranged from 90 (MiSHRS-18) to 350 bp (MiSHRS-39). The Principal Coordinate Analysis also exhibited more or less similar distribution of mango hybrids.

Vasugi *et al.* (2012) analyzed 43 accessions using 14 SSRs developed at the Indian Institute of Horticultural Research, Bangalore. The analysis of 211 bands detected 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. Grouping of accessions in cluster II was mainly based on the qualitative traits like high TSS, low titratable acidity, low pH and medium to high sugar, making these accessions suitable for fresh fruit consumption.

Twenty microsatellite loci in mango, an important commercial fruit tree in East Asia, were developed to evaluate the genetic diversity and identification of cultivars. The 20 new microsatellite markers were isolated from mango using a magnetic bead enrichment method and polymorphisms were identified in 22 mango cultivars. The number of alleles ranged from one to nine, with expected heterozygosity ranging from 0 to 0.826. The polymorphism information content ranged from 0 to 0.756 with a mean of 0.525. These new microsatellite loci should be useful and convenient for further studies of the genetic diversity and identification of cultivars in mango. (Yu- Chung Chiang *et al.*, 2012).

The genetic relationship of twenty two mango (*Mangifera indica* L.) cultivars/lines was examined based on 37 SSR markers. Number 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 (Chi-Chu Tsai *et al.*, 2013).

Dillon *et al.* (2013) analyzed 254 mango accessions and related *Mangifera* species originating from 12 diverse geographic areas using eleven known Simple Sequence Repeat (SSR) markers. A total of 133 alleles were detected, ranging from 8 to 16 alleles per locus with a mean value of 12.36 and average Polymorphism Information Content (PIC) of 0.72. Diversity analysis divided the accessions into four major nodes broadly representing their geographical origins.

Vasugi *et al.* (2013) investigated the mango germplasm to identify duplicates in the germplasm using molecular methods (SSR) resulted 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.

Genetic analysis of 90 mango genotypes including juicy, table, dual and pickle types from different parts of Andhra Pradesh of India was carried out 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 two major groups with a genetic similarity range of 47 to 88%. Grouping of the genotypes based on the utility type was observed only at sub-cluster level. Study of population structure by a model-based STRUCTURE analysis revealed the germplasm to exist in four gene pools. Overall F_{st} of 0.11 indicated genetic differentiation between the populations to be low. Analysis of molecular variance revealed that major proportion of the variation was within the individuals (62.25 %). The molecular marker-based study of genetic diversity suggests that the germplasm studied representing the kind of variability would be a valuable genetic resource for future breeding and association mapping in search for new and novel alleles (Malathi *et al.*, 2013).

Kumar *et al.* (2013) studied the genetic variation and investigated inter-relationship between ten mango genotypes. Twenty (20) SSR markers were tested with 10 genotypes produced a total of 240 amplified products, of which 184 were polymorphic and 56 monomorphic. The sizes of the alleles

detected ranged from 120 to 369 bp. SSR markers were highly polymorphic with an average of 2.70 alleles per primers. SSRs gave moderate values of Polymorphic Information Content (PIC) range of 0.320 to 0.774. Moderate degree of genetic diversity with Jaccard's similarity co-efficient values ranging from 0.075 to 0.285. 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.

Hammedunnisa Begum *et al.* (2014) assessed 30 accessions of 'Cherukurasam' (CKR Acc-I to CKR Acc-30) using 109 Simple Sequence Repeats (SSRs) out of which, 25 were highly polymorphic. Dendrogram based on unweighted pair group of arithmetic means indicated that the accessions were not grouped as per geographic separation. Jaccard's similarity coefficient ranged from 0.75 to 1.00 attesting that 'Cherukurasam' whatsoever cultivated throughout Andhra Pradesh state was not pure clone.

Thirty eight local mango trees in Eastern and Central Kenya were sampled and characterized with 19 Simple Sequence Repeat markers. Genetic relatedness between the mango samples was visualized using a dendrogram based on Nei's genetic distance and Neighbor Joining methods. Morphological characterization resulted in six distinct clusters, and molecular analysis in eight clusters, which partly supported the morphological classification. Four of the eight molecular clusters were consistent and molecular results confirmed with morphological classification (Sennhenn *et al.*, 2014).

2.6 Indigenous traditional knowledge documentation

Indigenous Knowledge is as old as human civilization but the term was first coined by an American botanist, John Harshburger (1896). Since then it has been defined as traditional knowledge on indigenous communities about surrounding plant diversity.

Indigenous knowledge can be defined as knowledge of the people living together in certain areas. Generated by their own and their ancestor's experience including knowledge originating from elsewhere which has to be internalized by the local people (Madisa, 1998). The knowledge of traditional uses is wild edible plants as a food source (Addis, 2009). Ethnobotanical studies conducted have indicated that wild plants are gathered and consumed by indigenous people (Asfaw, 2009). As a result local people knowledge has been a key element in several ethnobotanical studies to explore various aspects. This explores the knowledge of how plants are used for food, shelter, medicine, clothing, hunting and religious ceremonies (Qureshi *et al.*, 2007), this relationship between a given society and its environment and in particular the plant world (Aumeeruddy, 1996).

Buatathoki (2003) had identified several TK on citrus cultivation in Nepal such as (i) *in situ* manuring by keeping the animals in the orchards (ii) smoking of trees in the evenings and nights to repel the insect pest *etc.* Such information could be very useful in increasing fruit production and thereby enhancing farm income.

Idris *et al* (2011), had shown several examples in which TFT genetic resources are being used to as curatives / food supplements. For instance, in Malaysia, decoction of roots of pulasan has been reported to be used as vermifuge for fever; and the roots are boiled and used for bathing patients inflicted with fever.

Renisha (2012) attempted traditional knowledge documentation with respect to the processing of mango in two villages of Thrissur and Palakkad districts of Kerala as part of the biodiversity analysis of traditional mango types. In these studies, typical varietal specificity for each and every products prepared by the house maids of these regions and the simple traditional skills employed by them for making it unique.

Bhat *et al.* (2013) reported tropical fruit tree species form an important source of livelihood, medicine and income of local indigenous people. Several wild TFT genetic resources partially domesticated and deployed into farming systems in tropical Fruits in the Farmlands of Central Western Ghats. Several varieties of wild aromatic 34 pickle mango popularly known as Appemidi an important commodity of commerce, have been identified by the farmers from the riverine habitats and have been deployed under various agro forestry systems from Central Western Ghats (Vasudeava and Rajeswari, 2014).

III. MATERIAL AND METHODS

The investigation entitled “Studies on Diversity of Indigenous Mango (*Mangifera indica* L.) Genotypes” was carried out during 2013-14 and 2016-2018 at the Division of Fruit crops, ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru. The experimental site is located at an altitude of 890 m above mean sea level with 13⁰ N and 17.37⁰ E. Soil is red sandy loamy having pH 5.2 to 6.4.

3.1 Experimental details

The research work was carried out by studying the fruit and leaf morphology, biochemical, molecular and pollen characters to assess the extent of variability present in the selected population and the genetic diversity using SSR markers. The investigation was carried out in four broad sequential experiments *viz*,

1. To study the variability present in various morphological characters of indigenous mango.
2. To study the diversity within the population through molecular markers.
3. To study the chemical profile of fruit sap and leaf.
4. To document the traditional knowledge on south Indian mango.

3.1.1 To study the variability present in various morphological characters of indigenous mango

3.1.1.1 Experimental material

Based on the diversity richness a well planned survey on collection of indigenous types, which mainly included Appemidi mangoes was conducted in the western ghat regions of Chikmagalur district in Karnataka state. This included places *viz.*, Gonibeedu, Marebylu, Aldur, Balehonnur, Kudige, Soppinakadu, Arenur, Magundi, Bynduru, Elemadlu, Kanathi and Chimmankudige which are located on the banks of the river Hemavathi and Bhadra. The leaves, fruits, fruit saps and pollens were collected for characterization. The details of the genotypes collected and characterized are provided in table 1.

3.1.1.2 Observations recorded

Morphological observations were taken as per the IPGRI descriptor for mango. (IPGRI, 2006).

3.1.1.2.1 Leaf characteristics

Leaf shape, leaf length (cm), leaf width (cm), petiole length (cm), leaf color, leaf texture, leaf tip, leaf margin, color of young leaf, intensity of anthocyanin pigmentation, color of fully developed leaves, leafy bracts and leaf fragrance were recorded.

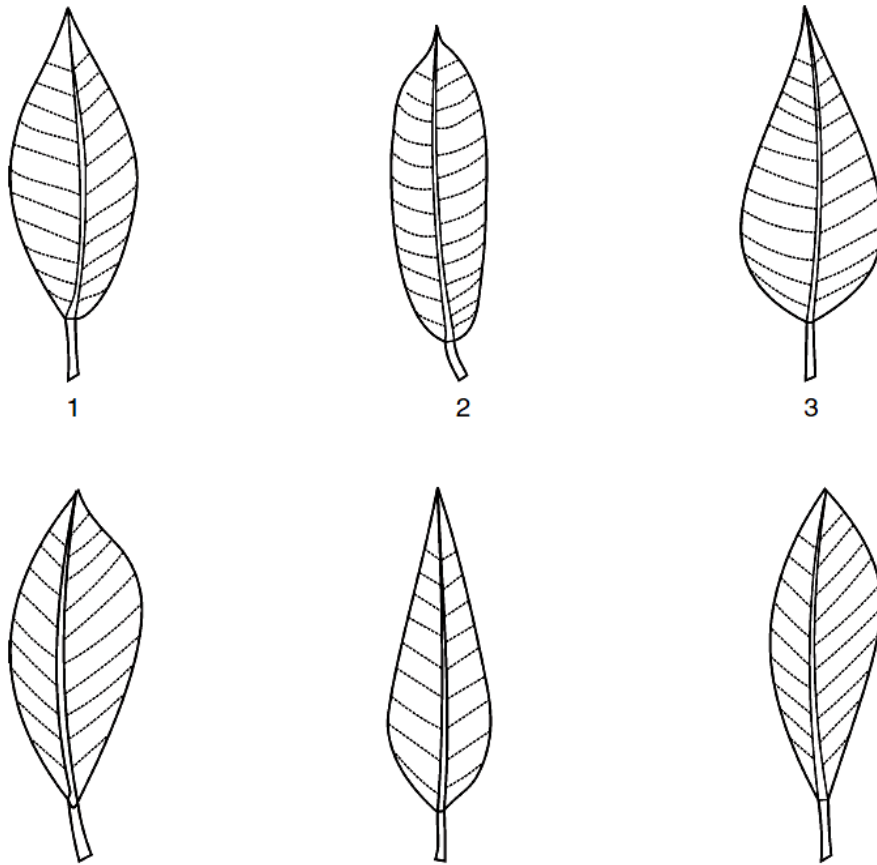


Figure 1: Leaf blade shape 1-Elliptic; 2-Oblong; 3-Ovate; 4-Obovate; 5-Lanceolate; 6-Oblanceolate

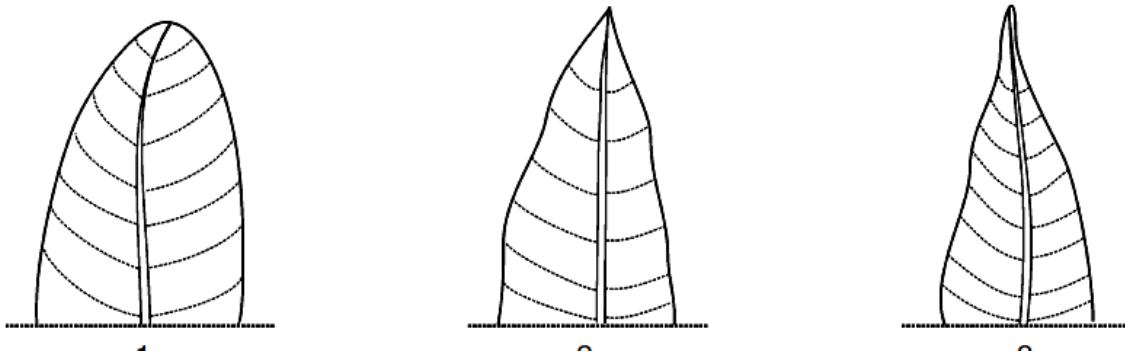


Figure 2: Leaf tip shape 1-Obtuse; 2-Acute; 3-Acuminate

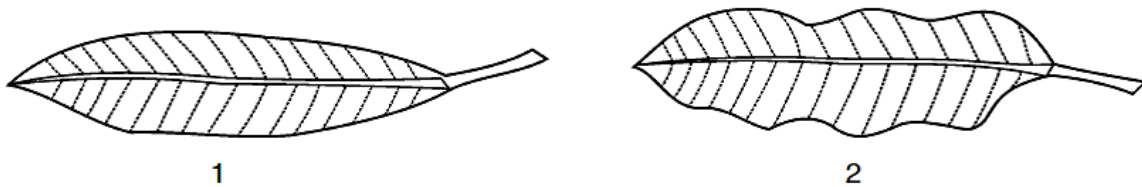


Figure 3: Leaf margin 1-Entire; 2-Wavy

3.1.1.2.2 Inflorescence characteristics

Inflorescence position, inflorescence shape, flower density, inflorescence length (cm), inflorescence diameter (cm), inflorescence color, hairiness, leafy bracts, flower diameter (mm) and flower type were recorded.

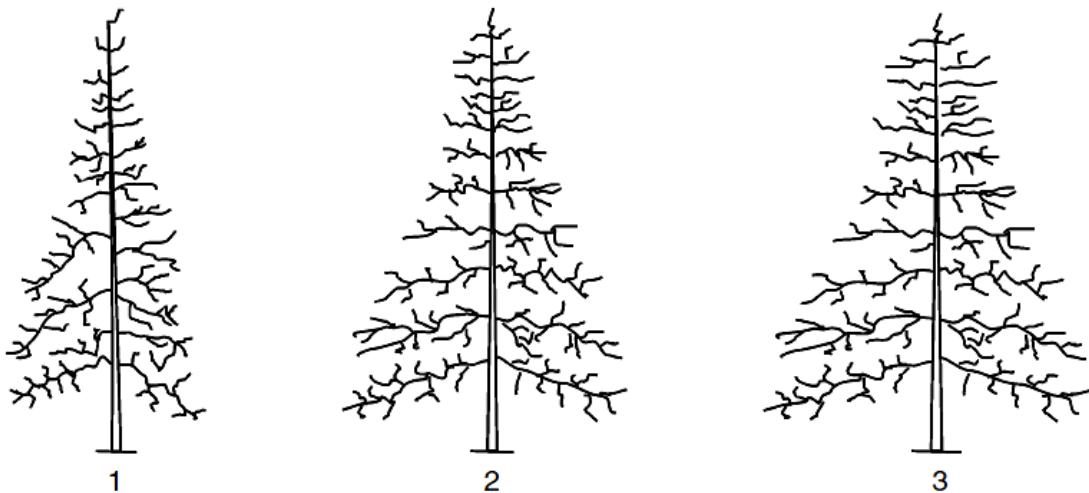


Figure 4: Inflorescence Shape 1-Conical; 2-Pyramidal; 3-Broadly pyramidal

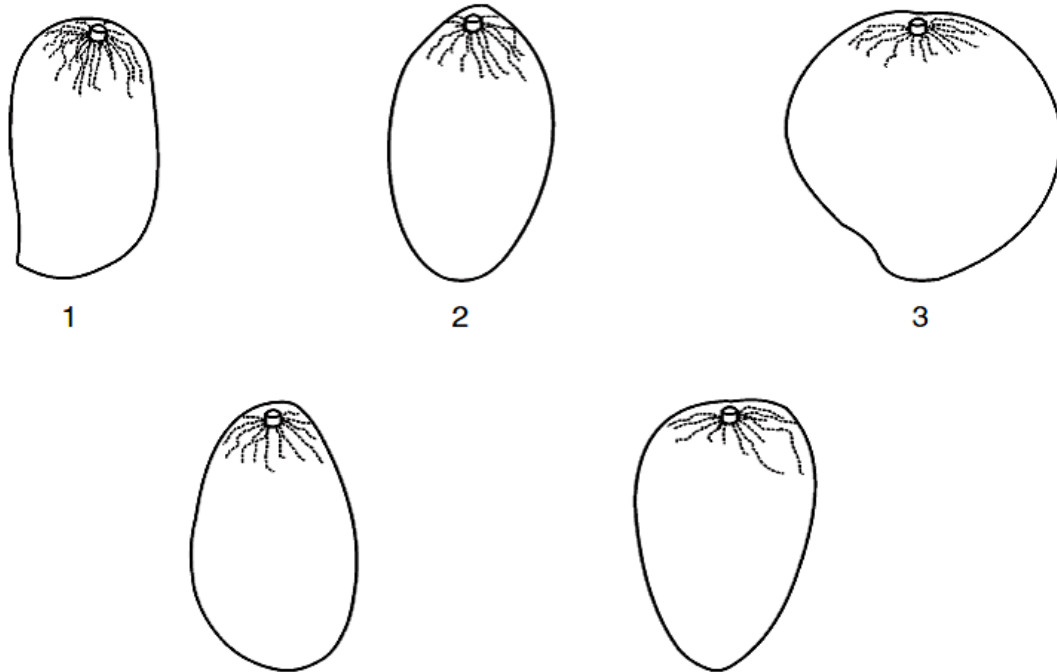


Figure 5: Fruit shape 1-Oblong; 2-Elliptic; 3-Roundish; 4-Ovoid; 5-Obovoid



Figure 6: Sap burning test

3.1.1.2.3 Fruit characteristics

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

3.1.1.2.4 Fruit length (cm)

Fruit length of five fruits from base of the fruit to top of the groove at the calyx end was recorded with the help of vernier calipers and average was expressed in centimeters.

3.1.1.2.5 Fruit width/diameter (cm)

Fruit width in centimeters was measured in five fruits at the broadest part of fruit for five randomly selected fruits and the average was recorded in centimeters.

3.1.1.2.6 Fruit thickness (cm)

Thickness was measured by using vernier calipers and expressed in centimeters.

3.1.1.2.7 Fruit weight (g)

Five fruits were weighed with the help of electronic balance separately and the average fruit weight was expressed in grams.

3.1.1.2.8 Fruit Shape: Fruit shape was grouped into anyone of the categories *viz.*, Oblong, Elliptic, Roundish, Ovoid and Obovoid as depicted in Fig-5.

3.1.1.2.9 Sap burning test (Vasudeva *et al.*, 2015)

It is one of the traditional practices being followed in the appemidi growing areas. The sap from the tender fruits was burnt for a longer period and such fruits were taken for pickling. Pickles prepared from these fruits can be stored for a longer period than less sap burnt fruits. Therefore, for all the surveyed fruits, the sap burning test was performed and the time interval was recorded in seconds (Fig. 6).

Table 1: GPS details of the samples surveyed in Western Ghats regions of Chikmagalur District

Sl. No.	Genotype Name	North	East	Elevation (m)
1	Agrahara-1	13 ⁰ 06'38.8	077 ⁰ 28'59.6	858
2	Horatti-1	13 ⁰ 03'53.4	075 ⁰ 42'03.5	861
3	Ittige gudu	13 ⁰ 04'38.6	075 ⁰ 41'46.8	901
4	Gonibeedu-5	13 ⁰ 04'40.6	075 ⁰ 41'47.0	900
5	Gonibeedu-4	13 ⁰ 04'40.6	075 ⁰ 41'47.0	900
6	Gonibeedu-3	13 ⁰ 04'39.6	075 ⁰ 41'47.4	896
7	Gonibeedu-2	13 ⁰ 04'35.2	075 ⁰ 41'45.2	898
8	Gonibeedu-1	13 ⁰ 04'35.4	075 ⁰ 41'45.3	912
9	Marebylu-4	13 ⁰ 02'19.1	075 ⁰ 42'38.3	910
10	Marebylu-3	13 ⁰ 05'03.2	075 ⁰ 41'36.9	910
11	Marebylu-2	13 ⁰ 05'04.9	075 ⁰ 41'33.8	913
12	Marebylu-1	13 ⁰ 05'05.1	075 ⁰ 41'33.2	915
13	Bankal	13 ⁰ 04'59.1	075 ⁰ 42'00.0	929
14	Heggudlu	13 ⁰ 07'17.0	075 ⁰ 36'29.2	910
15	Arenuru-1	13 ⁰ 16'57.6	075 ⁰ 36'10.3	693
16	Arenuru-2	13 ⁰ 16'57.6	075 ⁰ 36'11.3	695
17	Kanathi-1	13 ⁰ 17'11.1	075 ⁰ 35'24.2	677
18	Kanathi-2	13 ⁰ 17'11.1	075 ⁰ 35'25.0	679
19	Kanathi-3	13 ⁰ 17'11.1	075 ⁰ 35'25.5	680
20	Magodu-1	13 ⁰ 17'53.2	075 ⁰ 35'31.0	755
21	Byndur-1	13 ⁰ 20'37.2	075 ⁰ 29'02.7	648
22	Marigundi	13 ⁰ 20'38.0	075 ⁰ 29'03.2	717
23	Soppinakadu-1	13 ⁰ 20'47.6	075 ⁰ 29'54.5	717
24	Soppinakadu-2	13 ⁰ 20'43.4	075 ⁰ 29'53.1	736
25	Soppinakadu-3	13 ⁰ 20'43.4	075 ⁰ 29'53.1	731
26	Kumrumane-3	13 ⁰ 20'27.7	075 ⁰ 29'57.0	724
27	Kumrumane-2	13 ⁰ 20'21.1	075 ⁰ 30'00.0	723
28	Kumrumane-1	13 ⁰ 19'11.1	075 ⁰ 29'24.2	677
29	Kudige-1	13 ⁰ 21'49.8	075 ⁰ 23'55.8	676
30	Chimankudige-1	13 ⁰ 21'49.8	075 ⁰ 23'55.8	712
31	Chimankudige-2	13 ⁰ 21'49.6	075 ⁰ 23'56.0	715
32	Chimankudige-3	13 ⁰ 21'51.2	075 ⁰ 23'56.2	717
33	Chimankudige-4	13 ⁰ 21'50.5	075 ⁰ 23'56.8	719
34	Heruru-1	13 ⁰ 21'53.7	075 ⁰ 23'56.2	721
35	Heruru-2	13 ⁰ 21'53.7	075 ⁰ 23'56.2	722
36	Heruru-3	13 ⁰ 21'54.2	075 ⁰ 23'58.2	725
37	Shantipura	13 ⁰ 21'54.0	075 ⁰ 23'38.6	689
38	Kaman Mori-2	13 ⁰ 21'54.2	075 ⁰ 23'56.2	722
39	Kaman Mori-1	13 ⁰ 21'50.8	079 ⁰ 02'00.2	705
40	Elemadlu	13 ⁰ 22'44.2	075 ⁰ 23'18.6	671

3.1.1.3 Pollen morphology and germination study in Appemidi

The pollen morphometry was studied for 28 surveyed samples of Appemidi to study the diversity and evolutionary relationship as well as to record the morphological variabilities in the surveyed pollen samples.

3.1.1.3.1 Study material

Pollen grains of 28 surveyed samples (15 genotypes region specific selected) and Thirty eight genotypes including 31 Appemidi genotypes, two cut mango pickle cultivars (Amini and Hamlet), one unique type (Varategiduga) and Four *Mangifera species* (*Mangifera species* were *M. camptosperma*, *M. griffitti*, *M. zeylanica* and *M. odorata*) collected from Mango germplasm block at IHR were used to study the pollen morphology (Table 2).

The freshly bloomed mature male and hermaphrodite flowers were collected from the panicle and put into the petri dishes separately and kept under direct sunlight for about 10-15 minutes for dehiscence of the anther. Later the pollen was cryopreserved until they were subjected for Scanning Electron Microscopy (SEM evaluation) and also for pollen germination studies.

3.1.1.3.2 Scanning electron microscopy

Dehisced fresh pollen grains were shifted onto double sided copper tape on the disc surface of polished aluminum stabs and passed in a vacuum evaporator under SEM microscope version, HITACHI-TM3030Plus. The pollen grains were observed and photographed at 1000-5000X for whole grain. For each genotype 10-15 pollen grains were examined to determine the pollen size and certain morphological traits.

The grain size was estimated by measuring the length of the equatorial diameter (E) and polar axis (P), the P/E ratio was calculated and exine sculpture ornamentation was observed. Length of the colpi (C), Colpi per cent (CP) was also recorded. The terminology followed was that of Erdtman (1969).

3.1.1.3.3 Morphological characters studied

3.1.1.3.3.1 Length of the pollen (μm)

Polar length of the pollen grain was measured using image J software. The lengths of 10-15 pollen grains were measured and the mean value was expressed in micro meters.

3.1.1.3.3.2 Equatorial width of the pollen (μm)

Equatorial width of pollen grain was measured using image J software. The equatorial lengths of 10-15 pollen grains were measured and the mean value was expressed as width of the pollen grain in micro meters.

3.1.1.3.3.3 P/E ratio

The ratio between the polar axis and the equatorial diameter of pollen gives us the shape of the pollen. Pollen shapes have been classified as per Erdtman (1971).

Table 2 Selected ICAR-IIHR germplasm block Appemidi details

Sl. No.	Name of the genotype	Place of collection
1	Ananthabhata Appe	Sagara
2	Arunagowdana Appe	Sirsi
3	Gorana Appe	Sirsi
4	Gurumurthy Appe	Kumuta
5	Kaana Appe	Perla
6	Kalgundikoppa Appe	Sirsi
7	Adderi Jeerige	Sagara
8	Dedderi Jeerige	Sagara
9	Appemidi	Sagara
10	Karpoora Jeerige	Sirsi
11	Balekoppa Appe	Sirsi
12	Gaddemara	Sirsi
13	Dannalli Appe	Sirsi
14	Nandgar	Sirsi
15	Malange	Sirsi
16	Sadamidi	Sagara
17	Kalakai	Sirsi
18	Kaikai	Sirsi
19	Manibhatta Appe	Sirsi
20	Jeerige	Sirsi
21	Huliappekai	Siddapura
22	Giduganamavu	Sirsi
23	Hamsamamidi	Tamil Nadu
24	Tatamidi	Tamil Nadu
25	Sudur Appe	Ripponpet
26	Chansi Appe	Sirsi
27	Mahabalagiri Appe	Sirsi
28	Kutumba Appe	Sirsi
29	Varategiduga	Sirsi
30	Naagara Appe	Sirsi

3.1.1.3.3.4 Length of the colpi (μm):

Length of colpi which is equal to or shorter than total distance from colpi apices to poles was measured and expressed in micro meters (Erdtman 1971).

3.1.1.3.3.5 Colpi per cent (%):

It is an elongated aperture with a length/width ratio which is greater than 2:1.

3.1.1.3.3.6 Exine pattern

Pollen exine ornamentation was described for pollen in polar view. Sculpturing refers to the external surface feature of exine without reference to the internal structure. This is basically a three layered structure which is more constant compared to other pollen characters.

3.1.1.3.4 Statistical analysis

The significant differences between the characters studied in the micromorphology of pollen grains was statistically analyzed using the software XL statistics (<https://www.xlstat.com>) and IBM-SPSS (www.spss.com.hk/software/statistics/data_analysis). Pollen images were analyzed using Image J software (https://imagej.net/Downloads/win_64).

Morphological parameters were analyzed by using SAS 9.3 software available at ICAR-IIHR for genetic estimates and diversity studies. Programme codings were developed for each of these analyses.

3.1.1.4.5 Pollen germination and cryopreservation in Appemidi

To study the per cent of pollen germination in Appemidi, 20 genotypes were selected along with four *Mangifera species*.

Flowers were collected after anthesis between 10-11 am, just before pollen dehiscence. Anthers were carefully removed using forceps. The collected anthers were dehisced under sun light for about 10-15 minutes depending upon the intensity of sunlight. For cryo-preservation, after anthesis, dehisced anthers were collected and preserved in a capsule at -196⁰C in cryobank.

3.1.1.4.5.1 Pollen collection

Matured anthers were collected for pollen collection and storage in clean petridishes from healthy panicles of trees between 10 and 11 AM. The anthers were brought into the laboratory and transferred to empty gelatin capsules.

3.1.1.4.5.2 Pollen germination media preparation

A media consisting of 1mg boric acid, 1.5g each of sucrose (Polyethylene glycol) 3mg of calcium nitrite (CaNO₃) and 2 mg of magnesium sulphate (MgSO₄) per 10 ml of distilled water was prepared one day in advance and preserved in refrigerator for further use.

3.1.1.4.5.2.1 Slide preparation

Pollen germination was tried by sitting drop and hanging drop technique using a cavity slide. A drop of germination media was put on the surface of the cover slip where the edges of coverslips were glued with Vaseline. Dehisced anthers were dusted on to the media and spread using needle for few seconds. Later debris and anthers were removed using forceps. The cover slip was covered using a cavity slide and pasted properly and kept overnight in a petri dish containing moist blotting paper layer for germination.

3.1.1.4.5.3 Observations recorded

3.1.1.4.5.3.1 Per cent germination

Pollen germination was observed under compound microscope and germination per cent was calculated by manually counting the number of pollen grains which are germinated in a piece of microscopic area.

3.1.1.4.5.3.2 Pollen tube length (μm)

Germinated pollen tube length was measured in an electron microscope with embedded software and expressed in micrometers. 10-15 pollen grains were measured and mean value was taken to express pollen tube length.

3.1.1.4.5.3.3 Pollen head width and length (μm)

Germinated pollen head width and length was measured in an electron microscope with embedded software and expressed in micrometers. 10-15 pollen grains were measured and mean value was taken to express the width and length of pollen head.

3.1.1.4.5.3.4 Statistical analysis of diversity studies

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^2 statistics), phenotypic and genotypic correlations analysis were performed on the data obtained.

3.1.1.4.5.3.4.1 Genetic divergence analysis

The genetic diversity in 40 genotypes for all the morphological characters was analyzed using Mahalanobis D^2 statistic technique.

Genetic diversity between cultivars was estimated by using D^2 analysis given by Mahalanobis (1936).

3.1.1.4.5.3.4.2 Grouping of cultivars into various clusters

Grouping of the cultivars into various clusters was done by using Tocher's method as described by Rao (1952). The criterion used for clustering in this method was that any two variables belonging to the same cluster should on an average show a smaller D^2 value than those belonging to different clusters.

3.1.1.4.5.3.4.3 Cluster diagram

The clusters and their mutual relationships were presented diagrammatically. The square root of average D^2 , which was an approximate measure of divergence between groups, was used to denote the distance.

3.1.1.4.5.3.4.4 Contribution of individual characters towards divergence

In all the combinations, each character was ranked on the basis of their contribution towards divergence between two entries ($d_i = y_{it} - y_{ij}$). Rank I was given to the highest mean difference and rank 'P' to the lowest mean difference, where P is the total number of characters considered. The number of cases where a particular character ranked first was continued, the proportion of this to the total number

of combinations expressed in percentage was quantified as the contribution of character to the overall genetic divergence between the cultivars.

$$\text{Percentage contribution of the character} = X = \frac{N \times 100}{M}$$

Where,

X = Per cent contribution of character

N = Number of cultivar combinations where the character ranked first.

M = All possible combinations of the cultivars concerned.

3.2 To study the diversity within the population through molecular characterization

- DNA isolation and variability study using 12 SSR to study the genic diversity.
- Chloroplast marker and ITS markers were used for sequencing.

3.2.1 Extraction of plant DNA

DNA was extracted by modified CTAB (Cetyl Trimethyl Ammonium Bromide) (Ravishankar *et al.*, 2011) as detailed below.

- a. Healthy young mango leaves (5g) were collected and brought to laboratory. The leaves were surface sterilized with 70 per cent ethyl alcohol and cut into pieces and immediately used for further processing.
- b. Approximately 1 g of fresh leaf sample was weighed and transferred into a 2 ml eppendorf tube and homogenized completely with pre-heated (65⁰C) 1000 μ l of extraction buffer (containing 3% CTAB, 100 mM Tris, 20 mM EDTA, 1.4M NaCl, 3% PVP and 2% β -mercapto ethanol).
- c. The contents were mixed by vortexing for few minutes and subjected to beat beater for grinding and the reaction mixture was incubated for 60 minutes at 65⁰ C in hot air oven.
- e. The tubes were centrifuged at 10,000 rpm for 10 minutes at 16⁰C by using Eppendorf centrifuge 5430R.
- f. Supernatant was collected in a separate tube to which 500 μ l Chloroform: Isoamyl alcohol (24:1) mixture was added and centrifuged at 12,000 rpm for 15 minutes using a refrigerated centrifuge.
- g. The supernatant was taken out and 1/6th by volume of ice cold isopropanol was added and kept overnight at -20⁰ C.
- h. The next day tubes were centrifuged at 10,000 rpm for 15 minutes and pellet was collected.
- i. Pellet was washed twice with 70% ethanol and centrifuged at 10,000 rpm for 5 minutes.
- j. The pellet obtained was air dried and dissolved in TE buffer, RNAase (10 mg/ml) at 1mg/ml was added and incubated for 1hr at 37⁰C and stored at -80⁰C for further use.
- k. The concentration and quality of DNA was estimated by using Helma Nano Drop spectrophotometer at 260 nm and verified by running sample on 0.8 per cent agarose gel electrophoresis.

3.2.2 Simple sequence marker analysis

After quantification, DNA was diluted to a concentration of 50 ng/μl for SSR diversity analysis. A total of 16 SSR primers were used for the analysis (Table 2).

3.2.2.1 PCR amplification for SSR

PCR reaction was carried out in a total volume of 20 μl containing the following details.

Table 3: SSR primer sequence details along with allele size

Primers (5'-3') details	Primer number	Allele size range (bp)
F:GCCCCATCAATACGATTGTC R:ATTTCCCACCATTGTCGTTG	MillHR12	154–177
F:CCCAGTTCCAACATCATCAG R: TTCCTCTGGAAGAGGGAAGA	MillHR13	169–190
F:CTAACCATTGCGGCATCCTCT R:TCTGTGATAGAATGGCAAAGAA	MillHR15	111–168
F: GCTTGCTTCCAACACTGAGACC R: GCAAAATGCTCGGAGAAGAC	MillHR17	230–269
F: TCTGACGTACCTCCTTTCA R: ATACTCGTGCCTCGTCTGT	MillHR18	154–193
F:TGATATTTTCAGGGCCCAAG R: AAATGGCACAAGTGGGAAAG	MillHR19	185–209
F: TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCTCATCATC	MillHR23	108–153
F: GCTCAACGAACCCAACACTGAT R: TCCAGCATTCAATGAAGAAGTT	MillHR24	238–260
F: GCGAAAGAGGAGAGTGCAAG R: TCTATAAGTGCCCCCTCACG	MillHR26	131–165
F: AGCTATCGCCACAGCAAATC R: GTCTTCTTCTGGCTGCCAAC	MillHR30	190–213
F: TTCTGTTAGTGGCGGTGTTG R: CACCTCCTCCTCCTCCTCTT	MillHR31	207–260
F: TGGTGGTGTGTTGTTTGCAGT R: ACCACCCGCAGTATTGAAAG	MillHR32	177–196
F: CTGAGTTTGGCAAGGGAGAG R: TTGATCCTTACCACCATCA	MillHR34	222–244
F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAAGTAG	MillHR36	210–249

3.2.2.1.1 PCR reaction mixture for SSR

Water	=	6.8μl
10X reaction buffer (Buffer B)	=	1.5μl
MgCl ₂ (25mM)	=	0.8μl
10mM dNTP	=	1.0μl
5 pM Primer Forward	=	0.8μl
5 pM Primer Reverse	=	0.8μl
0.5 U Taq DNA polymerase	=	0.3μl
100 ng DNA	=	3 μl

Table 4: PCR amplification programme for SSR

Step 1	Initial denaturation	94° C for 4 minutes
Step 2	Denaturation	94° C for 30 seconds
Step 3	Annealing	55° C for 30 seconds
Step 4	Extension	72° C for 1 minute
Step 5	Repeat step 2 to step 4 for 35 cycles	
Step 6	Final extension	72° C for 7 minutes

3.2.2.2 Chloroplast and ITS (Internal transcribed spacer) marker analysis for species identification

The genomic DNA was amplified using universal ITS4 and ITS5 (White *et al.*, 1990) for the entire ITS regions. Thirty five cycles of PCR were conducted using thermal cycler under below mentioned PCR profile.

For ITS rDNA sequencing, both strands of the amplicons were sequenced using the primers ITS4 & ITS5 (White *et al.* 1990) and partial chloroplast psbA-trnH gene. After that the amplified products were sent for DNA sequencing to Eurofins Genomics Pvt. Ltd., Bangalore. After sequencing, the generated nucleotide sequences were edited and manually adjusted as necessary using MEGA v. 7 (Tamura *et al.*, 2011).

3.2.2.2.1 PCR reaction mixture for ITS and PsbA tRnH primers

Water	= 5.5µl	4.6µl
10X reaction buffer (Buffer B)	= 1.5µl	1.5µl
MgCl ₂ (25mM)	= 1.5µl	1.5µl
1 mM dNTP	= 1.0µl	1.0µl
5 pM Primer Forward	= 1.5µl	1.5µl
5 pM Primer Reverse	= 1.5µl	1.5µl
0.5 U Taq DNA polymerase	= 0.4µl	0.4µl
100 ng DNA	= 2µl	2 µl

Table 5: PCR amplification programme for ITS primers

Sl. No.	Condition	Temperature	Duration
1	Initial denaturation	94° C	4 minute
2	Denaturation	94° C	30 Seconds
3	Annealing	55° C	0.30 seconds
4	Extension	72° C	1 minute
5	Final extension	72° C	1 minutes
6	Final hold	4° C	Forever
7	Total number of cycles	35 Cycles	-

3.2.2.3 Gene scan analysis

The genetic information was assessed only for single- locus SSR using the following parameters: number of alleles per locus (A), observed heterozygosity (H_o , direct count), expected heterozygosity $H_e = (1 - \sum p^2)$, where p is the frequency of the i th allele) and polymorphic information content. The computations were performed using the program Cervus 3.0. Band size to binary software was used to convert SSR data to binary data¹³.

3.2.2.4 Diversity analysis

Genetic diversity was estimated using Diversity software. Cluster analysis was performed with NTSYS PC Software, a numerical taxonomy and multivariate analysis software package with UPGMA and a dendrogram was obtained. For testing the consistency or the statistical significance of the genetic relationships between genotypes shown by the dendrogram, bootstrapping analysis was performed using WINBOOT software¹⁴.

3.3 To study the chemical profile of fruit sap and leaf.

3.3.1 Material: Sap and leaves of Appemidi

Method: Gas Chromatography Mass Spectrometry (GCMS)

3.3.2 Varieties (sap and leaf)

34 genotypes were used for leaf volatile estimation, viz., Adderi Jeerige, Appemidi, Balekoppa Appe, Gaddalahalli Appe, Gidagana mavu, Gorana Appe, Haldota Appe, Huliappekayi, Isgur Appe, Kadikai, Kalakai, Kalkuni, Kovesara, Malange, Manibhatta Appe, Murgeer, Shidadakke Appe, Thumbe beelu, Jeerige, Chansi, Kaana Appe, Kutumba Appe, Dodderi Jeerige, Sadamidi, Sudur Appe, Hittalahalli Appe, Ananthabhata Appe, Karpoora Jeerige, Gurusurthy Appe, Gomavu, Nandgar and Gadde mara.

Whereas fruit sap of 26 genotypes were used for volatile profiling.

For Surveyed samples only sap was analyzed for 15 genotypes viz., Gonibeedu1, 2, Marebylu 1, 2, Arenuru 1,2, Kudige 1, Soppinakadu 1, Heruru 1, Chimmankudige 2, Elemadlu, Kaman Mori, Magundi, Bynduru,

Statistical analysis

Correlation between the sap and leaf volatiles was worked out. The genotypes were grouped based on the volatile composition and principal component analysis was done. (SPSS statistical software and excel stat were used)

3.3.3 Profiling of major fat soluble, water soluble vitamins and organic acids in fresh fruits, brined fruits and brine solution of 15 Appemidi genotypes

3.3.3.1 Experimental details

Study material

15 Appemidi genotypes viz., Ananthabhatta Appe, Kaana Appe, Kalgundikoppa Appe, Karpoora Jeerige, Dannalli Appe, Appemidi, Balekoppa Appe, Naagara Appe, Jeerige, Malange, Gorana Appe, Gurumurthy Appe, Holekoppada Appe, Isgoor Appe and Nandgar were selected.

Parameters analyzed

- Vitamin C, acidity, total phenols, total flavonoids, total antioxidant activity were recorded in fresh, brined fruits and brine solutions.
- Profiling of major fat soluble, water soluble vitamins and organic acids in fresh, brined fruits and brine solution.
- Observations recorded: fruit weight, length width and fruit firmness.

3.3.3.1.1 Procedure

Freshly harvested immature whole tender mango of all 15 genotypes were harvested and analyzed for morphological parameters like fruit weight, length, width, thickness etc. Same fruits were utilized for biochemical analysis of vitamin C, acidity, total flavonoids, phenols and antioxidants etc. Some of the fruits were kept for drying in a jar containing of layer of salt and fruit which is a traditional practice of pickle preparation. After 15-20 days of osmotic dehydration, brined fruits and brine solutions were also analyzed for all the above mentioned biochemical analysis as well as morphological parameters of fruits.

3.3.3.1.2 Observations recorded before and after salt treatment

Fruit weight (g), length (cm), width (cm), thickness (cm) and firmness, vitamin C (mg/100g), acidity (mg/100g), total phenols (mg/100g), flavonoids mg/100g), antioxidant activity mg/100g), fat and water soluble vitamins and organic acids (mg/100g) were recorded.

3.3.3.2 Estimation of vitamin C

3.3.3.2.1 Principle

Titrimetric estimation of vitamin C was conventionally done using 2, 6-dichlorophenol indophenol (DCPIP) dye solution (AOAC, 2006). This dye exhibits blue colour in alkaline medium and pink in acidic medium. Ascorbic acid reduces the dye to colourless form. Reaction is quantitative and specific for ascorbic acid at pH 1.0-3.5. The end point is the appearance of pink color.

Reagents used

- 4% Oxalic acid
- 2,6-Dichlorophenol indophenol sodium salt ($C_{12}H_6Cl_2NNaO_2 \cdot 2H_2O$) 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 was used as a standard.

3.3.3.2.2 Procedure

5g of sample was homogenized with 10 ml 4% oxalic acid in a pestle and mortar for 2-3 times, squeezed through a muslin cloth and made up the volume to 50 ml. Vitamin C content present in the solution was estimated by titrating a known quantity of the extract against standard DCPIP dye solution.

Vitamin C content was calculated and expressed as mg of ascorbic acid equivalents per 100g fresh weight using a standard curve of L-Ascorbic acid.

3.3.3.2.3 Calculation

$$\text{Vitamin C (mg/100g)} = \frac{\text{Titre value} \times \text{Standard Value } (\mu\text{g}) \times \text{Total volume of extract} \times 100}{\text{Assay volume} \times \text{weight of the sample (g)} \times 1000}$$

3.3.3.3 Acidity

Acidity was determined by titration method (AOAC, 2000). Fruits were homogenized in a blender to fine condition. 5 grams of finely ground fruit sample 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.

$$\text{Acidity mg/100g} = \frac{\text{Titre value} \times \text{standard Value } (\mu\text{g}) \times \text{Total volume of extract} \times 100}{\text{Assay volume} \times \text{Weight of the sample (g)} \times 1000}$$

3.3.3.4 Estimation total phenols

Principle: Phenols react with the oxidizing agent phosphomolybdate in Folin-Ciocalteu reagent and form a blue colored complex, molybdenum blue which is measured at 700nm.

3.3.3.4.1 Reagents

- 80% Methanol
- Folin Ciocalteu's Phenol Reagent (1N)
- 20% Sodium Carbonate (Na_2CO_3)
- Standard phenol (gallic acid) solution (20-100 $\mu\text{g/mL}$) prepared in water

3.3.3.4.2 Procedure for total phenols

2g of sample was homogenized in methanol (80%) using pestle and mortar 2-3 times. The extracts were pooled and the volume was made up to 50 ml. 0.5 ml of the aliquots was taken in test a tube and 0.2 ml of Folin Ciocalteu's Phenol reagent was added followed by 3.3 ml of distilled water and mixed well. After 2 min, 1 ml of sodium carbonate solution was added and mixed well. Allowed it to stand at room temperature for 30 minutes and the blue color was read in a spectrophotometer at 700 nm (Singleton and Rossi, 1965).

3.3.3.4.3 Calculation

$$\text{Total phenol content (mg Gallic acid equivalents/100g)} = \frac{\text{OD@700nm} \times \text{Standard Value } (\mu\text{g/OD}) \times \text{Total Volume of extract} \times 100}{\text{Assay volume} \times \text{Weight of the tissue (g)} \times 1000}$$

3.3.3.5 Estimation of total flavonoids

3.3.3.5.1 Principle

Flavonoids develop a brick red colour with AlCl_3 and NaNO_2 at alkaline pH. The absorbance of the complex is read at 510 nm (Dewanto *et al.*, 2002).

3.3.3.5.2 Reagents

- 80% methanol
- 10% Aluminium chloride (AlCl_3)
- 5% Sodium nitrite (NaNO_2)
- 4N Sodium hydroxide (NaOH)
- Standard quercetin in water (20 - 100 $\mu\text{g}/\text{mL}$)

3.3.3.5.3 Procedure

2g of sample was homogenized in methanol (80%) using pestle and mortar 2-3 times. The extracts were pooled and the volume was made up to 50 ml. 1 ml of extract was taken in a test tube and 0.3 ml of 5% NaNO_2 was added. After 2 minutes 0.3 ml of 10% AlCl_3 was added. After 2 mins 3.4 ml of 4N NaOH was added and allowed at room temperature for 10 minutes. The absorbance of pink colour was read at 510 nm against blank. (Use catechin or quercetin as standard).

3.3.3.5.4 Calculation

$$\text{Total flavonoid content (mg Catechin equivalents/100g)} = \frac{\text{OD}_{510} \times \text{Standard value (mg/OD)} \times \text{Total Volume of extract} \times 100}{\text{Assay volume} \times \text{weight of the sample (g)}}$$

3.3.3.6 Estimation of total antioxidant activity

3.3.3.6.1 Method: Ferric reducing antioxidant potential (FRAP) (Benzie *et al.*, 1996)

Principle

The reduction of ferric tripyridyltriazine complex to the ferrous form at low pH is monitored by measuring the change in absorption of the blue colored solution at 593 nm, the reaction is nonspecific, in that any half reaction that has a lower redox potential than that of the ferric/ferrous half-reaction under reaction conditions will drive the ferric to ferrous reaction. The change in absorbance therefore is directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture.

3.3.3.6.2 Reagents required

- Acetate buffer (0.3 M, pH 3.6): Mix 3.1g of sodium acetate trihydrate with 16 ml of glacial acetic acid and make up to 1000ml with distilled water.
- HCl (0.04M): Mix 1.8ml of Concentrated HCl with a small volume of distilled water and make up to 500 ml.
- TPTZ (Tripyridyl-s-triazine) solution (10mM): Dissolve 0.195g of TPTZ in 6.25 ml of HCL.
- $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in distilled water and make up to 1000 ml with distilled water.
- FRAP reagent: Mix 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of FeCl_3 solution (10:1:1).
- 80% methanol (extraction medium)

- Standard ascorbic acid solution (20-100 µg/ml).

3.3.3.6.3 Procedure

2g of sample was homogenized in methanol (80%) using pestle and mortar 2-3 times. The extracts were pooled and the volume was made up to 50 ml. 0.2 ml of extract was taken in test tubes and 1.8 ml of FRAP reagent was added. Incubated at room temperature for 30 min and the absorbance were read at 593 nm. A standard curve was developed by using different concentrations of ascorbic acid (20-100 µg/ml). The results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC).

3.3.3.6.4 Calculation

$$\text{Antioxidant capacity (mg AEAC/100g)} = \frac{\text{OD}_{593\text{nm}} \times \text{Standard value } (\mu\text{g/OD}) \times \text{Total volume of the extract} \times 100}{\text{Assay volume} \times \text{Weight of the tissue (g)} \times 1000}$$

3.3.3.7 Extraction of water soluble vitamin

3.3.3.7.1 Methodology

Water soluble and fat soluble vitamins extraction procedure was followed as described by Santos *et al*, (2012). 5g of fruit sample was extracted with 25 ml of 10 mM ammonium acetate: methanol 50:50 (v/v) containing 0.1% BHT. After homogenization for 5 mins in micro grinder and vigorously shaking, the samples were placed in a sonication bath maintained at 25°C for 15-20 minutes. The sample was centrifuged at 14000g for 15 min and the supernatant was withdrawn (residue to be kept for further analysis) and filtered through a 0.45 µm nylon filter and the volume was made up. 2 ml of the supernatant was dried into a nitrogen stream and reconstituted to the same in 1 ml of the mobile phase, filtered through 0.2 µm nylon filter and injected into a UPLC-MS/MS system to determine the water soluble vitamin content.

Note: During the extraction process, samples were always protected from direct exposure to light and kept on ice bath to minimize vitamins degradation.

3.3.3.7.2 LC and MS conditions

The initial gradient was composed of 60% (A) and 40% (B) with flow rate of 0.3 ml/min, brought down to 30% of (A) and 70% of (B) in a linear gradient in 14 mins and back to initial condition by 15 min. The analytical column is 2.1 X 50 mm UPLC BEH-C18 column with 1.7µm particles, protected by a vanguard BEH C-18 guard column with 1.7µm. Particle size (Waters, USA) was used with column temperature maintained at 25°C. The elution was monitored using a TQDMS/ MS (Waters, USA) system, optimized for the water soluble vitamins analysis.

Mobile phase

Solvent - A: 0.1% formic acid in water
Solvent - B: Acetonitrile

3.3.3.8 Extraction of fat soluble vitamins

3.3.3.8.1 Methodology

The solid residues from the water soluble vitamins were re-extracted twice with 25 ml of ethyl acetate containing 0.1% BHT for 15 mins, and 15 mins in the ultrasonic bath. Finally, the samples were

centrifuged (14000g, 15 min) and pooled supernatants were filtered through a 0.45 µm nylon filter, made up to known volume. 15 ml of the extract was evaporated to dryness under vacuum and then in N₂ stream. Dissolved the residue in 2 ml of mobile phase and filtered through 0.2 µm nylon filter prior to injection in a UPLC-MS/MS system to monitor the fat soluble vitamin contents.

3.3.3.8.2 LC and MS-MS conditions

The initial gradient composed of 70% (A) and 30% (B) with the flow rate of 0.1 mL/min, brought down to 10% of (A) and 90% of (B) in a linear gradient in 10 min and back to initial condition by 15 min. The analytical column is 2.1 X 50 mm UPLC BEH-C18 column (Waters, USA) with 1.7µm particles, protected by a vanguard BEH C-18 with 1.7µm guard column (Waters, USA) with column temperature maintained at 25°C. The elution is monitored using a TQD-MS/MS (Waters, USA) system, optimized for the fat soluble vitamins analysis.

Mobile phase

Solvent - A: Acetonitrile

Solvent - B: 0.2% formic acid in methanol

Table 6: MRM of vitamin standards

Water soluble vitamins	Formula /Mass	Parent m/z [M+H] ⁺	Cone Voltage	Daughters	Collision energy (CE)	Ion Mode
Thiamine(B1)	264	265.03	20	122.06(Q)	16	ES+
		265.03	20	144.02	14	ES+
Riboflavin(B2)	376	376.97	40	243.05	24	ES+
Niacin(B3)	123	123.9	34	80.523(Q)	20	ES+
		123.9	34	77.47	18	ES+
Pantathenic acid (B5)	219.03	220.01	28	202.21	12	ES+
		220.01	28	124.16	20	ES+
Pyridoxine(B6)	169	169.97	24	152.09(Q)	12	ES+
		169.97	24	134.04	20	ES+
Biotin (B7)	244	245.03	26	227.14	14	ES+
Folic acid (B9)	441	442.1	24	295.16	16	ES+
Cyanocobalamine (B12)	677	678.29	38	147.18	68	ES+
Fat Soluble Vitamins						
Tocopherol (E)	430.71	431.42	165.13	36	22	ES+
Vitamin K1	450.70	451.35	187.07	38	18	ES+
Vitamin K2	444.65	445.30	187.13	28	22	ES+
Vitamin D1	384.64	385.35	367.34 105.09	24	14 46	ES+
Vitamin D2	396.65	397.36	379.36 107.11	20	8 26	ES+

* "Q" as quantifier ion.

3.3.3.9 Profiling of organic acids by LCMS

3.3.3.9.1 Extraction procedure

The extraction procedure of organic acid was followed as described earlier by Oliveira *et al.* (2008), Ribeiro *et al.* (2007), with slight modification. 5g of sample was homogenized using 10 ml of 80% methanol and sonicated for 30 min. The extract was then allowed to cool at room temperature and centrifuged at 10000 rpm for 15 mins. The supernatant was collected and the trace of methanol was completely removed by using vacuum evaporator. The extract was then transferred to a separating funnel and then extracted 3-4 times with 25 ml ethyl acetate. The lower aqueous phase was collected and a trace of organic solvent was completely removed by using vacuum evaporator, reconstituted the concentrate sample with water for SPE purification.

SPE for organic acids (STRATA X SPE Cartridge)

1. Conditioning: With 15 mL of Methanol
2. Equilibration: With 3 mL of water
3. Loading: sample diluted, pH around 6-7
4. Washing: With 2 mL of water
5. Elution: With 2 mL of acidified methanol (2% formic acid in Methanol)

Volume was made up with acidified methanol. The eluted solution obtained from the cartridge was dried completely under the flow of nitrogen, reconstituted the same in the mobile phase (Solvent A and Solvent B; 50:50) and filtered through a nylon membrane filter with a pore size of 0.45 μ m followed by 0.2 μ m. 4 μ l of sample was then injected into the LC-MS/MS for analysis.

3.3.3.9.2 LC and MS-MS conditions

The initial gradient composed of 100% aqueous phase (A) and 0% organic phase (B), held for 0.5 min. At 5.0 min, the gradient was changed to 95% aqueous phase and 5% organic phase, held for 0.5 min then system was return to the initial conditions at 6 min and this condition was hold for 1 min to equilibrate before the next injection. The flow rate was 0.1 mL/min. The analytical column was used 2.1 X 50mm UPLC BEH- Amide column (Waters) with 1.7 μ m particles, protected by a Vanguard 2.1 X 5mm BEH-Amide with 1.7 μ m particle size guard column (Waters) and the column temperature was maintained at 25°C. The sample injected volume was 4 μ L. The eluted organic acids were monitor using a PDA detector and the UPLC column effluent was pump directly without any split into the TQD-MS/MS (Waters, USA) system, which was optimize for the identification and quantification of organic acids analysis.

Mobile phase

Solvent - A: 10mM Ammonium Acetate: Acetonitrile (50:50), pH: 8.5 (pH adjusted with Ammonia)

Solvent - B: Acetonitrile with 0.05% formic acid

Table 7: MRM of organic acid standards:

Water soluble organic acids	Formula /Mass	Parent m/z [M+H] ⁺	Cone Voltage	Daughters	Collision energy (CE)	Ion Mode
Citric acid	191.99	190.96	20	111.09	10	ES ⁻
Fumaric acid	116	114.84	20	70.99	8	ES ⁻
Maleic acid	116	114.84	18	70.98	12	ES ⁻
Malic acid	134	132.84	24	115.03	12	ES ⁻
Malonic acid	104	102.84	16	59.00	6	ES ⁻
Oxalic acid	90	88.78	20	44.98	8	ES ⁻
		88.78	20	60.95	8	
Oxaloacetic acid	132	130.84	12	87.02	8	ES ⁻
Pyruvic acid	88.03	86.87	22	43.02	6	ES ⁻
Shikimic acid	174.03	172.93	34	93.05	14	ES ⁻
Succinic acid	118	116.84	20	73.00	10	ES ⁻
Tartaric acid	150	148.84	22	87.01	10	ES ⁻
			22	73.00	12	

3.3.4 Profiling of volatile aroma compounds using GCMS

Aroma compounds are usually low molecular weight compounds belonging to terpenoids, esters, hydrocarbons, alcohols, aldehydes, lactones and acids. They are mostly nonpolar in nature however a few polar group compounds are also observed like acids. Aroma compounds are extracted using various methods like steam distillation, vacuum distillation, solvent extraction, simultaneous solvent and steam extraction, super critical fluid extraction method and solid phase micro extraction (SPME). The extracted the compounds were injected to GC or GCMS for estimation. The following were used for aroma extraction and estimation in our study.

3.3.4.1 Analysis and profiling of volatile/aroma compounds in fruits using GCMS

Gas Chromatography (GC) is a commonly used analytical technique for the identification of volatile compounds which are thermally stable by using different detector. The detectors such as Flame Ionization Detector (FID), Thermal Conductivity Detector (TCD) and Electron Capture Detector (ECD), Mass spectral Detector (MSD) is being used for identification and quantification of the component present in the essential oil/ flavour of fruits/flower/vegetable.

3.3.4.2 Principle

Like other chromatographic techniques, a mobile and a stationary phase are used in GC for separation of the compounds. The mobile phase is comprised of an inert gas e.g. helium, hydrogen, nitrogen, etc., and the stationary phase consists of a column packed with a solid support. Frequently

used columns are capillary columns in which the stationary phase coats the walls of a small-diameter tube directly e.g. 0.25 microm film in a 0.32 mm tube. Compounds are separated by the interaction of the compound with the stationary phase. Stronger interaction leads to longer retention time of the compound. Separation of compounds depends on the polarity/absorptivity of volatiles compounds.

3.3.4.3 Identification and Quantification

In GC equipped with FID/ECD/TCD/MSD detectors, the peak for the identification of the individual compound will be compared with the standard compounds available and their retention index or Kovats index** and the mass spectra available in literature.

3.3.4.4 Sap volatile aroma profiling (Liquid sample) (Solvent extraction method)

Volatiles from the Appemidi fruit sap were analysed using GCMS fitted with a DB-5 MS column, using helium as carrier gas. Temperature programme was used for GC separation. Volatiles from the sap were extracted using the solvent extraction method. Sap was mixed with pentane–ether mixture and the pentane–ether layer was carefully separated using a separating funnel. Extraction was repeated three times and the pentane–ether was pooled. The pooled extract was evaporated under vacuum and redissolved in dichloromethane before injection to GCMS (Varian 4000). Volatiles were identified using NIST mass library. They were also identified using retention indices of standards.

3.3.5 Solid phase micro extraction (SPME) method of volatile extraction from leaves

Solid phase micro extraction was based on the adsorption of analytes onto the coated phase of fused silica fibre and the partitioning of analytes between stationary phase of the fibre and the extraction medium as air. It consists of a 1-2 cm long fused silica fiber, coated with stationery phase such as poly dimethyl siloxane (PDMS), divinyl benzene(DVB), carbowaxan (CAR) or the mixture of all the three and bonded to a stainless steel plunger and holder. These fibres are to be first conditioned at 250°C for 2-3 hours in the injector port of GC with continued flow of N₂/He gas.

A known quantity (5g) of ground fresh leaf was transferred in to the flasks with silicon rubber caps. The flasks were kept on a stirrer for an incubation period of 20 minutes and then SPME fibre was inserted into the flask through the silicon rubber stopper and was allowed to adsorb the head-space volatiles for 2 hours with continuous stirring. SPME fibre device coated with DVB/CAR/PDMS (50/30 μm, highly crossed linked) was first conditioned at 250°C for 2 hours (Facundo *et al.*, 2013). Later fibre was removed and injected into a GCMS for separation and identification of compounds.

3.3.5.1 GC and GC-MS analysis

Subsequently, the fibre was allowed to remain in the injector port in the inlet for 15 min. The MS column was fused-silica capillary column of 30 m x 0.25 mm id, 0.25 mm film thickness for the analysis. The injector temperature was set at 250°C and all injections were either split-less or in split (1:20) mode for 0.5 min followed by split-less, detector temperature was 270°C, and the temperature programmes for column was as follows: 40°C for 3 min at an increment 3°C/min to 190°C, hold for 1 min, then 5°C/min to 220°C and maintained the constant temperature for 5 min. Spectrometer was in the external electron ionization mode with the carrier gas helium 1 ml/min; injector temperature, 250°C; trap temperature 180°C, ion source-heating at 190°C, transfer line temperature 260°C, EI-mode at 70 eV, with full scan-range 50-350 amu. The total volatile production was calculated by the sum of all GC peak areas in the chromatogram and expressed individual compounds as relative per cent area and the compounds were identified by comparing the retention index which were determined by using homologous series of n-alkanes (C₅ to C₃₂) as standard and compared the spectra using two spectral libraries available as Wiley and NIST-2007.

3.4 Traditional knowledge and documentation on south Indian mango

Survey was conducted to collect the data on traditional practices for pickle preparation and other practices.

3.4.1 Survey

Geographic area survey: Geographic information system was conducted to position the diversity spot exactly on the GPS instrument. Recording of Geo Co-ordinates for spotted mango landraces at different locations were carried. Diversity mapping and digitalizing using GIS software for processing the geographic data of located diversity spots and for integrating into geo referenced map was done. Representative homesteads with appreciable diversity of seedling mango races were located through sociometric survey by referring the existing study (Subedi *et al.*, 2005).

3.4.2 Documentation

The data related to selection of best Appemidi for pickling, method of pickle preparation, pickle quality and shelf life were recorded. Photographs were taken. Some of the indigenous traditional practices as well as some appemidi recipies were documented.

Five key persons and 10 different farmers explained the methodology they were practicing for selection of best midi and method of pickle preparation.

Collaboration with other divisions:

- Division of Biotechnology for molecular work.
- Division of Plant Physiology and Biochemistry for volatile profiling and quantification.
- Statistical lab for SAS analysis of experiment data.
- Indian Institute of Science - for pollen morphology study.

IV. RESULTS

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 diverse genepools will be useful to breeders. Thus, an attempt was made to study the diversity of indigenous mango genotypes of Western Ghat regions of Chikmagalur District, Karnataka, by conducting a survey and also using molecular markers such as SSRs in order to complement the morphological data and to throw more light on the genetic diversity of Appemidi. Western Ghat regions known for the rich diversity of unique pickle type mango (Appemidi), keeping this in view, and the present investigation entitled “**Studies on Diversity of Indigenous Mango Genotypes**” was conducted at ICAR-IIHR, Bengaluru. The data collected were statistically analyzed and the results obtained were presented in this chapter.

In this study the surveyed data of forty Appemidi genotypes were analyzed for Mahalanobi's 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, palynological, as well as molecular data to know the relationship among the genotypes collected. The major volatiles were profiled from appemidi leaves and were compared with the fruit sap volatile profile. The surveyed fruit saps were also subjected for profiling to study the diversity in its volatile composition. The results of all the parameters were presented in following headings.

4.1 To study the variability in various morphological characters of indigenous mango

A three phase survey was conducted at Gonibeedu, Marebylu, Aldur, Balehonnur, Kudige, Soppinakadu, Arenuru, Magundi, Bynduru, Elelmadlu, Kanathi and Chimankudige places of Chikmagalur district, western ghat regions of Karnataka. Forty indigenous types were collected from different regions and each genotype was named

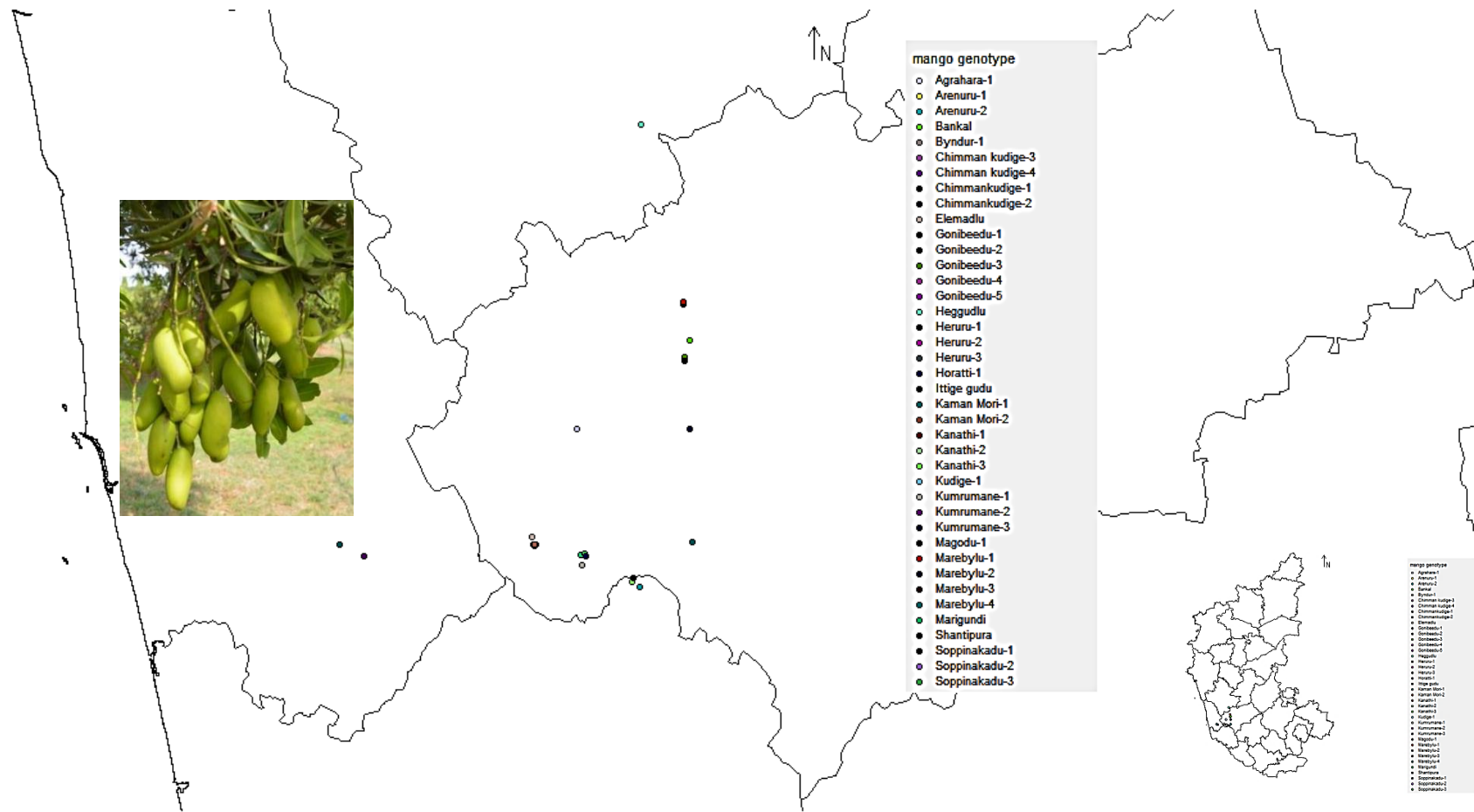


Fig. 7: Locations of the 40 surveyed Appemidi genotypes of western ghat regions of Chikmagalur



Plate 1: Surveyed Appemidi habitat



Plate 2: Variability in the canopy architecture of surveyed Appemidi genotypes from Chikamagalur

depending upon their place of collection. The leaf, inflorescence, fruit, sap and pollens were characterized (Table 8a and 8b & Fig 7).

4.1.1 Leaf characterization

Leaves of forty indigenous genotypes were collected and characterized from surveyed areas. Table 8a shows the leaf characteristics of surveyed genotypes.

4.1.1.1 Leaf altitude in relation to branch, leaf venation and texture

Based on orientation of leaf and associated morphological characters the genotypes were grouped in to two categories viz., horizontal and semi erect. Out of forty genotypes twenty were having horizontal and twenty were semi erect and leaves were of medium venation and coriaceous in texture (Table 8a).

4.1.1.2 Leaf Shape

The shape of the leaf in forty genotypes were predominantly of oblong, elliptic and oblanceolate (Table 8a).

4.1.1.3 Leaf margin and pubescence

All surveyed genotypes leaf margins were wavy and pubescent (Table 8a).

4.1.1.4 Colour of young leaf

Colour of young leaves was recorded from 5-10 days old newly emerged ones. A wide colour variation of young leaves was observed such as light green, light brown, brown or deep coppery tan in appemidi leaves. Out of forty genotypes surveyed, it was found that the young leaves of twenty six genotypes were light green in colour, nine were in light brown and five genotypes were having dark brown or coppery coloured young leaves.

4.1.1.5 Intensity of anthocyanin pigmentation in young leaves

The intensity of anthocyanin pigmentation in young leaves also varied significantly. Out of forty genotypes low pigmentation was observed in twenty



Plate 3: Kudige- Appemidi tree aged more than 100 years



A

B



Plate 4: Variability in young and matured leaf colour of surveyed Appemidi genotypes; A= young leaf (Light green and brown colour) B= matured leaf (Light Green and Dark green colour)

Table 8a: Leaf characteristics of forty surveyed Appemidi genotypes from western ghat regions of Chikmagalur

Genotypes	Leaf Attitude in relation to branch	Thickness of pelvinus	Leaf venation	Leaf texture	Leaf shape	Leaf margin	Leaf pubescence	Colour of young leaf	Intensity of anthocyanin pigmentation	Colour of fully developed leaf	Leafy bracts	Leaf tip	Leaf fragrance
Agrahara	Horizontal	T	Medium	Coriaceous	Elliptic	Wavy	Present	LG	Low	DG	Present	Pointed	Strong
Gonibeedu-1	Horizontal	T	Medium	Coriaceous	Oblong	Wavy	Present	LB	High	DG	Present	Pointed	Strong
Gonibeedu 2	Horizontal	T	Medium	Coriaceous	Oblong	Wavy	Present	LB	High	DG	Present	Pointed	Strong
Gonibeedu -3	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LB	Medium	DG	Present	No Pointed	Strong
Gonibeedu-4	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LB	Medium	DG	Present	No Pointed	Strong
Gonibeedu-5	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	Low	Green	Absent	Pointed	Mild
Kamanmori-1	Horizontal	T	Medium	Coriaceous	Oblong	Wavy	Present	LB	Medium	DG	Present	Pointed	Mild
Kamanmori-2	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	Low	DG	Present	Pointed	Mild
Chimankudige 1	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	Low	DG	Present	Pointed	Mild
Chimankudige 2	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	High	Green	Absent	Pointed	Mild
Chimankudige-3	Horizontal	T	Medium	Coriaceous	Oblong	Wavy	Present	Brown	High	DG	Present	No pointed	Strong
Chimankudige-4	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	Low	Green	Absent	Pointed	Mild
Heruru-1	Horizontal	T	Medium	Coriaceous	Oblanceolate	Wavy	Present	LG	Low	DG	Absent	No pointed	Mild
Heruru-2	Horizontal	T	Medium	Coriaceous	Oblanceolate	Wavy	Present	LG	Low	DG	Absent	No pointed	Mild
Elemadlu-1	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	Low	DG	Present	Pointed	Mild
Elemadlu-2	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	High	Green	Absent	Pointed	Mild
Marebylu-1	Horizontal	T	Medium	Coriaceous	Oblanceolate	Wavy	Present	LG	Low	DG	Absent	No pointed	Mild
Marebylu-2	Horizontal	T	Medium	Coriaceous	Oblanceolate	Wavy	Present	LG	Low	DG	Absent	No pointed	Mild
Marebylu-3	Horizontal	T	Medium	Coriaceous	Oblong	Wavy	Present	Brown	High	DG	Present	No pointed	Strong
Marebylu-4	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	Low	Green	Absent	Pointed	Mild
Soppinakadu-1	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	Low	DG	Present	Pointed	Mild
Soppinakadu-2	Horizontal	T	Medium	Coriaceous	Oblanceolate	Wavy	Present	LG	Low	DG	Absent	No pointed	Mild
Soppinakadu-3	Horizontal	T	Medium	Coriaceous	Oblanceolate	Wavy	Present	LG	Low	DG	Absent	No pointed	Mild

Genotypes	Leaf Attitude in relation to branch	Thickness of pelvius	Leaf venation	Leaf texture	Leaf shape	Leaf margin	Leaf pubescence	Colour of young leaf	Intensity of anthocyanin pigmentation	Colour of fully developed leaf	Leafy bracts	Leaf tip	Leaf fragrance
Kumrumane1	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	High	Green	Absent	Pointed	Mild
Kumrumane-2	Horizontal	T	Medium	Coriaceous	Oblanceolate	Wavy	Present	LG	Low	DG	Absent	No pointed	Mild
Marigundi	Horizontal	T	Medium	Coriaceous	Oblong	Wavy	Present	Brown	High	DG	Present	No pointed	Strong
Doobla	Horizontal	T	Medium	Coriaceous	Oblong	Wavy	Present	Brown	High	DG	Present	No pointed	Strong
Kudige	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	Low	Green	Absent	Pointed	Mild
Bynduru	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LB	Medium	DG	Present	No pointed	Strong
Arenuru-1	Horizontal	T	Medium	Coriaceous	Oblong	Wavy	Present	LB	High	DG	Present	Pointed	Strong
Arenuru-2	Horizontal	T	Medium	Coriaceous	Oblong	Wavy	Present	LB	Medium	DG	Present	Pointed	Mild
Maagodu	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	Low	DG	Present	Pointed	Mild
Horatti Jeerige-1	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	High	Green	Absent	Pointed	Mild
Horatti Jeerige-2	Horizontal	T	Medium	Coriaceous	Oblanceolate	Wavy	Present	LG	Low	DG	Absent	No pointed	Mild
Banakal	Horizontal	T	Medium	Coriaceous	Oblanceolate	Wavy	Present	LG	Low	DG	Absent	No pointed	Mild
Heggudlu	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	Low	DG	Present	Pointed	Mild
Kanathi-1	Horizontal	T	Medium	Coriaceous	Oblong	Wavy	Present	Brown	High	DG	Present	No pointed	Strong
Kanathi-2	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	Low	Green	Absent	Pointed	Mild
Kanathi-3	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LB	Medium	DG	Present	No pointed	Strong
Shantipura	Semi Erect	T	Medium	Coriaceous	Oblong	Wavy	Present	LG	High	Green	Absent	Pointed	Mild

Note: T= Thick, LG= light green, LB= light brown, DG= dark green

genotypes, medium pigmentation was seen in six and thirteen were with high pigmentation (Table 8a).

4.1.1.6 Colour of fully developed leaves

The colour of fully developed leaves varied from green to dark green, among forty genotypes thirty genotypes were of dark green and ten were green coloured (Table 8a).

4.1.1.7 Leafy bracts

Out of forty genotypes twenty-one were having leafy bracts and remaining genotypes lacked leafy bracts (Table 8a).

4.1.1.8 Leaf tip

Leaf tips of twenty genotypes were pointed and remaining eighteen genotypes were not pointed (Table 8a).

4.1.1.9 Leaf fragrance

The fragrance of leaves of forty genotypes was assessed and was grouped as absent, present, mild and strong. Leaf fragrance was present in all the forty genotypes in which thirteen were having strong leaf fragrance and remaining were with mild leaf fragrance (Table 8a).

4.1.1.10 Leaf length (cm)

Leaf length varied significantly among the genotypes studied and presented in Table 9. The leaf length was ranged from 18.77 cm to 31.31cm with mean of 24.36 cm. The maximum leaf length was recorded in genotype Kanathi-3 (31.31 cm) which was on par with Gonibeedu-4 (30.00 cm), Soppinakadu-3 (30.00 cm), Chimankudige-2 (29.83 cm), Elemadlu-2 (29.83 cm), Kanathi-1 (28.6 cm) and Kudige (28.23 cm). Whereas, the minimum leaf length was recorded in Chimankudige-1 (18.77 cm) followed by Elemadlu-1 and Soppinakadu-2 (18.83 and 18.93 cm).

Table 9: Leaf characterization of surveyed Appemidi genotypes from western ghat regions of Chikmagalur

Cultivars	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Leaf area (cm²)
Agrahara	22.30	3.93	2.60	87.70
Gonibeedu-1	20.53	4.87	1.73	100.10
Gonibeedu 2	24.00	5.07	2.87	121.59
Gonibeedu -3	25.33	5.07	3.73	128.42
Gonibeedu-4	30.00	6.83	3.03	206.44
Gonibeedu-5	20.63	6.90	3.03	142.05
Kaman Mori-1	27.33	5.23	4.67	142.85
Kaman Mori -2	27.57	7.23	4.63	200.88
Chimankudige-1	18.77	4.53	2.07	85.53
Chimankudige-2	29.83	6.93	3.03	208.43
Chimankudige-3	25.00	4.00	2.60	99.97
Chimankudige-4	23.97	3.93	2.60	94.25
Heruru-1	21.30	6.43	1.90	137.16
Heruru-2	22.80	5.37	3.20	122.12
Elemadlu-1	18.83	4.67	2.10	88.20
Elemadlu-2	29.83	7.07	3.03	212.43
Marebilu-1	23.20	4.23	2.80	98.09
Marebilu-2	20.97	4.10	2.60	85.87
Marebilu-3	24.30	6.43	1.87	156.68
Marebilu-4	23.97	5.30	3.37	125.62
Soppinakadu-1	27.57	7.23	4.63	200.88
Soppinakadu-2	18.93	4.60	2.07	87.53
Soppinakadu-3	30.00	6.87	3.13	207.46
Kumrumane 1	20.70	3.93	2.67	81.46
Kumrumane-2	21.63	4.13	2.57	89.20
Doobla	24.00	6.43	1.87	154.65
Marigundi	23.97	5.37	3.33	127.12
Kudige	28.23	7.23	4.80	205.98
Bynduru	25.53	5.07	3.73	129.49
Arenuru-1	27.33	5.43	4.97	148.65
Arenuru-2	19.00	4.53	2.37	86.57
Maagodu	21.65	3.93	2.60	85.17
Horatti Jeerige-1	23.40	6.43	2.00	150.92
Horatti Jeerige-2	23.97	5.30	3.43	125.62
Banakal	27.57	7.23	4.67	200.88
Heggudlu	19.60	6.83	3.10	134.10
Kanathi-1	28.60	6.50	2.60	186.98
Kanathi-2	29.70	6.93	3.77	206.26
Kanathi-3	31.31	7.57	3.90	236.34
Shantipura	21.26	3.90	2.80	83.03
Mean	24.36	5.59	3.06	139.32
CD @5%	3.731	1.13	0.794	44.796
±SE (m)	1.323	0.4	0.281	15.88
F Test	*	*	*	*
C.V.	9.401	12.408	15.917	19.743

4.1.1.11 Leaf width (cm)

Leaf width differed significantly with a mean of 5.59 cm and ranged from 3.90 to 7.57 cm among the genotypes studied (Table 9). The maximum leaf width was recorded in Kanathi-3 (7.57 cm) which was on par with Banakal, Kudige, Soppinakadu-1, Kamanmori-2 (7.23 cm), Soppinakadu-3 and Elemadlu-2 (7.07) which is followed by Chimankudige-2 (6.93 cm), Gonibeedu-4 (6.83 cm). The minimum leaf width was recorded in Shantipura (3.90 cm) which was followed by Chimankudige-4, Kumrumane-1 and Magodu (3.93 cm).

4.1.1.12 Petiole length (cm)

Significant difference was observed for petiole length among the genotypes and it ranged from 1.73 to 4.97 cm with the mean of 3.06 cm (Table 9). Maximum petiole length was recorded in Arenuru-1 (4.97 cm) which was on par with genotypes Kudige (4.80 cm), Banakal, Kamanmori-1 (4.67 cm), Kamanmori-2 and Soppinakadu-1 (4.63 cm), while the minimum petiole length was recorded in Gonibeedu-1 (1.73 cm).

4.1.1.13 Leaf area (cm²)

Leaf area differed significantly among the genotypes studied with the mean of 139.32 cm² (Table 9). Maximum leaf area was recorded in Kanathi-3 (236.34cm²) which was on par with Elemadlu-2 (212.43 cm²), Gonibeedu-4 (206.44 cm²), Soppinakadu-3 (207.46 cm²), Chimankudige-2 (208.43 cm²), Kanathi-2 (206.26 cm²) and Kudige (205.98cm²), whereas, the minimum leaf area was recorded in Shantipura (83.03 cm²).

4.1.2 Inflorescence characterization

The selected genotypes inflorescence characterization was presented in table 8b.

4.1.2.1 Regularity of flowering

Out of forty genotypes only five were regular in flowering habit and remaining were biennial (Table 8b).

4.1.2.2 Inflorescence position

All forty genotypes showed terminal borne inflorescence type (Table 8b).

4.1.2.3 Inflorescence axis growth habit

All forty genotypes showed semi erect type of growth habit (Table 8b).

4.1.2.4 Inflorescence shape

Three shapes of inflorescences were observed in the accessions viz., conical, pyramidal and broadly pyramidal. Majority of genotypes had conical shape of inflorescence (20) followed by broadly pyramidal (11) and pyramidal (9).

4.1.2.5 Inflorescence anthocyanin coloration

Ten genotypes had strong anthocyanin coloration out of forty genotypes followed by sixteen mild types and in fourteen genotypes inflorescence anthocyanin coloration was absent (Table 8b).

4.1.2.6 Presence of leafy bracts

Leafy bracts were absent in all the forty genotypes surveyed (Table 8b).

4.1.2.7 Density of flowers in inflorescence

The flower density in the inflorescence was classified based on IPGRI descriptor as medium, dense, sparse. Nineteen genotypes showed densely flowered inflorescence and twenty one with sparsely flowered types (Table 8b).

4.1.2.8 Flower type

All the genotypes were of pentamerous type of flowers (Table 8b).

4.1.2.9 Inflorescence colour

The inflorescence colour ranged from light green, green with red and yellow green. Majority of genotypes had green with red colored inflorescence (GWR=23), followed by yellowish green (6) and light green (11) (Table 8b).

Table 8b: Inflorescence characteristics of forty surveyed Appemidi genotypes from western ghat regions of Chikmagalur

Genotypes	Regularity of flowering	Inflorescence position	Inflorescence axis growth habit	Inflorescence shape	Inflorescence anthocyanin coloration	Presence of leafy bracts	Density of flowers in inflorescence	Flower type	Inflorescence colour	Nature of disc	Hairiness
Agrahara	Regular	Terminal	Semi Erect	Conical	weak	Absent	Dense	Penta	LG	Swollen	Absent
Gonibeedu-1	Regular	Terminal	Semi Erect	Pyramidal	Medium	Absent	Sparse	Penta	YG	Swollen	Absent
Gonibeedu-2	Regular	Terminal	Semi Erect	Pyramidal	Medium	Absent	Sparse	Penta	YG	Swollen	Absent
Gonibeedu -3	Biennial	Terminal	Semi Erect	Broadly pyramidal	Medium	Absent	Dense	Penta	GwR	Swollen	Sparse
Gonibeedu-4	Biennial	Terminal	Semi Erect	Broadly pyramidal	Medium	Absent	Dense	Penta	GwR	Swollen	Sparse
Gonibeedu-5	Biennial	Terminal	Semi Erect	Pyramidal	Absent	Absent	Sparse	Penta	LG	Swollen	Sparse
Kamanmori-1	Biennial	Terminal	Semi Erect	Broadly pyramidal	Absent	Absent	Sparse	Penta	YG	Swollen	Sparse
Kamanmori-2	Biennial	Terminal	Semi Erect	Conical	Absent	Absent	Sparse	Penta	YG	Swollen	Sparse
Chimankudige-1	Biennial	Terminal	Semi Erect	Conical	Absent	Absent	Sparse	Penta	YG	Swollen	Sparse
Chimankudige-2	Biennial	Terminal	Semi Erect	Conical	Strong	Absent	Dense	Penta	GwR	Swollen	Dense
Chimankudige-3	Biennial	Terminal	Semi Erect	Broadly pyramidal	Strong	Absent	Sparse	Penta	GwR	Swollen	Sparse
Chimankudige-4	Biennial	Terminal	Semi Erect	Pyramidal	Absent	Absent	Sparse	Penta	LG	Swollen	Sparse
Heruru-1	Biennial	Terminal	Semi Erect	Conical	Medium	Absent	Dense	Penta	GwR	Swollen	Absent
Heruru-2	Biennial	Terminal	Semi Erect	Conical	Medium	Absent	Dense	Penta	GwR	Swollen	Sparse
Elemadlu-1	Biennial	Terminal	Semi Erect	Conical	Absent	Absent	Sparse	Penta	YG	Swollen	Sparse
Elemadlu-2	Biennial	Terminal	Semi Erect	Conical	Strong	Absent	Dense	Penta	GwR	Swollen	Dense
Marebylu-1	Biennial	Terminal	Semi Erect	Conical	Medium	Absent	Dense	Penta	GwR	Swollen	Absent
Marebylu-2	Regular	Terminal	Semi Erect	Conical	Medium	Absent	Dense	Penta	GwR	Swollen	Sparse
Marebylu-3	Biennial	Terminal	Semi Erect	Broadly pyramidal	Strong	Absent	Sparse	Penta	GwR	Swollen	Sparse
Marebylu-4	Biennial	Terminal	Semi Erect	Pyramidal	Absent	Absent	Sparse	Penta	LG	Swollen	Sparse
Soppinakadu-1	Biennial	Terminal	Semi Erect	Conical	Absent	Absent	Sparse	Penta	YG	Swollen	Sparse

Soppinakadu-2	Biennial	Terminal	Semi Erect	Conical	Medium	Absent	Dense	Penta	GwR	Swollen	Sparse
Soppinakadu-3	Biennial	Terminal	Semi Erect	Conical	Medium	Absent	Dense	Penta	GwR	Swollen	Absent
Kumrumane-1	Biennial	Terminal	Semi Erect	Conical	Strong	Absent	Dense	Penta	GwR	Swollen	Dense
Kumrumane-2	Biennial	Terminal	Semi Erect	Conical	Medium	Absent	Dense	Penta	GwR	Swollen	Absent
Marigundi	Biennial	Terminal	Semi Erect	Broadly pyramidal	Strong	Absent	Sparse	Penta	GwR	Swollen	Sparse
Doobla	Biennial	Terminal	Semi Erect	Broadly pyramidal	Strong	Absent	Sparse	Penta	GwR	Swollen	Sparse
Kudige	Biennial	Terminal	Semi Erect	Pyramidal	Absent	Absent	Sparse	Penta	LG	Swollen	Sparse
Bynduru	Biennial	Terminal	Semi Erect	Broadly pyramidal	Medium	Absent	Dense	Penta	GwR	Swollen	Sparse
Arenuru-1	Regular	Terminal	Semi Erect	Pyramidal	Medium	Absent	Sparse	Penta	YG	Swollen	Absent
Arenuru-2	Biennial	Terminal	Semi Erect	Broadly pyramidal	Absent	Absent	Sparse	Penta	YG	Swollen	Sparse
Maagodu	Biennial	Terminal	Semi Erect	Conical	Absent	Absent	Sparse	Penta	YG	Swollen	Sparse
Horatti Jeerige-1	Biennial	Terminal	Semi Erect	Conical	Strong	Absent	Dense	Penta	GwR	Swollen	Dense
Horatti Jeerige-2	Biennial	Terminal	Semi Erect	Conical	Medium	Absent	Dense	Penta	GwR	Swollen	Absent
Banakal	Biennial	Terminal	Semi Erect	Conical	Medium	Absent	Dense	Penta	GwR	Swollen	Sparse
Heggudlu	Biennial	Terminal	Semi Erect	Conical	Absent	Absent	Sparse	Penta	YG	Swollen	Sparse
Kanathi-1	Biennial	Terminal	Semi Erect	Broadly pyramidal	Strong	Absent	Sparse	Penta	GwR	Swollen	Sparse
Kanathi-2	Biennial	Terminal	Semi Erect	Pyramidal	Absent	Absent	Sparse	Penta	LG	Swollen	Sparse
Kanathi-3	Biennial	Terminal	Semi Erect	Broadly pyramidal	Medium	Absent	Dense	Penta	GwR	Swollen	Sparse
Shantipura	Biennial	Terminal	Semi Erect	Conical	Strong	Absent	Dense	Penta	GwR	Swollen	Dense

LG= light green, GwR= green with Red patches, YG= Yellowish green



Plate 5: Variability in the inflorescence petiole colour of surveyed Appemidi genotypes from Chikmagalur (Light green, Green with red patches and pink)



Plate 6: Variability in the inflorescence length width and colour of surveyed Appemidi genotypes from Chikmagalur (Light green, Green with red patches and pink)

4.1.2.10 Nature of disc

The nature of disc observed in surveyed forty genotypes was swollen type (Table 8b).

4.1.2.11 Hairiness

The hairiness was absent in 9 genotypes, sparse in 26 genotypes and dense in 5 genotypes.

4.1.2.12 Inflorescence length (cm)

The results presented in table 10 revealed that, inflorescence length was ranged from 12.70 cm to 22.78 cm with the mean value of 16.57 cm. The maximum inflorescence length was recorded in Agrahara (22.78 cm) which is on par with Shantipura (22.68 cm) followed by genotypes Gonibeedu-5, Marebilu-1, Marebilu-2, Kumrumane-1 and Kumrumane-2 (22.27 cm), while the minimum was observed in Heruru-1, Heruru-2, Magodu, Soppinakadu-2 and Horatti Jeerige (12.70 cm).

4.1.2.13 Inflorescence width (cm)

Among the genotypes studied, inflorescence width varied significantly which ranged from 8.0 to 15.82 cm with the mean of 11.37 cm. The maximum inflorescence width was observed in the genotype Shantipura (15.82 cm) and it was on par with cultivars Kumrumane-1, Kumrumane-2, Arenuru-1, Bynduru, Marebilu-1, Marebilu-2 and Gonibeedu-5 (15.33 cm). The minimum inflorescence width was recorded in Gonibeedu-3, Marigundi (8.00 cm) (Table 10).

4.1.2.14 Flower diameter (cm)

Significant differences were observed among forty genotypes, the mean flower diameter was 0.51 cm and it was ranged from 0.47cm to 0.80 cm. The maximum flower diameter was observed in genotype Agrahara (0.80 cm) followed by Gonibeedu-1 (0.73 cm) and the minimum was recorded in the genotype Chimankudige-1 (0.47 cm) which was on par with Gonibeedu-3, Gonibeedu-2 (0.47 cm) followed by Chimankudige -2 (0.50 cm) (Table 10).

Table 10: Inflorescence characterization of surveyed Appemidi genotypes from western ghat regions of Chikmagalur

Cultivars	Inflorescence length (cm)	Inflorescence width (cm)	Flower diameter (cm)	Peduncle length (cm)	Peduncle width (cm)
Agrahara	22.78	13.05	0.80	5.30	0.73
Gonibeedu-1	18.83	12.17	0.73	4.37	0.73
Gonibeedu 2	14.50	9.00	0.47	5.10	0.60
Gonibeedu -3	14.67	8.00	0.47	6.37	0.60
Gonibeedu-4	13.67	10.73	0.47	6.23	0.60
Gonibeedu-5	22.27	15.33	0.53	5.67	0.60
Kamanmori-1	19.17	14.07	0.53	6.00	0.60
Kamanmori-2	18.95	11.23	0.53	5.10	0.57
Chimankudige-1	13.67	10.73	0.47	6.23	0.60
Chimankudige-2	15.67	10.73	0.50	6.47	0.67
Chimankodige-3	14.67	9.33	0.50	4.30	0.53
Chimankodige-4	13.84	10.57	0.50	6.30	0.70
Heruru-1	12.70	8.27	0.47	5.17	0.57
Heruru-2	12.70	8.27	0.47	4.87	0.57
Elemadlu-1	13.84	10.57	0.50	6.17	0.70
Elemadlu-2	13.67	10.73	0.47	6.10	0.60
Marebilu-1	22.27	15.33	0.53	6.20	0.60
Marebilu-2	22.27	15.33	0.53	6.30	0.60
Marebilu-3	15.67	10.73	0.50	6.43	0.67
Marebilu-4	14.67	9.33	0.50	5.00	0.53
Soppinakadu-1	19.17	14.07	0.53	6.00	0.60
Soppinakadu-2	12.70	8.27	0.47	5.17	0.57
Soppinakadu-3	13.67	10.73	0.47	6.40	0.60
Kumrumane-1	22.27	15.33	0.53	6.60	0.60
Kumrumane-2	22.27	15.33	0.53	6.30	0.60
Doobla	15.67	10.73	0.50	6.00	0.67
Marigundi	14.67	8.00	0.47	6.20	0.60
Kudige	19.17	14.07	0.53	6.21	0.60
Bynduru	22.27	15.33	0.53	6.09	0.60
Arenuru-1	22.27	15.33	0.53	5.67	0.60
Arenuru-2	15.67	10.73	0.50	6.21	0.67
Maagodu	12.70	8.27	0.47	4.67	0.57
Horatti Jeerige-1	13.84	10.57	0.50	5.79	0.70
Horatti Jeerige-2	12.70	8.27	0.47	5.25	0.57
Banakal	13.67	10.73	0.47	6.00	0.60
Heggudlu	14.67	9.33	0.50	5.17	0.53
Kanathi-1	13.84	10.57	0.50	6.00	0.70
Kanathi-2	13.84	10.57	0.50	6.00	0.70
Kanathi-3	14.67	9.33	0.50	5.22	0.53
Shantipura	22.68	15.82	0.58	6.07	0.60
Mean	16.57	11.37	0.51	5.77	0.61
C.D. @5%	1.84	2.00	0.11	0.70	0.10
±SE(m)	0.65	0.71	0.04	0.25	0.04
F- Test	*	*	*	*	*
C.V.	6.80	10.77	12.64	7.48	10.34

4.1.2.15 Peduncle length (cm)

The peduncle length ranged from 4.37 to 6.60 cm (Table 10). The maximum peduncle length was observed in the cultivar Kumrumane-1 (6.60 cm) which was on par with Chimankudige-2 (6.47 cm), Marebilu-3 (6.43 cm) and Soppinakadu-3 (6.40 cm) followed by Gonibeedu-3 (6.37 cm) and Chimankudige-4, Marebilu-2 and Kumrumane-3 (6.30 cm). The minimum length was noticed in Gonibeedu-1 (4.37 cm), which was on par with the genotype Kamanmori (5.10 cm).

4.1.2.16 Peduncle width (cm)

The peduncle width ranged from 0.53 cm to 0.73 cm. The maximum peduncle width was observed in the genotype Agrahara and Gonibeedu-1 (0.73 cm) which was on par with Chimankudige-4, Elemadlu-1 Horatti Jeerige and Kanathi genotypes (0.70 cm). The minimum length was registered in Chimankudige-3, Marebilu-4, Kanathi-3 and Heggudlu (0.53 cm) (Table 10).

4.1.3 Fruit characterization

4.1.3.1 Fruit length (cm)

Significant difference was observed in fruit length among the genotypes (Table 11). The mean fruit length recorded was 4.53 cm, and it ranged from 3.40 cm to 5.65 cm. The maximum fruit length was recorded in Chimankudige-3 (5.65 cm) which was on par with Banakal (5.54 cm), Marigundi (5.50 cm), Elemadlu-1 (5.23 cm) and Agrahara (5.22 cm) while, minimum fruit length was recorded in Chimankudige-2 (3.40 cm) followed by Kanathi-1 (3.60 cm).

4.1.3.2 Fruit diameter (cm)

The fruit diameter differed significantly with a mean of 3.00 cm and was ranged from 2.22 cm to 3.92 cm among the genotypes studied (Table 11). Maximum fruit diameter was recorded in Arenuru-1 (3.92 cm) followed by Horatti Jeerige (3.55 cm) and Agrahara (3.52 cm) while, the minimum fruit diameter was recorded in Heruru-1 (2.22 cm) which was on par with Heruru (2.30 cm).

Table 11: Fruit morphological characterization of surveyed Appemidi genotypes from western ghat regions of Chikmagalur

Cultivars	Fruit length (cm)	Fruit diameter (cm)	Fruit thickness (cm)	Fruit weight (g)	Sap burning time (seconds)
Agrahara	5.22	3.52	2.95	14.35	11.52
Gonibeedu-1	4.25	2.55	1.92	16.67	14.48
Gonibeedu 2	4.62	2.97	2.42	13.55	15.46
Gonibeedu -3	4.47	3.07	2.65	16.45	14.08
Gonibeedu-4	4.12	2.77	2.10	12.80	17.34
Gonibeedu-5	4.12	3.09	2.10	14.60	18.92
Kaman mori-1	5.23	2.98	2.18	13.35	16.98
Kaman mori-2	4.95	2.98	2.18	14.12	16.73
Chimankudige 1	4.15	3.48	2.25	13.22	16.55
Chimankudige 2	3.40	3.05	2.42	12.22	16.17
Chimankudige-3	5.65	2.95	1.52	15.75	16.15
Chimankudige-4	4.40	3.25	2.40	19.12	16.42
Heruru-1	4.50	2.22	2.59	18.32	16.32
Heruru-2	4.52	2.30	2.40	19.90	16.05
Elemadlu-1	5.23	2.55	2.60	17.30	16.58
Elemadlu-2	5.33	2.55	2.80	17.93	16.98
Marebilu-1	3.62	2.75	2.07	14.40	16.15
Marebilu-2	3.70	2.60	2.10	14.83	16.19
Marebilu-3	4.58	2.93	2.25	15.20	15.82
Marebilu-4	3.50	3.07	2.27	14.35	16.37
Soppinakadu-1	4.92	3.10	2.32	18.92	19.00
Soppinakadu-2	4.95	3.27	2.25	21.09	64.66
Soppinakadu-3	4.95	3.075	2.325	20.425	19.17
Kumrumane-1	4.27	3.10	2.40	20.15	17.31
Kumrumane-2	4.27	3.10	2.37	20.00	17.51
Doobla	4.60	2.70	2.15	16.19	25.95
Marigundi	5.50	3.10	2.70	16.74	12.27
Kudige	4.92	3.05	2.71	15.12	28.25
Bynduru	4.16	2.75	2.07	14.30	12.31
Arenuru-1	3.80	3.92	2.07	15.31	27.02
Arenuru-2	4.42	3.38	2.25	21.12	27.88
Maagodu	4.58	3.25	2.25	15.02	15.67
Horatti Jeerige-1	4.72	3.55	2.65	16.15	15.70
Horatti Jeerige-2	4.60	3.25	2.68	16.15	15.75
Banakal	5.54	3.10	2.90	13.75	14.05
Heggudlu	5.03	3.07	2.05	20.31	10.40
Kanathi-1	3.60	3.07	2.15	16.07	25.38
Kanathi-2	4.31	3.10	2.20	15.73	22.87
Kanathi-3	4.11	2.70	2.28	16.26	25.38
Shantipura	4.53	2.78	2.35	16.09	15.05
Mean	4.53	3.00	2.33	16.33	18.82
C.D. @5%	0.60	0.29	0.25	2.48	--
±SE (m)	0.22	0.10	0.09	0.89	7.26
F-Test	*	*	*	*	*
C.V.	9.51	6.82	7.76	10.84	77.32



Plate 7: Variability in the trunk diameter of surveyed Appemidi genotypes from Chikmagalur



Plate 8: Variability in fruit size and fruit shape of surveyed Appemidi genotypes from Chikmagalur

4.1.3.3 Fruit thickness (cm)

Fruit thickness differed significantly among the genotypes studied with the mean fruit thickness of 2.33 cm and ranged from 1.52 cm to 2.95 cm (Table 11). The maximum fruit thickness was recorded in the genotype Agrahara (2.95 cm) which was on par with Banakal (2.90 cm) and Elemadlu-2 (2.80 cm), the minimum fruit thickness was recorded in Chimankudige-3 (1.52 cm) followed by genotype Gonibeedu-1 (1.92 cm).

4.1.3.4 Fruit weight (g)

The fruit weight registered significant difference among the genotypes (Table 11). The fruit weight of mango cultivars studied in the present investigation ranged from 12.22g to 21.12 g with a mean fruit weight of 16.33 g (Table 11). In the present study, significantly maximum fruit weight was recorded in Arenuru-2 (21.12 g) followed by Soppinakadu-2 (21.09g), Soppinakadu-3 (20.42g) and Heggudlu (20.31 g), while minimum fruit weight was recorded in Chimankudige-2 (12.22 g) which was on par with Gonibeedu-3 (12.80 g).

4.1.3.5 Sap burning time (seconds)

The mean sap burning time was 18.82 seconds and varied significantly among the genotypes studied (Table 11). The maximum burning time was recorded in the cultivar Kudige (28.25 seconds) and the minimum was observed in Agrahara (11.52 seconds).

4.1.4 Pollen morphology and germination studies in Appemidi

4.1.4.1 General pollen morphometry of surveyed Appemidi genotypes from Western Ghats

Pollen grains of all investigated *Mangifera species* were symmetric, isopolar, monad, trizonocolpate (three marginal colpi are present) and small in size (Fig 11). In polar view, pollen grains were subtriangular and elliptical in equatorial view. The mean length of the polar axis varied from 42.19 μm to 59.92 μm and the equatorial diameter from 19.59 μm to 27.70 μm . The shape of the pollen grain varies among the genotypes selected for this study (Table 12).

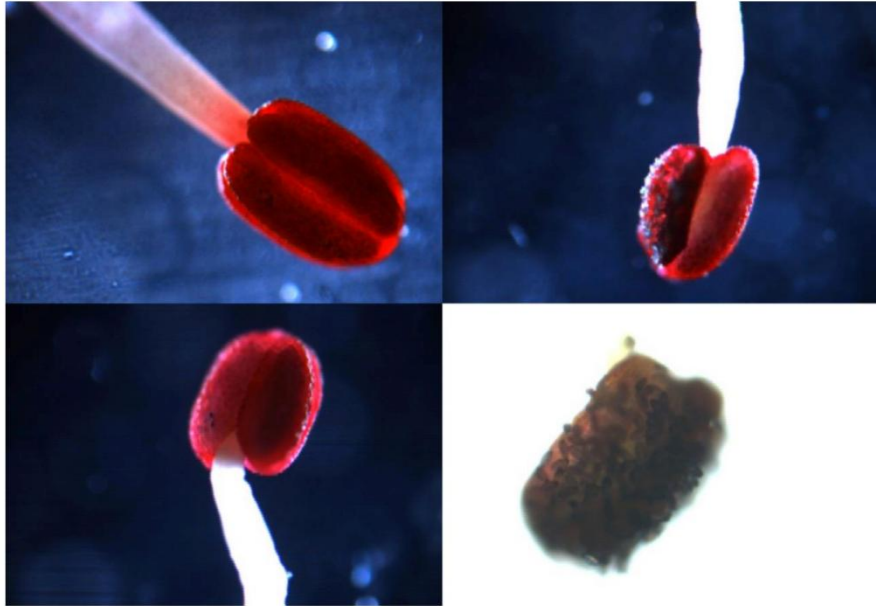


Plate 9: Anthers and dehiscing pollen grains of Appemidi

4.1.4.1.1 Pollen length (μm)

Length of the pollen along polar axis varied significantly and ranged from 42.19 μm to 59.92 μm with an average length of 50.65 μm among the genotypes studied (Table 12). The maximum pollen length was observed in the genotype Arenuru-2 (59.92 μm), which was on par with Kudige (58.89 μm) followed by Soppinakadu -2 (53.94 μm). The minimum pollen length was noticed in Soppinakadu-3 (42.19 μm).

4.1.4.1.2 Equatorial width (μm)

Significant differences were noticed for equatorial width of the pollen grains with an average width of 23.14 μm (Table 12). The maximum equatorial width was recorded in Marebilu-3 (27.70 μm) followed by Arenuru-2 (25.67 μm) and the minimum was observed in Elemadlu-1 (19.59 μm).

4.1.4.1.3 Colpi length (μm)

Genotypes registered significant differences for colpi length with an average length of 40.47 μm (Table 12). Maximum length of colpi was observed in Heruru -2 (48.60 μm) followed by Kudige (48.40 μm) and the minimum was noticed in the genotype Marebilu-3 (30.47 μm).

4.1.4.1.4 Colpi per cent (%)

Colpi per cent showed significant differences among the genotypes studied with an average of 80.02 per cent (Table 12), whereas, the highest colpi per cent was noticed in Heruru-2 (98.34%) and minimum in Doobla (70.60%).

4.1.4.1.5 P/E ratio

Among the genotypes studied, P/E ratio varied significantly and most of the genotypes recorded P/E ratio of more than 2, which decides the shape of the pollen grain (table 12). The highest P/E ratio (2.66) in genotypes Bynduru and Elemadlu-1 indicated that pollens were perprolate in shape. The genotype Soppinakadu-3 recorded the minimum ratio of 1.67 followed by Marebylu-3 (1.74) exhibited prolate pollen shape.

Table 12: Pollen morphometry of surveyed Appemidi genotypes from western ghat regions of Chikmagalur

Genotypes	Pollen length (µm)	Equatorial diameter (µm)	Colpi length (µm)	Colpi per cent	P/E ratio	Mean size (mm)	Pollen shape
Arenuru-1	52.22	20.91	46.43	88.92	2.50	1.09	Perprolate
Arenuru-2	59.92	25.67	47.83	79.83	2.33	1.54	Perprolate
Bynduru	54.14	20.36	47.49	87.72	2.66	1.10	Perprolate
Chimankudige-1	47.80	22.42	41.65	87.14	2.13	1.07	Perprolate
Chimankudige-2	47.74	20.91	40.99	85.85	2.28	1.00	Perprolate
Chimankudige-3	47.80	22.42	41.65	87.14	2.13	1.07	Prolate
Kaman Mori-1	47.03	24.05	37.18	79.06	1.96	1.13	Prolate
Kaman Mori-2	45.61	24.67	36.43	79.88	1.85	1.13	Prolate
Doobla	54.28	22.85	38.32	70.60	2.37	1.24	Perprolate
Elemadlu-1	52.11	19.59	39.79	76.36	2.66	1.02	Perprolate
Elemadlu-2	51.79	19.62	41.60	80.33	2.64	1.02	Perprolate
Gonibeedu-1	50.60	23.13	40.87	80.77	2.19	1.17	Perprolate
Gonibeedu-2	53.37	22.44	44.87	84.08	2.38	1.20	Perprolate
Gonibeedu-3	48.76	23.27	40.48	83.03	2.10	1.13	Perprolate
Gonibeedu-4	48.69	24.66	43.07	88.45	1.97	1.20	Prolate
Marebylu-1	51.94	24.95	31.10	59.87	2.08	1.30	Perprolate
Marebylu-2	54.32	22.48	45.68	84.10	2.42	1.22	Perprolate
Marebylu-3	48.19	27.70	30.47	63.22	1.74	1.33	Prolate
Marebylu-4	51.84	24.36	35.80	69.05	2.13	1.26	Perprolate
Kudige	58.89	23.70	48.40	82.19	2.49	1.40	Perprolate
Heruru-1	45.61	24.67	36.43	79.88	1.85	1.13	Prolate
Heruru-2	49.42	23.09	48.60	98.34	2.14	1.14	Perprolate
Kumrumane-1	47.83	20.60	39.59	82.76	2.32	0.99	Perprolate
Kumrumane-2	49.62	24.56	35.30	71.13	2.02	1.22	Perprolate
Magodu	51.84	24.36	35.80	69.05	2.13	1.26	Perprolate
Soppinakadu-1	50.84	22.37	39.52	77.74	2.27	1.14	Perprolate
Soppinakadu-2	53.94	23.01	39.59	73.39	2.34	1.24	Perprolate
Soppinakadu-3	42.19	25.19	38.27	90.72	1.67	1.06	Prolate
Mean	50.65	23.14	40.47	80.02	2.21	1.17	
± SE (m)	0.13	0.07	0.17	0.45	0.01	0.01	
CD@ 1%	0.38	0.19	0.48	1.27	0.03	0.03	
F-Test	*	*	*	*	*	*	
C.V.	0.47	0.52	0.75	1.02	0.85	1.42	

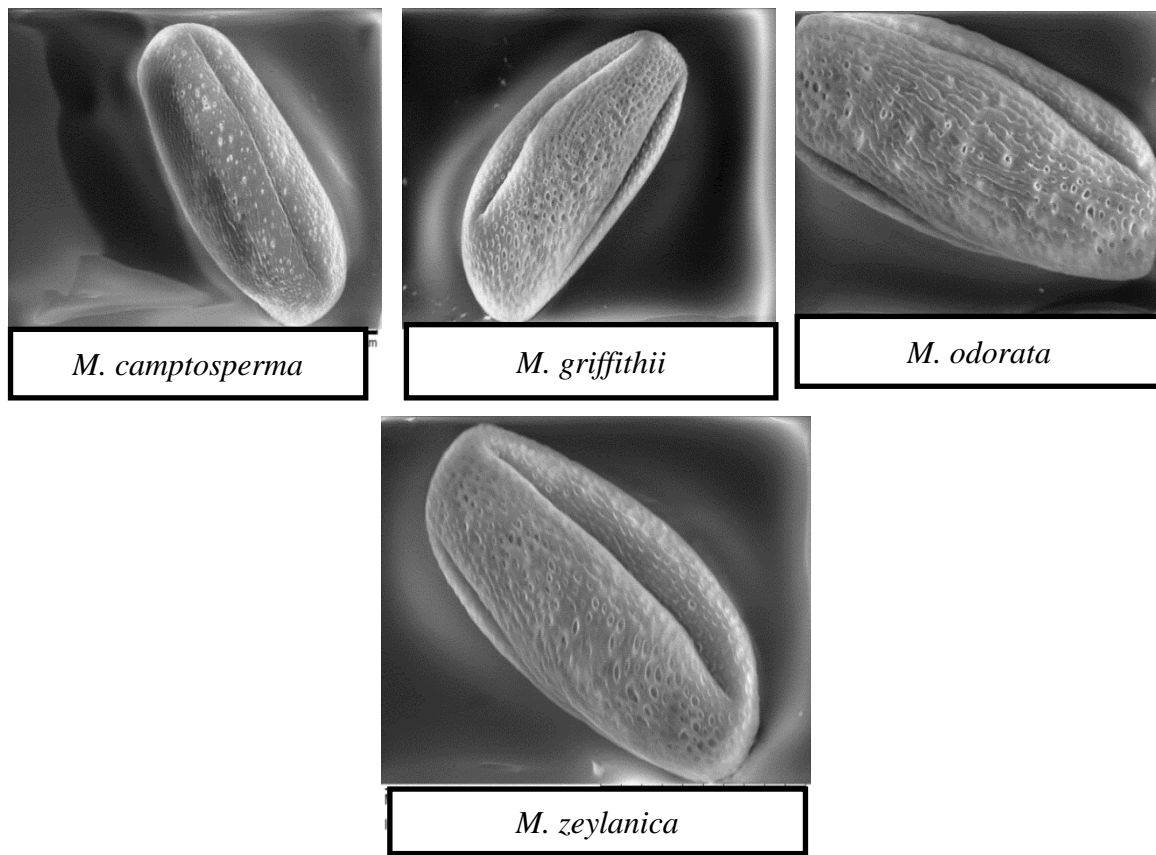


Fig. 8: Pollen morphometry and Exine ornamentation of *Mangifera* species

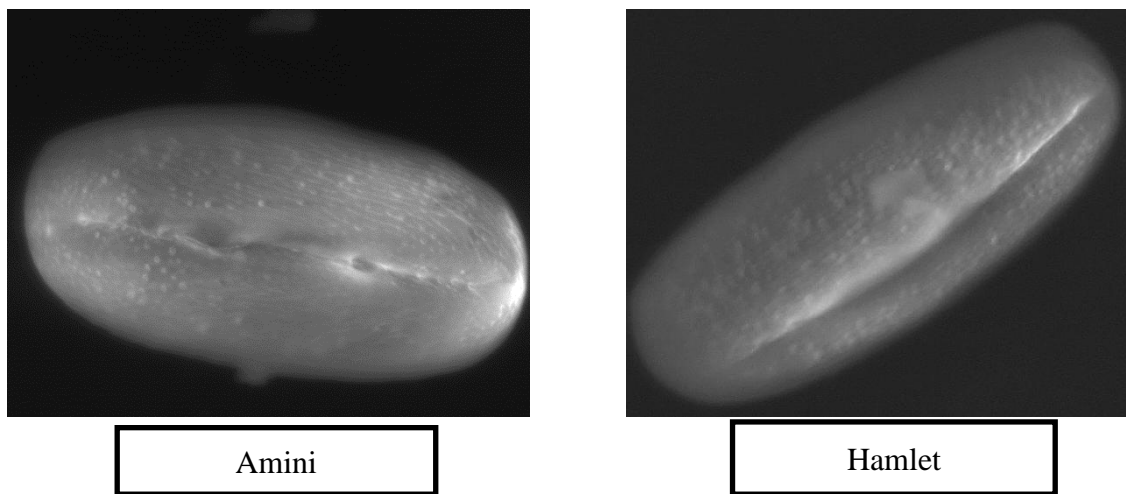


Fig. 9: Pollen morphometry and exine ornamentation of cut mango pickle types

4.1.4.1.6 Mean size of the pollen (PXE) (mm)

The mean size of the pollen also varied significantly among the surveyed genotypes with an average value of 1.17 mm (Table 12). The maximum pollen size was recorded in Arenuru-2 (1.54 mm) followed by Kudige (1.40 mm) and the minimum was observed in Kumrumane-1 (0.99mm) followed by Chimankudige-2 (1.00 mm).

4.1.4.1.7 Exine ornamentation

The exine ornamentation was thick, striate to reticulated type, were separated by grooves and we could see large perforation on the outer surface of some genotypes.

4.1.4.2 General pollen morphometry of Appemidi genotypes from ICAR-IIHR germplasm

Thirty six genotypes which include four *Mangifera species*, two cut mango pickle genotypes (Amini and Hamlet), one unique sucking type (Varategiduga) and twenty nine appemidi genotypes from ICAR-IIHR germplasm block were subjected for scanning electron microscopy to study the variability in morphology of pollen.

Pollen grains of all the investigated genotypes were symmetric, isopolar, monad, trizonocolpate (three marginal colpi are present) and small in size. In polar view pollen grains were subtriangular and in equatorial view it was elliptical. The mean length of the polar axis varied from 31.66 μm to 56.07 μm and the equatorial diameter from 17.15 μm to 36.27 μm . The shape of the pollen grain varies among the genotypes selected for this study (Table 13). The shape of pollen grains in *Mangifera* genus varied from prolate to perprolate depending upon the P/E ratio. The mean P/E ratio observed was 2.07. The exine ornamentation was thick, striate to reticulated type, were separated by grooves and we could see large perforation on the outer surface (Fig 8, 9 & 10).

4.1.4.2.1 Pollen length (μm)

Significant differences were obtained for the length of the pollen studied for 36 genotypes from ICAR-IIHR germplasm block were represented in table 13. The average length of pollen was 44.87 μm . The maximum length was observed in the genotype

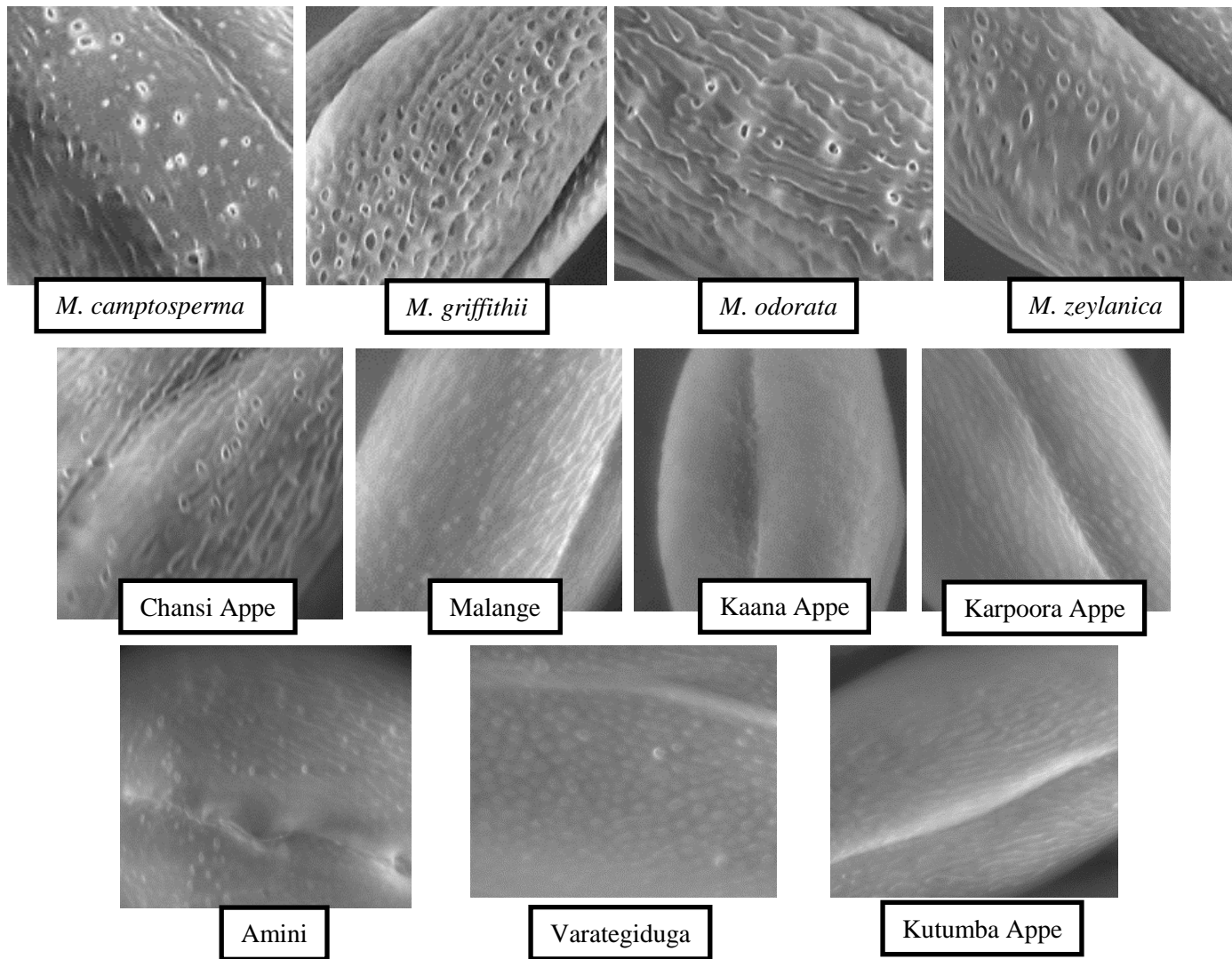


Fig. 10: Exine ornamentation of *Mangifera* species and Appemidi genotypes

Kashimidi (56.06 μm) and the minimum was recorded in cultivar Kalgundikoppa Appe (31.66 μm).

4.1.4.2.2 Equatorial diameter (μm)

The equatorial diameter showed significant differences among the genotypes with a mean diameter of 21.99 μm . The cultivar Kashimidi recorded the maximum diameter of 36.27 μm (table 13), which was on par with Karpooora Jeerige (32.55 μm) and the minimum diameter was observed in cultivar Naagara Appe (17.15 μm).

4.1.4.2.3 Colpi length (μm)

Thirty six genotypes showed significant differences for colpi length (Table 13). The average length of the colpi was 32.43 μm (Table 13), while the maximum length was observed in Gaddalahalli Appe (46.32 μm) followed by Holekoppada Appe (45.25 μm) and the minimum colpi length was noticed in the genotype Mahabalagiri Appe (20.15 μm).

4.1.4.2.4 Colpi per cent (%)

Colpi per cent also showed significant differences among the genotypes studied with an average per cent of 72.27 (table 13), whereas the highest per cent of colpi was noticed in Gaddalahalli Appe (95.62%) followed by Gaddemara (86.75) and the minimum was noticed in Mahabalagiri Appe (41.44%).

4.1.4.2.5 P/E ratio

Majority of the genotypes were observed to be perprolate with a P/E >2.0 and prolate ≤ 2.0 , the maximum ratio was noticed in Balekoppa Appe (2.40) and the minimum ratio was recorded in Kashimidi (1.55) (Table 13).

4.1.4.2.6 Mean size of the pollen (PXE) (mm)

The significant differences among the genotypes mean pollen shape values were showed in table 13, with an average value of 1.00 mm. The maximum was recorded in

Table 13: Pollen morphometry of 36 genotypes (*Mangifera Species* and Appemidi genotypes) from ICAR-IIHR germplasm

Genotypes	Pollen length (µm)	Equatorial diameter (µm)	Colpi length (µm)	Col per cent	P/E ratio	Mean size (P X E) (mm)	Pollen shape
Amini	43.12	18.83	34.50	80.00	2.29	0.81	Perprolate
Appemidi	42.08	18.25	30.46	72.38	2.31	0.77	Perprolate
Hamsamamidi	36.63	18.49	24.21	66.10	1.98	0.68	prolate
Hamlet	42.89	18.05	34.39	80.18	2.38	0.77	Perprolate
Kaana Appe	34.07	17.90	21.90	64.29	1.90	0.61	Prolate
Karigal Appe	46.35	20.30	37.00	79.83	2.28	0.94	Perprolate
Karpoora Appe	40.10	19.16	31.47	78.49	2.09	0.70	Prolate
Kalgundikoppa Appe	31.66	20.02	21.72	68.58	1.58	0.63	Prolate
Kutumba Appe	42.69	18.74	32.68	76.55	2.28	0.80	Perprolate
Malange	39.43	20.02	23.18	58.79	1.97	0.79	Prolate
Naagara Appe	36.66	17.15	24.30	66.28	2.14	0.63	Perprolate
Varate Giduga	41.12	17.65	30.48	74.13	2.33	0.73	Perprolate
Ananthabhatta Appe	40.10	19.16	31.50	78.55	2.09	0.77	Perprolate
Aruna gowda Appe	46.18	24.24	37.88	82.03	1.91	1.12	Prolate
Adderi Jeerige	46.04	22.48	37.95	82.43	2.05	1.03	Perprolate
Balekoppa Appe	54.05	22.50	40.16	74.30	2.40	1.22	Perprolate
Chansi appe	39.43	20.09	23.20	58.55	1.97	0.79	Prolate
Dannalli Appe	48.25	22.34	38.29	79.36	2.16	1.08	Perprolate
Dodderi Jeerige	46.57	23.47	38.62	82.93	1.98	1.09	Prolate
Gaddemara	49.05	23.56	42.55	86.75	2.08	1.16	Perprolate
Gaddala halli Appe	48.44	26.24	46.32	95.62	1.85	1.27	Prolate
Giduganamavu	50.19	21.41	33.41	66.57	2.34	1.07	Perprolate
Hittalahalli Appe	48.56	21.80	33.21	68.39	2.23	1.06	Perprolate
Holekkoppada Appe	56.07	26.66	45.25	80.70	2.10	1.49	Perprolate
Isgoor Appe	46.24	22.68	33.19	71.78	2.04	1.05	Perprolate
Jeerige	48.03	24.55	36.17	75.31	1.96	1.18	Prolate
Kashimidi	56.06	36.27	30.56	54.51	1.55	2.03	Prolate
Karpoora Jeerige	52.47	32.55	32.08	61.14	1.61	1.71	Prolate
Kalgundikoppa Appe	50.73	25.83	30.59	60.30	1.96	1.31	Prolate
Kovesara	49.31	24.16	30.79	62.45	2.04	1.19	Perprolate
Kutumba Appe	44.68	23.88	25.25	56.51	1.87	1.07	Prolate
Mahabalagiri Appe	48.62	26.84	20.15	41.44	1.81	1.30	Prolate
<i>M. camptosperma</i>	40.98	17.17	34.24	83.56	2.39	0.70	Perprolate
<i>M.grifitti</i>	41.37	18.99	31.67	76.54	2.18	0.79	Perprolate
<i>M. odorata</i>	46.29	20.95	37.00	79.93	2.21	0.97	Perprolate
<i>M. zeylanica</i>	40.67	19.08	31.06	76.38	2.13	0.78	Perprolate
Mean	44.87	21.99	32.43	72.27	2.07	1.00	
± SE (m)	0.171	0.12	0.187	0.312	0.007	0.01	
CD @1%	0.483	0.34	0.528	0.881	0.019	0.03	
F-Test	*	*	*	*	*	*	
C.V.	0.661	0.95	0.996	0.743	0.568	1.60	

Kashimidi (2.03 mm) and the minimum was observed Naagara Appe (0.63 mm) and Kalgundikoppa Appe (0.63 mm).

4.1.4.2.7 Exine ornamentation

The exine ornamentation is thick, striate to reticulated type and was separated by grooves with large perforation on the outer surface of some genotypes. In *M. camptosperma* exine ornamentation pattern was irregular, while, *M. griffithii* appeared to be more irregular with thick striate to reticulate, *M. zeylanica* and *M. odorata* had more irregular with thick striae. Among Appemidi genotypes exine sculpture ornamentations appeared to be irregular, small sized perforations which were sparsely distributed on the surface of the pollen grain with striae (Fig 10).

4.1.4.3 Pollen germination study

In vitro pollen germination study was carried out to see the response of Appemidi genotypes. Twenty genotypes which include sixteen Appemidi cultivars and four *Mangifera species* (Table 14) were selected for pollen germination study. Considerable differences were obtained in germination per cent, pollen tail length, pollen head length and width. The results of pollen germination were presented in table 14.

4.1.4.3.1 Pollen germination (%)

Significant differences were obtained with respect to per cent pollen germination among the genotypes and species studied with an average pollen germination per cent of 36.29 (Table 14). The maximum pollen germination per cent was observed in Naagara Appe (70.25%) and the minimum was recorded in *M. camptosperma* (12.75%).

4.1.4.3.2 Pollen head length (µm)

Significant differences were obtained in the pollen head length among twenty genotypes studied (Table 14). The maximum pollen head length was observed in Kutumba Appe (26.49 µm) followed by *M. odorata* (26.42 µm) and Karpooora Jeerige (25.77 µm). The genotypes *M. griffithii* (22.41 µm), *M. zeylanica* (22.75 µm), Gaddemara (22.70 µm), Amini (23.49 µm) and Ananthabhattera Appe (23.97 µm) were

Table 14: *In vitro* pollen germination, pollen head length, width and tail length of Appemidi and *Mangifera* species

Genotypes	Pollen head length (µm)	Pollen head width (µm)	Tail length (µm)	Pollen germination per cent
<i>M. griffithii</i>	22.41	19.78	53.08	17.71
<i>M. zeylanica</i>	22.75	21.35	80.92	18.70
<i>M. odorata</i>	26.42	26.61	55.61	20.40
<i>M. camptosperam</i>	23.27	23.56	51.67	12.75
Gaddemara	22.70	18.14	169.92	36.68
Hamsamamidi	21.28	22.36	102.52	31.08
Amini	23.49	24.35	89.24	59.50
Karpoora Jeerige	25.77	24.23	141.71	45.44
Naagara Appe	10.00	9.97	131.14	70.25
Hamlet	9.47	9.62	55.72	44.42
Amrapali	16.81	17.37	104.49	54.36
Ananthabhattera Appe	23.97	21.37	117.70	39.22
Hittalhalli Appe	8.82	12.03	70.90	49.40
Kutumba Appe	26.49	22.51	168.37	35.45
Arunagowdana Appe	23.77	22.51	115.64	19.90
Karigal Appe	20.11	24.85	94.09	50.40
Kalgundi Koppa Appe	10.13	10.30	117.81	24.94
Karpoora Appe	20.11	24.51	104.09	34.80
Chansi	18.98	19.00	71.97	26.14
Malangi	19.01	19.00	73.10	34.40
Mean	19.79	19.67	98.49	36.29
± SE (m)	1.79	1.28	18.55	3.50
CD@ 1%	5.15	3.69	5.31	10.08
F-Test	*	*	*	*



Plate 10: Scanning electron microscope

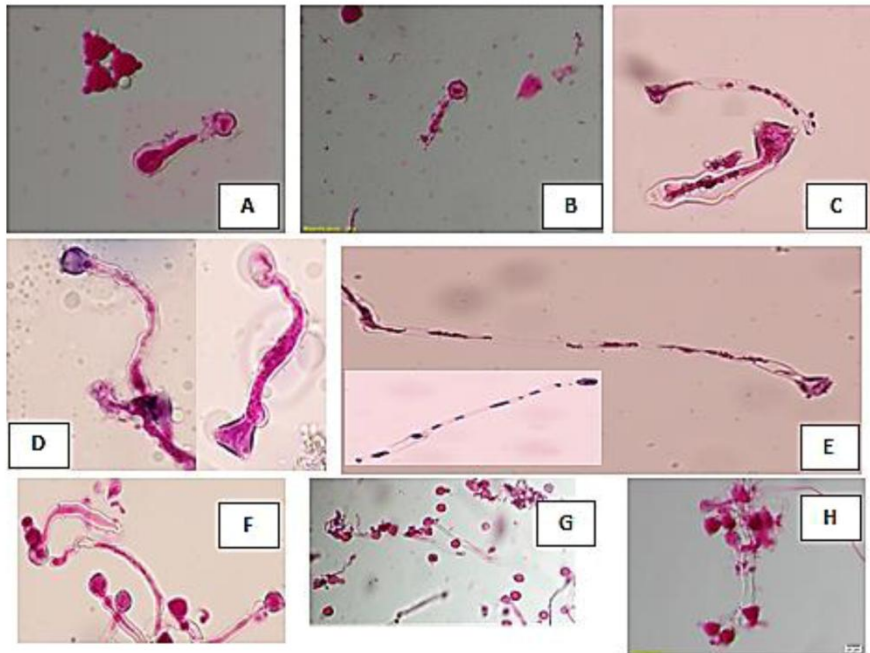


Plate 11: Variability in pollen head length, width and tail length after pollen germination appemidi genotypes and *Mangifera* species (A= *Mangifera camptosperma*, B= *M. griffithii*, C= *M. odorata*, D= Amini, E= Gaddemara, F= Naagara Appe, G= Kutumba Appe and H= Ananthabhatta Appe)

found to be on par. The minimum pollen head length was recorded in Hittalahalli Appe (8.82 μm).

4.1.4.3.3 Pollen head width (μm)

The maximum pollen head width was observed in cultivar *M. odorata* (26.61 μm) followed by Karigal Appe (24.85 μm) and Karpooora Appe (24.51 μm). The minimum width was observed in Hamlet (9.62 μm) followed by Naagara Appe (9.97 μm). The average pollen head width was 19.67 μm (Table 14).

4.1.4.3.4 Tail length (μm)

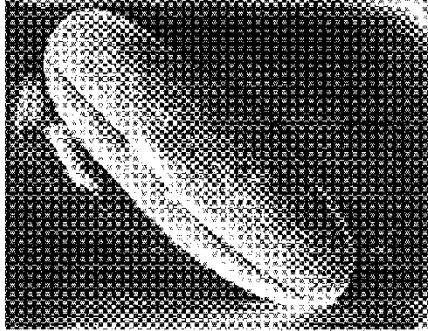
The tail length of the pollen showed significant differences among the twenty genotypes studied (table14). The average pollen tail length was 98.49 μm and it ranged from 51.67 to 169.92 μm . The maximum length of the tail was observed in cultivar Gaddemara (169.92 μm) and the minimum was observed in *M. camptosperma* (51.67 μm).

4.1.5 Genetic variability studies for morphological characters of surveyed Appemidi genotypes

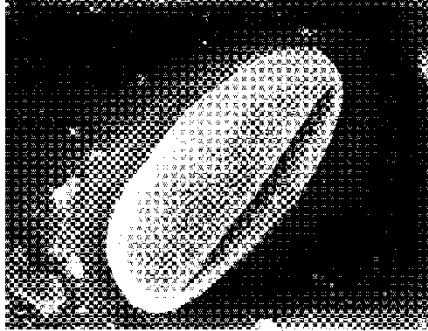
The genetic parameters, viz., phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (broad sense) and genetic advance as per cent mean were estimated for all the characters in this study and the results were presented in table 15. Further, the variability studied in different characters was presented character wise under the following heads.

4.1.5.1 Leaf length

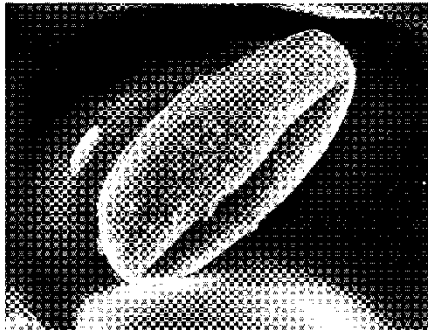
The genetic estimates showed moderate PCV (16.89%), GCV (14.04%), high genetic advance as per cent mean (24.04%) and high heritability (69.10%) were observed for leaf length (Table 15).



ARENUR0327 2018/01/18 13.31 NL MD11.3 x4.0k 20 µm
ICAR-IIHR Virology Lab



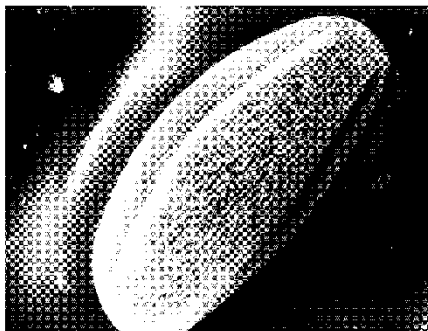
CM0321 2018/01/18 13.06 NL MD11.3 x4.0k 20 µm
ICAR-IIHR Virology Lab



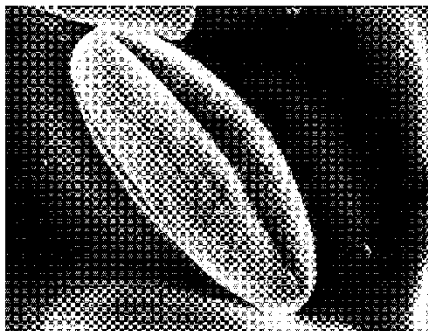
GNBD0317 2018/01/18 12.40 NL MD11.6 x4.0k 20 µm
ICAR-IIHR Virology Lab



KMRU0323 2018/01/18 13.18 NL MD11.6 x4.0k 20 µm
ICAR-IIHR Virology Lab



MAGODU0315 2018/01/18 12.28 NL MD11.8 x4.0k 20 µm
ICAR-IIHR Virology Lab



KUDGE0318 2018/01/18 12.48 NL MD11.5 x4.0k 20 µm
ICAR-IIHR Virology Lab

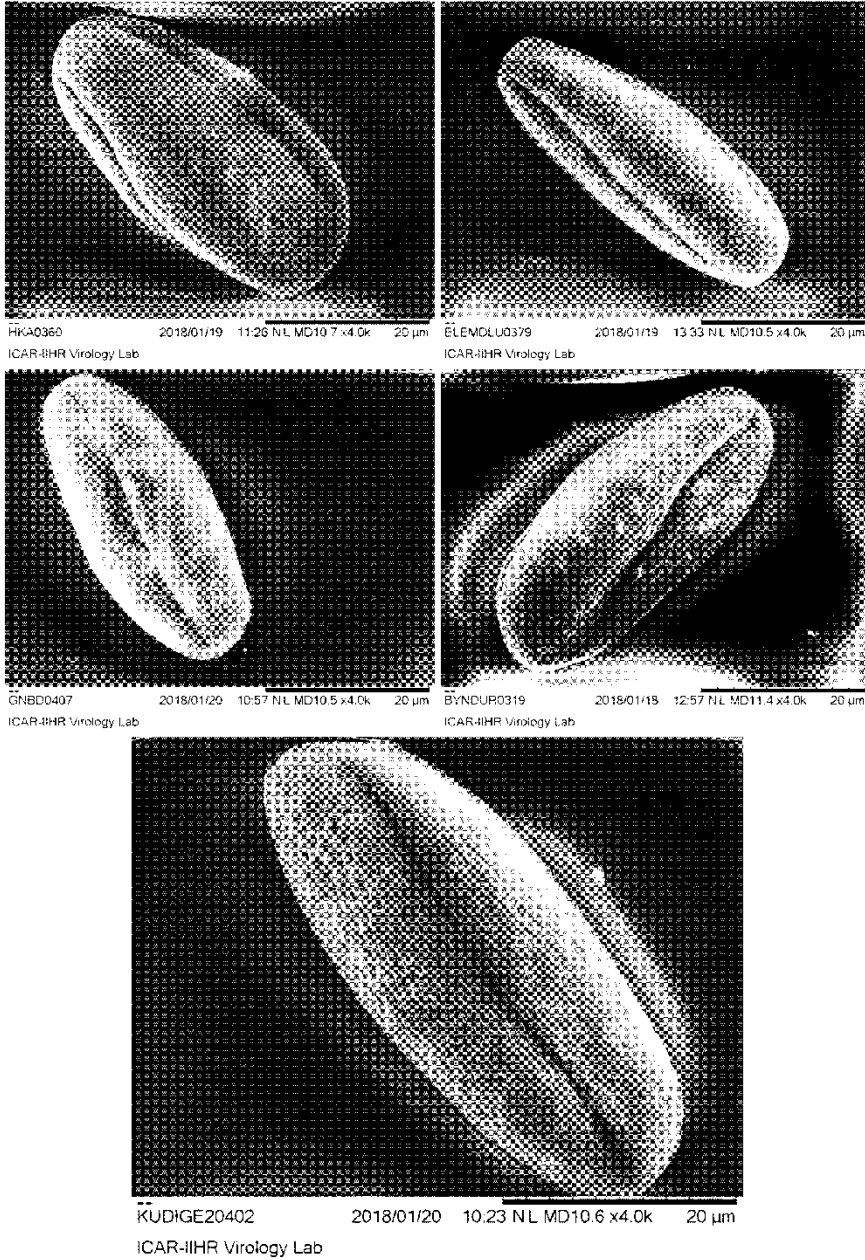


Plate 12: Scanning electron microscopic images of surveyed Appemidi pollen

4.1.5.2 Leaf width

Phenotypic coefficient of variation (24.22%), genotypic coefficient of variation (20.80%), genetic advance as per cent mean (36.80%), and heritability (73.76%) were found to be high among the genotypes studied for leaf width and indicated the presence of additive gene action in the inheritance of this trait (Table 15).

4.1.5.3 Petiole length

Significant differences were observed for the petiole length with respect to genetic parameters viz., PCV (32.11%), GCV (27.89%), per cent means of genetic advance (49.995) and heritability (75.43%) proved the presence of additive gene action which will be highly rewarding for improvement of this character (Table 15).

4.1.5.4 Inflorescence length

The phenotypic coefficient of variability (22.67), genotypic coefficient of variability (21.63), genetic advance in per cent means (42.50) and the heritability (91.00) were found to be very high for inflorescence length which showed the presence of additive gene action in inheritance of this trait where as simple selection would be more efficient on the basis of phenotypic performances of cultivars.

4.1.5.5 Inflorescence width

The estimates of phenotypic coefficient of variation (24.14%), genotypic coefficient of variation (21.61%), genetic advance as per cent mean (39.83%) and heritability (80.10%) were observed for inflorescence width which indicated the presence of additive gene action in the inheritance of this trait (Table 15).

4.1.5.6 Flower diameter

Flower diameter (Table 15) recorded moderate estimates for phenotypic and genotypic coefficient of variation (16.50 and 10.60%) respectively and high estimates for heritability (41.31%) and moderate genetic advance (14.04%) as per cent mean were observed for this trait which indicated the presence of both additive and non-additive gene action.

4.1.5.7 Peduncle length and width

Peduncle length and width showed moderate phenotypic (12.29% and 12.36%), low genotypic coefficient of variation (9.75 and 6.77%) respectively and high estimates for heritability (62.92% and 29.98%) and moderate and low genetic advance (15.93 and 7.63%) as per cent means indicated the presence of both additive and non-additive gene action (Table 15).

4.1.5.8 Fruit length

The moderate estimates of phenotypic coefficient of variation (14.62%), genotypic coefficient of variation (11.55%), genetic advance as per cent mean (18.80%) and heritability (62.43%) were observed for fruit length showed the presence of both additive and non-additive gene action (Table 15).

4.1.5.9 Fruit diameter

The variability parameters studied for this trait (Table 15) viz., moderate phenotypic coefficient of variation (12.04%), low genotypic coefficient of variation (9.91%), medium genetic advance as per cent

mean (16.80%) and high heritability (67.74%) were found for fruit diameter indicating presence of additive and non-additive gene effect.

4.1.5.10 Fruit thickness

Fruit thickness (Table 15) recorded moderate estimates for phenotypic and genotypic coefficient of variation (13.66 and 11.38%) respectively and high estimates for heritability (69.54%) and medium genetic advance as per cent mean (19.54%) were observed for this trait which indicated the presence of both additive and non-additive gene action.

4.1.5.11 Fruit weight

The moderate estimates of phenotypic coefficient of variation (18.27%), genotypic coefficient of variation (14.55%), genetic advance as per cent mean (23.87%)

Table 15: Estimates of variability and genetic parameters for morphological characters of surveyed Appemidi genotypes

Characters	Mean	Range	Phenotypic coefficient of variation	Genotypic co-efficient of variation	Heritability (%)	Genetic advance	Genetic advance as % means
Leaf Length	24.36	17.70-38.50	16.89	14.04	69.10	5.86	24.04
Leaf width	5.59	3.60-8.00	24.22	20.80	73.76	2.06	36.80
Petiole Length	3.06	1.50-6.10	32.11	27.89	75.43	1.53	49.99
Inflorescence Length	16.57	11.60-25.25	22.67	21.63	91.00	7.04	42.50
Inflorescence Width	11.37	7.00-16.84	24.14	21.61	80.10	4.53	39.83
Inflorescence diameter	0.51	0.40-0.90	16.50	10.60	41.31	0.07	14.04
Peduncle Length	5.77	3.20-6.80	12.29	9.75	62.92	0.92	15.93
Peduncle width	0.61	0.50-0.80	12.36	6.77	29.98	0.05	7.63
Fruit Length	4.58	3.00-6.45	14.62	11.55	62.43	0.86	18.80
Fruit diameter	3.02	2.10-4.30	12.04	9.91	67.74	0.51	16.80
Fruit thickness	2.36	1.40-3.20	13.66	11.38	69.44	0.46	19.54
Fruit weight	16.34	10.50-26.00	18.27	14.55	63.44	3.90	23.87

Note: 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%

and high heritability (63.44%) were observed for fruit weight indicated the presence of additive gene action in the inheritance of this trait (Table 15).

4.1.5.12 Genotypic (r_g) and phenotypic correlation (r_p) coefficients for morphological characters

Leaf length was significantly and positively associated with leaf width, petiole length, and peduncle length both at genotypic and phenotypic levels (table 16). At genotypic level a strong positive and highly significant association of leaf length, petiole length, inflorescence length with leaf width were recorded. Petiole length showed highest positive correlation with inflorescence width at genotypic level. Inflorescence length was significantly positively correlated with inflorescence width, flower diameter and peduncle length whereas fruit thickness was significantly associated with peduncle length, fruit length and fruit width. Significant negative correlations were observed for the traits, fruit weight with leaf length, petiole length, inflorescence length and width.

At phenotypic level strong positive and significant association of petiole length with leaf length, leaf width, inflorescence length and fruit length was observed (table 16). Fruit thickness was having strong positive and significant correlation with peduncle length, fruit length and fruit width was recorded. Significant negative correlations were observed for leaf width with inflorescence length and inflorescence width. On the other hand fruit length had a significant positive correlation with inflorescence diameter and pedicle length but negative association with petiole length. Fruit diameter showed more nonsignificant association with the characters studied. Fruit weight was significantly negatively correlated with leaf length.

4.1.6 Genetic divergence study

The genetic divergence among 40 genotypes of Appemidi was quantitatively assessed by adopting Mahalanobi's D^2 statistics and principal component analysis, while the divergence at molecular level was also studied by using SSR markers.

Table 16: Genotypic (rg) and phenotypic (rp) correlation coefficients among the morphological characters of surveyed appemidi genotypes

Characters		Leaf length	Leaf width	Petiole length	Inflorescence length	Inflorescence width	Flower diameter	Peduncle length	Fruit length	Fruit width	Fruit diameter	Fruit thickness	Fruit weight
Leaf Length	rg rp	1 1											
Leaf width	rg rp	0.700** 0.581**	1 1										
Petiole length	rg rp	0.634** 0.577**	0.406** 0.416**	1 1									
Inflorescence Length	rg rp	-0.139NS -0.125NS	-0.299** -0.246**	0.226* 0.185*	1 1								
Inflorescence width	rg rp	-0.014 0.058NS	-0.180* -0.152NS	0.256** 0.157NS	0.968** 0.837**	1 1							
Flower diameter	rg rp	-0.141 -0.230*	-0.351** -0.193*	-0.058NS -0.119NS	0.732** 0.526**	0.479** 0.499**	1 1						
Peduncle length	rg rp	0.237** 0.112NS	0.112NS 0.056NS	0.079NS 0.065NS	0.249** 0.168NS	0.463** 0.309**	-0.228* -0.166NS	1 1					
Fruit length	rg rp	-0.015NS -0.077NS	-0.118NS -0.040NS	-0.523** -0.225*	0.073NS 0.085NS	0.199* 0.109NS	0.511** 0.451**	0.359** 0.262**	1 1				
Fruit width	rg rp	-0.094NS -0.101NS	-0.007NS 0.008NS	-0.014NS -0.009NS	-0.325** -0.270**	-0.352** -0.281**	-0.053NS -0.050NS	-0.205* -0.109NS	-0.089NS -0.021NS	1 1			
Fruit diameter	rg rp	0.020NS -0.036NS	-0.171NS -0.147NS	0.157NS 0.143NS	0.103NS 0.059NS	0.086NS 0.084NS	0.076NS 0.067NS	0.140NS 0.087NS	0.155NS 0.126NS	-0.167NS 0.058NS	1 1		
Fruit thickness	rg rp	0.015NS 0.017NS	0.049NS 0.099NS	0.052NS 0.075NS	-0.120NS -0.101NS	-0.115NS -0.124NS	0.050NS 0.024NS	0.394** 0.205*	0.238** 0.175NS	0.289** 0.352**	0.031NS 0.068NS	1 1	
Fruit weight	rg rp	-0.269** -0.242**	-0.122NS -0.135NS	-0.243** -0.178NS	-0.197* -0.111NS	-0.191* -0.127NS	-0.153NS -0.011NS	-0.014NS 0.048NS	-0.036NS 0.022NS	0.329** 0.148NS	0.067NS -0.068NS	0.152NS 0.050NS	1 1

*, ** Significant at 0.05 and 0.01 levels respectively.

4.1.6.1 Relative contribution of different characters towards divergence

A. Morphological characters of forty surveyed genotypes towards diversity

The relative ranking of various character components to divergence D^2 was furnished in Table 17a and Fig 12. The result on character wise contribution towards the total genetic divergence showed that no single trait had a greater contribution to the total divergence.

Among the characters studied, fruit diameter had a maximum contribution (13.0%) to the total diversity by ranking first in 101 times out of 778 combinations, followed by fruit weight and Inflorescence diameter (11.20% with 87 times ranked first). The characters *viz.*, fruit length and petiole length contributed 10.80% and 10.70% to the diversity. The leaf length, inflorescence width, leaf area, inflorescence length, peduncle length, peduncle width and fruit thickness contributed 7.8, 0.5, 6.0, 3.1, 5.8, 3.0, and 8.5 per cent to the total divergence.

B. Palynological characters of surveyed pollen samples contribution towards diversity

Among the pollen characters studied colpi per cent showed the maximum per cent contribution of 21.88% towards total divergence by appearing 86 times first rank out of 393 different combinations followed by equatorial diameter which contributed 19.85% and colpi length which contributed 16.79% towards total diversity (Table 17b & Fig 13).

4.1.6.2 Grouping of cultivars into different clusters (D^2 analysis)

A. Cluster analysis depending upon the morphological characters of surveyed genotypes

The entire forty genotypes were grouped mainly into two clusters. These two clusters were further divided into six sub clusters (table 18a & Fig 14). The cluster I consisted of two sub clusters which included cultivars Kamanmori-1, Kamanmori-2, Soppinakadu-1, Kudige and Arenuru-1 in Cluster IA. The cultivars Gonibeedu-4, Chimankudige-2, Elemadlu-2, Soppinakadu-3, Banakal and Kanathi-3 grouped under sub cluster IB.

Table 17a: Contribution of each morphological character to divergence in surveyed appemidi genotypes

Character	No of times appearing I in ranking	Per cent contribution
Leaf blade length	61	7.8
Leaf blade width	4	0.5
Leaf Area	47	6.0
Petiole Length	83	10.7
Inflorescence Length	24	3.1
Inflorescence width	45	5.8
Flower diameter	87	11.2
Peduncle length	23	3.0
Peduncle width	66	8.5
Fruit Length	84	10.8
Fruit Diameter	101	13.0
Fruit thickness	66	8.5
Fruit weight	87	11.2

Table 17b: Contribution of each of pollen character to divergence in surveyed appemidi genotypes

Character	No of times appearing I in ranking	Per cent contribution
Pollen Length	59	15.01
Equatorial diameter	78	19.85
Colpi Length	66	16.79
Colpi percent	86	21.88
P/E ratio	54	13.74
Mean size	50	12.72

The cluster II was divided into two sub clusters, in which sub cluster IIA consists Agrahara, Gonibeedu-1, Gonibeedu-5, Marebilu-1, Elemadlu-2, Bynduru, Kumrumane-1, Kumrumane-2 and Shantipura. The sub-cluster II B1 consists Gonibeedu-3, Horatti Jeerige-2, Magodu, Soppinakadu-1, Chimankudige-1, Gonibeedu-3, Marigundi, Chimankudige-3, Heruru-1, Heruru-2, Soppinakadu-2 and Heggudlu whereas sub- cluster II B2 consists of Chimankudige-4, Arenuru-2, Elemadlu-1, Marebilu-3, Doobla and Horatti Jeerige-1.

B. Cluster analysis depending upon the palynological attributes of surveyed genotypes

The surveyed 28 pollen samples were also subjected to diversity study, which were grouped into two different main clusters (Table 18b & Fig 15). The cluster I consists of genotypes Arenuru-2, Kudige, Doobla, Soppinakadu-2, Marebylu-1, Marebylu-4, Marebylu-3, Kumrumane-2 and Magodu. The cluster II divided into two main sub clusters in which sub cluster II A consists of Chimanludige-1, Chimankudige-3, Gonibeedu-3, Gonibeedu-4, Gonibeedu-1, Soppinakadu-1, Heruru-2, Kamanmori-1, Kamanmori-2, Heruru-1 and Soppinakadu-3. The sub cluster IIB consists of cultivars Arenuru-1, Bynduru, Gonibeedu-2, Marebylu-2, Chimankudige-3, Kumrumane-1, Elemadlu-1 and Elemadlu-2.

4.1.6.3 Mean intra and inter cluster distances

A. Morphological characteristics

The mean inter and intra-cluster distances among six clusters were presented in Table 19a. The inter cluster D^2 values ranged from 18.428 to 147.997 indicating the presence of substantial amount of genetic diversity in the genetic material. The highest inter- cluster D^2 value was recorded between clusters 2 and 4 (149.997) while the lowest inter- cluster D^2 value was recorded between clusters 5 and 1 (18.428). This suggested wide genetic diversity between the cultivars of these clusters to obtain suitable hybrids and desirable segregants. The highest inter cluster distance indicated the presence of wide genetic diversity among the genotypes present.

Table 18a: Distribution of 40 Appemidi genotypes in 6 different clusters based on the morphological characterization

Cluster	No. of genotypes	Name of genotype
I	2	Agrahara and Gonibeedu-1
II	7	Gonibeedu-5, Marebylu-1, Marebylu-2, Kumrumane-1, Kumrumane-2, Byndur, Shantipura
III	12	Gonibeedu-2, Maagodu, Horatti Jeerige-2, Marebylu-4, Soppinakadu-2, Chimankudige-1, Gonibeedu-3, Marigundi, Chimankudige-3, Heruru-1, Heruru-2 and Heggudlu
IV	6	Chimankudige-4, Elemadlu-1, Marebylu-3, Doobla, Arenuru-1, Horatti Jeerige-1
V	8	Gonibeedu-4, Chimankudige-2, Elemadlu-2 Soppinakadu-3, Banakal, Kanathi 1, 2, 3
VI	5	Kamanmori-1, Kamanmori-2, Soppinakadu-1, Kudige-1, Arenuru-2

Table 18b: Distribution of Appemidi genotypes in 6 different clusters based on the pollen morphometry

Cluster	No. of genotypes	Name of genotype
I	4	Arenuru-1, Bynduru, Gonibeedu-2, Marebylu-2
II	4	Chimankudige-2, Kumrumane-1, Elemadlu-1, Elemadlu-2
III	7	Chimankudige-1, chimankudige-3, Gonibeedu-3, Gonibeedu-4, Gonibeedu-1, Soppinakadu-1, Heruru-2
IV	4	Kumrumane-1, Kumrumane-2, Heruru-1, Soppinakadu-3
V	2	Arenuru-2 and Kudige
VI	5	Doobla, Soppinakadu-2, Marebylu-1, Maarebylu-4, Maagodu, Kumrumane-2, Marebylu-3

Table 19a: Mean intra and inter cluster distances based on morphological traits

Cluster	1	2	3	4	5	6
1		51.142	57.816	96.947	18.428	37.275
2	51.142		108.855	147.997	33.002	88.141
3	57.816	108.855		39.316	76.010	21.479
4	96.947	147.997	39.316		115.075	59.987
5	18.428	33.002	76.010	115.075		55.176
6	37.275	88.141	21.479	59.987	55.176	

Table 19b: Mean intra and inter cluster distances based on pollen characters

Cluster	1	2	3
1		14.287	9.130
2	14.287		23.090
3	9.130	23.090	

B. Palynological characteristics

The mean intra and inter-cluster distances with respect to pollen characters showed that, the D^2 value ranged between 14.287 to 23.090. The maximum D^2 value was recorded between cluster 2 and 3 (23.09) whereas the minimum D^2 value was observed between the cluster 3 and 1 (9.13). This suggested wide genetic diversity between the cultivars of these clusters (Table 19b).

4.1.6.4 Cluster means of 40 genotypes

A comparison of cluster means for the different characters was presented in table 20. Cluster I comprising of (2) genotypes which had a high value for peduncle width (0.73 cm), fruit length (4.74 cm) and fruit thickness (2.44 cm). The second highest values were observed in inflorescence length and fruit diameter. The cluster-2 consists of 7 genotypes with extreme mean values for the characters *viz.*, inflorescence length, inflorescence width and highest values for flower diameter. The cluster which shared maximum number of genotypes (12) had only second extreme value for fruit length and fruit weight. Cluster-IV had highest mean value for the trait fruit weight. Whereas cluster V consisting of 8 genotypes were contributing more for leaf length, leaf width and leaf area. Whereas cluster VI consisted of only 5 genotypes which had a second extreme value for leaf length, leaf width, leaf area, inflorescence width and highest mean value for petiole length.

4.1.6.5 Principal Component Analysis

A. PCA for morphological characteristics

The principal component analysis for all morphological characters was performed by adopting correlation matrix method. The character loading values for principal components represented the weights defining the contribution of different traits for respective principal components. Further, the loading signs (+/-) were indicative of the direction of contribution, similar to that of regression coefficients.

The principal components, eigen values, per cent variability, cumulative per cent of variability and component loading of different morphological characters are furnished

Table 20: Cluster means of 40 surveyed Appemidi genotypes

Character /Cluster mean	Leaf length (cm)	Leaf width (cm)	Leaf Area (cm²)	Petiole Length (cm)	Inflorescence Length (cm)	Inflorescence width (cm)	Flower diameter (cm)	Peduncle length (cm)	Peduncle width (cm)	Fruit Length (cm)	Fruit Diameter (cm)	Fruit thickness (cm)	Fruit weight (g)
Cluster 1	21.42	4.40	93.82	2.17	20.81	12.61	0.77	4.83	0.73	4.74	3.04	2.44	15.51
Cluster 2	22.01	4.51	99.32	2.84	22.31	15.39	0.54	6.19	0.60	4.00	2.84	2.18	15.96
Cluster 3	22.44	5.15	115.27	2.86	13.75	8.76	0.48	5.29	0.57	4.67	3.00	2.34	16.74
Cluster 4	22.25	5.41	121.59	2.13	14.75	10.65	0.50	6.15	0.68	4.66	3.06	2.38	17.52
Cluster 5	29.61	6.99	207.10	3.40	14.09	10.52	0.48	6.05	0.63	4.42	2.93	2.40	15.65
Cluster 6	25.35	6.26	160.29	4.11	19.24	13.55	0.52	5.84	0.60	4.59	3.23	2.26	14.95

Table 21: Per cent variance contributions by principal components for the dimension and morphological characteristics

Factors	Eigen value	Variability (%)	Cumulative (%)
F1	3.426	26.352	26.352
F2	2.644	20.338	46.691
F3	1.580	12.152	58.843
F4	1.283	9.867	68.710
F5	1.181	9.088	77.798
F6	1.033	7.943	85.741
F7	0.741	5.699	91.439
F8	0.482	3.709	95.148
F9	0.336	2.582	97.730
F10	0.170	1.304	99.034
F11	0.086	0.665	99.699
F12	0.036	0.280	99.979
F13	0.003	0.021	100.000

in the Table 21. The principal components with eigen values less than one were considered as non-significant as per the procedure. In the present investigation, the first six principal components with eigen values more than one contributed to 85.741 per cent of cumulative variability among 40 genotypes of Appemidi for 13 morphological characters. The first principal component (F1) contributed highest variability (26.352%). The character loading values for principal components represented the weights defining the contribution of different characters for the respective principal components (Table 22). Characters like leaf length (0.817), leaf width (0.852), leaf area (0.916), petiole length (0.566), peduncle length (0.081), fruit length (0.165) and fruit thickness (0.221) showed positive loadings and were coupled with negative loadings for inflorescence length (0.492), inflorescence width (0.386), inflorescence diameter (0.532), peduncle width (0.272) fruit diameter (0.091) and fruit weight (0.183).

The second principal component (F2) had positive loadings for the characters includes, leaf length (0.394), leaf width (0.154), leaf area (0.284), Petiole length (0.495), inflorescence length (0.788), inflorescence width (0.837), inflorescence diameter (0.469), peduncle length (0.430), peduncle width (0.231) and fruit diameter (0.197). The negative loadings were observed for the characters fruit length (0.340), fruit thickness (0.066) and fruit weight (0.451), the second principal component contributed 20.338% variability (table 22).

The third principal component F3 contributed 12.152 per cent to the total variability with positive loadings for the traits like leaf width (0.047), leaf area (0.038), inflorescence diameter (0.122), peduncle length (0.532), peduncle width (0.687), fruit length (0.290), fruit diameter (0.259), fruit thickness (0.744) and fruit weight (0.191) whereas the negative loadings were recorded for the traits like leaf length (0.017), petiole length (0.191), inflorescence length (0.151) and inflorescence width (0.071) (table 22).

The fourth principal component contributed 9.867 per cent to the total variability; whereas fifth and sixth principal component contributed 9.088 per cent and 7.943 per cent towards total variability coupled with positive and negative loadings for the different morphological traits studied is represented in the table 22.

Table 22: PCA loadings and contribution of each PCA components

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13
Leaf length (Cm)	0.817	0.394	-0.017	-0.066	0.115	0.032	0.070	0.080	-0.332	-0.185	0.050	0.020	-0.019
Leaf width (Cm)	0.852	0.154	0.047	-0.116	0.151	-0.176	0.193	-0.096	0.360	0.057	-0.024	0.012	-0.025
Leaf Area	0.916	0.284	0.038	-0.112	0.143	-0.106	0.155	-0.032	0.061	-0.065	0.005	-0.010	0.041
Petiole Length (Cm)	0.566	0.495	-0.191	0.506	-0.154	0.075	-0.024	-0.069	-0.176	0.271	-0.046	-0.013	0.000
Inflorescence Length (cm)	-0.492	0.788	-0.151	0.193	-0.075	-0.201	-0.018	-0.050	0.069	-0.052	-0.011	0.143	0.004
Inflorescence width (cm)	-0.386	0.837	-0.071	0.055	-0.190	-0.236	0.034	0.071	0.094	-0.009	0.167	-0.093	-0.003
Flower diameter (cm)	-0.532	0.469	0.122	0.207	0.601	-0.106	0.109	-0.146	-0.047	-0.077	-0.147	-0.066	-0.003
Peduncle length (cm)	0.081	0.430	0.532	-0.309	-0.588	-0.111	-0.100	0.197	-0.005	-0.025	-0.150	-0.021	-0.001
Peduncle width (cm)	-0.272	0.231	0.687	-0.362	0.403	0.072	0.184	0.132	-0.095	0.188	0.069	0.039	0.002
Fruit Length (cm)	0.165	-0.340	0.290	0.689	0.164	-0.255	-0.019	0.447	0.072	-0.034	0.006	0.008	0.001
Fruit Diameter (cm)	-0.091	0.197	0.259	0.329	-0.205	0.767	0.358	-0.010	0.116	-0.079	0.009	0.000	0.000
Fruit thickness (cm)	0.221	-0.066	0.744	0.287	-0.041	-0.048	-0.404	-0.370	0.009	-0.047	0.064	0.003	-0.001
Fruit weight (g)	-0.183	-0.451	0.191	0.145	-0.346	-0.451	0.570	-0.199	-0.134	-0.003	0.008	0.004	0.000

B. PCA for palynological characteristics

The principal component analysis was also carried out for the pollen morphological characteristics of 28 surveyed genotypes to study the contribution of pollen traits towards diversity. The eigen values, per cent variability, cumulative per cent of variability and component loading of different morphological characters studied are furnished in table 23.

In the present study, the first three principal components with eigen values more than one contributed to 99.89 per cent of cumulative variability among 28 Appemidi genotypes for six pollen morphological characters. The first principal component (F1) contributed highest variability (47.912 %). The character loadings values for principal components represented the weights defining the contribution of different characters for the respective principal components (Table 24). The principal component (F1) had positive loadings for the characters equatorial diameter (0.859) and mean size of the pollen grain (0.356) coupled with negative loadings for the traits pollen length (0.419), colpi length (0.841), colpi per cent (0.632) and P/E ratio (0.356).

The second principal component of pollen studies showed 34.291% variability along with the positive loadings for the characters pollen length (0.906), equatorial width (0.263), colpi length (0.172), P/E ratio (0.369) and mean size of the pollen grain (0.883) and having only one negative loading for the trait colpi per cent (0.472) (Table 24).

The third principal component of the pollen studies contributed 17.697% variability. The positive loadings were observed for the characters equatorial diameter (0.436), colpi length (0.512), colpi per cent (0.614) and mean size of the pollen grain (0.306) and the negative loadings were recorded for traits pollen length (0.053) and P/E ratio (0.368) (Table 24).

4.1.6.6 Cluster analysis

Based on the Ward's minimum variance method, the entire genotypes were grouped into two main clusters and six sub clusters, the main cluster I composed of two sub clusters which shared 13 genotypes showed maximum peduncle length and fruit

Table 23: Per cent variance contributions by principal components for the dimension and characteristics of pollen

Factors	Eigen value	Variability (%)	Cumulative (%)
F1	3.162	52.696	52.696
F2	2.188	36.469	89.165
F3	0.628	10.459	99.625
F4	0.020	0.326	99.950
F5	0.002	0.039	99.989
F6	0.001	0.011	100.000

Table 24: PCA loadings and contribution of PCA component

	F1	F2	F3	F4	F5	F6
Pollen Length	0.875	0.354	0.324	-0.063	-0.021	-0.009
E. diameter	0.981	-0.158	-0.094	0.054	-0.021	0.015
Colpi length	0.373	0.916	-0.135	-0.055	0.021	0.012
Col Per.	-0.200	0.885	-0.418	0.047	-0.016	-0.009
P/E	-0.514	0.645	0.563	0.060	-0.001	0.005
Mean size of pollen grain (P X E)(mm)	0.995	0.012	0.066	0.063	0.028	-0.010

length. Similarly the main cluster II having Sub cluster A consisted of 9 cultivars and sub cluster B of 18 cultivars which are having highest leaf length, leaf width, leaf area, petiole length, inflorescence length, inflorescence width, fruit thickness and fruit weight.

Based on the pollen morphological characters, the 28 genotypes were grouped into three different clusters. Cluster I consisted 9 genotypes which were having maximum pollen length, colpi Length, colpi per cent and P/E ratio. However, mean size of the pollen grain was found highest in cluster II consisting of 11 genotypes. Similarly, equatorial diameter contributed maximum in cluster III composed of 8 genotypes.

4.2 To study the diversity within the population through molecular characterization

Molecular diversity among 40 genotypes of Appemidi was studied by using SSR markers. The DNA was isolated from young healthy leaves using the modified CTAB method, yielded good amount of DNA. The DNA quantity in ng/ μ l, per gram of fresh leaf tissue and ratio of DNA to protein were estimated. The purity was determined by A260:A280 ratio which varied from 1.61 to 1.80 indicating that the DNA was relatively of high purity and was suitable for PCR amplification.

4.2.1 SSR analysis

SSR markers were used to estimate the extent of molecular diversity among 40 Appemidi genotypes. Fourteen selected primers were used to analyse inter and intra cultivar diversity among 40 Appemidi genotypes. All the primers were initially evaluated for their annealing temperature using a gradient PCR technique. The annealing temperatures and PCR conditions for these SSRs were first standardized by using a PCR with temperature gradient technique, which indicated that annealing temperatures of 42 to 56^oC (Table 4).

4.2.2 Gene scan analysis

The fourteen selected SSR primers amplified alleles across the 40 genotypes showed varied degrees of polymorphism. High level of polymorphism was observed with

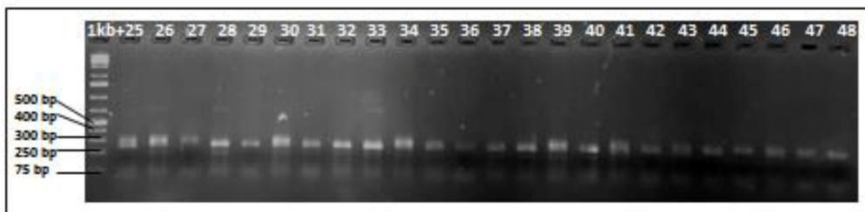
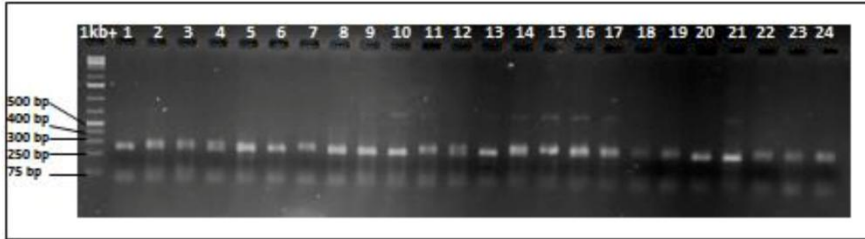


Plate 13: PCR amplified products showing polymorphic bands using SSR primer MiIHR 17

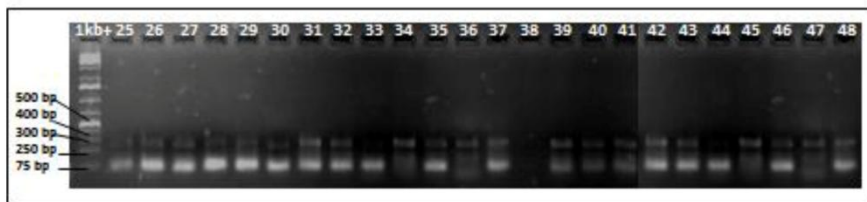
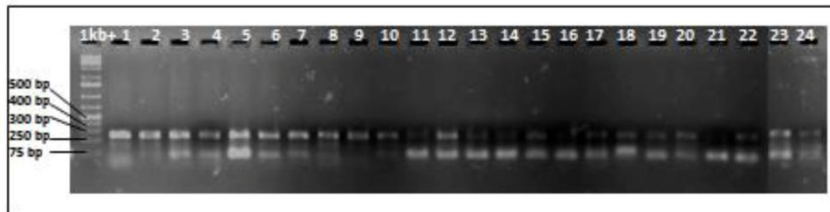


Plate 14: PCR amplified products showing polymorphic bands using SSR primer MiIHR 36

MillHR 18 primer. The PCR product size obtained by amplification of SSR primers ranged from 50 to 800 bp and these primers were labelled with four different dyes namely, FAM, PET, NED and VIC at their 5' ends and used as probes.

4.2.3 Polymorphic information content (PIC) of SSR marker analysis

The SSR primer pairs used in this study showed relatively higher values of heterozygosity and PIC. The expected heterozygosity (H_e) values ranged from 0.976 to 0.985, PIC content ranged from 0.964 to 0.973 with a mean of 0.969 and the majority of the genotypes showed high PIC values (0.700) showing that the markers selected were highly informative. Observed heterozygosity ranged from 0.091 to 0.273. The probability identity (PI) for all the microsatellite loci was $9.104E-0023$. The maximum PI value obtained in the loci MillHR 34 (0.0023) with 41 numbers of alleles per locus and the minimum was identified in MillHR 18 (0.0014) with 43 numbers of alleles per locus (Table 25).

4.2.4 Diversity analysis

Based on the Ward's minimum variance method for genetic dissimilarity, the genotypes were grouped into three major clusters (Fig 19) Cluster I consisted of Arenuru-1, Kamanmori-2, Maagodu, Gonibeedu-5, Gonibeedu-4, Chimankudige-4, Chimankudige-2, Kanathi-3, Banakal, Soppinakadu-3 (Sub-cluster IA) and Kanathi-1, Chimankudige-4, Marigundi, kanathi-2, Chimankudige-3 and Soppinakadu-2 (Sub-cluster IB). Cluster II consists of genotypes Marebylu-3, Mareblu-2, Kumrumane-2, Gonibeeu-3, Heggudlu, Marebylu-4, Heruru-1, Agrahara, Kudige, Chimankudige-1, Heruru-2, Arenuru-2, Elemadlu-1, Kumrumane-1, Elemadlu-2, Gonibeedu-1 (Sub Cluster IIA) and Kamanmori-1, Gonibeedu-2 and Soppinakadu-1 (Sub Cluster II B). Similarly, Cluster III composed of Shantipura, Doobla, Horatti Jeerige-2, Bynduru, Marebylu-1 and Horatti Jeerige-1.

4.2.5 Species identification using chloroplast and ITS markers

Molecular characterization by using ITS Primer amplified product with restriction enzyme of ten different Appemidi genotypes collected from survey was done.

Table 25: List of SSR primers and their characteristics based on genetic analysis of 40 surveyed Appemidi genotypes

Locus	Primers (5'-3') details	Allele Size (bp)	K (No. of alleles per locus)	N	H _o	H _e	PIC	PI
MiIIIHR 23	F:TCTGACCCAACAAAGAACCA R: TCCTCCTCGTCCTCATCATC	108-153	41	40	0.273	0.983	0.971	0.0015
MiIIIHR 26	F:GCGAAAGAGGAGAGTGCAAG R: TCTATAAGTGCCCCCTCACG	131-165	38	40	0.273	0.981	0.969	0.0018
MiIIIHR 18	F: TCTGACGTCACCTCCTTTCA R: AACTCGTGCCTCGTCCTGT	154-193	43	40	0.273	0.985	0.973	0.0014
MiIIIHR 30	F: AGCTATCGCCACAGCAAATC R: GTCTTCTTCTGGCTGCCAAC	190-213	41	40	0.273	0.983	0.971	0.0016
MiIIIHR 31	F: TTCTGTTAGTGGCGGTGTTG R: CACCTCCTCCTCCTCCTCTT	207-260	39	40	0.091	0.980	0.968	0.0019
MiIIIHR 17	F: GCTTGCTTCCAAGTGGAGACC R:GCAAAATGCTCGGAGAAGAC	230-269	37	40	0.091	0.980	0.968	0.0018
MiIIIHR 34	F: CTGAGTTTGGCAAGGGAGAG R: TTGATCCTTCACCACCATCA	224-244	41	40	0.273	0.976	0.964	0.0023
MiIIIHR 36	F: TCTATAAGTGCCCCCTCACG R: ACTGCCACCGTGGAAAGTAG	210-249	39	40	0.182	0.980	0.968	0.0019

N= total number of samples, H_o= observed heterozygosity, H_e= expected heterozygosity, PIC= polymorphic information content, PI= probability identity

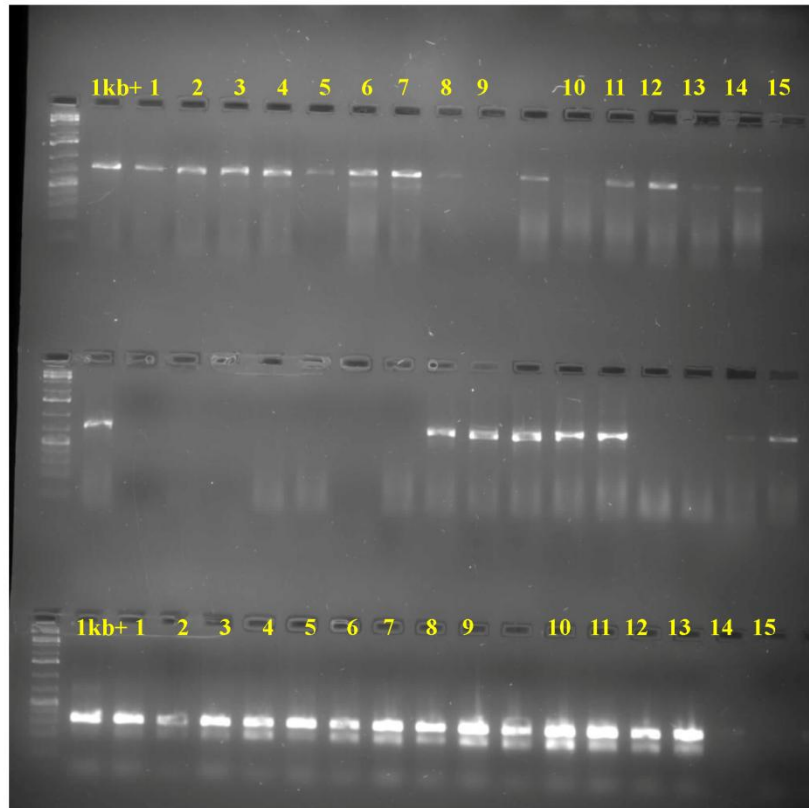


Plate 15: PCR amplified products showing similar polymorphic banding pattern among the genotypes surveyed using Psb and tRnH primer

Restriction digestion results that ITS region in these genotypes have restriction site for Taq 1 and also restricted product did not show any polymorphism between them. This confirms there was no variation in the sequence of ITS region. Further, the cloning and sequencing of amplicons generated by ITS region in ten genotypes were approximately 450 bp using the ITS4 and ITS5 primer combination. For the partial chloroplast psbA-trnH rDNA analysis was also done and the product sequences of these Appemidi genotypes were cross checked by searching in National Centre for Biotechnological Information (NCBI), USA and the results showed that all those ten genotypes were similar to *Mangifera indica*. There was no variation with respect to other mangifera species.

4.3 To study the chemical profile of fruit sap and leaf

Appemidi is also known as aromatic pickle mango and it is well known for its unique flavor. Therefore in this study an attempt was made to profile the leaf and fruit sap volatile fragments through GC/MS and the components were tabulated. The fruit sap and leaf volatiles were compared to see the correlation existing between them. The principal component 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.

4.3.1 Leaf volatile aroma composition

The leaf aroma components of 34 appemidi genotypes were determined independently with HS-SPME-GC-MS technique. Altogether 80+ volatile components were detected (Table 26). The monoterpenoids and sesuiterpenoids contributed to a major portion among the volatile compounds (Table 27 and 28). The results revealed that, Appemidi leaves were rich in volatile components and the genotype Ananthabhattacha Appe showed the highest area of volatiles with 55+ volatile components. In contrast, Gidaganamavu had the least volatile components sharing less proportion of aroma compounds. Shidadakke Appe was having 64 volatile components in less proportion.

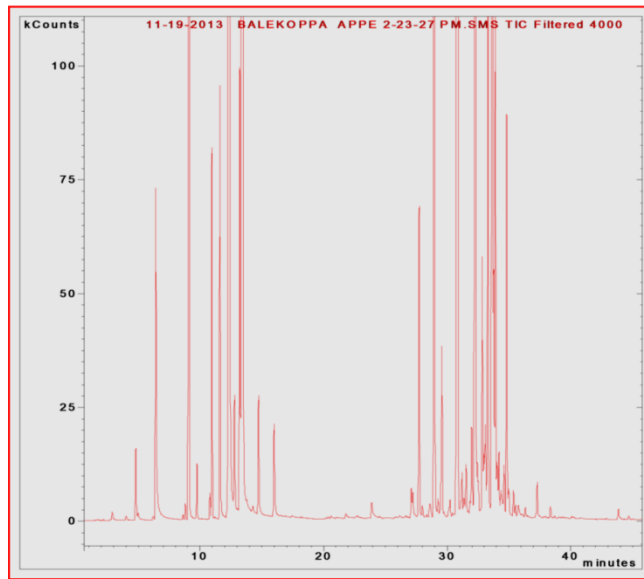
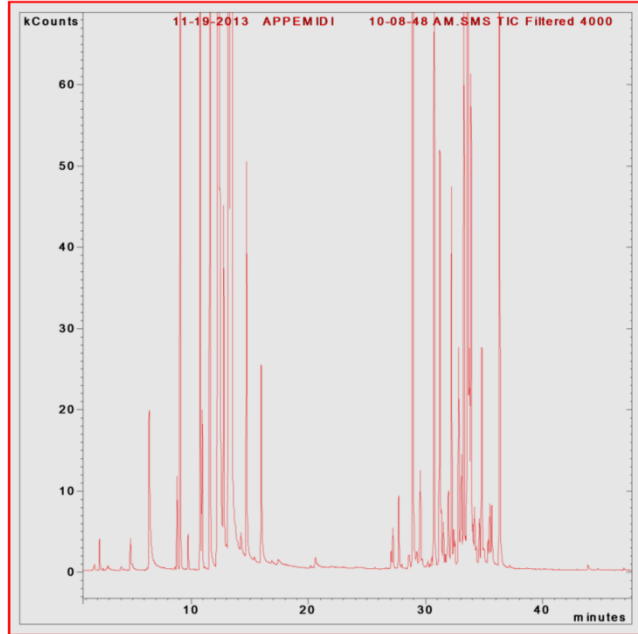


Plate 16: Leaf volatile profile of Appemidi genotypes

RT	Hydrocarbons	KI Value	Dannalli Appe	Chauthi	Sadamidi	Gidaganamavu	Balekoppa Appe	Kalakai	Kovesara	Malange	Shidadakke Appe	Kalwaguda
6.22	E-2-Hexenal	857	1.17	0.02	0.00	0.08	0.00	0.01	1.01	0.14	0.64	0.05
16.354	p-Isopropenyl toluene	1080	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.04	0.00
17.43	2-Methylenebornane	1021	0.00	0.00	0.07	0.00	0.01	0.02	0.00	0.02	0.00	0.00
16.871	Cinerone	1238	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.03
20.562	1,3,4-Trimethyl-3-cyclohexen-1-carboxaldehyde	1204	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00
	Alcohol											
2.762	1-Pentyn-3-ol	678	0.01	0.00	0.00	0.01	0.00	0.02	0.00	0.02	0.02	0.00
4.207	cis-2-Penten-1-ol	767	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
4.785	2-Hexenol	854	0.18	0.09	0.00	0.57	0.22	0.07	0.07	0.04	0.03	0.09
21.788	Sabinol	1137	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
23.89	4-Ethyl, 4-Dimethyl-2-cyclohexen-1-ol	1147	0.01	0.01	0.01	0.00	0.08	0.02	0.00	0.00	0.00	0.00
20.595	4-terpineol	1179	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00
37.305	Veridiflorol	1567	0.03	0.04	0.03	0.00	0.00	0.00	0.00	0.00	0.43	0.03
37.16	Spathulenol	1576	0.00	0.02	0.04	0.10	0.12	0.02	0.33	0.02	0.03	0.03
37.918	11-Hexadecyn-1-ol/ Santalol	1668	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	others											
25.181	Santolina epoxide	991	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01
17.354	1,3,8-p-Menthatriene	1111	0.00	0.01	0.40	0.00	0.00	0.03	0.18	0.34	0.22	0.03
21.098	4,8-Epoxy-para-menth-1-ene	1183	0.00	0.01	0.09	0.01	0.01	0.01	0.01	0.01	0.01	0.54
	8-Hydroxy-p-cymene	1158	0.00	0.00	0.16	0.02	0.00	0.00	0.00	0.00	0.00	0.00
37.3	Caryophyllene oxide	1567	0.09	0.01	0.06	0.01	0.01	0.03	0.00	0.03	0.02	0.05

Table 27: Major monoterpenoids components found in leaves (per cent) of 34 Appemidi genotypes

Monoterpenoids (Area %)	α -Pinene	β -Phellandrene	α -Phellandrene	Limonene	cis-Ocimene	α -Terpinolene
DannalliAppe	1.27	2.11	17.34	8.78	0.11	0.17
Chauthi	4.92	2.26	1.19	27.92	0.35	1.01
Sadamidi	0.94	0.90	1.66	11.50	7.43	7.41
Gidaganamavu	11.35	9.22	14.78	16.31	2.23	3.56
BalekoppaAppe	8.21	1.30	3.33	16.31	0.08	0.32
Kalakai	1.42	1.45	39.95	21.42	0.00	0.26
Kovesara	0.43	0.15	0.32	0.93	12.54	0.26
MalangeAppe	3.38	0.52	14.33	5.25	26.70	3.49
ShhidadakkeAppe	3.46	0.67	0.32	0.44	0.49	0.07
Gaddalahalli Appe	1.94	1.84	42.38	9.31	0.00	0.20
Murgeer	1.62	1.78	5.51	27.28	0.55	0.86
Nandgar	5.61	1.30	36.73	19.64	0.72	0.61
Kalwaguda	2.76	2.04	2.88	2.46	0.28	0.23
GurumurthyAppe	0.03	0.83	14.05	7.06	0.03	0.38
KaanaAppe	1.38	0.45	3.95	2.07	0.62	0.34
HaldotaAppe	1.95	1.94	6.34	10.08	0.11	0.09
Kadikai	7.50	14.00	1.02	1.30	5.29	0.23
Kalkuni	14.63	1.20	34.62	16.96	0.12	0.17
GoranaAppe	0.65	0.58	20.26	9.29	29.96	0.63
Isgoorappe	6.48	4.73	0.77	16.63	0.06	2.15
Huliappe kai	21.02	1.23	0.67	2.32	1.42	0.05
Jeerige	8.94	24.38	0.38	13.64	0.09	0.03
KutumbaAppe	4.63	7.79	0.00	1.47	0.15	1.26
Adderijeerige	14.48	21.29	0.80	2.65	4.35	0.35
Gomavu	6.53	1.16	11.25	11.61	0.12	1.28
Karigal Ape	3.43	1.08	2.00	29.15	0.02	0.03
DodderiJeerige	5.78	1.55	9.13	20.59	0.11	0.48
Gaddemara	5.37	1.06	8.49	0.86	0.08	0.86
Kangarumatha	4.56	0.92	36.80	2.95	0.20	2.95
Appemidi	2.13	2.18	5.59	7.15	0.33	0.06
Hittalahalli Appe	1.25	0.18	32.62	29.50	0.08	2.33
ManibhattaAppe	0.16	2.73	15.81	17.74	0.10	1.29
Thumbbeelu	1.30	22.76	0.15	12.31	0.19	0.35
Ananthabhatta Appe	1.39	10.41	12.82	6.73	0.19	0.10
Mean	4.73	4.35	11.71	11.46	2.80	1.00

Table 28: Major Sesquiterpenoids components (per cent) found in leaves of 34 Appemidi genotypes

Sesquiterpenoids	δ -Elemene	α -cubebene	α -copaene	β -Elemene	Germaacrene D	Isolongifolene	Caryophyllene	γ -Gurjunene	α -Humulene	α -Selinene	γ -Selinene	γ -Cadinene
DannalliAppe	0.196	0.119	0.171	0.455	1.410	1.249	24.946	0.417	11.106	11.743	7.518	0.420
Chauthi	1.180	0.350	0.663	1.007	16.811	6.332	13.963	0.120	6.953	0.456	0.000	2.494
Sadamidi	0.000	0.091	0.111	0.100	0.000	1.441	20.207	0.026	8.970	0.916	0.103	0.259
Gidaganamavu	0.016	0.103	0.402	0.065	0.432	0.055	16.060	0.040	6.726	0.139	0.029	0.099
Balekoppaappe	0.050	0.065	0.054	0.634	2.425	0.523	26.890	0.616	13.454	0.130	1.265	0.151
Kalakai	0.197	0.023	0.043	2.423	1.543	3.632	6.845	2.142	2.261	1.381	1.200	0.071
Kovesara	0.794	0.069	0.177	1.992	3.951	1.371	7.978	0.148	2.822	2.967	0.108	0.110
MalangiAppe	0.003	0.076	3.123	0.783	0.094	0.814	17.521	0.049	7.755	1.057	0.153	0.000
Shhidadakke Appe	0.087	0.971	0.145	0.926	0.894	5.969	4.933	0.067	23.058	23.579	0.076	0.000
Gaddalahalli appe	0.242	0.254	1.837	0.684	9.644	0.597	15.584	0.057	4.238	0.125	0.000	0.000
Murgeer	0.006	0.255	12.181	0.202	1.656	0.999	14.596	0.013	6.780	0.495	0.000	0.000
Nandgar	0	0.250	1.447	0.201	0.828	3.236	7.625	0.009	2.725	0.262	0.141	0.000
Kalwaguda	0.444	0.068	0.250	0.207	0.201	0.199	0.224	0.186	23.510	0.113	0.423	0.000
Gurumurthy Appe	0.072	0.046	0.909	0.440	0.326	3.812	3.015	0.029	13.566	0.837	2.764	1.024
KaanaAppe	2.669	0.571	0.270	2.385	2.814	5.307	8.212	0.255	38.472	2.934	0.000	1.287
HaldotaAppe	0.000	0.088	5.282	0.024	0.395	0.623	34.756	0.000	18.213	0.326	11.524	0.057
Kadikai	0.567	0.081	0.160	0.146	3.352	0.748	25.836	0.101	12.843	1.228	0.000	0.031
Kalkuni	0.075	0.040	0.276	0.053	1.194	0.585	7.708	0.012	4.894	0.302	0.000	0.075
GoranaAppe	0.061	0.061	0.320	0.546	1.082	0.004	18.804	0.042	2.552	0.767	0.000	0.008
Isgoorappe	1.881	0.767	0.135	0.615	0.065	0.050	23.735	0.015	12.339	1.638	0.000	0.000
Huliappe Kai	0	0.017	0.020	0.033	0.000	0.027	9.564	0.058	6.876	8.327	0.000	0.000
Jeerige	0.039	0.583	0.057	0.249	0.085	0.013	16.717	0.011	8.443	6.417	0.000	0.017
KutumbaAppe	0	0.082	0.004	0.696	0.095	0.016	16.483	0.000	7.462	1.330	0.000	0.895
Adderijeerige	0	0.032	0.000	0.567	0.000	0.046	10.814	3.893	4.940	20.079	0.000	0.000
Karigal Ape	0.005	0.014	0.147	0.000	0.307	0.379	5.470	0.000	8.663	0.588	0.000	0.000
DodderiJeerige	0.072	0.035	0.199	0.599	2.298	0.465	13.181	0.000	4.196	8.110	5.005	1.297
Gaddemara	0.086	0.116	4.358	1.069	0.346	0.000	21.080	0.028	10.841	14.725	1.457	0.036
Kangarumatha	0.112	0.095	1.221	0.639	0.422	3.217	6.740	0.005	2.886	20.147	0.000	0.160
Appemidi	0.091	0.210	6.168	0.204	4.876	0.229	2.294	0.019	0.320	5.114	0.000	0.223
Hittalahalli Appe	0.111	0.043	0.649	0.172	0.722	0.000	5.693	0.000	2.178	12.057	0.000	0.007
ManibhattaAppe	0.204	0.480	2.764	1.794	12.901	0.000	2.754	0.075	5.599	22.128	0.000	0.804
Thumbbeelu	0.682	0.000	1.342	0.000	0.000	0.042	6.517	0.053	0.020	9.238	2.906	0.003
Anantha Bhatta Appe	0.06	0.04	0.09	7	0.36	0.53	10.73	0.12	0.04	24.05	1.79	0.06
Mean	0.30	0.18	1.36	0.82	2.17	1.29	12.95	0.26	8.66	6.17	1.10	0.29

4.3.1.1 Genotypes variability in leaf volatile composition

Significant differences in the composition and proportion of leaf volatile components were observed among the 34 appemidi genotypes studied (Table 29). The genotype Kalkuni had the highest per cent monoterpenoids proportion (71.41%) followed by Nandgar (71.18%) and Kalakai (69.81%). The least monoterpenoids proportion was observed in genotype Kaana Appe (10.98%) followed by Kalwaguda (14.06%). Among the monoterpenoids, β -phellandrene, α -pinene, limonene and Cis-ocimene contributed highest proportion to leaf volatiles of Appemidi genotypes (Fig 7). Sesquiterpenoids are the major group of compounds showed significant differences in composition of total sesquiterpenoids among 34 genotypes as shown in table 18. The highest per cent of sesqui terpenoids composition was observed in genotype Gaddemara (90.39%) followed by Kalwaguda (78.73%). The least content was noticed in genotype Kalkuni (22.82%) followed by Appemidi (23.35%). Among sesqui terpenoids caryophyllene and α -humulene contributed maximum to the leaf volatiles in Appemidi genotypes.

4.3.1.2 Principal component (PC) analysis for leaf volatiles

The correlation matrix for monoterpenoids showed the variables that mostly define six principal components (Table 30): F1 was strongly correlated with β -phellandrene (0.73), F2 with cis-ocimene (0.79), F3 with α -terpinolene (0.70), F4 with α -pinene (0.63), F5 with β -phellandrene (0.54) and F6 with limonene (0.38) (Table 28). The projections of the PC loadings defined by first two principal components shows 50% of variability and allowed us to visualize the position of different mango genotypes as shown in plot (Fig 21). The components one and two (α -pinene and β -phellandrene) were contributing more towards total variability having highest eigen value (1.70 and 28.34% and 1.350 and 22.50% respectively) (Table 31). The results of PCA analysis and dendrogram interpreted that, the leaf α -pinene, β -phellandrene, limonene and cis-ocimene were important monoterpene compounds which clearly distinguish the variability among 34 appemidi genotypes.

In case of sesquiterpenoids the correlation matrix showed the variables that mostly define 12 principal components (Table 32). The projections of the PC loadings

Table 29: Relative contents of mono and sesqui terpene classes in different Appemidi genotypes

Genotype	Total volatiles	Total monoterpenoids (%)	Total sesquiterpenoids (%)
DannalliAppe	36543500	30.63	62.82
Chauthi	19428025	39.85	53.498
Sadamidi	22873283	40.19	45.518
Gidaganamavu	7648359	63.36	24.560
BalekoppaAppe	30877497	32.63	60.731
Kalakai	26702231	68.93	24.797
Kovesara	34813997	15.63	77.709
MalangiAppe	45545122	61.37	32.952
ShhidadakkeAppe	30125834	30.69	62.591
Gaddalahalliappe	22951974	58.86	35.562
Murgeer	28052274	40.06	54.580
Nandgar	59831536	71.18	23.490
Kalwaguda	25001040	14.06	78.732
GurumurthyAppe	50659286	59.47	34.500
KaanaAppe	22697993	10.98	76.899
HaldotaAppe	22230235	22.40	71.901
Kadikai	17006218	38.55	54.041
Kalkuni	25371054	71.41	22.822
GoranaAppe	39572382	67.46	26.841
Isgoorappe	23174834	35.40	59.196
Huliappe kai	20889422	52.56	39.996
Jeerige	49798587	53.26	39.381
KutumbaAppe	15480487	18.15	63.895
Adderijeerige	31458388	48.61	45.249
Gomavu	17548318	35.97	55.547
Karigal Ape	27021458	57.69	36.042
DodderiJeerige	18637803	41.01	47.273
Gaddemara	24261300	18.64	90.339
Kangarumatha	24807664	52.31	40.256
Appemidi	21546881	50.20	23.335
Hittalahalli Appe	24347111	67.84	25.000
ManibhattaAppe	21208387	39.44	54.704
Thumbebeelu	35274550	37.72	43.101
Ananthabhatta Appe	80500075	41.23	50.96
Mean	29526091.32	43.76	48.20

defined by first four principal components showed around 70% of variability and allowed us to visualize the position of different mango genotypes as shown in plot (Fig 23). The factor 1 is contributing more towards total variability having a highest eigen value of 2.835 and 23.62 per cent variability. The factors 2, 3, 4 and 5 also having more eigen value and contributing positively towards total variability (Table 33). The results of PCA analysis and the cluster analysis revealed that, caryophyllene, germacrene-D, α -selinene, α -humulene and α -copaene were important sesquiterpene compounds which clearly distinguish the 34 appemidi genotypes (Table 30).

4.3.1.3 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 34 appemidi genotypes, and the result revealed that, Adderi jeerige, Kadikai, Kutumba Appe, Thumbe beelu, Jeerige, Huliappekai and Shidadakke Appe were clustered together with diverse proportion of β -phellandrene, α -pinene and limonene as major composition. Genotype Gorana Appe, Malange, Kovesara and Sadamidi were clustered separately, as members of this group were rich in cis-ocimene. Cluster 1 grouped with 21 genotypes which were analogous in their volatile composition and proportions, containing mainly limonene and β -phellandrene. Similarly, cluster 2 consists of genotypes rich in cis-ocimene, α -pinene and β -phellandrene and cluster 3 with limonene and β -phellandrene rich genotypes (Fig 20).

The cluster analysis was conducted for the sesqui terpenoids composition of 34 genotypes separately and was grouped into three different clusters based on the diverse proportion of sesquiterpene molecules in them. The cluster 1 consists of 21 genotypes which are caryophyllene rich in nature, the cluster 2 consists of α -selinene rich genotypes viz., Hittlahalli Appe, Adderi Jeerige, Kangaramatha, Thumbebeelu, Manibhatta Appe, Shidadakke Appe and Appemidi. The cluster 3 grouped with α -humulene rich genotypes viz., Kaana Appe, Kalwaguda, Gurumurthy Appe and Karigal Appe (Fig 22).

Table 30: Principal component analysis loadings and contributions of the principal components.

	F1	F2	F3	F4	F5	F6
α -Pinene	0.568	-0.144	0.443	0.631	-0.147	0.201
β Phellandrene	0.736	0.005	0.249	-0.204	0.540	-0.250
α Phellandrene	-0.629	-0.347	-0.060	0.519	0.335	-0.313
Limonene	-0.413	-0.617	0.423	-0.289	0.204	0.381
cis-Ocimene	-0.262	0.791	-0.008	0.178	0.382	0.359
α -Terpinolene	-0.428	0.440	0.701	-0.106	-0.189	-0.290

Table 31: Percentage variance contributions by principal components for the monoterpenoids composition among the leaf of 34 Appemidi genotypes

	Eigenvalue	Variability (%)	Cumulative %
F1	1.683	28.046	28.046
F2	1.341	22.348	50.394
F3	0.932	15.537	65.931
F4	0.836	13.926	79.858
F5	0.649	10.817	90.675
F6	0.560	9.325	100.000

Table 32: Percentage variance contributions by principal components for the sesquiterpenoids composition among the leaf of 34 Appemidi genotypes

	Eigen value	Variability (%)	Cumulative %
F1	2.835	23.626	23.626
F2	1.818	15.153	38.779
F3	1.401	11.675	50.455
F4	1.303	10.858	61.312
F5	1.117	9.305	70.618
F6	0.930	7.753	78.371
F7	0.774	6.448	84.818
F8	0.637	5.307	90.126
F9	0.469	3.910	94.036
F10	0.306	2.552	96.588
F11	0.236	1.966	98.554
F12	0.174	1.446	100.000

Table 33: Per cent contribution of principal components to the variability

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
δ -Elemene	19.559	3.614	2.847	0.424	2.708	2.801	4.797	2.743	30.810	20.226	0.859	8.614
α -cubebene	15.187	0.567	2.062	9.678	18.815	0.686	2.871	14.414	2.061	4.186	0.009	29.464
α -copaene	1.268	2.788	16.830	0.810	34.225	1.593	10.198	28.500	0.273	0.103	3.315	0.095
β -Elemene	2.335	8.630	5.080	17.494	1.068	31.544	0.211	21.423	3.446	0.007	4.661	4.101
Germacrene D	10.557	1.385	29.217	2.577	0.074	0.243	11.515	8.654	0.188	4.206	27.390	3.995
Isolongifolene	20.554	0.186	0.002	0.027	0.014	15.101	3.949	3.727	37.120	8.169	0.032	11.120
Caryophyllene	1.702	23.542	1.624	12.959	0.011	6.615	15.272	8.230	14.202	0.024	9.063	6.755
γ -Gurjunene	0.421	9.811	10.902	2.283	3.758	27.388	41.512	0.495	0.015	0.040	0.018	3.357
α -Humulene	10.057	15.380	15.718	0.632	0.726	2.115	0.539	2.478	0.842	48.013	3.102	0.397
α -Seliene	0.158	23.506	5.636	2.623	28.903	0.033	1.057	8.637	4.182	1.402	5.485	18.380
γ -Selinene	1.345	10.428	0.274	35.174	6.581	11.015	5.806	0.658	5.472	9.037	11.275	2.936
γ -Cadinene	16.857	0.164	9.807	15.320	3.119	0.866	2.273	0.041	1.388	4.586	34.791	10.786

4.3.2 Fruit sap volatile profiling

The fruit sap aroma of 26 appemidi genotypes was determined independently with HS-SPME-GC-MS technique. The volatile profile was separated from the water fraction of the sap (Table 34) indicated that there is a clear distinction between the genotypes in volatile profiles.

The sap constituted mainly terpenoids compounds in all the genotypes. The highest per cent of volatile composition was observed in genotype Ananthabhattera Appe.

4.3.2.1 Genotypes variability in fruit sap volatile composition

Significant differences in the composition and proportion of sap volatile components were observed among the 26 appemidi genotypes studied (Table 35). Unlike leaves, sap of the fruit contains highest proportion of monoterpenoids. α -phellandrene, β -phellandrene, α -pinene β -pinene 3-carene, limonene, β -terpinene and trans-ocimene are the major monoterpenoid compounds identified in fruit sap of Appemidi genotypes (Fig 25&26). The maximum total monoterpenoids content was observed in Ananthabhattera Appe (14.743 mg/ml).

4.3.2.2 Per cent similarity between major monoterpenoids of fruit sap and leaf

Fruit sap per cent monoterpenoids composition was compared with leaf monoterpenoids to study the relationship between fruit sap and leaf presented in table 36.

This study also observed the relationship of per cent volatile composition between sap and leaf mainly monoterpenoids (Table 36). For example in cultivar Balekoppa Appe, the per cent contribution of β -pinene (8.21 and 36.40%), α -phellandrene (3.33 and 48.97%) was found maximum in leaf as well as in fruit sap respectively. Similar trend has been followed in the genotype Kalakai for α -phellandrene (3.33 and 48.97%) in leaf and fruit sap respectively. Both the sap and leaf volatiles followed the similar per cent contributions from major monoterpenes.

Table 34: Per cent fruit sap volatile composition of 26 Appemidi genotypes from ICAR-IIHR germplasm

Compounds/ Genotypes	Ananthabbhatta Appe	Sudur Appe	Shidadakke Appe	Karpoora Jeerige	Holekoppada Appe	Kashimidi	Gurumurthy Appe	Isgoor Appe	Murgeer	Huliappekai	Kalakai	Gaddalhalli Appe
Compounds	%	%	%	%	%	%	%	%	%	%	%	%
α -Thujene	0.93	2.08	0	0	ND	0.00	1.08	0.26	0.00	0.11	0.00	0.00
α -Pinene	2.80	6.12	0.53	10.00	0.79	0.00	0	7.29	3.68	0.81	3.41	1.83
Camphene	ND	0.41	ND	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.00
1R- α -Pinene	ND	ND	ND	0.50	ND	0.00	53.23	0.00	0.00	0.09	0.22	0.00
Sabinene	ND	4.40	ND	0.00	0.64	0.13	3.06	0.99	0.69	0.00	0.00	0.40
β -Pinene	ND	2.85	1.07	0.25	0.34	0.00	0.00	0.00	0.00	0.08	0.00	0.00
2-Carene	ND	1.23	ND	13.21	0.65	0.00	1.08	0.00	0.00	0.00	0.00	0.00
α -Phellandrene	36.57	1.14	0.19	0.00	3.04	0.00	53.23	45.08	47.95	0.00	59.48	82.76
3-Carene	ND	0.19	ND	0.24	0.00	0.00	2.75	6.77	0.00	0.00	0.00	0.00
α -Terpinene	ND	0.21	ND	0.33	0.00	0.00	0.00	11.96	33.22	0.00	0.00	0.00
β -Phellandrene	38.57	72.18	0.53	59.45	2.12	0.00	11.78	16.36	0.88	0.98	0.91	0.55
Limonene	9.52	0.36	0.19	0.26	53.87	0.00	15.46	0.30	0.00	0.06	22.18	2.79
cis-Ocimene	ND	0.26	ND	0.00	0.00	0.00	0.10	0.25	0.13	84.82	0.00	0.62
trans-Ocimene	ND	ND	8.07	ND	27.69	0.00	0.10	0.06	0.00	0.40	0.06	2.30
β -Terpinene	ND	ND	ND	ND	0.00	0.46	0.00	1.28	0.32	0.07	0.00	0.00
Terpinolene	0.53	0.00	0.62	ND	0.19	ND	ND	0.26	0.65	0.10	0.12	1.33
γ -Terpinene	ND	0.00	ND	ND	0.20	ND	0.06	0.00	0.00	0.00	0.00	0.00
1,3,8-p-menthatriene	ND	0.00	ND	ND	ND	ND	1.58	0.00	0.00	0.00	0.00	0.00
p-Cymene	ND	ND	ND	ND	ND	ND	0.78	0.00	0.00	0.00	0.28	0.00
Allo-Ocimene	1.52	0.28	ND	ND	ND	ND	ND	0.10	0.17	0.08	0.00	0.00
α -Cubebene	0.00	ND	ND	ND	0.05	36.18	0.05	0.00	0.00	0.07	0.00	0.14
Copaene	0.29	0.00	6.62	4.47	0.36	0.00	0.62	0.39	0.00	0.09	0.00	0.00
β -Cubebene	0.00	2.32	0.21	1.57	0.04	0.00	0.39	0.02	0.00	0.00	3.42	0.00
β -Elemene	ND	0.00	ND	ND	0.09	0.00	0.26	0.00	0.35	0.79	0.14	0.27
α -Gurjunene	ND	0.88	ND	0.44	ND	0.26	0.09	0.00	0.00	0.02	0.36	0.00
cis- α -Bergamotene	ND	ND	ND	0.00	ND	ND	ND	0.16	0.06	0.00	0.00	0.04
β -Caryophyllene	ND	0.09	0.53	ND	0.07	0.41	0.09	0.08	0.05	0.06	0.08	0.00

Table 35: Fruit sap volatile composition of 26 Appemidi genotypes from ICAR-IIHR germplasm (per cent)

Compounds/ Genotypes	Balekoppa Appe	Chansi	Adderi Jeerige	Kalkuni	Thumbbeedu	Hittalahalli appe	Kana appe	Malange	Gidugana Mavu	Jeerige	Dannalli appe	Nandgar	Gaddemar
Compounds	%	%	%	%	%	%	%	%	%	%	%	%	%
α -Thujene	0.00	1.95	0.00	32.73	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.077	N.D
α -Pinene	3.64	0.18	3.07	0.01	9.03	1.039	3.590	3.490	0.629	5.284	3.398	3.496	4.450
Camphene	0.00	0.00	0.00	0.00	0.17	N.D	N.D	N.D	N.D	N.D	0.071	N.D	0.068
1R- α -Pinene	0.00	0.00	0.00	0.00	0.49	N.D	N.D	0.246	N.D	0.247	0.098	0.204	0.181
Sabinene	0.00	0.00	0.07	0.00	0.60	N.D	N.D	0.102	N.D	0.277	0.074	0.084	0.414
β -Pinene	0.00	0.22	1.00	0.00	1.12	1.078	1.156	1.180	1.359	1.203	1.090	1.124	2.100
2-Carene	0.00	0.00	0.00	0.00	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
α -Phellandrene	48.98	0.00	0.00	0.40	63.64	N.D	69.709	66.138	0.351	63.770	60.550	62.848	0.665
3-Carene	0.43	0.09	0.00	0.00	N.D	60.253	N.D	N.D	N.D	N.D	N.D	N.D	70.398
α -Terpinene	0.00	0.00	2.51	0.00	0.14	0.349	N.D	N.D	N.D	0.194	0.322	0.297	N.D
β -Phellandrene	0.88	1.81	58.17	0.89	N.D	N.D	N.D	0.349	N.D	N.D	0.959	0.204	0.633
Limonene	11.15	134.10	12.74	0.81	11.10	29.410	11.157	10.976	91.224	10.561	11.549	12.186	1.927
cis-Ocimene	0.00	4.86	0.00	58.50	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
trans-Ocimene	6.68	0.00	10.85	0.00	N.D	N.D	N.D	N.D	N.D	N.D	0.104	0.078	0.108
β -Terpinene	0.00	0.00	0.00	0.00	9.34	N.D	9.442	12.056	N.D	12.092	10.503	10.080	0.890
Terpinolene	0.00	0.10	0.00	0.00	N.D	0.301	N.D	N.D	N.D	N.D	N.D	N.D	0.387
γ -Terpinene	0.00	1.74	0.24	0.00	0.13	3.003	0.087	0.120	0.038	0.185	0.163	0.150	4.728
1,3,8-p-menthatriene	0.00	0.00	0.00	0.00	N.D	N.D	N.D	N.D	N.D	0.033	0.046	0.024	0.201
p-Cymene	0.00	0.00	0.10	0.00	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
Allo-Ocimene	0.00	0.00	0.00	0.00	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
α -Cubebene	16.15	0.00	0.00	0.00	0.14	N.D	N.D	N.D	N.D	0.155	0.007	0.004	0.065
Copaene	0.00	0.08	0.57	0.13	1.30	N.D	N.D	N.D	0.446	1.324	N.D	N.D	1.552
β -Cubebene	0.00	1.08	0.00	0.00	N.D	N.D	N.D	N.D	N.D	N.D	0.024	N.D	1.048
β -Elemene	0.25	0.04	0.40	0.00	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D
α -Gurjunene	0.00	0.14	0.40	1.31	N.D	N.D	N.D	N.D	N.D	N.D	0.009	0.040	0.112
cis- α -Bergamotene	0.12	0.00	0.00	0.00	N.D	N.D	N.D	N.D	N.D	N.D	0.036	0.236	N.D
β -Caryophyllene	0.00	0.02	0.12	0.34	0.28	0.123	0.293	1.249	2.273	0.393	0.260	0.090	0.510

Compounds/ Genotypes	Balekoppa Appe	Chansi	Adderi Jeerige	Kalkuni	Thumbbeedu	Hittalahalli appe	Kana appe	Malange	Gidugana Mavu	Jeerige	Dannalli appe	Nandgar	Gaddemar
β -Gurjunene	0.00	0.00	0.16	0.00	N.D	N.D	N.D	N.D	N.D	N.D	0.023	0.044	0.542
α -Guaiene	0.04	0.09	0.00	0.00	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.491
trans- α -Bergamotene	0.00	0.01	0.11	0.00	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	1.508
(+)-Aromadendrene	0.21	0.03	0.00	0.00	N.D	N.D	N.D	N.D	N.D	N.D	0.053	N.D	N.D
γ -Gurjunene	0.00	0.08	0.00	0.00	N.D	N.D	N.D	N.D	N.D	0.060	0.164	0.010	0.044
α -Humulene	0.05	0.08	1.61	0.18	0.15	0.159	0.157	0.746	1.227	0.233	0.098	0.132	0.315
2,6-di-tert-Butyl-para- benzoquinone	0.00	0.00	0.07	0.00	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	0.037
Allo-Aromadendrene	0.10	0.04	0.17	0.00	N.D	N.D	N.D	N.D	N.D	N.D	0.271	N.D	N.D
γ -Elemene	0.00	0.00	0.12	0.00	N.D	N.D	N.D	N.D	N.D	N.D	1.272	0.019	N.D
Germacrene D	0.00	0.00	0.00	0.00	N.D	N.D	N.D	N.D	N.D	0.054	N.D	0.014	N.D
β -Selinene	0.11	0.52	0.03	0.00	N.D	1.834	0.968	N.D	N.D	N.D	1.904	0.161	0.031
Valencene	0.00	0.09	0.01	0.00	N.D	N.D	N.D	N.D	N.D	N.D	0.104	0.006	N.D
α -Muurolole	0.00	0.00	0.09	0.00	N.D	0.203	N.D	N.D	N.D	0.224	0.179	0.007	N.D
δ -Guaiene	0.04	0.10	0.00	0.00	N.D	N.D	N.D	N.D	N.D	N.D	0.018	0.023	2.528
β -Bisabolene	2.20	2.66	0.05	0.018	N.D	N.D	N.D	N.D	N.D	N.D	0.444	N.D	0.061
γ -cadinene	0.00	1.54	0.08	0.00	N.D	N.D	N.D	N.D	N.D	N.D	1.537	0.092	N.D
δ -Cadinene	0.04	0.00	0.00	0.00	0.13	0.161	N.D	0.112	0.054	0.127	0.054	0.014	0.178
α -Bisabolene	0.00	0.00	0.00	0.00	N.D	N.D	N.D	N.D	N.D	N.D	0.003	0.047	N.D
Cadina-1,4-diene	0.16	0.00	0.00	0.00	N.D	N.D	N.D	0.062	N.D	0.427	0.007	2.093	0.058
Viridiflorol	2.43	0.00	1.46	0.00	N.D	0.106	N.D	N.D	N.D	N.D	2.194	0.044	0.366
δ -Cadinol	1.33	0.00	0.80	0.00	N.D	N.D	N.D	0.102	N.D	0.558	0.011	0.278	N.D
1-Tetradecanol	0.00	0.00	0.00	0.00	N.D	N.D	N.D	N.D	N.D		0.289	0.451	0.033
Total	95.00	151.65	95.00	95.32	97.8	97.800	96.700	96.800	97.600	97.400	97.900	97.500	97.700

Table 36: Volatile composition similarity of major monoterpenoids between sap and leaf (per cent)

Volatile component	Balekoppa Appe		Kalakai		Shhidadakke Appe		Gaddalahalli Appe		Thumbe beedu		Gaddemara		Huliappe kai	
	Leaf	Sap	Leaf	Sap	Leaf	SAP	Leaf	Sap	Leaf	Sap	Leaf	Sap	Leaf	Sap
α -Pinene	8.21	3.64	1.42	3.40	3.46	0.42	21.02	0.81	1.30	9.03	5.37	4.45	5.37	4.45
β -Pinene	1.12	0.00	0.20	0.00	0.34	0.00	0.74	0.08	22.76	63.64	1.07	0.67	1.07	0.67
β -Phellandrene	1.30	0.88	1.45	0.91	0.67	0.52	1.23	0.98	0.01	N.D	8.94	70.40	8.94	70.40
I-Phellandrene	3.33	48.97	39.95	59.48	0.32	0.10	0.67	0.00	0.12	N.D	1.06	0.11	1.06	0.11
3-Carene	0.01	0.42	0.07	0.00	0.00	0.00	0.04	0.00	0.43	9.34	0.07	0.89	0.07	0.89
Limonene	16.31	11.15	21.42	22.17	0.44	0.19	2.32	0.06	12.31	11.10	0.86	1.93	0.86	1.93

Volatile component	Murgeer		Kalkuni		Adderi jeerige		Gidagana Mavu		Ananthabhata Appe		Hittalahalli Appe		Gurumurthy Appe	
	Leaf	Sap	Leaf	Sap	Leaf	Sap	Leaf	Sap	Leaf	Sap	Leaf	Sap	Leaf	Sap
α -Pinene	1.62	3.68	14.63	14.66	14.48	3.07	11.35	0.63	1.39	2.8	1.25	0.78	0.03	0.00
β -Pinene	0.17	0.00	0.08	0.01	1.04	0.90	14.78	0.35	0.36	0.96	0.05	0.30	0.75	0.00
β -Phellandrene	1.78	0.00	1.20	0.00	21.29	58.17	1.55	N.D	10.41	35.57	0.18	0.64	0.83	1.08
I-Phellandrene	5.51	47.95	34.62	0.40	0.80	0.00	0.43	N.D	12.82	38.57	32.62	3.06	14.05	53.23
3-Carene	0.00	0.68	0.07	0.00	0.12	0.00	0.01	N.D	8.49	0.53	0.96	0.19	0.00	0.00
Limonene	27.28	33.21	16.96	0.36	2.65	11.83	16.31	91.22	6.73	10.58	29.50	27.69	7.06	15.45

Volatile component	Manibhatta Appe	Kadikai	Sadamidi	Dodderi Jeerige	Gorana appe
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	Leaf	Sap	Leaf	Sap	Leaf	Sap	Leaf	Sap	Leaf	Sap
α -Pinene	0.16	1.47	7.5	8.18	0.94	0.89	5.78	3.67	0.65	3.61
α -Phellandrene	0.1	N.D	1.02	N.D	1.66	0.61	9.13	64.83	20.26	65.30
β -Phellandrene	15.81	52.35	0.01	0.00	2.8	5.16	ND	ND	0	0
3-Carene	0.096	0.01	0.01	N.D	0	0	1.06	N.D	0.07	N.D
trans-Ocimene	0.11	N.D	5.59	2.44	2.49	1.34	0.28	N.D	1.56	N.D
β -Terpinene	0.01	N.D	0.8	0.09	0.05	0.95	0.01	9.99	4.22	11.84
Limonene	17.74	34.43	1.3	0.26	11.5	1.08	20.59	12.28	9.29	11.32

Volatile component	Malange		Kana Appe		Dannalli Appe		Nandgar	
	Leaf	Sap	Leaf	Sap	Leaf	Sap	Leaf	Sap
α -Pinene	3.38	3.49	1.38	3.59	1.27	3.40	5.61	3.50
α -Phellandrene	14.33	66.14	3.95	69.71	17.34	60.55	36.73	62.85
3-Carene	0.00	N.D	0	N.D	0.01	N.D	0	N.D
trans-Ocimene	4.48	N.D	0.5	N.D	0.11	0.10	3.64	0.08
β -Terpinene	0.00	12.06	0.77	9.44	0	10.50	0.02	10.08
Limonene	5.25	10.98	2.07	11.16	8.78	11.55	19.64	12.19

4.3.2.3 Correlation analysis of leaf and fruit sap volatiles

In this investigation leaf flavour volatiles were compared with fruit sap and obtained highly significant positive correlation with respect to some of the major monoterpenoids (Table 37). Among the monoterpenoids, leaf β -phellandrene had the highest correlation index of 0.89 with fruit sap and *vice versa*. Whereas in case of β -pinene, limonene, ocimene and carene also showed the significant positive correlation of 0.75, 0.51, 0.477 and 0.41 respectively when compared to leaf and fruit sap. Therefore this showed that there was a significant positive correlation existing between appemidi leaf and fruit sap volatiles particularly for major monoterpenoids.

4.3.2.4 Profiling of major fruit sap volatiles from surveyed Appemidi genotypes

Fruit sap collected from survey were emitting different aroma it may be region or cultivar specific in nature therefore surveyed fruit sap were also subjected for profiling by GC/MS to study the variability in surveyed fruit sap volatile composition.

The volatile compounds separated from water fraction of the sap (Table 38) indicated that there was a clear distinction between the mango types in volatile profiles of surveyed Appemidi genotypes. Mainly terpenoids contributed to the aroma of fruit sap in all the genotypes. The total concentration of aroma compounds was maximum in genotype Kudige (4640.46 $\mu\text{g/g}$), followed by Arenuru-2 (2836.93 $\mu\text{g/g}$), Arenuru-1 (2478.82 $\mu\text{g/g}$) and Kumrumane-2 (1922.25 $\mu\text{g/g}$). The minimum amount of aroma components were found in Kamanmori (237.64 $\mu\text{g/g}$) followed by Marigundi (241.02 $\mu\text{g/g}$). The total aroma components indicated that genotype Kudige has a very strong aroma when compared to other genotypes (Table 39).

4.3.2.5 Relative contribution of monoterpenoids and sesquiterpenoids from the fruit sap for the volatile profile of surveyed Appemidi genotypes

There was a significant contribution of monoterpenoids towards the volatile profile of appemidi genotypes (table 40a). The per cent contribution varied from 46.16% to 93.27%. The maximum per cent of monoterpenoids were found in genotype Doobla (93.27%) followed by Kudige (93.14%) and Kumrumane-1 (92.87%). The major

Table 37: Correlation between major monoterpenoids of leaf and fruit sap of appemidi

Variables	Sap- α -Pinene	Sap- β -Pinene	Sap- β -Phellandrene	Sap- α -Phellandrene	Sap-Limonene
Leaf- α -Pinene	0.269*				
Leaf- β -Pinene		0.768**			
Leaf- β -Phellandrene			0.899**		
Leaf- α -Phellandrene				0.477**	
Leaf-Limonene					0.512*

**= highly significant, *= significant

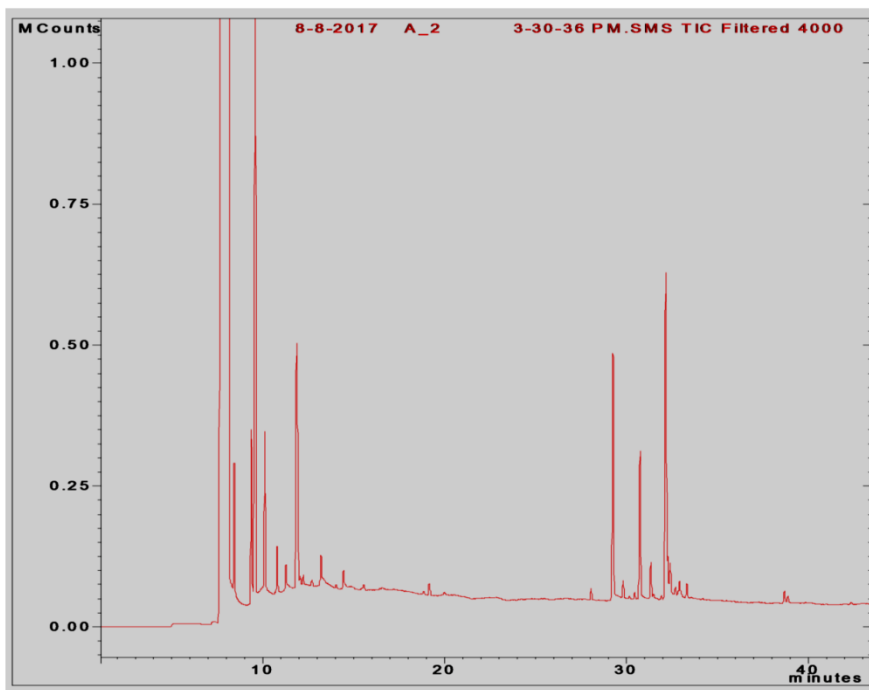


Plate 17: Fruit sap volatile profile of surveyed Appemidi genotype

Table 38: Composition and relative contents of volatiles of fruit sap from 10 surveyed Appemidi genotypes (Per cent)

	KI Value	Marebylu-1	Gonibeedu-1	Arenuru-2	Chimankudige-2	Elemadlu-2	Kamanmori	Doobla	Kumrumane-1	Soppinakadu-2	Kudige-1
Hydrocarbons											
Cyclopentene	582	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00
2-Methyl Furan	618	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
Benzene	654.8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.00	0.00
2-Ethyl furan	705	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.18	0.00	0.00
3-Hexene	740	0.00	0.00	4.87	0.00	0.03	0.00	0.00	0.00	0.43	0.00
Toluene	770.8	0.00	0.00	2.41	0.00	0.01	0.00	0.00	0.00	0.20	0.00
Tricyclene	924	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2-(1-Methyl-2-propenyl) bicyclo[2.2.1]heptane	1027	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3-Isopropyl-2,3-dimethyl -1-cyclopentene	2666	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Monoterpenoids											
α -Thujene	928	1.21	7.09	17.46	479.89	1.38	0.14	0.00	7.25	2.54	0.00
α -Pinene	940	334.86	320.98	1167.72	866.44	345.17	32.48	0.21	1162.83	2.10	521.66
Cyclofenchene	957	0.00	0.00	0.00	0.00	0.97	0.00	58.74	0.00	0.00	4.76
Sabinene	974	0.00	35.79	0.00	11.35	12.35	0.87	0.00	4.83	0.04	2.10
β -Pinene	976	0.51	25.07	10.94	7.67	0.00	29.06	1585.08	0.00	0.08	0.00
β -Phellandrene	1005	10.68	16.33	75.09	91.47	2.39	21.19	22.94	64.49	1.92	3982.92
α -Phellandrene	1007.1	0.00	5.60	4.87	22.05	0.98	0.00	0.87	14.40	17.41	9.57
3-Carene	1010	0.89	1.17	2.41	6.26	0.11	17.29	0.97	0.08	809.20	3.36
α -Terpinene	1019	0.11	0.00	0.93	0.00	6.01	2.22	0.00	4.73	4.73	0.59
Para cymene	None	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Limonene	1033	0.26	0.56	1414.54	52.75	0.19	1.31	0.00	33.76	0.19	22.05
β -Terpinene	1038	0.20	0.00	0.00	1.25	0.08	2.22	9.80	0.73	8.76	0.90
cis-Ocimene	1040	5.23	91.85	0.00	1.87	0.13	8.50	0.14	0.07	15.75	0.91
trans-Ocimene	1051	0.00	0.00	36.25	2.21	0.02	0.00	0.00	1.29	6.06	0.52
γ -Tepinene	1088	0.18	1.44	2.58	2.01	0.26	0.00	0.97	0.54	0.36	0.14
α -Terpinolene	1086	0.03	0.67	0.00	1.37	0.03	0.18	0.15	0.19	11.15	0.32
Allo-Ocimene	1130	0.70	0.05	2.38	3.84	0.02	0.00	0.84	0.58	0.00	0.00
Sesquiterpenoids											

	KI Value	Marebylu-1	Gonibeedu-1	Arenuru-2	Chimankudige-2	Elemadlu-2	Kamanmori	Doobla	Kumrumane-1	Soppinakadu-2	Kudige-1
E-2-Hexenal	857	0.00	0.00	1.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00
p-Isopropenyl toluene	1080	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.62	0.00	0.14
2-Methylenebornane	1021	0.00	0.79	0.00	0.00	0.03	0.00	0.00	6.48	0.00	0.16
Cinrone	1238	0.00	0.16	0.00	0.27	0.01	0.12	0.00	0.00	0.00	0.20
1,3,4-Trimethyl-3-cyclohexen-1-arboxaldehyde	1204	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Alcohol											
1-Pentyn-3-ol	678	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.81	0.00	0.27
cis-2-Penten-1-ol	767	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.31
2-Hexenol	854	0.00	0.00	0.68	0.00	0.00	0.00	0.00	0.44	0.00	0.16
Sabinol	1137	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-Ethyl, 4-Dimethyl-2-cyclohexen-1-ol	1147	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4-terpineol	1179	0.10	0.16	0.74	0.27	0.00	0.00	0.00	0.00	0.00	0.00
Veridiflorol	1567	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Spathulenol	1576	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11-Hexadecyn-1-ol/ Santalol	1668	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Others											
Santolina epoxide	991	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1,3,8-p-Menthatriene	1111	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4,8-Epoxy-para-menth-1-ene	1183	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8-Hydroxy-p-cymene	1158	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Caryophyllene oxide	1567	0.00	0.00	0.00	0.00		0.00				
Total Volatiles (µgm/gm)		369.91	994.83	2836.93	1874.92	381.80	237.64	1711.93	1325.52	1083.62	4640.46

Table 39: Relative contents of mono and sesqui terpene classes in surveyed Appemidi fruit sap from western ghat regions of Chikmagalur

Genotypes	Total volatiles	% monoterpenoids	% sesquiterpenoids
Marebylu-1	369.91	90.66	3.47
Marebylu-2	746.46	46.15	3.84
Marebylu-4	312.16	91.14	2.52
Gonibeedu-1	994.83	48.38	46.52
Gonibeedu-2	339.16	86.18	7.95
Gonibeedu-3	1139.46	91.70	2.48
Arenuru-1	2478.82	87.62	7.27
Arenuru-2	2836.93	91.59	3.06
Chimankudige-1	1046.03	86.67	8.29
Chimankudige-2	1874.92	78.56	16.41
Chimankudige-3	385.12	91.97	3.02
Elemadlu-1	1470.60	88.36	6.56
Elemadlu-2	381.80	46.16	48.79
Kamanmori	237.64	73.03	17.77
Doobla	1711.93	93.27	1.72
Kumrumane-1	1325.52	92.87	1.49
Kumrumane-2	1922.25	69.60	24.09
Soppinakadu-1	301.77	92.09	1.83
Soppinakadu-2	1083.62	91.18	3.82
Kudige	4640.46	93.14	21.47
Marigundi	241.02	89.12	2.89
Maagodu	657.29	77.17	5.87

Table 40a: Major monoterpenoids content in surveyed Appemidi fruit sap from western ghat regions of Chikmagalur

Genotypes/ Micro gram	α-Thujene	α-Pinene	β-Pinene	β-Phellandrene	α-Phellandrene	3-Carene	Limonene	cis-Ocimene
Marebylu-1	1.21	334.86	0.51	10.68	0.00	0.89	0.26	5.23
Marebylu-2	0.18	55.52	0.00	0.00	601.51	0.44	31.17	0.64
Marebylu-4	0.03	13.47	34.65	64.00	7.85	4.49	15.59	0.79
Gonibeedu-1	7.09	320.98	25.07	16.33	5.60	1.17	0.56	91.85
Gonibeedu-2	161.95	14.21	1.12	0.00	8.09	0.08	25.13	1.17
Gonibeedu-3	583.93	5.74	1.37	411.14	0.17	8.89	9.03	8.89
Arenuru-1	0.77	459.66	2.27	15.22	0.93	0.19	35.20	6.20
Arenuru-2	17.46	1167.72	10.94	75.09	4.87	2.41	1414.54	0.00
Chimankudige-1	0.00	863.27	2.14	38.72	9.15	2.88	20.05	0.84
Chimankudige-2	479.89	866.44	7.67	91.47	22.05	6.26	52.75	1.87
Chimankudige-3	0.00	349.93	0.94	11.28	2.24	0.81	5.30	0.15
Elemadlu-1	0.10	266.78	48.71	15.03	1027.33	6.80	0.28	0.00
Elemadlu-2	1.38	345.17	0.00	2.39	0.98	0.11	0.19	0.13
Kamanmori	0.14	32.48	29.06	21.19	0.00	17.29	1.31	8.50
Doobla	0.00	0.21	1585.08	22.94	0.87	0.97	0.00	0.14
Kumrumane-1	7.25	1162.83	0.00	64.49	14.40	0.08	33.76	0.07
Kumrumane-2	3.03	11.30	0.00	267.71	1442.30	84.16	5.28	5.05
Soppinakadu-1	11.15	22.21	0.00	93.43	22.73	0.09	50.95	0.00
Soppinakadu-2	2.54	2.10	0.08	1.92	17.41	809.20	0.19	15.75
Kudige-1	0.00	521.66	0.00	3982.92	9.57	3.36	22.05	0.91
Marigundi	0.10	32.77	0.00	0.62	19.04	9.48	112.13	9.09
Maagodu	2.51	569.12	0.00	4.69	1.86	0.28	0.41	0.28

Table 40b: Major sesquiterpenoids content in surveyed Appemidi fruit sap from western ghat regions of Chikmagalur

Genotype/μgm/gm	δ-Elemene	β-Elemene	α-Gurjunene	Caryophyllene	α-Himachalene	Aromadendrene	α-Humulene
Marebylu-2	0.00	0.07	0.00	6.05	0.16	12.07	0.14
Gonibeedu-2	0.00	0.08	0.00	15.10	0.08	0.02	9.17
Gonibeedu-3	3.87	0.16	0.23	2.52	0.00	0.30	2.85
Arenuru-1	3.69	1.00	0.00	3.23	0.55	123.03	5.93
Arenuru-2	0.00	1.18	0.00	28.01	0.36	2.16	16.17
Chimankudige-1	49.87	0.77	13.75	0.24	0.73	0.00	0.35
Chimankudige-2	0.88	10.17	88.15	41.12	1.84	0.00	39.69
Chimankudige-3	0.02	0.87	0.60	0.00	0.08	0.00	0.02
Gonibeedu-1	2.03	0.00	0.00	1.63	0.05	0.00	2.73
Marebylu-1	0.12	0.00	0.00	3.43	0.30	0.00	1.90
Arenuru	0.00	0.64	3.69	6.78	0.44	0.00	25.83
Elemadlu	0.21	0.30	29.47	17.38	0.95	0.00	0.50
Marebylu-4	0.22	0.00	0.00	0.30	2.22	1.40	121.02
Doobla	0.11	0.00	0.00	0.02	0.93	0.37	23.54
Kumrumane	1.42	0.18	0.13	1.97	3.61	0.00	0.25
Chimankudige-2	3.63	0.08	0.81	9.24	3.34	0.00	0.00
Chimankudige-1	0.60	0.03	0.00	23.27	0.05	0.00	0.00
Kudige-1	0.00	0.04	0.00	17.00	4.42	0.00	0.58
Chimankudige-3	0.00	0.84	0.00	0.15	8.32	0.00	0.02
Maagodu	0.00	0.28	0.00	0.13	25.10	0.00	1.85
chimankudige-4	0.67	0.00	1.67	167.89	2.81	0.00	0.52
Chimankudige-5	0.30	0.00	0.78	55.38	0.79	0.00	0.22

monoterpenoids identified were α -Thujene, α -Pinene, α -Phellandrene, β -Phellandrene, Limonene, 3-Carene etc. (Table 37 a).

The per cent contribution of sesiterpenoids was also varied significantly among 22 genotypes (Table 40b). The highest per cent was identified in genotype Elemadlu-2 (48.79%) followed by genotype Gonibeedu-1 (46.52%) and the minimum was recorded in Kumrumane -1(1.49%) followed by Doobla (1.72%) and Soppinakadu-1 (1.83%). The major sesiterpenoids identified were δ -Elemene, α -Gurjunene, β -Elemene, Caryophyllene, α -Himachalene, Aromadendrene and α -Humulene (Table 40b).

4.3.3 Profiling of major fat soluble, water soluble vitamins and organic acids in fresh, brined fruits and brine solution of 15 appemidi genotypes

To study the loss or gain of major nutraceuticals present in Appemidi fruits after and before pickling of 15 Appemidi genotypes viz., Ananthabhatta Appe, Kaana Appe, Kalgundikoppa Appe, Karpoora Jeerige, Dannalli Appe, Appemidi, Balekoppa Appe, Naagara Appe, Jeerige, Malange, Gorana Appe, Gurumurthy Appe, Holekoppada Appe, Isgoor Appe and Nandgar were selected for all the biochemical component analysis in immature fresh fruits, brined fruits and brine solution obtained after 30 days of salt treatment. The morphological characterization was also done for fresh immature fruits and also for salt cured fruits viz., fruit length width, thickness, fruit firmness and fruit weight were recorded to address the effect of osmosis.

The biochemical parameters like vitamin C, acidity, total phenols, total flavonoids, total antioxidant activity, profiling of major fat soluble and water soluble vitamins as well as major organic acids were profiled and interesting results obtained were represented in following heads.

4.3.3.1 Morphological characterization of genotypes

4.3.3.1.1 Fruit weight (g)

Significant difference was observed with respect to fresh fruit weight among the Appemidi genotypes and was recorded maximum in Dannalli Appe (27.02g) and minimum in Malange (15.64g) with an average fruit weight of 24.19g. There was no significant difference obtained for salt cured fruit weight (Table 41).

The per cent weight loss observed due to brining indicated that, 81.79 per cent of fruit weight loss was observed in the genotype Appemidi followed by Karpoora Jeerige (81.43%) and Naagara Appe (76.47%), whereas, minimum was noticed in the genotype Ananthabhatta Appe (61.11%). However, no significant differences were recorded for factors interaction.

4.3.3.1.2 Fruit length (cm)

Maximum fruit length was observed in Ananthabhatta Appe (6.94 cm) among fresh fruits. The minimum fruit length for fresh fruits was observed to be 3.82 cm in Malange (Table 41). Non-significant differences were obtained for salt cured fruits length, the highest was recorded in Dannalli Appe (4.86cm) followed by Hittalhalli Appe (4.76 cm). The significant per cent variability observed among all the genotypes, the maximum per cent reduction in fruit length was noticed in Appemidi (46.06%) followed by Holekoppada Appe (37.03%) and the minimum were observed in Balekoppa Appe (9.43%). But the non-significant differences were noticed for the factors interaction.

4.3.3.1.3 Fruit width (cm)

Significant differences were noticed for fresh fruit width among all the genotypes studied. The maximum fruit width was recorded in Appemidi (3.98 cm) and the minimum was observed in Malange (2.92cm), while the mean fruit width recorded was 3.26 cm (Table 41).

Non-significant differences were obtained for salt cured fruits width, the highest was recorded in Gorana Appe (2.58 cm) followed by Dannalli Appe (2.52 cm), but the non-significant differences were noticed for the treatment levels interaction.

The significant per cent variability was observed among the genotypes studied with a mean per cent reduction in fruit width of 32.67% (Table 38). The highest per cent reduction was noticed in cultivar Appemidi (69.11%) and the least was recorded in Gorana Appe (13.42%).

4.3.3.1.4 Fruit thickness (cm)

Thickness of the fruits was also measured before and after salt treatment. Fresh fruits recorded the significant differences with an average fruit thickness of 2.56cm (Table 41). The maximum fruit thickness was observed in Appemidi (2.98 cm) followed by Karpoora Jeerige (2.86cm), where as in case of salt cured fruits non-significant differences were noticed with a mean of 1.06 cm (Table 38). The highest brined fruit thickness of 1.34 cm was recorded by genotype Jeerige followed by Isgoor Appe (1.24 cm).

The per cent reduction in fruit thickness was also calculated. The average per cent reduction in thickness of fruit was 57.15%. The maximum was 68.49% in Balekoppa Appe (Table 38) followed by Appemidi (65.77%).

4.3.3.1.5 Fruit firmness (kg/cm²)

Significant differences were noticed among the genotypes in fresh fruit form for fruit firmness (Table 41). The average fruit firmness recorded for fresh form was 6.50 kg/cm², the maximum firmness was observed in the cultivar Gorana Appe (8.21 kg/cm²), followed by Appemidi (6.83 kg/cm²). The minimum firmness was recorded by Jeerige (6.10 kg/cm²).

Non-Significant differences were noticed in brine fruit firmness. The maximum firmness was observed in genotype Appemidi (14.82 kg/cm²) and the minimum was recorded in Balekoppa Appe (10.49 kg/cm²) while the average firmness observed was 12.62 kg/cm² (Table 41).

In case of per cent variation, there was an increase in fruit firmness considerably. The average increase in the per cent fruit firmness was observed to be 94.85%. The maximum per cent increase was noticed in Isgoor Appe (123.58%) and the minimum was in Balekoppa Appe (60.89%).

Table 41: Fruit morphological variability in 15 Appemidi genotypes before and after salt treatment

Genotypes	Fruit weight			Fruit length (cm)			Fruit width (cm)			Fruit thickness (cm)			Fruit firmness (kg/cm ²)		
	Fresh fruits (g)	Brined fruits (g)	% loss of weight	Fresh fruits (g)	Brined fruits (g)	% reduction in fruit length	Fresh fruits (g)	Brined fruits (g)	% reduction in fruit width	Fresh fruits (g)	Brined fruits (g)	% reduction in fruit thickness	Fresh fruits (g)	Brined fruits (g)	% increase in firmness
Gurumurthy Appe	21.64	5.72	73.57	4.72	3.94	16.53	3.32	2.14	35.54	2.64	0.92	65.15	6.39	13.61	112.99
Appemidi	21.86	3.98	81.79	4.94	2.66	46.15	3.82	1.18	69.11	2.98	1.02	65.77	6.83	14.82	116.98
Malange	15.64	4.68	70.08	3.82	3.30	13.61	2.92	2.14	26.71	2.42	1.04	57.02	6.38	11.53	80.72
Balekoppa Appe	18.9	7.08	62.54	4.24	3.84	9.43	3.08	2.36	23.38	2.38	0.75	68.49	6.52	10.49	60.89
Naagara Appe	23.12	5.44	76.47	4.66	3.66	21.46	3.48	2.2	36.78	2.62	1.04	60.31	6.27	12.16	93.94
Jeerige	18.92	6.90	63.53	4.14	3.90	5.80	3.18	2.42	23.90	2.62	1.34	48.85	6.10	12.79	109.67
Nandgar	17.44	6.36	63.53	4.54	4.50	0.88	2.98	2.12	28.86	2.28	1.14	50.00	6.21	10.83	74.40
Holekoppada Appe	26.86	7.46	72.23	6.54	4.10	37.30	3.26	2.12	34.97	2.74	1.04	62.04	6.30	12.42	97.14
Ananthabhata Appe	23.86	9.28	61.11	6.94	4.44	36.02	3.08	1.98	35.71	1.88	1.00	46.81	6.44	12.76	98.14
Gorana appe	20.08	6.44	67.93	4.92	4.34	11.79	2.98	2.58	13.42	2.24	0.90	59.82	8.21	12.74	55.18
Kaana Appe	22.62	5.42	76.04	4.58	3.58	21.83	3.26	1.82	44.17	2.64	1.14	56.82	6.35	10.63	67.40
Hittalahalli Appe	21.68	6.78	68.73	5.5	4.76	13.45	3.16	2.12	32.91	2.12	1.08	49.06	6.38	12.98	103.45
Isgoor Appe	18.58	6.16	66.85	4.22	3.24	23.22	3.3	2.14	35.15	2.5	1.24	50.40	6.36	14.22	123.58
Dannalli Appe	27.02	7.56	72.02	6.42	4.86	24.30	3.26	2.52	22.70	2.52	1.06	57.94	6.35	13.54	113.23
Karpoora Jeerige	24.56	4.56	81.43	4.22	3.32	21.33	3.36	2.46	26.79	2.86	1.18	58.74	6.38	13.72	115.05
Mean	24.19	6.25	70.52	5.03	4.03	17.05	3.26	2.15	32.67	2.56	1.06	57.15	6.50	12.62	94.850
	±SEm	CD@5%	F-test	±SEm	CD@5%	F-test	±SEm	CD @5%	F-test	±SEm	CD @5%	F-test	±SEm	CD @ 5%	F-test
Fresh fruit	0.91	2.537	*	0.14	0.38	*	0.07	0.20	*	0.06	0.18	*	0.98	1.49	*
Brined fruits	0.33	0.926	NS	0.049	0.138	NS	0.026	0.073	NS	0.023	0.065	NS	0.022	0.54	NS
Fresh X Brined	1.28	3.588	NS	0.19	0.53	NS	0.10	0.28	NS	0.09	0.25	NS	1.02	2.11	NS

4.3.3.2 Biochemical analysis of 15 Appemidi genotypes before and after salt treatment

4.3.3.2.1 Vitamin C (mg/100g)

In the present investigation, significant differences were observed in the Vitamin C content among the selected Appemidi genotypes (Table 42 and Fig. 1). The content was ranged from 20.66 to 263.00 mg/100g, with a mean of 111.57 mg in fresh fruits. The highest vitamin C content was found in genotype Appemidi (263.00 mg/100g) followed by Dannalli Appe (231.33 mg/100g) and minimum was recorded in genotype Malangi (20.66 mg/100g). A wide range of variability was observed for vitamin C content among the fifteen appemidi genotypes in this investigation.

Data pertaining to the vitamin-C content of brined fruits were represented in Table 23. The content in brined fruits ranged from 4.47 mg to 39.68 mg per 100 gram fruit with an average content of 16.45 mg/100g compared to fresh fruit form.

Brine solution obtained after 30 days of brining contains traceable amount of vitamin-C (Table 39). The mean vitamin C content in brine solution was 8.34 ml, the maximum was observed in Appemidi genotype brine solution (26.22 ml/100ml) and the minimum vitamin C content was observed in Nandgar genotype brine solution (1.73 ml) which was on par with cultivar Malangi brine solution (2.41 ml) and Naagara Appe (3.94 ml).

In total the cultivar Appemidi contained maximum vitamin C (102.52 mg/100g) followed by Dannalli Appe (94.57 mg/100g).

But the per cent loss of vitamin C was also observed during the process of brining. The loss in vitamin C content varied among the fifteen genotypes. The maximum per cent loss was observed in genotype Gurumurthy Appe (91.73%) followed by Kaana Appe (89.50 %) and the minimum per cent loss was observed in genotype Gorana Appe (13.07%) followed by Ananthabhattacha Appe (27.47%).

Table 42: Vitamin C concentration of 15 Appemidi genotypes at three different forms

Genotypes	Vitamin C (mg/100g)				
	Fresh fruit	Brine fruit	Brine solution	Mean	% Loss
Ananthabhatta Appe	40.66	18.51	10.98	23.38	27.47
Gorana Appe	46.00	23.54	16.45	28.66	13.07
Holekoppada Appe	110.00	11.80	12.61	44.80	77.81
Nandgar	65.33	13.30	1.73	26.79	76.99
Appemidi	263.00	18.33	26.22	102.52	83.06
Gurumurthy Appe	195.00	10.88	5.25	70.38	91.73
Malangi	20.66	4.47	2.41	9.18	66.70
Jeerige	54.66	18.05	6.49	26.40	55.10
Naagara appe	30.66	5.07	2.94	12.89	73.87
Balekoppa Appe	69.33	23.07	4.76	32.39	59.86
Karpoora Jeerige	142.67	14.51	12.00	56.39	81.42
Dannalli Appe	231.33	39.68	12.71	94.57	77.35
Hittalahalli Appe	186.66	17.67	4.07	69.47	88.35
Kaana Appe	175.00	15.23	3.15	64.46	89.50
Isgoor Appe	42.66	12.67	3.35	19.56	62.45
Mean	111.57	16.45	8.34	45.45	68.32
	± SE (m)	CD	F-Test	CV	
Genotype	1.325	3.729	*	8.72	
FBS	0.838	1.668	NS		
Genotype X FBS	3.245	6.458	NS		

Note: FBS: F=fresh fruit, B= Brined fruit, S= brine solution, NS= Non significant,
 *=Significant

4.3.3.2.2 Acidity (mg/100g)

The acidity content showed significant differences among the genotypes studied in all three different phases of pickle preparation. In fresh fruits the acidity ranged from 1.25 to 2.21 mg/100g (Table 43). The cultivar Appemidi was having the highest acidity in fresh fruit form (2.21 mg/100g) whereas Balekoppa Appe was containing less acidity (1.25 mg/100g) among the fifteen genotypes studied. The average acidity content in fresh fruit form of appemidi was 1.70 mg/100g (Table 43).

In case of brined fruits the acidity was ranged from 0.40 to 0.90 mg/100g. The maximum acidity in brined fruit form was observed in genotype Nandgar (0.90 mg/100g) followed by Gorana Appe (0.89 mg/100g) and the minimum was noticed in Naagara Appe (0.40 mg/100g).

Whereas the brine solution obtained was also containing some amount of acidity, it was ranged from 0.05 to 0.39 ml. The average acidity in brine solution was 0.16 ml, while the genotype Appemidi was having the maximum acidity (0.39 ml) and the minimum was recorded in cultivar Balekoppa Appe (0.05 ml).

The per cent loss in acidity from fresh fruit to brined fruit form was observed among all the fifteen genotypes in this experiment. The maximum per cent loss was noticed in genotype Gurumurthy Appe (67.15%) followed by Naagara Appe (65.11%) and Karpoora Jeerige (63.67%).

4.3.3.2.3 Total Phenols (g GAE/100g)

The average total phenols content was 0.97 g in fresh fruit form of appemidi genotypes (table 44), where it was ranged from 0.57 g to 1.64 g GAE/100g. The fresh fruit form has showed the maximum total phenol content in genotype Balekoppa Appe (1.64 g GAE/100g) followed by Gurumurthy Appe (1.38 g GAE/100g) and the minimum total phenol content was observed in Naagara Appe (0.57 g GAE/100g) followed by Appemidi (0.62 g GAE/100g).

Table 43: Acidity concentration of 15 Appemidi genotypes at three different forms

Genotypes	Acidity (mg/100g)				
	Fresh fruit	Brined fruit	Brine solution	Mean	% Loss
Ananthabhatta Appe	1.65	0.86	0.16	0.89	37.69
Gorana Appe	1.57	0.89	0.15	0.87	33.63
Holekoppada Appe	1.6	0.69	0.30	0.86	37.94
Nandgar	1.54	0.90	0.09	0.84	35.82
Appemidi	2.21	0.43	0.39	1.01	62.92
Gurumurthy Appe	2.01	0.53	0.13	0.89	67.15
Malangi	1.95	0.64	0.07	0.89	63.62
Jeerige	1.65	0.86	0.11	0.87	40.91
Naagara appe	1.66	0.40	0.18	0.75	65.11
Balekoppa Appe	1.25	0.75	0.05	0.68	36.01
Karpoora Jeerige	1.46	0.44	0.09	0.66	63.67
Dannalli Appe	1.88	0.61	0.17	0.89	58.49
Hittalahalli Appe	1.92	0.79	0.16	0.96	50.81
Kaana Appe	1.59	0.57	0.13	0.76	56.02
Isgoor Appe	1.62	0.84	0.14	0.87	39.69
Mean	1.70	0.68	0.16	0.85	49.97
	SE (m)	CD	F-Test	CV	
G	0.017	0.049	*	6.47	
FBS	0.008	0.022	NS		
GX FBS	0.03	0.085	NS		

Note: FBS: F=fresh fruit, B= Brined fruit, S= brine solution, NS= Non significant,
 *=Significant

The brined fruits also recorded significant amount of total phenol content among the genotypes studied. The average total phenol content noticed in brined fruit was 0.70 g GAE/100g and the maximum amount was recorded in cultivar Gorana Appe (0.94g GAE/100g) followed by Nandgar (0.93 g GAE/100g). The minimum value was observed in Holekoppada Appe (0.35 g GAE/100g) followed by Gurumurthy Appe (0.37 g GAE/100g).

The brine solution obtained after 30 days of treatment was also analysed for total phenols content which showed a significant differences among the different genotypes studied, whereas the average total phenols recorded in brine solution was 0.27 g GAE/100g (Table 44), the highest total phenols content was found in Appemidi brine solution (0.59 g GAE/100g) followed by Gorana Appe (0.54 g GAE/100g) where as these cultivars recorded comparatively less amount of phenol in fresh and brine fruit. Therefore the amount of total phenols transferred to brine solution was comparatively higher in these genotypes.

The per cent loss from fresh fruit to brined fruit form was observed to be maximum in genotype Appemidi (63.31%) followed by Gurumurthy Appe (53.33%) and Balekoppa Appe (53.01%) whereas the minimum per cent loss was observed in the genotype Nandgar (1.18%) which retained more compare to other genotypes.

4.3.3.2.4 Total Flavonoids (g catechin equivalent/100g)

Significant differences were obtained in total flavonoids content in three different forms among fifteen appemidi genotypes in this study. The fresh form of fruits showed an average of 0.05 g of total flavonoids content and it ranges from 0.033 to 0.072 g catechin equivalent/100g (Table 45). The maximum amount was recorded in Gurumurthy Appe (0.072 g catechin equivalent/100g) and the minimum was in Kaana Appe (0.033 g catechin equivalent/100g).

The brined fruits were also containing a significant amount of total flavonoids with a mean value of 0.036 g catechin equivalent/100g (Table 45). The highest amount was recorded in genotype Balekoppa Appe (0.052 g) and the less amount was observed in

Table 44: Total phenol concentration in 15 Appemidi genotypes at three different forms

Genotype	Total phenols (g GAE/100g)				
	Fresh fruits	Brined fruits	Brine solution	Mean	% Loss
Ananthabhata Appe	0.78	0.69	0.27	0.58	23.16
Gorana Appe	1.34	0.94	0.54	0.94	9.82
Holekoppada Appe	0.84	0.35	0.36	0.52	14.90
Nandgar	0.98	0.93	0.04	0.65	1.18
Appemidi	0.62	0.41	0.59	0.54	63.31
Gurumurthy Appe	1.38	0.37	0.28	0.68	53.33
Malangi	0.86	0.71	0.10	0.56	6.44
Jeerige	0.96	0.97	0.31	0.75	32.68
Naagara appe	0.57	0.40	0.21	0.39	6.03
Balekoppa Appe	1.64	0.48	0.29	0.80	53.01
Karpoora Jeerige	1.12	0.53	0.17	0.61	38.28
Dannalli Appe	0.82	0.70	0.32	0.61	23.88
Hittalahalli Appe	0.95	0.65	0.10	0.57	21.21
Kaana Appe	0.67	0.50	0.13	0.43	5.87
Isgoor Appe	1.19	0.84	0.27	0.77	7.16
Mean	0.97	0.70	0.27	0.65	24.02
	±SE (m)	CD @ 1%	F-Test	CV	
Genotypes	0.011	0.032	*	5.06	
FBS	0.005	0.014	NS		
Genotypes X TP	0.02	0.055	NS		

Note: FBS: F=fresh fruit, B= Brined fruit, S= brine solution, NS= Non significant,
 *=Significant TP= total phenols

Table 45: Total flavonoids concentration in 15 Appemidi genotypes at three different forms

Genotype	Total flavonoids (g catechin equivalent/100g)				
	Fresh fruits	Brined fruits	Brine solution	Mean	% Loss
Ananthabhatta Appe	0.046	0.044	0.018	0.036	2.99
Gorana Appe	0.055	0.038	0.016	0.036	29.72
Holekoppada Appe	0.047	0.025	0.022	0.031	46.77
Nandgar	0.050	0.048	0.022	0.040	4.29
Appemidi	0.047	0.020	0.028	0.032	58.66
Gurumurthy Appe	0.072	0.040	0.036	0.049	45.18
Malange	0.048	0.046	0.023	0.039	4.38
Jeerige	0.059	0.041	0.021	0.040	30.56
Naagara appe	0.069	0.012	0.015	0.032	83.20
Balekoppa Appe	0.056	0.052	0.018	0.042	7.14
Karpooora Jeerige	0.035	0.013	0.022	0.023	63.71
Dannalli Appe	0.050	0.048	0.026	0.041	4.14
Hittalahalli Appe	0.045	0.032	0.015	0.031	28.67
Kaana Appe	0.033	0.031	0.029	0.031	5.44
Isgoor Appe	0.054	0.047	0.015	0.039	12.63
Mean	0.049	0.036	0.022	0.036	28.50
	±SE (m)	CD @ 1%	F-TEST	CV	
Genotypes	0.001	0.003	*	59.42	
FBS	0	0.001	NS		
GX TF	0.002	0.005	NS		

Note: FBS: F=fresh fruit, B= Brined fruit, S= brine solution, NS= Non significant, *=Significant TF= total flavonoids

ultivar Naagara Appe (0.012 g) followed by Karpoora Jeerige (0.013 g catechin equivalent per 100g).

The brine solution also showed significant amount of total flavonoids content (Table 45). The average total flavonoids found was 0.22 g with a maximum amount was found in Gurumurthy Appe (0.036 g). The flavonoids content in brine solution was ranged from 0.015 to 0.036 g catechin equivalent/100g. Wide range of variability was observed among the genotypes studied in this investigation.

Nonsignificant effects were noticed in interaction of genotypes and three different forms. On an average the per cent loss observed in total flavonoids content was 28.50%. The minimum per cent loss in total flavonoid content was observed in the genotype Ananthabhatta Appe (2.99%) followed by Dannalli Appe (4.14%) and Nandgar (4.29%) whereas the maximum loss was noticed in genotype Naagara Appe (83.20%).

4.3.3.2.5 Total antioxidants activity (g AEAC/100g)

Significant differences were noticed for the total antioxidant activity of fifteen appemidi genotypes. The total antioxidant activity was varied from 0.38 g to 1.21 g AEAC/100g (Table 46), with an average content of 0.72 g AEAC/100g in fresh fruit form. The maximum total antioxidant activity was observed in the genotype Gurumurthy Appe (1.21 g AEAC/100g) and the minimum was in Naagara Appe (0.38 g AEAC/100g).

Non-Significant differences were obtained in total antioxidant activity of brined fruits with an average content of 0.26 g AEAC/100g (Table 46). The range of total antioxidant activity was from 0.11 g to 0.42 g AEAC/100g. The highest amount was noticed in cultivar Balekoppa Appe (0.42 g AEAC/100g) and the minimum was in Naagara Appe (0.11 g AEAC/100g).

The brine solution also showed differences in total antioxidant activity among the cultivars studied, the maximum amount was noticed in Ananthabhatta appe brine solution (0.35 g AEAC/100g), whereas the average content was found to be 0.16 g AEAC/100g in brine solution and the minimum was observed in Kaana Appe and Karpoora Jeerige (0.08

Table 46: Total antioxidant activity in 15 Appemidi genotypes at three different forms

Genotype	Total antioxidants activity (g AEAC/100g)				
	Fresh fruits	Brined fruits	Brine solution	Mean	% Loss
Ananthabhata Appe	0.59	0.37	0.35	0.44	22.03
Gorana Appe	0.99	0.34	0.26	0.53	39.28
Holekoppada Appe	0.54	0.14	0.18	0.29	40.45
Nandgar	0.87	0.39	0.07	0.44	47.08
Appemidi	0.50	0.15	0.26	0.30	17.06
Gurumurthy Appe	1.21	0.26	0.14	0.54	66.87
Malangi	0.84	0.23	0.07	0.38	63.16
Jeerige	0.82	0.29	0.14	0.42	47.28
Naagara appe	0.38	0.11	0.13	0.21	34.76
Balekoppa Appe	1.02	0.42	0.13	0.52	46.74
Karpoora Jeerige	0.60	0.17	0.09	0.29	56.21
Dannalli Appe	0.67	0.22	0.15	0.35	44.91
Hittalahalli Appe	0.70	0.18	0.26	0.38	37.03
Kaana Appe	0.42	0.15	0.08	0.22	45.36
Isgoor Appe	0.80	0.28	0.11	0.40	51.00
Mean	0.72	0.26	0.16	0.38	41.01
	SE (m)	CD	F-Test	CV	
G	0.008	0.022	*	8.33	
FBS	0.004	0.01	NS		
GX TAA	0.014	0.038	NS		

Note: FBS: F=fresh fruit, B= Brined fruit, S= brine solution, NS= Non significant,
 *=Significant , TAA=total antioxidant activity

and 0.09 g AEAC/100g). Non-significant differences were found for treatment interaction (table 46).

The per cent loss in total antioxidant activity was also varied among the genotypes studied the highest loss was noticed in Gurumurthy Appe (66.87%) whereas the minimum loss was observed in genotype Appemidi (17.06%).

4.3.3.2.6 Water soluble vitamins (ng/g DW)

Major water soluble vitamins were profiled and quantified from fifteen appemidi genotypes in three different phases' viz., fresh fruits, brined fruits and brine solution obtained when salt treatment imposed after 30 days. Majority of water soluble vitamins were also obtained in the brine solution of respective genotypes but the concentration was varied significantly among them.

The eight water soluble vitamins were profiled and quantified viz., thiamine, niacin, pyridoxine, pantothenic acid, biotin, riboflavin, folic acid and cyanocobalamin (Table 47 a & 47b). Significant differences were obtained among all the genotypes, water soluble vitamins, fresh, brined fruits and brine solution and their interaction. The results are elaborated hereunder.

4.3.3.2.6.1 Thiamine (ng/g DW)

Thiamine concentration among the fresh fruits of fifteen appemidi genotypes showed significant differences with an average concentration of 108.88 ng/g of dry weight (Table 47a). The maximum concentration of thiamine was found in the genotype Isgoor Appe (185.78 ng/g dry weight) followed by Malange (158.00 ng/g dry weight) and the minimum amount of thiamine was observed in Holekoppada Appe (30.33 ng/g dry weight). The thiamine concentration was ranged from 30.33 ng to 185.78 ng/g dry weight.

Brine fruit forms were also estimated for thiamine content and the significant differences were obtained among all the genotypes studied with an average value of 29.95 ng/g dry weight. The amount of thiamine in brined fruits ranged from 14.91 ng to 63.11 ng /g dry weight. The highest concentration of thiamine in brined fruit was recorded in genotype Gorana Appe (63.11 ng/g dry weight) followed by Dannalli Appe (49.06 ng/g dry weight). The less concentration was observed in genotype Jeerige (14.91 ng/g dry weight) (Table 47a).

The amount of thiamine in brine solution varied significantly from 0.20 ng to 12.23 ng/g dry weight (Table 47a), the maximum amount of thiamine was recorded in Ananthabhattera Appe cultivar brine solution (12.23 ng/g dry weight) and the minimum amount was observed in Karpoora Jeerige brine solution (0.20 ng/g dry weight).

4.3.3.2.6.2 Niacin (ng/g dry weight)

The amount of Niacin varied significantly from 16.55 ng to 148.99 ng/g dry weights (Table 47a). The average niacin content in fresh fruit of appemidi was 67.47 ng/g dry weight. The highest amount was noticed in Balekoppa Appe 148.99 ng/g dry weight which is followed by Isgoor Appe (118.93 ng/g dry weight) while the less amount of niacin was found in cultivar Appemidi (16.55 ng/g dry weight).

Significant variabilities were observed in niacin concentration in brined fruits of all fifteen appemidi genotypes. The average niacin content in brined fruit of appemidi genotypes was 13.26 ng /g dry weight and the niacin content was ranged from 4.37 ng to 26.41 ng/g dry weight (Table 47a) whereas the genotype Balekoppa Appe has recorded the highest niacin content in brined fruit form (26.41 ng) and the less amount was found in Isgoor Appe (4.37 ng/g dry weight) followed by Gurumurthy Appe (4.38 ng).

Brine solution obtained by respective appemidi genotypes were also containing niacin in significant concentration was estimated. The average niacin content in brine solution was 103.20 ng/g dry weights (Table 47a). The maximum niacin content was obtained in brine solution of cultivar Isgoor Appe (399.33 ng/g dry weight) which was followed by Jeerige (206.98 ng/g dry weight) and the minimum amount was observed in Karpoora Jeerige (26.91 ng / g dry weight) followed by Naagara Appe (27.67 ng).

4.3.3.2.6.3 Pyridoxine (ng/d dry weight)

The concentration of pyridoxine varied significantly among fresh fruits of all fifteen genotypes studied (Table 47a). The average content of pyridoxine in fresh fruits of appemidi was 15.46 ng/g dry weights whereas the concentration was varied from 1.82 ng to 47.33 ng/g dry weight. The maximum concentration of pyridoxine was found in cultivar Malange (47.33 ng/g dry weight) followed by cultivar Jeerige (44.25 ng/g dry weight) and the minimum concentration was recorded in cultivar Hittalahalli Appe (1.82 ng/g dry weight).

The brine fruits were also showed significant differences in pyridoxine concentration among all the fifteen genotypes studied. The average pyridoxine content was 7.98 ng/g dry weight where as it was ranged from 1.13 ng to 20.23 ng / g dry weight. The highest amount was found in Dannalli Appe (20.23 ng/g dry weight) and the minimum amount was recorded in Ananthabhata Appe Appe (1.13 ng/g dry weight) followed by Hittalahalli Appe (1.42 ng/g).

The amount of pyridoxine found in brine solution was varied from 0.02 ng to 25.78 ng/ ml dry weight (table 47a), the average concentration recorded was 4.13 ng/ml of dry weight. The highest amount of pyridoxine was found in cultivar Dannalli Appe brine solution (25.78 ng/ml of dry weight) and the minimum in cultivar Holekoppada Appe (0.02 ng/ml of dry weight).

4.3.3.2.6.4 Pantothenic acid (ng/g dry weight)

The concentration of pantothenic acid was significantly varied from fresh, brined fruits and brine solution (table 47a). The amount of pantothenic acid showed significant differences among the fifteen cultivars. The average pantothenic acid found in fresh fruits of Appemidi was 55.49 ng/g dry weight. Genotype Dannalli Appe recorded maximum amount of pantothenic acid (19.86 ng/g dry weight) and the cultivar Kaana Appe recorded minimum (1.96 ng/g dry weight).

Among the brined fruits also the concentration of pantothenic acid varied significantly with a mean value of 4.23 ng/g dry weight whereas the concentration was ranged from 0.45 ng to 10.93 ng/g dry weight. The maximum and minimum concentration was recorded in cultivar Gorana Appe (10.93 ng/g dry weight) and Kaana Appe (0.45 ng/g dry weight).

The amount of pantothenic acid in brine solution of fifteen Appemidi genotypes was a varied from 0.20 to 1.46 ng/ml dry weight. The average pantothenic acid content in brine solution was 0.97 ng/ml dry weight (Table 47a).

4.3.3.2.6.5 Biotin (ng/g dry weight)

Biotin content among the fifteen Appemidi genotypes varied significantly in fresh, brined fruits as well as in brine solution of respective cultivars (Table 47b).

Among fresh fruits the biotin concentration was varied from 0.97 ng/g to 8.84 ng/g dry weight. The average biotin content among fresh fruits of Appemidi was 2.77 ng/g of dry weight. The maximum amount was recorded in Dannalli Appe (8.84 ng/g dry weight) and the minimum concentration was recorded in Karpoora Jeerige (0.97 ng/g dry weight).

In case of brined fruit the biotin concentration was varied from 0.33 ng to 2.42 ng/g of dry weight with an average amount of 2.07 ng /g of dry weight (Table 47b). The highest amount of biotin was found in Gorana Appe (2.42 ng/ g of dry weight) brined fruit followed by Hittalahalli Appe (1.54 ng/g).

The brine solution also showed significant amount of biotin content (Table 47b). The concentration was varied from 0.14 ng/ml (Hittalahalli Appe) to 4.43 ng/ml (Nandgar) while the average content in brine solution was 1.57 ng/ml.

4.3.3.2.6.6 Riboflavin (ng/g dry weight)

Riboflavin one of the important water soluble vitamins showed a significant difference among the fifteen genotypes studied (Table 47b). The fresh fruit contains an average amount of riboflavin of 15.45 ng/g dry weight. The riboflavin concentration was varied from 6.38 ng to 28.93 ng/g of dry weight, whereas the maximum amount of riboflavin was recorded in cultivar Jeerige (28.93 ng/g dry weight) followed by Dannalli Appe (22.91 ng/g dry weight) and the minimum amount of riboflavin was found in cultivar Holekoppada Appe (6.38 ng/g dry weight) followed by Hittalahalli Appe (6.80 ng/ g dry weight).

The riboflavin concentrations among brined fruits were varied from 0.74 ng (Isgoor Appe) to 9.39 ng/g dry weight (Appemidi), whereas the average content found in brined fruits was 4.07 ng/g dry weight.

Brine solution also showed a varied concentration of riboflavin. The maximum amount of riboflavin was found in brine solution of genotype Jeerige (2.09 ng/ml) followed by Ananthabhata Appe (2.02 ng/ml) and the minimum was recorded in Gurumurthy Appe (0.06 ng/ml) followed by Malange (0.10 ng/ml).

4.3.3.2.6.7 Folic acid (ng/g dry weight)

The fresh fruits of appemidi genotypes showed significant amount of folic acid content. The maximum amount of folic acid was found in cultivar Gurumurthy Appe (7.47 ng/g dry weight) and the minimum was observed in cultivar Kaana Appe (1.15 ng/g dry weight).

The brined fruits also showed significant amount of folic acid content (Table 47b), whereas the range of folic acid was varied from 0.21 ng/g dry weight (Kaana Appe) to 2.36 ng/g dry weight (Karpooora Jeerige). The average folic acid content was 3.95 ng/g dry weight.

The brine solution contains around 5.45 ng/ml of folic acid content. The highest concentration was found in Nandgar (12.03 ng/g dry weight) and the minimum was recorded in Kaana Appe (1.04 ng/g dry weight).

4.3.3.2.6.8 Cyanocobalamin (ng/g dry weight)

The concentration of cyanocobalamin was varied from 0.60 ng to 1.86 ng/g dry weight among fresh fruits of Appemidi. The average content was 1.91 ng/g dry weight in fresh fruits (Table 47b).

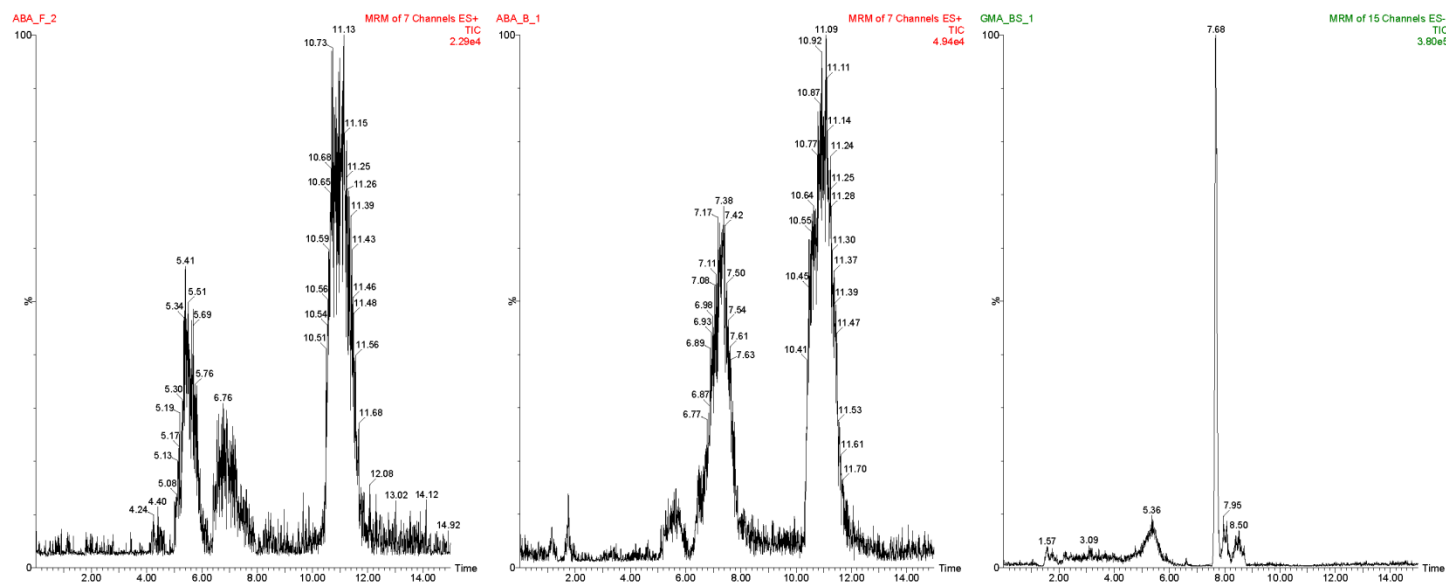


Plate 18: Chromatograms of water soluble vitamins in fresh, brined fruit and brine solution of Apendidi

Table 47a: Major water soluble vitamins concentration in 15 Appemidi genotypes at three different forms

Genotypes	Water soluble vitamins (ng/g DW)															
	Thiamine				Niacin				Pyridoxine				Pantothenic acid			
	F	B	BS	Mean	F	B	BS	Mean	F	B	BS	Mean	F	B	BS	Mean
Gurumurthy Appe	125.82	43.34	0.29	56.48	80.26	4.38	45.13	43.26	3.7	2.89	1.02	2.54	12.65	1.44	0.42	4.84
Appemidi	101.82	34.81	6.38	47.67	16.55	25.77	161.19	67.84	20.67	9.22	4.00	11.30	8.47	6.63	1.26	5.45
Malange	158.00	19.03	2.11	59.71	64.8	13.07	126.11	67.99	47.33	3.11	2.56	17.67	12.38	8.62	0.60	7.20
Balekoppa Appe	64.48	23.22	1.13	29.61	148.99	26.41	51.45	75.62	16.91	8.90	5.51	10.44	4.61	4.17	0.99	3.26
Naagara Appe	109.97	28.76	3.24	47.32	64.05	11.88	27.67	34.53	7.58	4.78	0.58	4.31	16.18	1.38	0.86	6.14
Jeerige	97.86	14.91	3.15	38.64	26.96	4.58	206.98	79.51	44.25	5.99	2.42	17.55	5.92	1.70	0.95	2.86
Nandgar	109.12	48.06	2.09	53.09	64.46	25.07	84.82	58.12	7.66	8.02	7.27	7.65	16.24	2.29	0.88	6.47
Holekoppada Appe	30.33	20.36	2.53	17.74	55.67	21.00	85.58	54.08	4.94	4.82	0.02	3.26	15.73	1.80	0.58	6.04
Ananthabhata Appe	79.26	17.78	12.23	36.42	29.89	9.74	180.61	73.41	2.4	1.13	2.46	2.00	11.79	0.56	0.20	4.18
Gorana appe	144.28	63.11	2.02	69.80	108.12	5.06	29.27	47.48	29.19	12.34	1.90	14.48	13.51	10.93	1.46	8.63
Kaana Appe	126.28	18.79	0.82	48.63	40.61	6.03	32.19	26.28	2.42	2.72	0.26	1.80	1.96	0.45	0.54	0.98
Hittalahalli Appe	112.67	15.42	0.77	42.95	65.27	15.42	48.19	42.96	1.82	1.42	0.56	1.27	8.63	5.42	0.28	4.78
Isgoor Appe	185.78	23.62	6.85	72.08	118.93	4.37	399.33	174.21	6.54	5.15	4.15	5.28	5.98	2.05	0.89	2.97
Dannalli Appe	132.64	49.06	1.47	61.06	62.49	16.65	42.60	40.58	28.82	20.23	25.78	24.94	19.86	6.91	3.48	10.08
Karpoora Jeerige	54.87	29.05	0.20	28.04	64.96	9.42	26.91	33.76	27.61	9.92	0.48	12.67	15.2	9.15	1.23	8.53
Mean	108.88	29.95	3.02	47.28	67.47	13.26	103.20	61.31	15.46	7.98	4.13	9.14	55.49	4.23	0.97	5.49

Table 47b: Major water soluble vitamins concentration in 15 Appemidi genotypes at three different forms

Genotypes	Water soluble vitamins (ng/g DW)															
	Biotin				Riboflavin				Folic acid				Cyanocobalamin			
	F	B	BS	Mean	F	B	BS	Mean	F	B	BS	Mean	F	B	BS	Mean
Gurumurthy Appe	2.64	0.80	1.00	1.48	16.89	2.71	0.06	6.55	7.47	0.63	0.71	2.94	0.67	0.12	0.07	0.29
Appemidi	1.4	0.43	0.97	0.93	13.71	9.39	0.82	7.97	3.13	1.92	1.24	2.10	1.28	0.40	0.80	0.83
Malange	2.82	0.48	2.10	1.80	10.58	1.21	0.10	3.96	2.48	0.24	0.44	1.05	1.84	0.40	1.27	1.17
Balekoppa Appe	2.05	1.03	1.07	1.38	11.87	2.72	0.41	5.00	2.15	1.90	0.70	1.58	0.8	0.22	0.60	0.54
Naagara Appe	2.49	0.38	0.95	1.27	15.14	2.51	0.76	6.14	1.44	0.90	0.81	1.05	1.75	0.24	1.34	1.11
Jeerige	0.98	0.46	0.17	0.54	28.93	2.77	2.09	11.26	4.69	0.40	0.52	1.87	0.84	0.17	0.58	0.53
Nandgar	2.6	0.63	1.52	1.58	17.24	4.20	0.89	7.44	2.55	1.94	0.32	1.60	1.14	0.20	0.72	0.69
Holekoppada Appe	5.11	0.47	1.02	2.20	6.38	1.58	0.94	2.97	1.45	0.59	0.23	0.76	1.73	0.65	0.48	0.95
Ananthabhata Appe	2.02	1.06	0.81	1.30	21.44	2.74	2.02	8.73	2.3	1.60	0.40	1.43	1.61	0.42	1.16	1.06
Gorana appe	2.71	2.42	0.14	1.76	18.06	4.46	0.15	7.56	3.3	1.30	0.38	1.66	1.57	0.89	0.77	1.08
Kaana Appe	1.56	0.33	1.03	0.97	11.98	1.41	0.15	4.51	1.15	0.21	0.21	0.52	1.34	0.19	0.59	0.71
Hittalahalli Appe	3.41	1.54	0.49	1.81	6.8	5.42	0.14	4.12	1.58	0.54	1.20	1.11	0.97	0.15	0.96	0.69
Isgoor Appe	2.99	0.38	1.14	1.50	17.11	0.74	0.34	6.06	1.69	0.34	1.17	1.07	1.54	0.15	0.56	0.75
Dannalli Appe	8.84	1.22	3.23	4.43	22.91	3.63	0.21	8.92	3.7	1.90	1.17	2.26	1.86	0.32	1.27	1.15
Karpoora Jeerige	0.97	0.54	0.29	0.60	12.73	5.50	0.27	6.17	2.77	2.36	0.32	1.82	1.81	0.09	1.12	1.01
Mean	2.77	2.07	2.15	1.57	15.45	4.07	0.62	6.49	2.39	3.95	0.81	1.52	1.91	1.53	1.07	0.84
	± SE (m)				CD @ 1%				F-TEST				CV			
Genotypes	0.35				0.96				*				5.85			
WSVT	0.252				0.702				*							
FBS	0.977				2.718				*							
G X WSVT	0.155				0.43				*							
G X FBS	0.598				1.665				NS							
WSVT X FBS	0.437				1.216				*							
G X WSVT X FBS	1.693				4.708				NS							

Note: WSVT= Water soluble vitamins, FBS= fresh fruit, brined fruit and brine solution, G= genotypes, *= Significant, NS= Non significant

The brined fruits also showed significant differences for cyanocobalamin content. The highest amount was found in Gorana Appe (0.89 ng/g dry weight) and the minimum was in cultivar Karpoora Jeerige (0.09 ng/g dry weight).

The content of cyanocobalamin in brine solution was varied from 0.07 ng/ml to 1.34 ng/ml. the average content was found to be 1.04 ng/ml in brine solution. The highest amount was recorded in cultivar Naagara Appe (1.34 ng/ml) and the minimum amount was found in Gurumurthy Appe (0.07 ng/ml).

There was significant loss of major water soluble vitamins from fresh fruit form to brined fruit form during the process of salt treatment. The losses were varied with genotypes as well as with vitamins.

4.3.3.2.7 Fat soluble vitamins (FSVT) (ng/g)

Major Fat soluble vitamins were profiled from 15 Appemidi genotypes in fresh and brine fruit form.

Mainly Vitamin D1, Vitamin D2, Tocopherol, Vitamin K2 and Vitamin K1 were profiled and the results were presented in table. Significant differences were obtained among the genotypes studied for major fat soluble vitamins in fresh form and significant differences were obtained for interaction effect of genotypes, major fat soluble vitamins and fresh brined fruits, whereas non-significant differences were obtained for brined fruits and also for the interaction effects of genotypes and major fat soluble vitamins, genotypes and fresh, brined fruits as well as for fresh and brined fruit forms (Table 48).

4.3.3.2.7.1 Vitamin D1 (ng/g)

The average Vitamin D1 content was found to be 1.31 ng/g (Table 48). The maximum amount was recorded in the genotype Kaana Appe (2.84 ng/g) which is on par with genotypes Gorana Appe (2.35 ng/g), Ananthabhatta Appe (2.17 ng/g), Karpoora Jeerige (2.33 ng/g), Balekoppa Appe (1.79 ng/g) and Jeerige (1.69 ng /g). The minimum amount was observed in the genotype Malange (0.30 ng/g) which was on par with Appemidi (0.34 ng/g).

The brined fruits were also contained significant amount of vitamin D1 (Table 48). The average vitamin D1 observed in brined fruit was 0.20 ng/g and the maximum content was recorded in Kaana Appe (1.00 ng/g) and the minimum value was observed in Holekoppada Appe (0.04 ng/g). There was a wide variability among the cultivars in fresh and brined fruit vitamin D1 content. The total per cent loss in vitamin D1 was more than 80% compare to fresh and brined fruit mean value.

The total average content of vitamin D1 in both fresh and brine fruit form was also calculated in fifteen genotypes (Table 48). On an average 0.76 ng/g of vitamin D1 content was found in Appemidi genotypes studied, whereas the treatments and the factors interaction showed non-significant differences but the interaction of all three components showed significant effect on vitamin D1 content.

4.3.3.2.7.2 Vitamin D2 (ng/g)

Significant differences were obtained for the vitamin D2 content in fresh fruit form among fifteen genotypes studied. The maximum vitamin D2 was observed in Jeerige (90.25 ng/g) and the minimum amount was recorded in Kaana Appe (1.46 ng/g).

Brined fruit were also showed significant differences for vitamin D2 content (table 48). The highest amount was found in Karpoora Jeerige (62.59 ng/g). The average vitamin D2 content was 30.79ng/g in brined fruits and the minimum amount was recorded in Malange 16.27 ng/g. The brined

fruit has retained vitamin D2 during the process of brining therefore the mean per cent loss was comparatively lesser.

4.3.3.2.7.3 Tocopherol (ng/g)

Among fresh fruits form of the 15 Appemidi genotypes the tocopherol content was ranged from 1.72 µg to 6.50 µg/g (table 48). The maximum tocopherol content was recorded in Nandgar (6.50 µg) followed by Ananthabhata Appe (5.86 µg) and Balekoppa Appe (5.59 µg/g) whereas the minimum amount was observed in Gorana Appe (1.72 µg) followed by Appemidi (1.98 µg/g).

Brined fruit were also having significant amount of tocopherol content which ranged from 1.36 µg to 5.41µg/g with average value of 2.65µg (Table 48). The highest amount of tocopherol was found in the genotype Ananthabhata Appe (5.41 µg/g) and the less amount was found in Malange (1.36 µg/g) followed by Kaana Appe (1.40 µg/g).

The amount of loss from fresh fruit to brine fruit form in case of tocopherol content was found to be less.

4.3.3.2.7.4 Vitamin K2 (ng/g)

Vitamin K2 was ranged from 1.00 to 5.21ng/g among the fresh fruits studied (table 48), the average Vitamin K2 content was found to be 2.97 ng in fresh fruits, while the highest amount was recorded in Jeerige (5.21 ng/g) and the minimum was observed in Isgoor Appe (1.00 ng/g).

The brined fruit vitamin K2 content was varying significantly with an average value of 1.67 ng/g. The maximum amount was recorded in the genotype Dannalli Appe (2.31 ng/g) and the minimum amount was noticed in the genotype Isgoor Appe (0.88 ng/g) while the non-significant differences were obtained for the interaction of genotypes and fat soluble vitamins but the interaction effect of all three factors found significant (table 48).

In case of vitamin K2 also the brined fruits were retained comparatively good amount after salt treatment.

4.3.3.2.7.5 Vitamin K1 (ng/g)

Vitamin K1 also showed a varied concentration among the cultivars studied (Table 48), with an average content of 71.18 ng/g in fresh fruits. The highest amount was found in cultivar Dannalli Appe (126.55 ng) and the minimum concentration was recorded in Gurusurthy Appe (17.48 ng/g).

Significant differences were obtained for the brined fruits concentration of vitamin K1. It ranges from 5.40 ng/g to 61.42 ng/g with an average concentration of

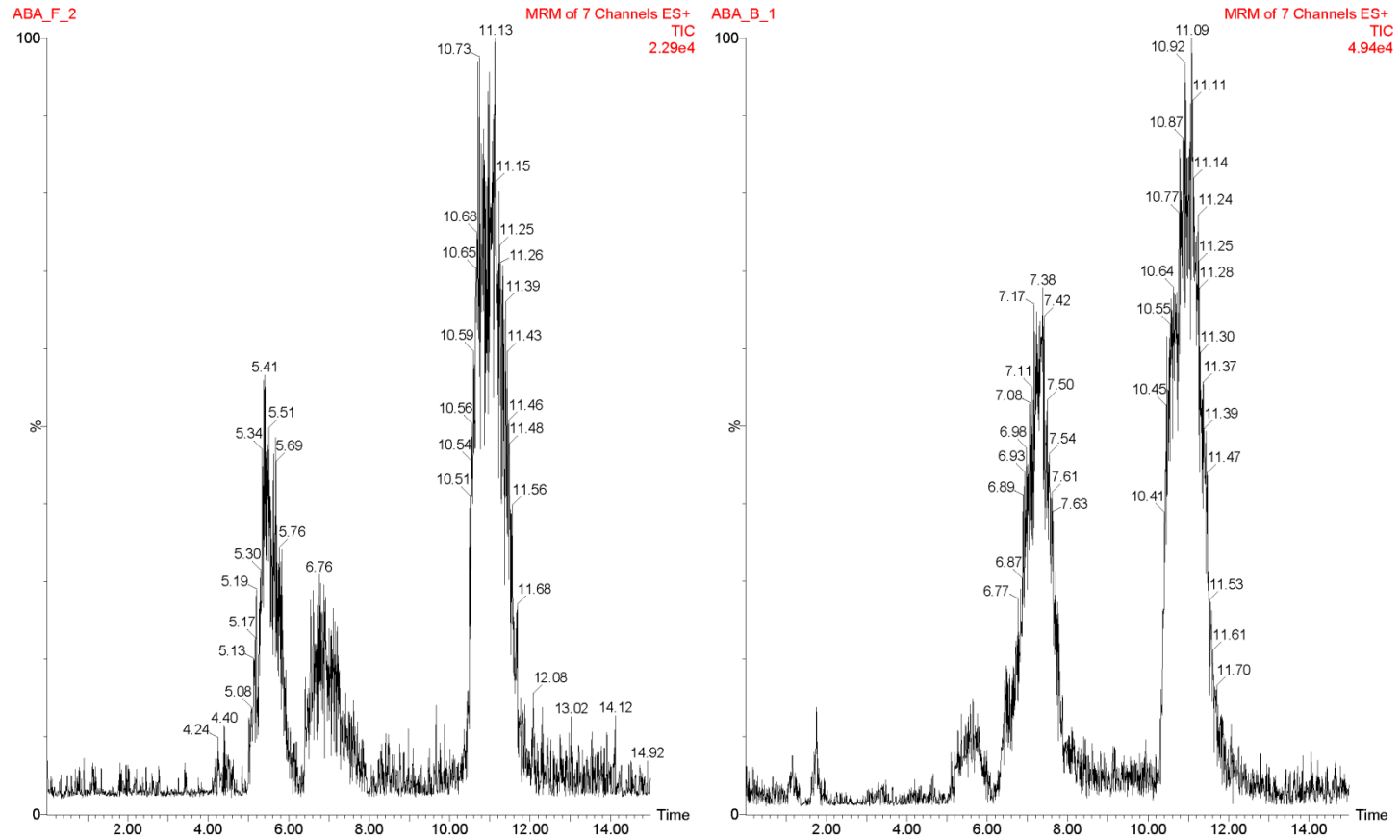


Plate 19: Chromatograms of Fat soluble vitamins in fresh and brined fruit of Appemidi

Table 48: Major fat soluble vitamins concentration in fresh and brined fruit form of 15 Appemidi genotypes

Genotypes/ Vitamins	Fat Soluble Vitamins (FSVT) (ng/g)														
	Vitamin D1			Vitamin D2			Tocopherol			Vitamin K2			Vitamin K1		
	Fresh fruit	Brined fruit	Mean	Fresh fruit	Brined fruit	Mean	Fresh fruit	Brined fruit	Mean	Fresh fruit	Brined fruit	Mean	Fresh fruit	Brined fruit	Mean
Gurumurthy Appe	0.60	0.08	0.34	5.73	36.52	21.12	1984.34	1688.50	1836.42	1.80	1.09	1.45	17.48	5.40	11.44
Appemidi	0.34	0.18	0.26	4.59	35.76	20.18	5089.71	5014.73	5052.22	2.41	1.67	2.04	23.65	31.74	27.70
Malange	0.30	0.07	0.19	62.59	16.27	39.43	3480.57	1366.91	2423.74	2.41	1.02	1.71	42.94	11.66	27.30
Balekoppa Appe	1.79	0.15	0.97	43.71	28.55	36.13	5581.88	5234.57	5408.23	2.41	1.97	2.19	75.07	57.85	66.46
Naagara Appe	1.19	0.18	0.69	4.01	19.61	11.81	3102.36	1988.02	2545.19	2.81	1.97	2.39	14.40	4.35	9.38
Jeerige	1.69	0.13	0.91	90.25	26.69	58.47	4693.81	1646.92	3170.36	5.21	1.75	3.48	80.47	15.56	48.02
Nandgar	0.89	0.29	0.59	51.91	32.22	42.07	6496.48	1623.36	4059.92	2.24	1.24	1.74	90.24	7.15	48.70
Holekoppada Appe	0.45	0.04	0.25	12.12	37.31	24.72	2113.10	1722.58	1917.84	2.41	1.10	1.75	105.15	56.43	80.79
Ananthabhata Appe	2.17	0.44	1.30	4.89	35.26	20.08	5864.81	5541.40	5703.10	1.80	1.04	1.42	119.29	47.25	83.27
Gorana appe	2.35	0.10	1.23	2.93	24.93	13.93	1719.10	967.34	1343.22	4.41	2.10	3.26	116.72	17.24	66.98
Kaana Appe	2.84	1.00	1.92	1.46	30.15	15.81	2243.76	1402.51	1823.13	1.80	1.04	1.42	55.53	21.15	38.34
Hittalahalli Appe	0.90	0.05	0.48	3.24	31.58	17.41	2847.92	2625.44	2736.68	4.61	1.80	3.20	33.42	20.60	27.01
Isgoor Appe	0.93	0.07	0.50	3.30	26.04	14.67	2752.19	2338.29	2545.24	1.00	0.88	0.94	98.21	61.42	79.82
Dannalli Appe	0.85	0.14	0.50	15.63	18.43	17.03	4474.74	3838.57	4156.65	4.81	2.31	3.56	126.55	46.34	86.45
Karpoora Jeerige	2.33	0.12	1.23	3.25	62.59	32.92	2830.24	2802.95	2816.59	4.41	1.99	3.20	68.59	53.01	60.80
Mean	1.31	0.20	0.76	20.64	30.79	25.72	3685.00	2653.47	3169.24	2.97	1.67	2.25	71.18	45.54	50.83
			± SE (m)			C.D@ 1%					F-Test			CV	
Genotypes			1.45			4.03					*			33.36	
FSVT			1.06			2.94					*				
Fresh & Brined Fruits			0.65			1.80					NS				
G X FSVT			4.10			11.40					NS				
G X Fresh & Brined fruits			2.51			6.98					NS				
G X FSVT X Fresh& Brined Fruits			7.10			19.74					*				
FSVT X Fresh & Brined Fruits			1.83			5.10					*				

Note: FSVT= Fat soluble vitamins, G= genotypes, *=Significant, NS=Non significant

45.54 ng/g in brined fruits of appemidi. The maximum content of vitamin K2 was 61.42 ng found in Isgoor Appe and the minimum concentration of 5.40 ng/g was observed in Gurumurthy appe.

4.3.3.2.8 Organic acids (micro gram/ gram)

Major organic acids namely, citric acid, fumaric acid, maleic acid, malic acid, malonic acid, pyruvic acid, shikimic acid, succinic acid and tartaric acid were profiled by LC/MS method in 15 Appemidi genotypes at three different phases viz., fresh fruits, brined fruits and brine solution. Significant differences were obtained for the major organic acids among three different phases in each genotypes studied in this experiment (Table 49 a,b,c).

4.3.3.2.8.1 Citric acid ($\mu\text{g/g}$)

In fresh fruits of fifteen appemidi genotypes the citric acid concentration was varied significantly with an average content of 142.98 $\mu\text{g/g}$ (Table 49a). The range of citric acid concentration in fresh fruits was varied from 59.90 $\mu\text{g/g}$ to 301.75 $\mu\text{g/g}$. The maximum concentration of citric acid content was obtained in the genotype Appemidi (301.75 $\mu\text{g/g}$) and the minimum was recorded in Isgoor Appe (59.90 $\mu\text{g/g}$).

The concentration of citric acid in brined fruits of all fifteen genotypes also showed significant differences with an average value of 42.49 $\mu\text{g/g}$ (Table 49a). The highest concentration was found in Karpoora Jeerige (87.04 $\mu\text{g/g}$) which is followed by the genotype Ananthabhatta Appe (81.38 $\mu\text{g/g}$) and the minimum concentration was recorded in the genotype Appemidi (11.41 $\mu\text{g/g}$).

The brine solution also showed significant concentration of citric acid (Table 49a). The average amount of citric acid found in brine solution of appemidi genotypes was 4.31 $\mu\text{g/g}$ whereas the concentration was varied from 1.54 $\mu\text{g/g}$ (Gorana Appe) to 14.42 $\mu\text{g/g}$ (Gurumurthy Appe).

4.3.3.2.8.2 Fumaric acid ($\mu\text{g/g}$)

The concentration of fumaric acid was varied significantly among the fresh fruits of appemidi genotypes studied (Table 49a). The average concentration of fumaric acid in fresh fruit of appemidi was 27.35 $\mu\text{g/g}$ whereas the concentration was varied from 10.20 $\mu\text{g/g}$ to 60.77 $\mu\text{g/g}$. The maximum concentration was recorded in the genotype Holekoppada Appe (60.77 $\mu\text{g/g}$) which was on par with the genotype Gorana Appe (59.34 $\mu\text{g/g}$) followed by Dannalli Appe (54.87 $\mu\text{g/g}$) and the minimum amount was found in genotype Nandgar (10.20 $\mu\text{g/g}$) followed by Isgoor Appe (10.68 $\mu\text{g/g}$).

The Fumaric acid concentration among brined fruits was also varied significantly with an average content of 9.74 $\mu\text{g/g}$. The highest concentration of fumaric acid observed was 19.46 $\mu\text{g/g}$ in genotype Balekoppa Appe (Table 49a) which was followed by Hittalhalli Appe (15.42 $\mu\text{g/g}$). The minimum amount was observed in the genotype Kaana Appe (5.02 $\mu\text{g/g}$).

The concentration of fumaric acid in brine solution was varied significantly from 0.31 $\mu\text{g/g}$ (Kaana Appe) to 1.31 $\mu\text{g/g}$ (Malange). The average amount of fumaric acid in appemidi brine solution was found to be 0.84 $\mu\text{g/g}$ (Table 49a).

4.3.3.2.8.3 Maleic acid ($\mu\text{g/g}$)

The immature fresh fruits of fifteen appemidi genotypes showed significant concentration of maleic acid (Table 49a). The concentration of maleic acid was varied from 4.19 $\mu\text{g/g}$ to 45.35 $\mu\text{g/g}$ with an average content of 18.21 $\mu\text{g/g}$. The maximum content was recorded in genotype Gorana Appe (45.35

$\mu\text{g/g}$) which was on par with Holekoppada Appe (42.23 $\mu\text{g/g}$) followed by Dannalli Appe (40.82 $\mu\text{g/g}$) and the minimum concentration was found in genotype Naagara Appe (4.19 $\mu\text{g/g}$).

The brined fruits of respective appemidi genotypes also showed significant concentration of maleic acid (Table 49a). The average maleic acid concentration was found to be 6.38 $\mu\text{g/g}$. The highest amount of maleic acid content was recorded in the genotype Hittalahalli Appe (15.42 $\mu\text{g/g}$) followed by Balekoppa Appe (12.21 $\mu\text{g/g}$) and the minimum content was recorded in Nandgar (1.75 $\mu\text{g/g}$) followed by Gorana Appe (1.80 $\mu\text{g/g}$) and Malange (1.86 $\mu\text{g/g}$).

Maleic acid concentration was also observed in brine solution of respective appemidi genotypes with varied concentration (Table 49a). The range was varied from 0.26 $\mu\text{g/g}$ (Kaana Appe) to 0.94 $\mu\text{g/g}$ (Gurumurthy Appe) whereas the average content was 0.58 $\mu\text{g/g}$.

4.3.3.2.8.4 Malic acid ($\mu\text{g/g}$)

The concentration of malic acid in fresh immature fruits of all fifteen genotypes showed significant differences with an average value of 82.87 $\mu\text{g/g}$ (Table 49b). The highest concentration was found in Hittalahalli Appe (190.27 $\mu\text{g/g}$) and the minimum concentration was recorded in genotype Kaana Appe (11.94 $\mu\text{g/g}$).

The Malic acid concentration among brined fruits was also varied significantly with an average content of 33.77 $\mu\text{g/g}$. The highest concentration of malic acid observed was 62.02 $\mu\text{g/g}$ in genotype Malange (Table 49b) and the minimum amount was observed in genotype Karpoora Jeerige (9.68 $\mu\text{g/g}$).

The range of malic acid in brine solution was varied from 0.47 $\mu\text{g/g}$ (Ananthabhatta Appe) to 14.13 $\mu\text{g/g}$ (Gurumurthy Appe) whereas the average content was 3.44 $\mu\text{g/g}$ (table 49b).

4.3.3.2.8.5 Malonic acid ($\mu\text{g/g}$)

Malonic acid concentration was varied significantly among the fifteen genotypes of appemidi fresh fruits (Table 49b). The average content was 65.33 $\mu\text{g/g}$, whereas it was ranged from 13.76 $\mu\text{g/g}$ to 186.94 $\mu\text{g/g}$. The highest concentration of malonic acid was observed in Holekoppada Appe (186.94 $\mu\text{g/g}$) and the minimum amount was found in Nandgar (13.76 $\mu\text{g/g}$).

The brined fruits of all respective genotypes also showed varied concentration of malonic acid (Table 49b). The average malonic acid concentration in brined fruit was 30.85 $\mu\text{g/g}$ whereas the minimum concentration was noticed in genotype Malange (12.99 $\mu\text{g/g}$) and the maximum content was recorded in genotype Balekoppa Appe (67.01 $\mu\text{g/g}$).

The brine solution also showed significant concentration of malonic acid (Table 49b). The average content was 3.83 $\mu\text{g/g}$ in brine solution whereas it was ranged from 0.95 $\mu\text{g/g}$ (Karpoora Jeerige) to 6.48 $\mu\text{g/g}$ (Naagara Appe).

4.3.3.2.8.6 Pyruvic acid ($\mu\text{g/g}$)

The concentration of pyruvic acid was varied significantly in fresh fruits, brined fruits as well as in brine solution (Table 49b).

The fresh fruits recorded an average content of 23.33 $\mu\text{g/g}$ (Table 49b) of pyruvic acid, while it was ranged from 7.42 $\mu\text{g/g}$ to 53.99 $\mu\text{g/g}$. The genotype Hittalahalli Appe fresh fruits showed the highest concentration of pyruvic acid (53.99 $\mu\text{g/g}$) and the minimum amount was observed in Naagara Appe (7.42 $\mu\text{g/g}$).

The brined fruits concentration of pyruvic acid was varied from 4.68 µg/g to 18.98 µg/g with an average concentration of 10.07 µg/g (Table 49b). The genotype Balekoppa Appe showed the highest concentration of pyruvic acid (18.98 µg/g) and Dannalli Appe recorded the minimum amount of pyruvic acid concentration (4.68 µg/g).

Among brine solution of respective genotypes the highest amount of pyruvic acid was obtained genotype Dannalli Appe (2.04 µg/g) and the minimum amount in genotype Balekoppa Appe (0.36 µg/g).

4.3.3.2.8.7 Shikkimic acid (µg/g)

Significant differences were observed in shikkimic acid concentration of all 15 appemidi genotypes at three different levels. The shikkimic acid content was varied from 227.92 µg/g to 4555.78 µg/g in fresh fruit form, whereas the average concentration of shikkimic acid in fresh fruit was 2017.37 µg/g (Table 49c). The genotype Nandgar recorded minimum amount of shikkimic acid and the maximum concentration was found in Hittalahalli Appe (4555.78 µg/g) which was on par with the genotype Kaana Appe (4451.68 µg/g) followed by Gorana Appe (4084.08 µg/g).

The brined fruits were also showed varied amount of shikkimic acid with an average amount of 783.98 µg/g (Table 49c), whereas the cultivar Dannalli Appe showed very high concentration of shikkimic acid (2485.81 µg/g) followed by Isgoor Appe (2182.85 µg/g) and the minimum amount was noticed in Malange (37.91 µg/g).

In brine solution of respective appemidi genotypes also recorded significant concentration of shikkimic acid (Table 49c), with an average value of 146.13 µg/g. The range was varied from 11.44 µg/g (Appemidi) to 333.09 µg/g (Dannalli Appe).

4.3.3.2.8.8 Succinic acid (µg/g)

In fresh fruits of fifteen appemidi genotypes the succinic acid concentration was varied significantly with an average content of 8.01 µg/g. The range of succinic acid concentration in fresh fruits was varied from 2.35 µg/g to 16.08 µg/g. The maximum concentration was obtained in cultivar Nandgar (16.08 µg/g) and the minimum was recorded in Malange (2.35 µg/g) followed by Isgoor Appe (2.46 µg/g).

The concentration of succinic acid in brined fruits of all fifteen genotypes also showed significant differences with an average value of 4.36 µg/g (Table 49c). The highest concentration was found in Hittalahalli Appe (11.42 µg/g) followed by Karpoora Jeerige (10.23 µg/g) and the minimum concentration was recorded in cultivar Malange (0.11 µg/g).

The brine solution also showed significant concentration of succinic acid (Table 49c). The average amount of succinic acid found in brine solution of appemidi genotypes was 1.08 µg/g whereas the concentration was varied from 0.27 µg/g (Nandgar) to 2.73 µg/g (Dannalli Appe).

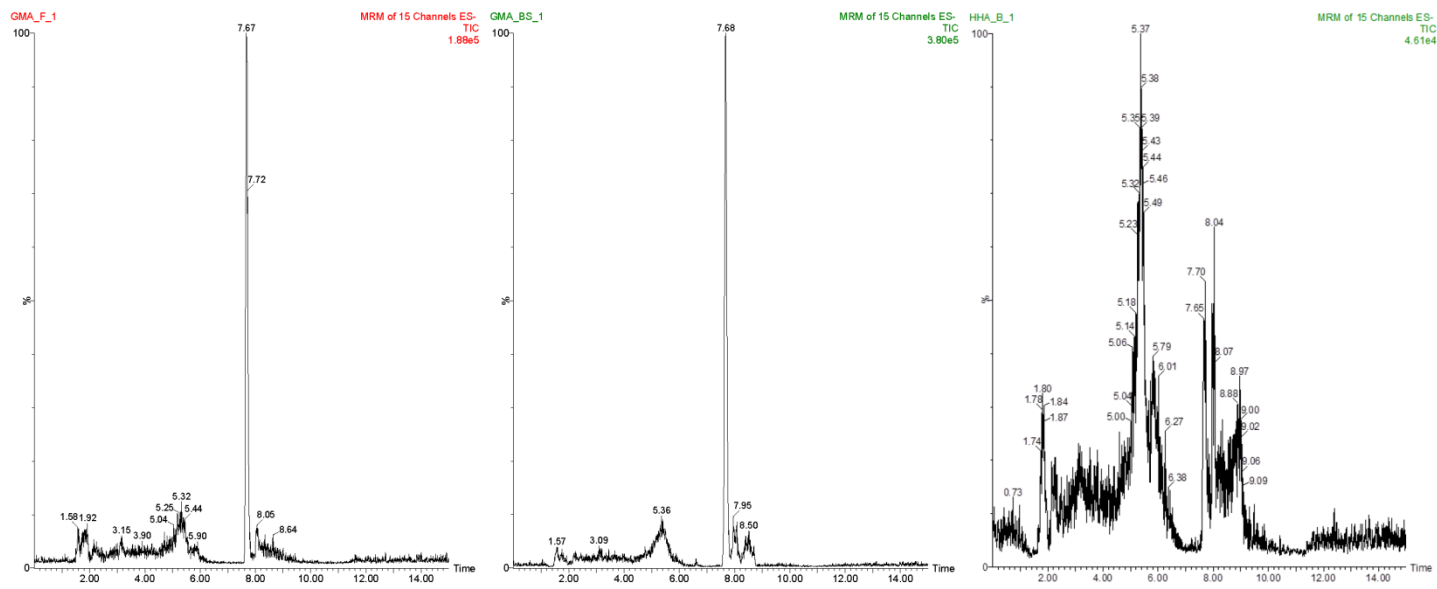


Plate 20: Chromatograms of major organic acids in fresh, brined fruit and brine solution of Appemidi

Table 49a: Major organic acids concentration in 15 Appemidi genotypes at three different forms

Genotypes	Organic acids (micro gram/gram)											
	Citric acid				Fumaric acid				Maleic acid			
	F	B	BS	Mean	F	B	BS	Mean	F	B	BS	Mean
Gurumurthy Appe	235.04	38.36	14.42	95.94	29.67	9.41	1.21	13.43	22.68	7.12	0.94	10.25
Appemidi	301.75	11.41	2.65	105.27	22.38	10.78	0.83	11.33	17.66	8.54	0.33	8.84
Malange	154.66	28.15	3.59	62.13	14.64	4.04	1.31	6.66	6.09	1.86	0.79	2.91
Balekoppa Appe	176.00	64.19	5.68	81.96	18.98	19.46	1.19	12.21	14.97	12.21	0.71	9.30
Naagara Appe	119.00	23.62	2.29	48.30	17.42	8.72	1.00	9.08	4.19	6.94	0.60	2.58
Jeerige	134.46	35.54	3.29	57.76	12.92	8.98	1.00	7.70	8.49	6.35	0.79	5.21
Nandgar	123.30	37.64	1.76	54.23	11.82	6.92	0.46	6.40	5.85	1.75	0.34	2.65
Holekoppada Appe	116.96	48.02	1.75	55.58	60.77	13.03	0.79	24.86	42.23	9.21	0.57	17.34
Ananthabhatta Appe	111.00	81.38	10.95	67.78	19.91	8.04	0.85	9.60	13.48	6.60	0.58	6.89
Gorana appe	170.09	36.95	1.54	69.53	59.34	8.38	0.76	22.83	45.35	1.80	0.48	15.88
Kaana Appe	125.80	15.86	3.30	48.32	16.56	5.02	0.31	7.30	6.29	3.42	0.26	3.32
Hittalahalli Appe	134.24	15.42	2.65	50.77	50.02	15.42	0.40	21.95	33.16	15.42	0.28	16.29
Isgoor Appe	59.90	74.38	3.55	29.28	10.68	8.13	1.15	6.65	5.11	6.35	0.84	3.37
Dannalli Appe	119.14	39.34	5.18	54.55	54.87	8.11	0.45	21.14	40.82	4.78	0.53	15.38
Karpoora Jeerige	63.33	87.04	2.06	30.81	10.20	11.60	0.87	6.78	6.76	3.30	0.59	3.55
Mean	142.98	42.49	4.31	60.81	27.35	9.74	0.84	12.53	18.21	6.38	0.58	8.25

Table 49b: Major organic acids concentration in 15 Appemidi genotypes at three different forms

Genotypes	Organic acids (micro gram/gram)											
	Malic acid				Malonic acid				Pyruvic acid			
	F	B	BS	Mean	F	B	BS	Mean	F	B	BS	Mean
Gurumurthy Appe	71.02	15.81	14.13	33.65	70.72	29.20	4.65	34.86	12.10	9.16	1.07	7.44
Appemidi	103.75	20.20	1.47	41.81	50.49	40.57	4.92	31.99	11.32	11.10	1.38	7.53
Malange	89.97	90.47	3.41	53.95	17.42	12.99	1.12	10.51	13.49	12.76	1.30	8.85
Balekoppa Appe	74.30	62.02	2.14	46.15	45.15	67.01	4.53	28.90	19.25	18.98	0.36	12.53
Naagara Appe	55.65	54.98	4.49	31.71	15.37	23.45	6.48	10.77	7.42	5.45	1.07	4.65
Jeerige	83.59	68.83	9.99	54.14	20.43	34.79	4.58	13.27	14.25	10.73	0.87	8.62
Nandgar	12.50	10.51	1.54	8.18	13.76	22.02	2.79	9.52	26.28	6.27	0.82	11.12
Holekoppada Appe	166.38	25.10	1.14	64.21	186.94	55.20	1.33	81.16	29.40	8.87	0.45	12.91
Ananthabhatta Appe	46.68	16.70	0.47	21.28	64.29	31.65	5.78	33.91	17.76	6.09	1.15	8.33
Gorana appe	144.72	60.03	1.55	68.77	133.90	28.13	2.54	54.86	24.20	8.77	0.46	11.14
Kaana Appe	11.94	10.75	2.25	7.65	53.71	18.16	4.66	25.51	26.93	7.37	0.52	11.61
Hittalahalli Appe	190.27	15.42	1.81	69.17	156.63	15.42	5.80	59.28	53.99	15.42	0.64	23.35
Isgoor Appe	16.73	13.00	1.74	10.49	14.96	24.73	2.72	9.49	20.30	11.96	1.17	11.14
Dannalli Appe	158.61	33.01	3.79	65.14	118.16	39.72	4.55	54.14	42.02	4.68	2.04	16.25
Karpoora Jeerige	16.89	9.68	1.74	9.44	17.99	19.77	0.95	11.79	31.22	13.46	0.43	15.04
Mean	82.87	33.77	3.44	39.05	65.33	30.85	3.83	31.33	23.33	10.07	0.92	11.37

Table 49c: Major organic acids concentration in 15 Appemidi genotypes at three different forms

Genotypes	Organic acids (micro gram/gram)											
	Shikimic acid				Succinic acid				Tartaric acid			
	F	B	BS	Mean	F	B	BS	Mean	F	B	BS	Mean
Gurumurthy Appe	1965.04	117.27	304.41	795.57	6.19	3.83	1.38	3.80	0.14	0.15	0.02	0.09
Appemidi	2380.84	910.32	11.44	1100.87	5.70	5.14	0.82	3.55	0.14	0.19	0.01	0.08
Malange	539.85	37.91	102.35	226.70	2.35	0.11	0.78	1.08	0.32	0.13	0.02	0.16
Balekoppa Appe	1488.56	1793.25	109.36	897.06	5.46	4.65	1.20	3.57	0.20	0.16	0.01	0.12
Naagara Appe	422.42	1643.96	176.24	880.87	4.60	2.22	1.04	2.62	0.11	0.11	0.03	0.07
Jeerige	1461.00	742.33	141.19	781.51	6.89	5.07	2.02	4.25	0.19	0.11	0.02	0.11
Nandgar	227.92	72.99	38.91	113.27	16.08	4.02	0.27	6.79	0.65	0.22	0.01	0.29
Holekoppada Appe	3681.99	491.15	106.29	1426.48	10.80	4.39	0.50	5.23	0.32	0.14	0.01	0.16
Ananthabhatta Appe	534.64	75.15	329.51	313.10	4.50	3.30	0.35	2.72	0.14	0.06	0.01	0.07
Gorana appe	4084.08	112.95	165.84	1454.29	12.14	3.87	1.26	5.76	0.27	0.15	0.01	0.14
Kaana Appe	4451.68	225.72	77.07	1584.82	3.60	2.42	0.45	2.16	0.59	0.09	0.04	0.24
Hittalhalli Appe	4555.78	215.42	83.28	1618.16	13.75	11.42	1.32	8.83	0.24	0.14	0.01	0.13
Isgoor Appe	461.70	2182.85	158.20	867.58	2.46	0.26	1.50	1.41	0.24	0.16	0.01	0.14
Dannalli Appe	3429.58	2485.81	333.09	1749.49	13.13	4.53	2.73	6.80	0.54	0.12	0.01	0.22
Karpoora Jeerige	575.41	652.58	54.74	760.91	12.52	10.23	0.61	7.79	0.70	0.16	0.01	0.29
Mean	2017.37	783.98	146.13	971.38	8.01	4.36	1.08	4.42	0.32	0.14	0.01	0.15
	± SE (m)				CD @ 1%				F TEST			CV
Genotypes	3.116				8.663				*			106.08
Organic acids	2.413				6.71				*			
FBS	9.347				25.988				NS			
G X O	1.393				3.874				*			
G X FBS	5.397				15.004				NS			
OA X FBS	4.18				11.622				*			
G X OA X FBS	16.19				45.013				NS			

Note: G=genotypes, OA= organic acids, FBS= fresh fruit, brined fruit and brine solution, *= significant, NS= non-significant

4.3.3.2.8.9 Tartaric acid ($\mu\text{g/g}$)

The tartaric acid concentration was varied significantly among all the fifteen genotypes (Table 49c). The fresh immature fruits showed an average tartaric acid concentration of 0.32 $\mu\text{g/g}$ and it was ranged from 0.11 $\mu\text{g/g}$ (Naagara Appe) to 0.70 $\mu\text{g/g}$ (Karpooora Jeerige).

The brined fruits average concentration of tartaric acid was 0.14 $\mu\text{g/g}$ and the maximum content was observed in cultivar Nandgar (0.22 $\mu\text{g/g}$) which was on par with Appemidi (0.19 $\mu\text{g/g}$).

The amount of tartaric acid content in brine solution of appemidi genotypes was varied from 0.01 $\mu\text{g/g}$ to 0.04 $\mu\text{g/g}$ (table 49c) with an average concentration of 0.01 $\mu\text{g/g}$. comparatively less amount of tartaric acid concentration was observed in brine solution of all appemidi genotypes.

4.4 Traditional knowledge documentation

An attempt was also made to document the traditional knowledge/ skills practiced by the villagers specifically to appemidi production and processing at the homesteads. It was noted that the local mango landraces occupies a prime position in this regard and is associated with many a rural cuisine of this region. Accordingly the available information collected from the different villages is consolidated and presented below.

ITK associated with appemidi of the region is predominantly /exclusively related to preparation of different products. In all the surveyed areas mango fruits at different stages of maturity find use in many culinary items and preparation of processed products.

4.4.1 Appemidi whole immature fruit pickle (Midi uppinakayi/ Jeerige mavu uppinakayi/ Kaadu mavina uppinakayi)

Preparation of tender fruit appemidi pickle, popularly known as kaadu mavu or Jeerige mavu uppinakayi is a systematic activity taken up by many village households of these regions in which the lead role taken by the elderly women after getting the fruits harvested either from their own compounds or from forest areas or the river banks.

The fruits were harvested in bunches at the marble / premature stage of development. Normally strong flavoured ones are preferred for this preparation since flavour will interfere with the flavour of the final product.

Usually they select the midi (tender fruit) depending upon the sap content, flavour, sap burning period and also they see thickness of the peduncle. According to the ITK practice the selection of fruits for whole tender mango pickle preparation were based on the following criterias,

- if the sap burns for longer period,
- if the thickness of peduncle is more means the sap content is also more,
- if the flavor of midi (tender fruit) is dominant and
- if the sap content is higher

Then those midis are the best ones for pickle preparation and also they can be kept for longer period without any contamination.

After harvesting, the fruits were separated from the bunch retaining half or one centimetre stalk along with the fruit, so as to allow some sap in the retained stalk. The presence of sap is supposed to act

as preservative during storage. Fruits are washed in water thereafter, and thoroughly wiped off with cloth to remove completely the adhering moisture. Further, the fruits are placed on a cloth spread on the floor.

Salt should be added in layers. Half the quantity is spread at the bottom and intercepting layers and the remaining half is spread at top layer. Normally vessels with narrow mouth are used so as to have a minimum space exposed to the air at top, which will also reduce spoilage.

After 10-15 days due to the osmotic release of water from the fruits, all the fruits get shrunken and get immersed in the salt water mixture. These fruits are taken out with a wooden ladle and entire contents are transferred to wide flat basin like vessel. The fruits are sorted thereafter to cull out the spoiled ones.



Plate 21: Containers used for preparation and preservation of Appemidi pickle



Plate 22: ITK followed in tender mango pickle preparation in western ghat regions of Chikmagalur

Good shrunken fruits were used for final product preparation. Chilli powder and mustard powder were added in the right proportions. The chilli powder is prepared after removing the stalk and seeds – a practice further enhancing the storage life of the final product.

The contents are then transferred to narrow mouth containers locally called as Bharani or Uppinakayi Jaadi. After transferring to the container mango leaves are spread on the top surface or keep it in dark room. White cloth soaked in gingelly oil is also used for this purpose. Such oil coated material will provide insulation for the product from the outer air space. As far as possible the container should be filled up to the brim. The mouth of the container is then closed with a wooden lid. The lid will be tightly fastened with a dry cloth and there after the top will be protected with mud caps. The prepared product can be stored without spoilage and quality loss, for a minimum period of three to five years.

4.4.2 Other recipes of appemidi

1. **Appehulli or juice:** The ripened appemidi fruits are used for this recipe. The thin curry made out of this fruit is called as appehuli which act as a refreshing drink in summer by providing sufficient amount of vitamin C which also gives a relaxed cool feeling.
2. **Appemidi sambar/ curry:** A curry is prepared out of ripened appemidi fruits, which can be consumed by mixing with rice during the meal. Because of the flavour and aroma it has been popularised in the household and it also increase the appetite.
3. **Cut mango pickle;** the matured appemidi fruits were cut into small pieces and were used for pickle preparation following the same practices of tender mango pickle. But they are not kept in salt for longer period as that of whole tender fruits.
4. **Dhideer uppinakayi:** Appemidi fruits are peeled out and cut into small pieces. The boiled and cooled salt water is used to dip the cut pieces of fruits. Later jeera, mustard, chilli powder was mixed properly by giving a light fry. Methi powder was also added it is optional.
5. **Mavinakayi gojju:** in this recipe the whole fruits are cooked in water till it get smoothen later upper peel was separated and the fruit pulp was smashed. In a pan fry little amount of cutlery, dill, peeper and grind it, later fry with oil add mustard, curry leaves, green chilli, fruit paste and masala paste add some jaggery and salt according to the taste.
6. **Mavinakayi chutni:** peeled out the appemidi fruit is used. Whole fruit is scraped or cut into thin layers, coconut, green chilli, salt was ground into paste.
7. **Appemidi fruit juice:** the appemidi fruit juice was extracted and little salt is added and preserved in a container or bottle for a year and can be used for many other culinary preparations.
8. **Mavinakayi chitranna:** For the same chitranna recipe instead of lemon or tamarind here they use scraped mango pieces or mango juice.
9. **Containers used for preservation of pickle:** Glass containers or Ceramic containers popularly known as Bharani (Uppinakayi Jaadi).
10. **Oil used:** Sunflower oil, Mustard oil and Coconut oil.

Not only the listed recipes in western ghat regions particularly in mango season they add mango fruit for most of the recipe and increase its taste and flavour.

4.4.3 Conservation

Conservation of our genetic diversity plays an important role, therefore in this study, ten unique genotypes scion samples were brought and grafted. These plants were conserved in germplasm block of IIHR.

As a part of exsitu conservation, pollen samples of 28 surveyed Appemidi genotypes were conserved *invitro* at -196°C cryo bank of IIHR.



A

B

C

Plate 23: ITK followed to select best Appemidi fruits for pickling. A&B= Fruits with thick petiole and more sap content, C= Sap burning test



Plate 24: Appemidi fruit sap collection

V. DISCUSSION

Conservation and maintenance of species and its landraces as a part of the cultural heritage of a region or country is yet to be given a serious thought by crop breeders and horticulturists worldwide. Landraces of a geographical region are the unique symbols which in many ways are comparable with monuments, regional dresses, and folk songs - a few telling examples of the regional or local cultural heritage (Zeven, 1998).

Western Ghats region of Karnataka is rich in the diversity of indigenous mango genotypes especially unique pickle type mango which is commonly called Appemidi type of mango. Keeping this in view, the present investigation entitled “**Studies on Diversity of Indigenous Mango Genotypes**” was conducted at ICAR-IIHR, Bengaluru, during 2013-2014 and 2016-18.

An attempt was made to study the diversity of indigenous mango genotypes with special reference to Appemidi genotypes from Western Ghat regions of Chikmagalur District, Karnataka, by conducting a survey. The surveyed areas are the habitat of aromatic pickle mango, spreads its diversity near river banks and forest areas. Using GPS waypoints the survey was conducted and the locations were marked to visualize the results in clear maps. Molecular markers such as SSRs were used in order to complement the morphological data and to throw more light on the genetic diversity of Appemidi. Profiling of major volatile compounds which are responsible for unique flavour was done to identify those aroma fragments from leaves and was compared with the volatile profile of fruit sap. An attempt was also made to profile the major nutraceuticals present in Appemidi fruits.

5.1 To study the variability in various morphological characters of indigenous mango

Morphological characterization is the simplest and formal standardised 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).

In the present study, various morphological characterizations were done for 40 Appemidi genotypes and they were described systematically as per the standard descriptor for mango by IPGRI (2006) comprising of leaf, inflorescence, fruit and other parameters. The tree growth observed were erect, spreading and drooping in habit which is in conformity with the observations of Simi (2006). The locations were marked by GPS and the area map was drawn which are showing the diversity of Appemidi (Fig 7).

5.1.1 Leaf characterization

In the present study, leaf characters *viz.*, orientation of leaf shape, tip, leaf margin, texture, color of young flush, fragrance, leaf venation, leaf length, width, petiole length etc. were studied among the 40 surveyed genotypes. The leaf orientation was horizontal and semi erect type with medium venation and coriaceous texture. Leaf shape was predominantly oblong type, with wavy margins. Majority of genotypes showed light green color of young leaves with dark green mature or fully developed leaves with mild fragrance and some of the genotypes leaves were high in aroma. Simi (2006) also reported that lanceolate type of leaf shape and acuminate character of leaf tip and light green with brown tinge colour of young flush predominantly appeared in traditional mango types of southern Kerala. Naik and Gangolly (1950) used leaf characters as secondary and tertiary characters for varietal identification

The leaf length ranged from 18.77 cm to 31.31 cm and the maximum leaf length and leaf width was recorded in Kanathi-3 (31.31 cm and 7.57 cm respectively). In a study carried out by Laroussihe (1980), the leaf characteristics for other cultivars of mango, with leaf length ranging from 150 to 400 mm and leaf width ranging from 15 mm to 40 mm. The leaf length/width ratio had an average of 4.37. Mature

leaves were considered coriaceous in texture, free of pubescence and presented a mild fragrance when crushed.

Petiole length also varied significantly with a mean of 3.06 cm while, the petiole length ranged from 1.73 to 4.97 cm. The majority of accessions showed petioles smaller than 4 cm and no petioles larger than 8 cm were found in a study carried out by Carla *et al.*, 2013. Leaf area varied significantly among forty genotypes, the mean leaf area was 139.32 cm² and the maximum leaf area was recorded in genotype Kanathi-3 (236.34cm²). Thus, the leaf characterization in the present study revealed that, leaf characters would also contribute towards the genetic divergence among the indigenous Appemidi genotypes based on the variability present among the genotypes studied.

5.1.2 Inflorescence characterization

The inflorescence characters were predominantly terminal in position, pyramidal in shape of inflorescence, light green in colour and with densely flowered panicles. Simi (2006) also reported dominance of terminal inflorescences, pyramidal shape of inflorescence and densely flowered panicles in southern Kerala. Jyothi *et al.*, (2009) also reported more than 70 per cent of the mango trees had broadly pyramidal inflorescence and are densely flowered which give them a bunch like appearance.

The maximum Inflorescence length was recoded in genotype Agraphara (22.78 cm) and inflorescence width which ranged from 8.0 to 15.82 cm and the mean inflorescence width 11.37 cm. The maximum inflorescence width was observed in genotype Shantipura (15.82 cm). According to Timmappaiah and Suman (1987), length of panicle varied from 11.25 cm to 42.20 cm. Simi (2006) also reported 12 cm to 41 cm of inflorescence length.

The mean flower diameter 0.51 cm was observed and it ranged from 0.47cm to 0.80 cm. The maximum flower diameter was observed in genotype Agraphara (0.80 cm). The peduncle length ranged from 4.37 to 6.60 cm, the maximum peduncle length was observed in the cultivar Kumrumane-1 (6.60 cm) whereas the peduncle width ranged from 0.53 cm to 0.73 cm and the maximum peduncle width was observed in the cultivar Agraphara and Gonibeedu-1 (0.73 cm).

The variability present among the indigenous genotypes with respect to inflorescence characteristics indicated the presence of genetic variability in terms of inflorescence length and width. Thus, these characters could play a major role in fruit setting *viz.*, number of hermaphrodite flowers, male: hermaphrodite flowers ratio, bearing habit and per cent fruit set. Hence, diversity found among these characters in the genotypes studied could be an alternative to fruit characteristics to study the variability. Similar work has been done by Yadav and Singh (1985), who evaluated mango cultivars, based on these characters and classified Indian mango cultivars into north and south ecotypes.

5.1.3 Fruit characterization

Fruit characteristics have been the major descriptors for identification of different varieties of fruit crops (Toili *et al.*, 2013). Variability in mango types showed 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. Presence of beak, fruit size, sinus, cavity of stalk insertion is also important for studying variability in mango germplasm (Ram and Rajan, 2003). Fruits have been exclusively used as the major descriptors for identification of different varieties of fruit crops (Toili *et al.*, 2013).

In the present study different fruit shapes were observed, *i.e.* round, elliptic, obovoid, ovoid and oblong. Simi,(2006) observed four types of fruit shapes such as round, oblong, ellipsoid, and oblong ellipsoid. NavPrem, (2014) characterized nine sucking mango genotypes and observed fruit shapes such as ovate, oblong and ovate oblong.

The fruit length ranged from 3.40 cm to 5.65 cm and the maximum fruit length was recorded in Chimankudige-3 (5.65 cm). Significant variations in length and width of fruit (cm) were noted in 30 mango varieties evaluated by Singh *et al.* (2011). The higher length of fruit was noticed in Mallika, Fazli. Hammedunnisa Begum *et al.* (2014) assessed 30 accessions of 'Cherukurasam' (CKR Acc-I to CKR Acc-30) and reported that fruit length ranged from 7.60 to 14.00 cm with an average of 11.20 cm. Dinesh (2004) evaluated 130 mango varieties for fruit characteristics and reported that Maharaja Pasand, Sora and Tenneru had higher fruit length (>20 cm). It was found to be least in the varieties Chandrakaran and Pacharasi (<6 cm).

The fruit diameter differed significantly with a mean of 3.00 cm while fruit diameters ranged between 2.22 cm to 3.92 cm among the cultivars studied. Maximum fruit diameter was recorded in Arenuru-1 (3.92 cm). Hammedunnisa Begum *et al.* (2014) assessed 30 accessions of 'Cherukurasam' (CKR Acc-I to CKR Acc-30) and reported that the fruit width ranged from 5.40 to 8.00 cm with an average of 6.31 cm. Dinesh (2004) evaluated 130 mango varieties for fruit characteristics and reported that the varieties Sora, Tenneru and Maharaja Pasand also had higher fruit breadth.

The mean fruit thickness was 2.33 cm, while it ranged from 1.52 cm to 2.95 cm. The maximum fruit thickness was recorded in Agraphara (2.95 cm). The fruit weight of mango cultivars studied in the present investigation ranged between 12.22g to 21.12 g with a mean fruit weight of 16.33 g. Significantly maximum fruit weight was recorded in Arenuru-2 (21.12 g) followed by Soppinakadu-2 (21.09g). Vasugi *et al.*, (2008), evaluated the unique pickle types and reported that fruit weight ranged from 17.43 g in Kanappe-1 to 191.75g in Gaddalalli Appe which is attributed to the inherent nature of different accessions and are heritable under all environments. Similarly, Simi (2006) reported variability in fruit size, length and width ranged from 37.5 g to 826 g, 4.4 cm to 18.1 cm and 3.9 to 12 cm respectively.

The variability pertaining to fruit characters in the present study indicated the high genetic divergence and fruit diameter contributed maximum (13.00%) towards total divergence followed by fruit weight (11.20 %). Such divergence present in terms of fruit size, sinus, cavity of stalk insertion and presence of beak as studied by the other authors indicated the variability present in the mango germplasm (Ram and Rajan, 2003) as well as used to differentiate cultivars in different fruit crops (Toili *et al.*, 2013). In addition, fruits shape indicated the presence of variability and contributed significantly towards genetic divergence among the 40 genotypes studied and most of the genotypes are oblong (30) and round (10) shape.

Sap burning is one of the traditional practices being followed to select the best tender fruits for pickle purpose. Therefore we also carried out sap burning test for surveyed fruit samples. Out of forty genotypes, the mean sap burning time was 18.82 seconds, the maximum was recorded in the cultivar Kudige (28.25 seconds) (Fig 6). However, a positive correlation was observed among the fruit shape and sap burning time in the present study. Sap from the oblong shape fruits merely burned for a longer time compared to other shapes. As indicated in the present study, the cultivars, Kudige, Arenuru 1& 2 and Kanathi are being routinely used by the local communities for pickling and these cultivars exhibited the oblong fruit shape and maximum sap burning time. Hence, fruit shape could be one of the important pre-selection criteria for selecting tender fruits for pickle purpose.

5.1.4 Pollen morphology and germination studies in Appemidi

5.1.4.1 General pollen morphometry of surveyed Appemidi samples

Pollen grains of all investigated *Mangifera* species were symmetric, isopolar, monad, trizonocolpate (three marginal colpi are present) and relatively small in size (Fig 8). In polar view pollen grains were subtriangular and in equatorial view it was elliptical. The mean length of the polar axis varied from 42.19 μm to 59.92 μm and the equatorial diameter from 19.59 μm to 27.70 μm . The shape of the pollen grain varies among the genotypes selected for this study. These results were in confirmation with the earlier works carried out by Bhattacharya *et al.* (2009), Singh (1954); Randhawa and Damodaran,

(1961). Pollen grains have several common features, including being prolate or perprolate; however, they differed in exine ornamentation (Yang *et al.*, 2015).

The length of the pollen along polar axis was recorded for all the genotypes, varying from 42.19 μm to 59.92 μm . The maximum pollen length was observed in genotype Arenuru-2 (59.92 μm). Significant differences were noticed for equatorial width of the pollen grains studied with an average diameter of 23.14 μm . The maximum equatorial diameter was recorded in Marebilu-3 (27.70 μm) followed by Arenuru-2 (25.67 μm).

Surveyed samples showed significant differences for colpi length. The average length of the colpi was 40.47 μm , while the maximum length was observed in Heruru -2 (48.60 μm) followed by Kudige (48.40 μm). Colpi per cent also showed significant differences among the genotypes studied with an average per cent of 80.02, whereas the highest per cent of colpi was noticed in Heruru-2 (98.34%) (Fig 11).

Majority of the surveyed genotypes recorded P/E ratio more than two, which decides the shape of the pollen grain. 2.66 was the highest P/E ratio recorded in Bynduru and Elemadlu-1. Depending upon the P/E ratio the shape of the pollen grains was perprolate in shape ($P/E > 2$) where as Chimakudige-3, Kamanmori-1&2, Gonibeedu-4. Marebylu-3, Heruru-1 and Soppinakadu-3 were having prolate type of pollen shape with a P/E ratio of ≤ 2 . Similar reports on pollen characters were given by Renisha (2012).

The significant differences among the surveyed genotypes for mean pollen shape values were depicted in table 11, with an average value of 1.17 mm. The maximum was recorded in Arenuru-2 (1.54 mm) followed by Kudige (1.40 mm).

Pollen grains showed striate to reticulate type exine sculpture with perforations in the grooves (Fig 10) and these results were in confirmation with the earlier works carried out by Bhattacharya *et al.*, 2012, Singh, 1954; Randhawa and Damodaran, 1961. Pollen grains have several common features, including being prolate or perprolate; however, they differed in exine ornamentation (Yang *et al.*, 2015). The major evolutionary trend of exine sculpture is postulated to be from reticulate through regulate to striate- reticulate or vice versa, in Alstroemeriacese reported by Agesen and Sanso (2003). There is an existence of a general relationship between pollen morphology and pollen vectors which was suggested by Wood house (1935), that the reticulate exine sculpture might represent the mode of pollination.

5.1.5 General pollen morphometry of Appemidi samples from ICAR-IIHR germplasm

The length of pollen grain observed among the Indian mango cultivars were ranged from 25.3 μm to 28.3 μm . Singh (1961), Parveen and Qaiser (2010) noted that the size of the pollen in the family Anacardiaceae ranges of 22.5 μm to 32.31 μm . The mean length of the polar axis varied from 31.66 μm to 56.07 μm and the equatorial diameter from 17.15 μm to 36.27 μm . The mean P/E ratio observed was 2.07 (Fig 11). The findings were in association with the results obtained by Mukherjee, 1950 and Singh, 1954. They also observed the genus *Mangifera* has an average length of polar axis 31- 46

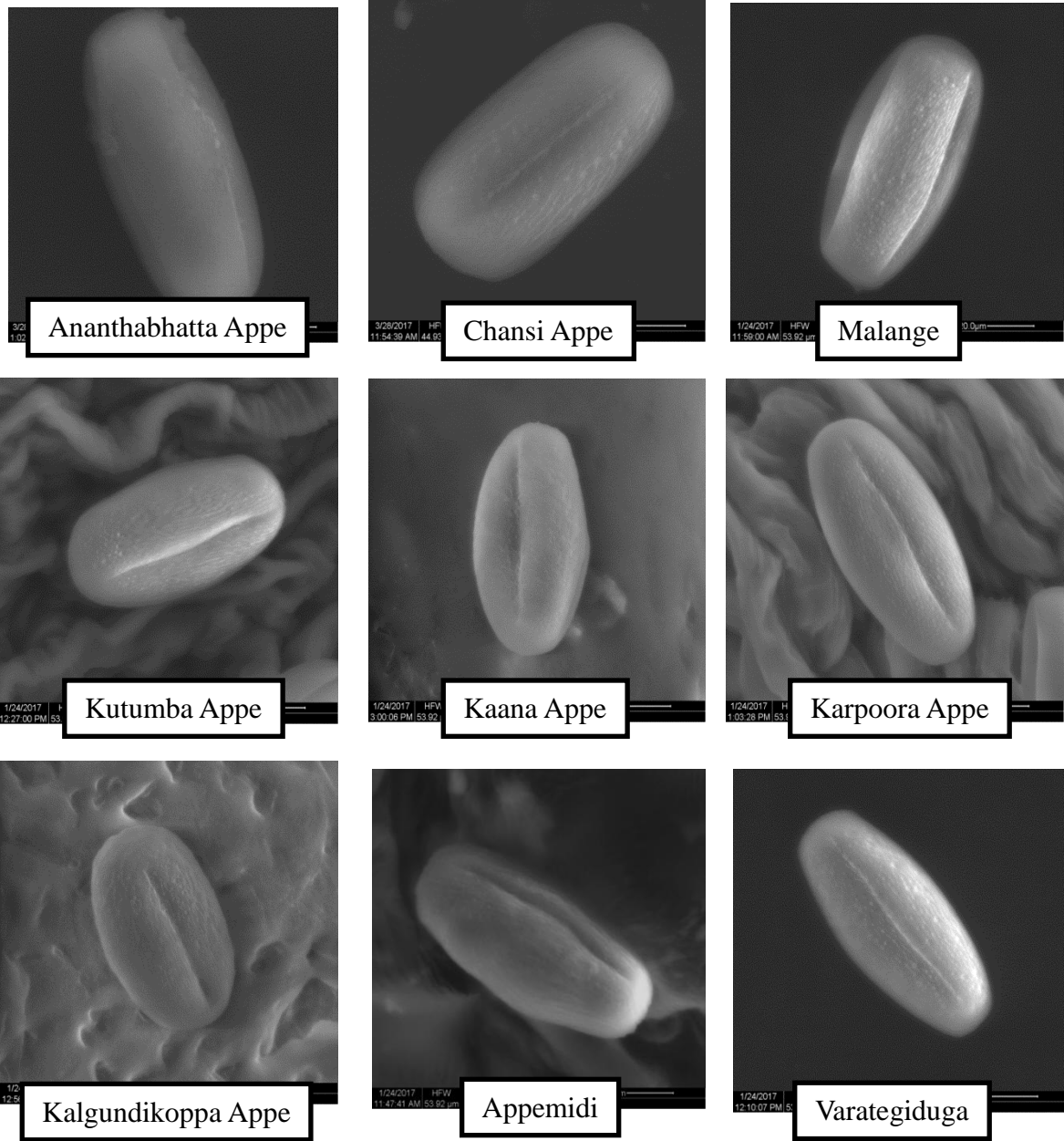


Fig. 11: Pollen morphometry and exine ornamentation of Appemidi genotypes

μm (Mukherjee, 1953) and the average size of pollen grains of mango cultivar Langra and Dashehari as 27.3 and 28.11 μm respectively.

The exine ornamentation is thick, striate to reticulated type, were separated by grooves and we could see large perforation on the outer surface of some genotypes. These results were in confirmation with the earlier works carried out by Bhattacharya *et al.*, 2012, Singh, 1954; Randhawa and Damodaran, 1961. The measurements of pollen grains showed a clear variability in most of the morphological traits.

Majority of the analyzed cultivars in the present study, the colpi per cent and equatorial diameter contributed significantly towards the genetic diversity among the indigenous genotypes studied. Based on the pollen morphological characters, the genotypes are grouped into 2 different main clusters and are similar to morphological characters based grouping of genotypes. A positive correlation can be arrived among the pollen morphological characters and fruit morphology. The fruits exhibiting oblong shape showed perprolate type of pollen and round shape with prolate type indicate the similar way of differentiation of genotypes as like fruit morphological characteristics in majority of the genotypes. Hence, pollen morphological characters could be used as an alternative tool to assess the genetic diversity and to differentiate the cultivars. These results are in corroboration with Bhattacharya *et al.*, (2013), Singh, (2004); Randhawa and Damodaran (1961) and Yang *et al.*, (2015) who differentiated the genotypes and grouped into different clusters based on pollen morphological characteristics.

5.1.6 Genetic variability studies for morphological characters of surveyed Appemidi genotypes

Among the morphological characters studied, high phenotypic coefficient of variation (32.11) was observed in petiole length followed by leaf width (24.22) and similarly, high genotypic coefficient of variation was observed in petiole length (27.89) followed by inflorescence length (21.63) indicating a higher degree of genetic variability among different genotypes for these characters. Besides, for all the characters phenotypic component of variation was higher than their respective genotypic variation indicating that, phenotypic variability is a reliable measure of genetic variability. In this study the estimates of PCV for most of the characters were found to be slightly higher than the GCV. This shows the influence of environment to certain extent. But the difference between PCV and GCV was narrow showed that the presence of sufficient genetic variability can be exploited.

These results are in consonance with the findings of Yadav *et al.* (1995), Attri *et al.* (1999), Singh (2002), Simi (2006), Rathod (2007) and Rajan *et al.* (2009) who found high phenotypic coefficient of variation compared to genotypic coefficient of variation for most of the traits studied and indicated the presence of additive gene action in the inheritance of these traits.

High GCV was observed for the characters petiole length, inflorescence length, inflorescence width and leaf length compare to other parameters. However, GCV alone is not sufficient for the determination of amount of heritable variation. Therefore, heritability in a broad sense was estimated for all the characters.

Heritability is the transmissibility of the characteristics from parents to off-springs. In the present study, high heritability estimates were observed for all the traits which ranged from 29.98 to 91.00 per cent. High heritability for different traits and closeness of their genotypic and respective phenotypic variation indicated that, reliable selection could be made for these traits on the basis of phenotypic expression being less influenced by environmental effects. Hence, in the present study, traits namely leaf width (73.76%), inflorescence length (91.00%), peduncle length (62.92%) and fruit thickness (69.44%) could be used for direct selection as these traits are less influenced by environment with high heritability. Similar results were obtained by Yadav *et al.* (1995), Attri *et al.* (1999), Singh (2002), Simi (2006), Rathod (2007) and Rajan *et al.* (2009) in different mango genotypes.

Although estimates of heritability were 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. 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. The highest values of genetic advance as per cent means were obtained for petiole length (49.90), inflorescence length (42.50) and fruit weight (23.87) with rest of the traits exhibiting near moderate values indicating that the influence of environment on expression of these characters to certain extent in rigid selection might bring about improvement in these characters.

In the present study, the traits with moderate to high PCV, GCV and genetic advance and high value of heritability in traits such as inflorescence width, peduncle length and fruit thickness revealed the low influence of environment, so that fairly rapid genetic gain could be expected for these traits.

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. Leaf length was significantly and positively associated with leaf width, petiole length, peduncle length both at genotypic and phenotypic levels. Fruit thickness was significantly associated with peduncle length, fruit length and fruit width. A significant negative correlation was observed for fruit weight with leaf length, petiole length, inflorescence length and width.

At phenotypic levels also strong positive and significant association of petiole length with leaf length, leaf width, inflorescence length and fruit length was observed. Fruit thickness was having a strong positive and significant correlation with peduncle length, fruit length and fruit width. 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 maximized their expression at phenotypic level. Non-significant association was also observed for the traits studied. In the earlier studies carried out by Biswas *et al.*, (2007), Olsen *et al.*, (1985) and Mir *et al.*, (2009) also observed significant positive association of the morphological characters they studied mainly with fruit yield.

Therefore the present study illustrated that existence of wide range of variations for most of the characters among Appemidi genotypes provide opportunities for genetic gain through selection. Leaf length, inflorescence length and fruit thickness showed strong significant correlations with most of the characters studied. Thus selection may be possible for these characters. The thickness of the fruit plays an important role in Appemidi whole tender mango pickle preparation as well as for consumer preference. Therefore this trait can be considered as an important selection criteria.

5.1.7 Genetic divergence study

For any successful breeding programme, presence of genetic diversity among the parents is the utmost important, since the crosses made between these parents are more likely to yield desirable recombinants in the resulted progenies. However, it is desirable to select suitable and genetically divergent parents, based on genetic variability and genetic diversity present among the available germplasm (Singh, 1998).

Among the characters studied, fruit diameter had a maximum contribution (13.0%) to the total diversity by ranking first in 101 times out of 778 combinations, followed by fruit weight and inflorescence diameter (11.20% with 87 times ranked first) (Fig 12). The characters *viz.*, fruit length and petiole length contributed 10.80% and 10.70% to the diversity. Similar findings with regard to contribution of fruit weight towards divergence were reported by Majumder *et al.* (2013), Barholia and Sangeetha (2014) in mango. The character which contributed the maximum to the divergence should be given greater emphasis for selection (Jagadev *et al.*, 1991).

Among the pollen characters studied Colpi per cent showed the maximum per cent contribution of 21.88 per cent towards total divergence by appearing 86 times first rank out of 393 different combinations followed by equatorial diameter which contributed 19.85 per cent and colpi length which

contributed 16.79 per cent towards diversity (Fig 13). Hence, either the leaf, inflorescence and fruit morphology or pollen morphological characters which contributed maximum to the divergence can be given greater emphasis for selection in breeding.

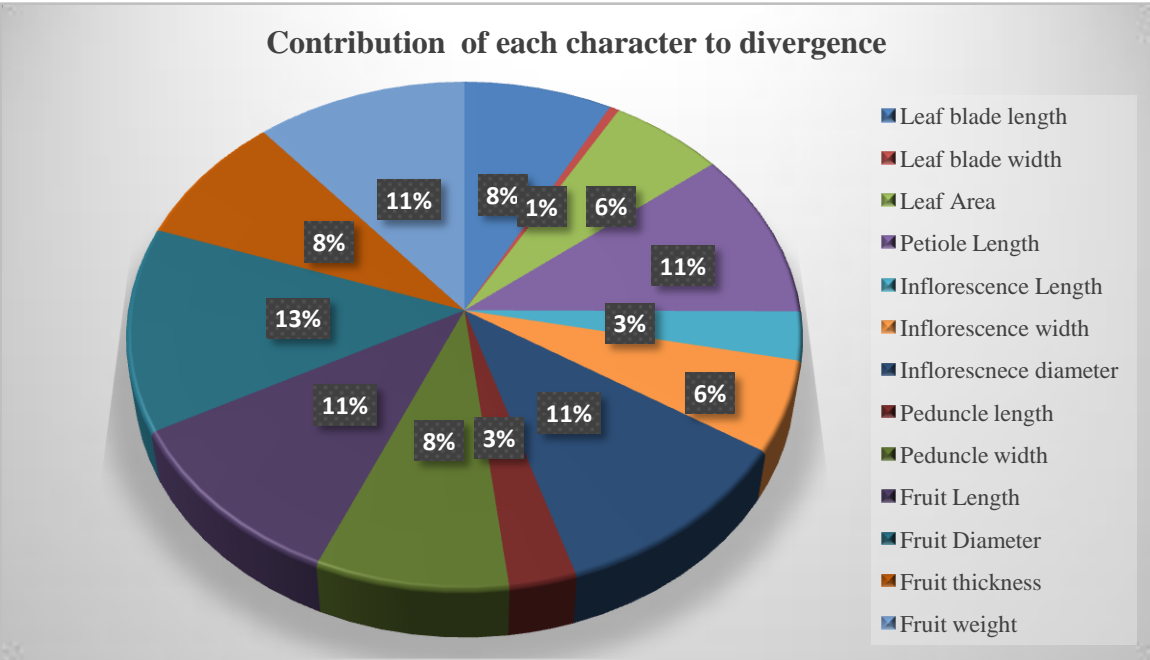


Fig. 12: Contribution of each morphological character of surveyed Appemidi genotypes towards divergence

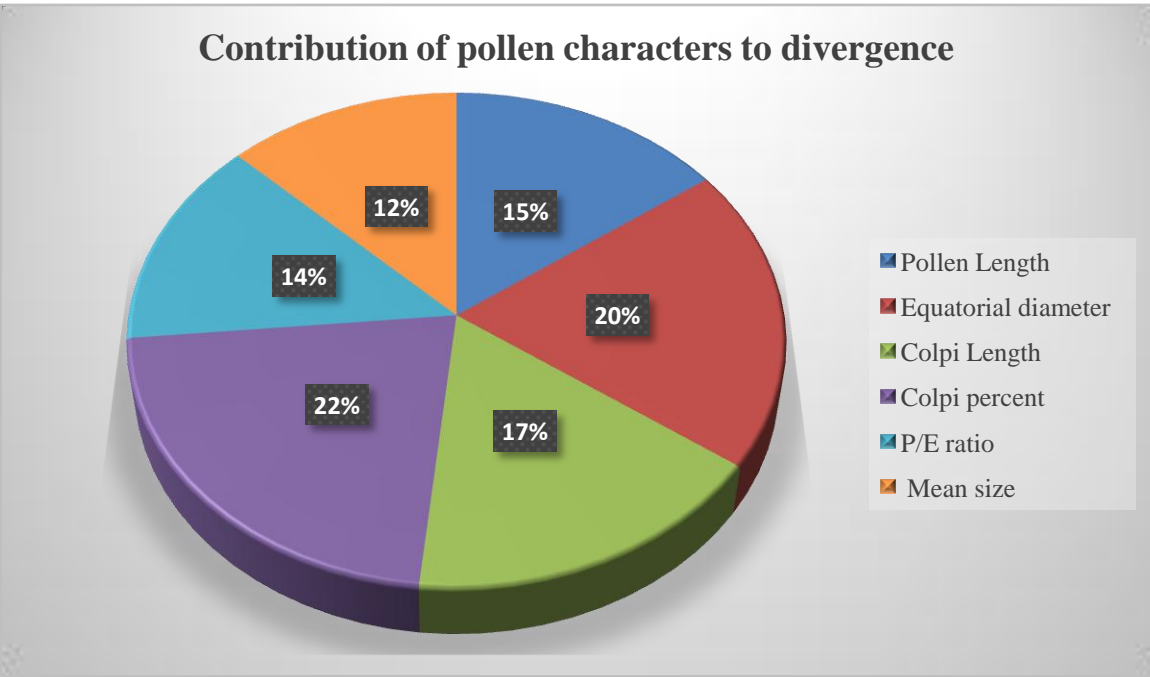


Fig. 13: Contribution of pollen morphological characters of surveyed Appemidi genotypes towards divergence

5.1.8 Grouping of cultivars into different clusters (D^2 analysis)

The forty genotypes based on morphological characteristics and twenty eight genotypes based on pollen characteristics were grouped mainly into three clusters and these were further grouped into six important sub clusters (Fig 14 & 15). Each main cluster consists of two sub clusters. The formation of different solitary clusters might be due to the fact that, geographic barriers prevented the gene flow or intensive natural and human selection for diverse and adoptable gene complexes which must be responsible for this genetic diversity. The genotypes were grouped into different clusters irrespective of its geographical origin in which they were located or place of collection. The clustering pattern of the genotypes revealed that the genotypes collected from the same region did not fall in a single cluster which indicated variability in the genotypes irrespective of their place of collection. 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 formation of large sized table fruit cultivars into a cluster were reported by Kumar *et al.* (2006) and Rathod (2007) in mango. 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.

5.1.8.1 Mean intra and inter cluster distances

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 distant clusters for getting highly heterotic crosses which are likely to yield wide range of segregants on which selection can be practiced.

The inter cluster D^2 values ranged from 18.428 to 147.997 indicating the presence of substantial amount of genetic diversity in the genetic material. The highest inter cluster D^2 value was recorded between clusters 2 and 4 (149.997) while the lowest inter cluster D^2 value was recorded between clusters 5 and 1 (18.428), the highest inter cluster distance indicted the presence of wide genetic diversity among the cultivars.

The mean intra and inter cluster distances with respect to pollen characters studied showed maximum D^2 value between cluster 2 and 3 (23.090) whereas the minimum D^2 value was observed between the cluster 3 and 1 (9.130). This suggested wide genetic diversity between the cultivars of these clusters.

5.1.8.2 Comparison of cluster means

A comparison of cluster means for the different characters presented in the table showed that the Cluster I comprising of (2) genotypes had a high value for peduncle width (0.73 cm), fruit length (4.74 cm) and fruit thickness (2.44 cm). The second highest values were for inflorescence length and fruit diameter. The cluster-2 consisted of 7 genotypes with the extreme mean values for the character inflorescence length, inflorescence width and highest values for flower diameter. The cluster-III shared maximum number of genotypes (12) had only second extreme value for fruit length and fruit weight. Cluster-IV had highest mean value for the trait fruit weight. Whereas cluster V consisting of 8 genotypes which are contributing more for leaf length, leaf width and leaf area. Cluster VI consisting of only 5 genotypes had a second extreme value for leaf length, leaf width, leaf area, inflorescence width and highest mean value petiole length. Similar results were also noticed by Ismail (2008) in case of lemon. Cluster V and III had the lowest mean value therefore they were expressing secondary highest value for the characters studied.

5.1.9 Principal component analysis

PCA 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.

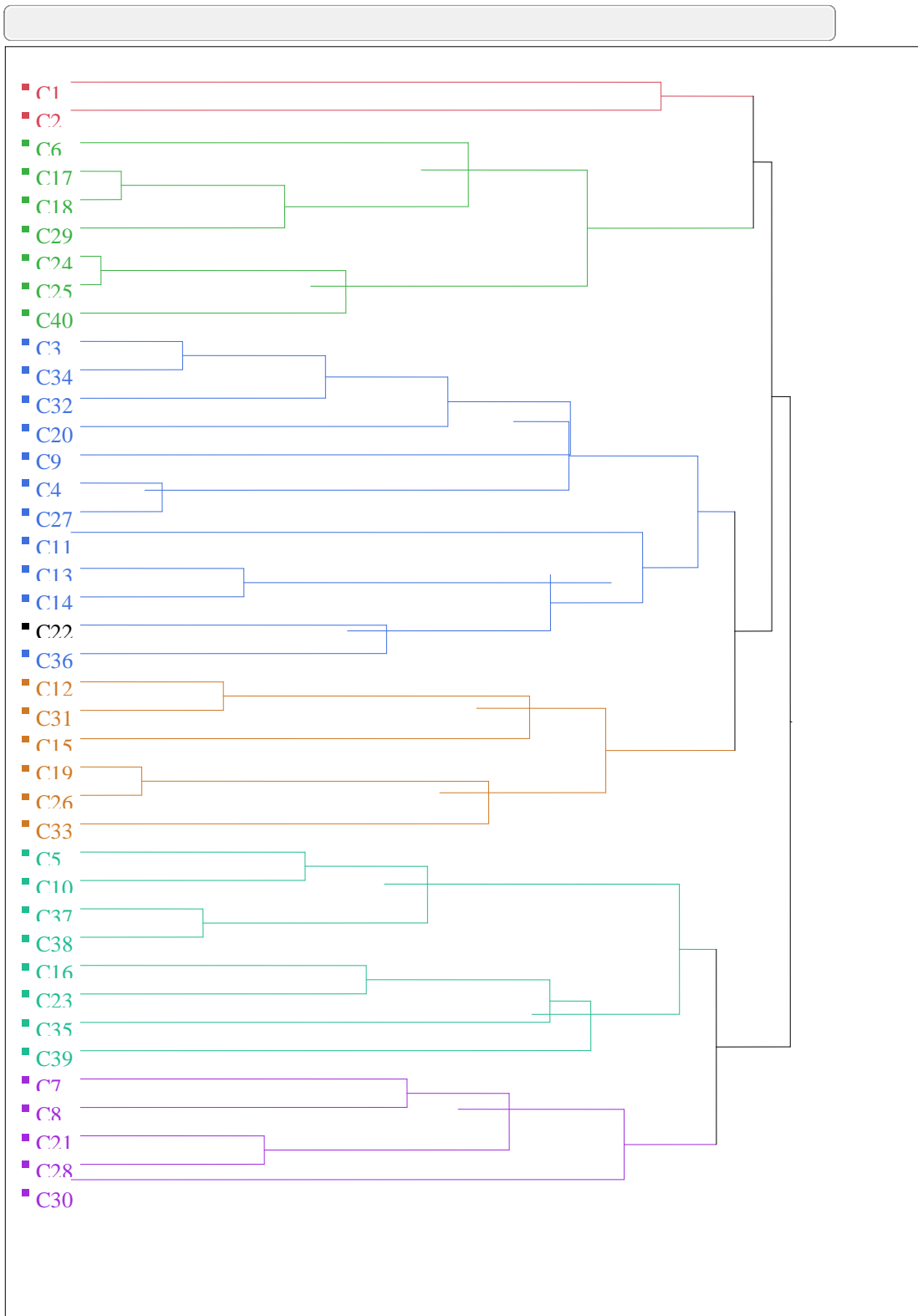


Fig. 14: Cluster analysis based on morphological parameters of surveyed Appemidi genotypes using Ward's method

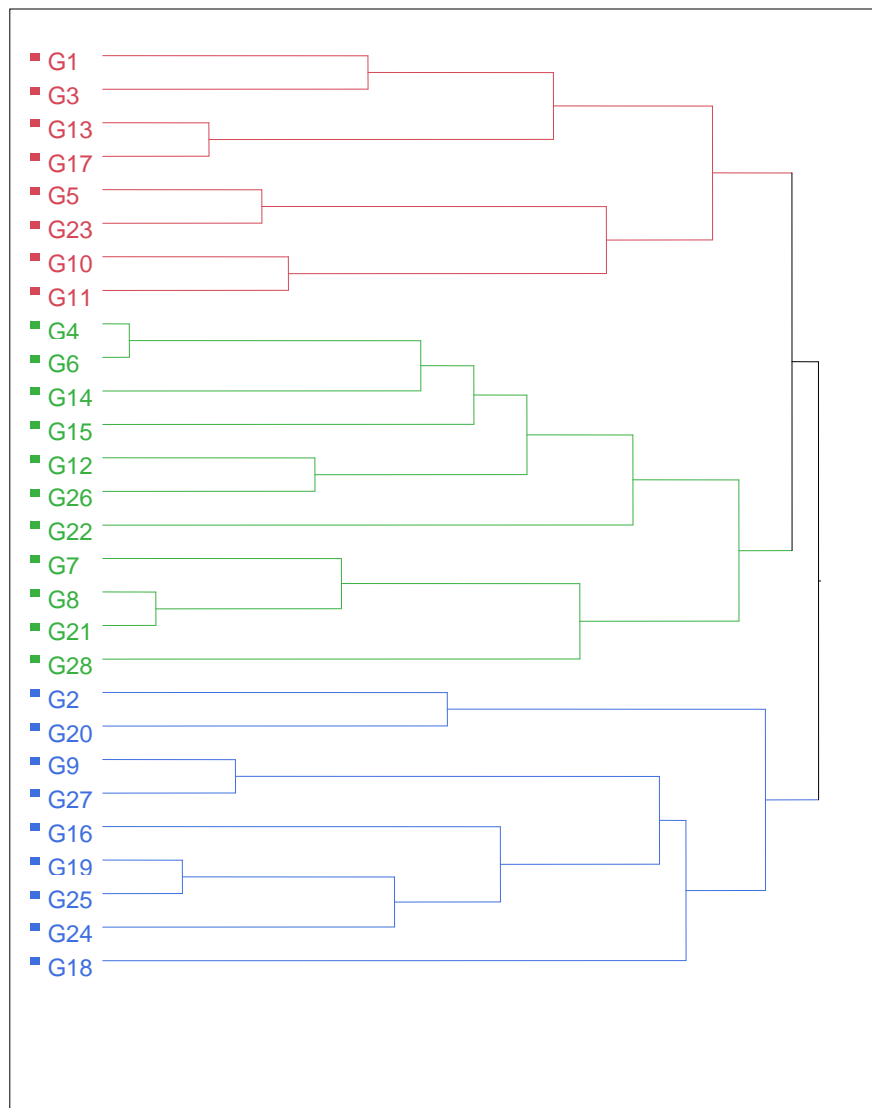


Fig. 15: Cluster analysis of pollen morphological parameters of surveyed Appemidi genotypes using Ward's method

The principal component analysis sorted only significant principal components out of 13 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 facilitates the in-depth analysis for genetic diversity.

The pattern grouping obtained by cluster analysis was confirmed by PCA by dividing it into six important principal components contributing more towards variability which were having eigen value more than 1.

In D^2 analysis, fruit diameter followed by fruit weight, inflorescence diameter, fruit length and petiole length contributed maximum towards diversity, while in PCA the characters viz., leaf length, leaf area, petiole length, inflorescence diameter, peduncle length, fruit length and fruit thickness contributed more towards variability. It could be concluded that the characters which contributed more towards divergence in D^2 analysis were loaded in PC-I except leaf length, leaf width, leaf area which showed significant high values in PC-I (Fig 16, 17 & 18).

5.1.9.1 Comparative study of D^2 analysis and principal component analysis

Mahalanobi's' D^2 statistic and Jackson's principal component analysis were used for analysing multivariate data. In D^2 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. This standardization made the principal component analysis to support the cluster analysis.

The 40 Appemidi mango cultivars were grouped into 3 main clusters whereas they were sub clustered into 6 different groups in both D^2 analysis and Ward's minimum variance method. In D^2 analysis the cluster II which was having two sub clusters sharing 18+9 genotypes was the largest consisting of 27 cultivars followed by cluster I with 13 cultivars in 2 different sub clusters. As per Ward's method, cluster II was the largest consisting of 27 cultivars followed by cluster I with 13 cultivars.

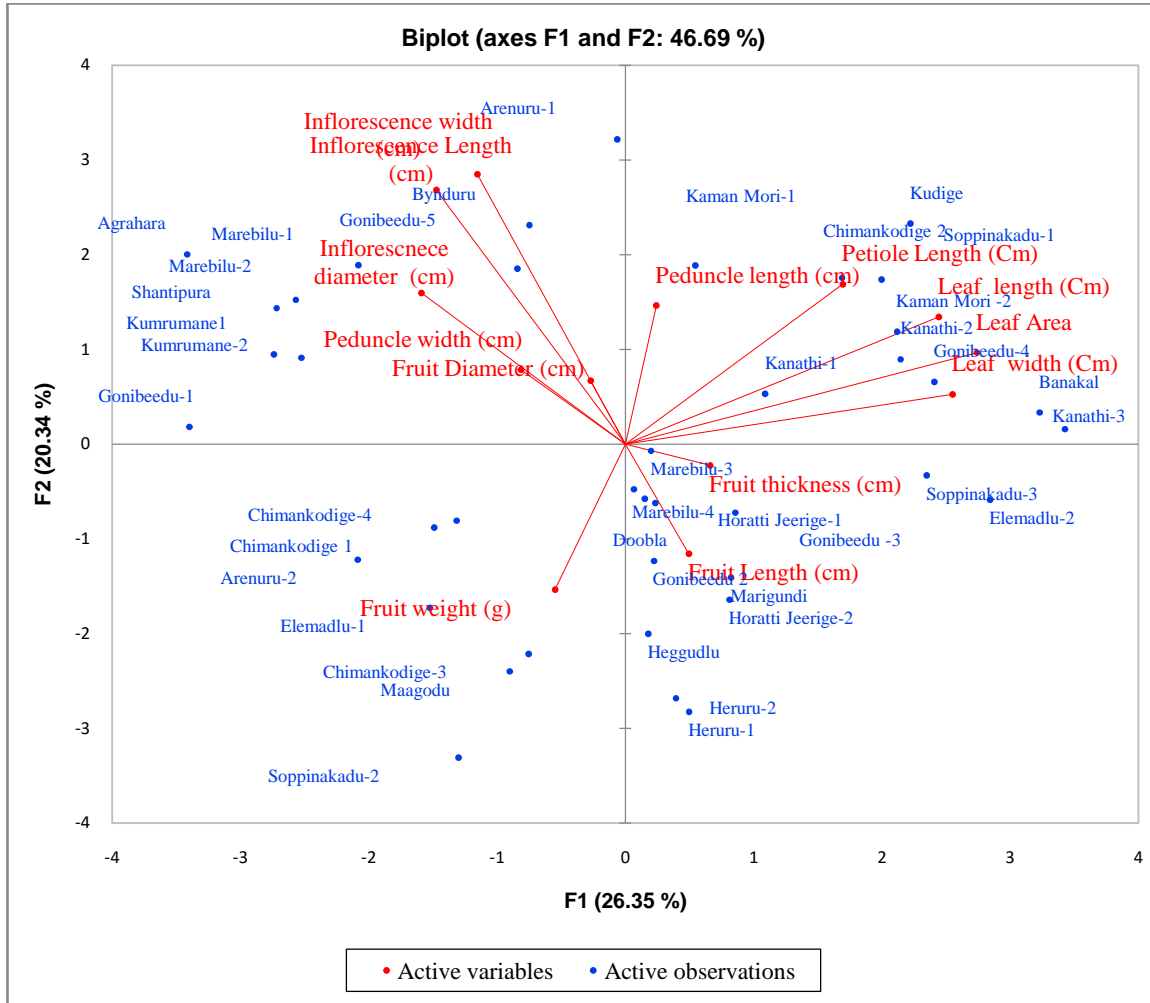


Fig. 16: Scatter diagram of surveyed Appemidi genotypes morphological parameters along with 6 principal components.

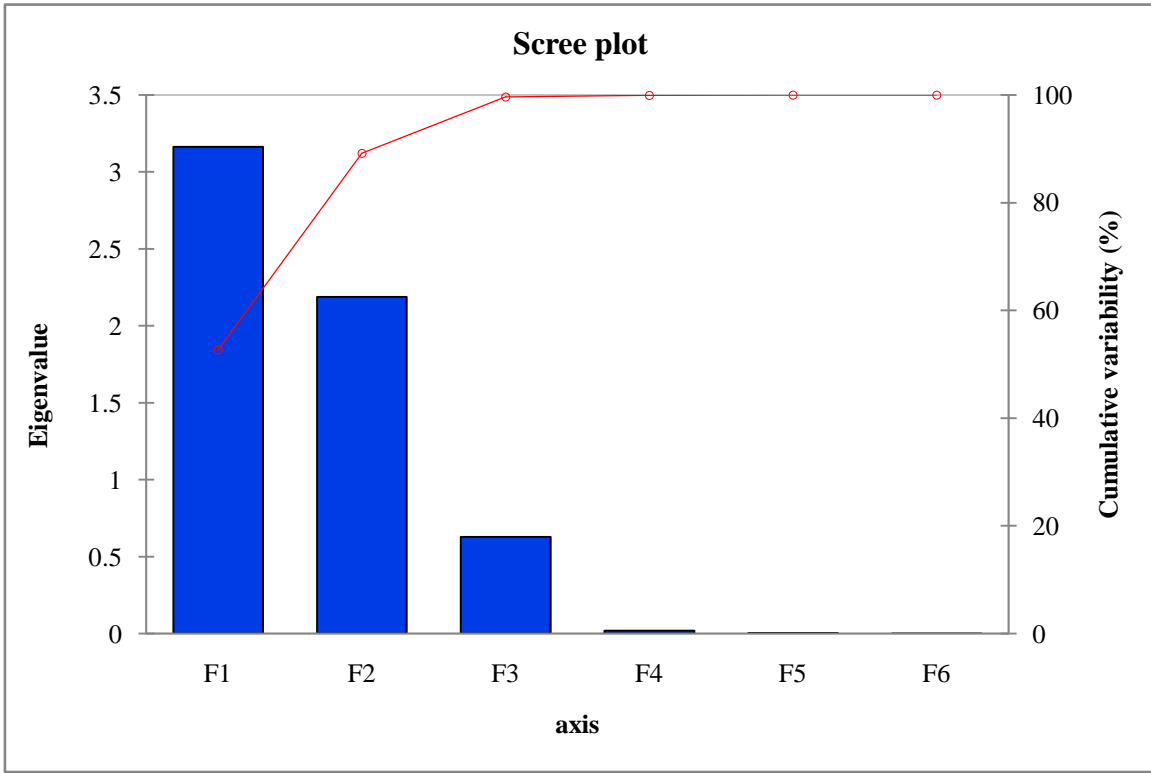


Fig. 17: Scree plot showing principal components contribution towards eigen value

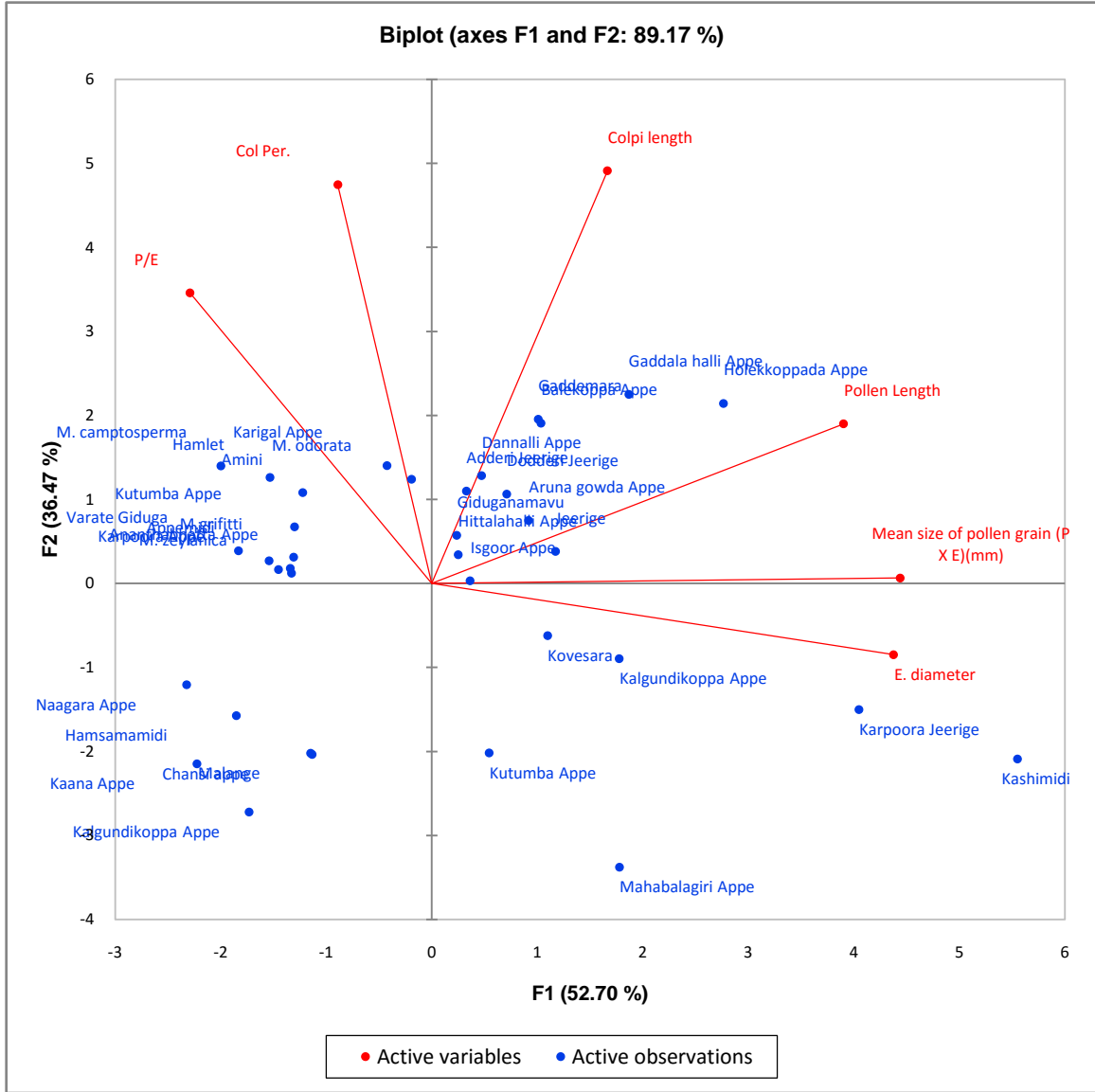


Fig. 18: Biplot of surveyed Appemidi genotypes pollen characters along with 3 principal components.

The pattern of distribution of cultivars into different clusters was at random. Furthermore, the two clustering methods were grouped differently and clustering pattern was somewhat similar. Irrespective of its place of collection or region of location the genotypes were grouped separately and the genotypes collected from same region or location did not fall in the same cluster which revealed that the variability in the genotypes is irrespective of their place of collection. Genetic diversity was the outcome of several factors along with geographical diversity. Hence, the selection for hybridization should be more based on genetic diversity rather than geographic diversity.

In case of D^2 analysis, one could only know the intra-cluster distance but not the cultivars relative position in the respective cluster.

5.2 To study the diversity within the population through molecular means

Characterization of diversity is a necessary requirement for crop improvement, use and conservation of plant genetic resources (Krishna and Singh 2007). Though morphological markers have been used to assess the genetic diversity, they had a limited application in breeding as they were few in numbers as well as stage specific. However molecular approaches offer an efficient alternative tool to help identify and develop genetically unique germplasm that complements the existing cultivars. They are very useful in characterizing the diversity and relatedness among different genotypes. Therefore in this investigation SSR markers were used to assess the diversity of surveyed Appemidi genotypes.

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 fourteen selected SSR primers were used to analyse inter and intra cultivar diversity among 40 Appemidi genotypes. This is in accordance with the findings of Viruel *et al.* (2005), Schnell *et al.* (2006), Singh and Bhat (2009), Anshuman *et al.* (2012), Kumar *et al.* (2013) and Malathi *et al.* (2013) who reported 5.50, 6.96, 5.78, 3.47, 2.70 and 2.87 alleles per locus, respectively in mango. High level of polymorphism was observed with MillHR 18 primer. It was comparable with the results generated by polymorphic bands ranging from 90 bp to 370 bp (Hammedunnisa Begum *et al.*, 2012), 100 bp to 480 bp (Anshuman *et al.*, 2012), 130 bp to 245 bp (Hammedunnisa Begum *et al.*, 2013b) in mango.

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.

5.2.1 Polymorphic information content (PIC) of SSR marker analysis

The SSR primer pairs 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 0.976 to 0.985, observed heterozygosity from 0.091 to 0.273 and polymorphic information content from 0.964 to 0.973 with a mean of 0.969 and the majority of them showed high PIC values (0.700). 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).

The low PI value of the markers implied that these can be easily employed universally for DNA fingerprinting in mango (Table 36). This low PI value clearly showed the potential of these SSR loci in discriminating the cultivars. Therefore these markers can qualify as universal markers for cultivar identification and characterization in mango (Ravishankar *et al.*, 2015).

5.2.2 Diversity analysis

Genetic relationships among the genotypes were calculated by computing the dissimilarities using simple matching coefficient. The dendrogram obtained through Ward's minimum variance method using DARWin 5 Software resulted in the formation of three major clusters which divided into six important sub clusters as that of morphological cluster analysis (Fig 19). Cluster I consisted of 16 genotypes viz., Arenuru-1, Kamanmori-2, Maagodu, Gonibeedu-5, Gonibeedu-4, Chimankudige-4, Chimankudige-2, Kanathi-3, Banakal, Soppinakadu-3 (Subcluster IA) and Kanathi-1, Chimankudige-4, marigundi, kanathi-2, Chimankudige-3 and Soppinakadu-2 (Subcluster IB). Cluster II consists of 19 genotypes namely, marebylu-3, Mareblu-2, Kumrumane-2, Gonibeeu-3, Heggudlu, Marebylu-4, Heruru-1, Agrahar, Kudige, Chimmankudige-1, Heruru-2, Arenuru-2, Elemadlu-1, Kumrumane-1, Elemadlu-2, Gonibeedu-1 (Sub Cluster IIA) and Kamanmori-1, Gonibeedu-2 and Soppinakadu-1 (Sub Cluster II B). The Cluster III comprised of 6 genotypes which includes Shapntipura, Doobla, Horatti Jeerige-2, Bynduru, Marebylu-1 and Horatti Jeerige-1. Here also irrespective of their place of collection or location genotypes were clustered depending upon the SSR genotyping results which indicated that each genotype are having wider variability among them.

Region- based grouping of Indian cultivars was earlier reported by Lopez *et al.* (1997) and Ravishankar *et al.* (2000). The results are in agreement with Saxena *et al.* (2005) who analyzed genetic diversity among Indian papaya cultivars by using RAPD, ISSR and DAMD methods. It can be seen from this study that genotypes from nearby places are grouped in same cluster to a larger extent. Similar to the evaluation of Appemidi types from western ghat regions were studied by Vasugi *et al.*, (2012), who concluded that genotypes belonging to different geographic region might have evolved from the existing mango gene pool from which they were selected by local people to domesticate them.

The morphological, palynological and molecular diversity analysis showed that the grouping of genotypes into three different main clusters and six important sub clusters irrespective of their place of collection. Some of the genotypes also fall under same cluster of molecular, palynological and morphological analysis. For example genotypes Arenuru-1, Gonibeedu-4, Chimankudige-4, Chimankudige-2, Soppinakadu-1, Soppinakadu-3, Kamanmori-2 and 3 were grouped in same cluster of morphological and molecular diversity analysis. Meanwhile genotypes like Agrahara, Gonibeedu-1&3, Elemadlu-1, Kumrumane1 and 2, Soppinakadu-2 were grouped similarly which indicated it's their genetic identity and they may be the duplicate accessions, whereas genotype

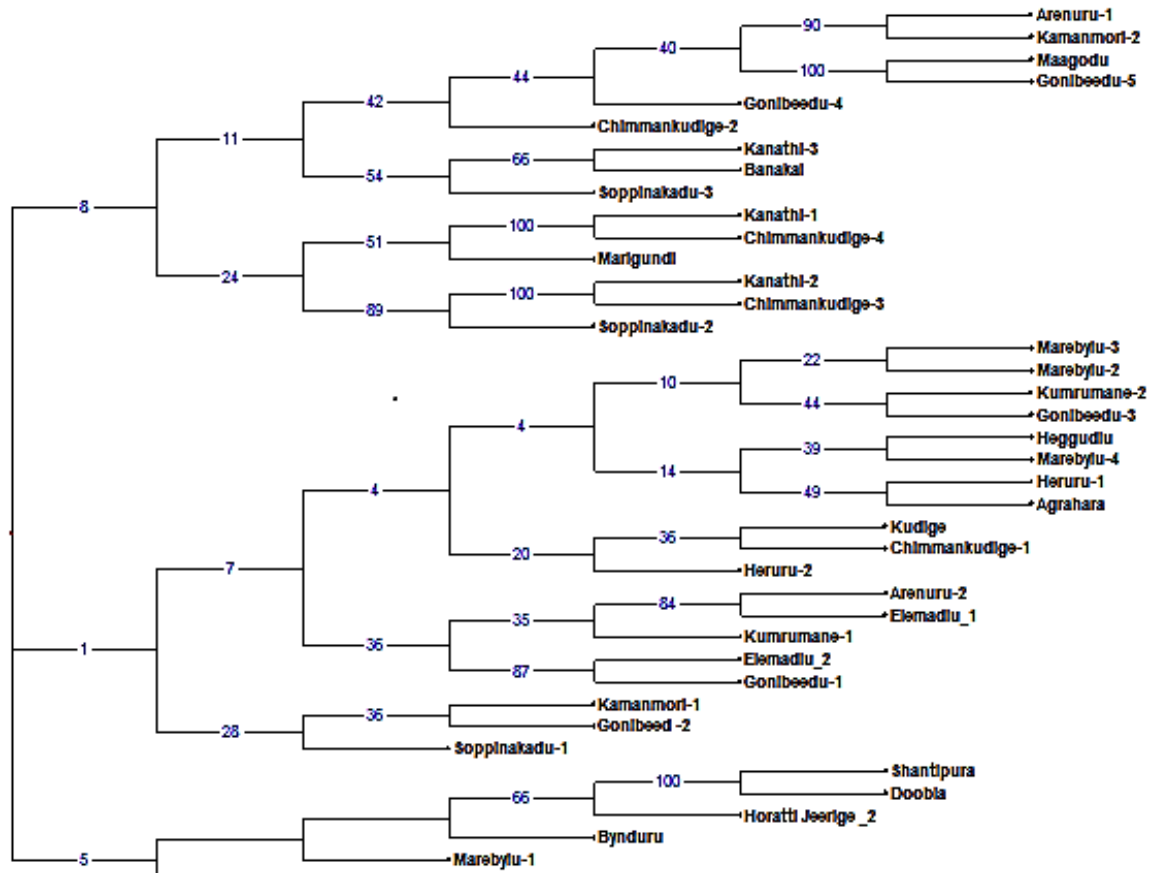


Fig. 19: Molecular clustering results according to the Neighbor joining method for 40 Appemidi genotypes from western ghat regions of Chikmagalur

Arenuru-2 and Kudige were fall in same cluster in morphological, palynological and molecular analysis may be because of their genetic identity and they may be duplicate accessions known by different local names. Although they belong to different regions according to place of collection which promotes the notion that the varieties spread within a locality by single plant selection by farmers. Eventhough they differ morphologically in terms of size and shape etc. It proved to be genetically similar in the present study. This also showed that geographic diversity might not necessarily be related with genetic diversity. Hence the present investigation clearly proves that the morphological characterization can be complemented by molecular characterization. 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 as well as the low PI values for these markers indicated their usefulness for fingerprinting mango genotypes (Ravishankar *et al.*, 2011).

5.2.3 ITS, Psb and tRnH markers for species identification

The rDNA ITS sequences were determined for ten different surveyed genotypes. Based on the ITS 4/5 primer amplified gel image showed that there was no polymorphism between the genotypes. Therefore further we purified the PCR product and subjected for sequencing. The sequence results obtained proved that, there was no different species identified and all the selected genotypes were matching with *Mangifera indica* only. Nantiya Kwanda *et al.*, (2013) reported that using both *rbcl* and *psbA trnH* chloroplast regions to identify the species- specific taxonomy and genetic distance in parasite plant species. Srichandan Padhi *et al.*, (2016) revealed that the genetic resemblance between endophytes and pathogenic strain of *Fusarium* species, using internal transcribed spacer 2 (ITS2) sequence and secondary structure analysis. Shafiquzzaman *et al.*, (2012) also reported that using ribosomal DNA internal transcribed spacer region (ITS1-5.8S -IST2) to give accurate species level identification in *Termitomyces*.

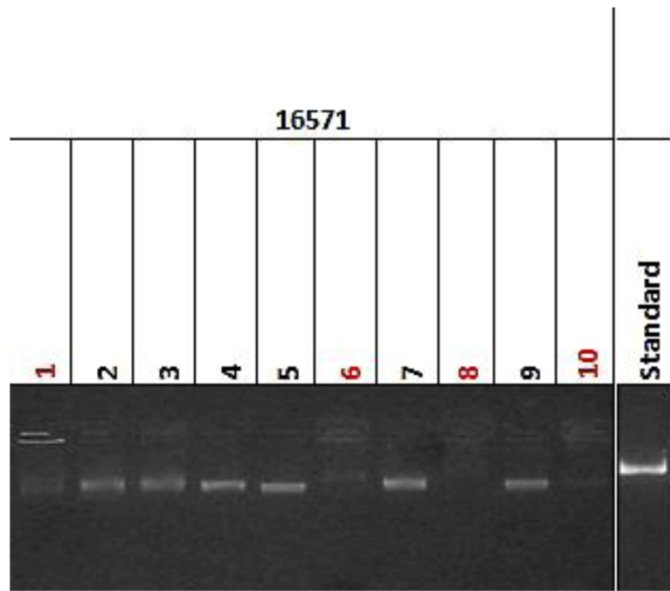


Plate 25: Sequence result also showed that all the genotypes studied were belonging to *Mangifera indica* only.

5.3 Chemical profiling from fruit sap and leaf.

The Appemidi genotypes are unique in nature with respect to its flavour and taste of pickle. The genotypes are high in aroma therefore there is a huge demand by the local people for Appemidi. Hence in this investigation fruit sap as well as leaf were subjected for profiling of major volatile compounds responsible for its unique aroma and were correlated.

5.3.1 Leaf volatile aroma composition

The aroma components of 34 appemidi genotypes were determined independently with HS-SPME-GC-MS technique. The major proportion was from monoterpenoids and sesquiterpenoids composition in individual Appemidi leaves. The genotype Ananthabhattacha Appe had the highest area of volatiles and 55+ volatile components were detected. The composition and concentrations of aromatic volatiles in mango fruit are influenced by the cultivar (Pandit *et al.*, 2009 and Pino *et al.*, 2005). In contrast, Gidaganamavu had the least volatile components sharing less proportion to aroma compounds. Shidadakke Appe was having 64 volatile components in less proportion. Similar differences in the compositions and concentrations of terpenoid compounds in the sap of seven Indian mango varieties were reported by Johna *et al.* (1999).

5.3.1.1 Genotypes variability in leaf volatile composition

Significant differences in the composition and proportion of leaf volatile components were observed among the Appemidi genotypes studied. The genotype Kalkuni had the highest per cent of monoterpenoids proportion 71.41 per cent followed by Nandgar (71.18%) and Kalakai (69.81%). Appemidi leaves were rich in β -phellandrene, α -pinene, limonene and Cis-ocimene (Fig 21). These 34 Appemidi genotypes showed a varying proportion of α and β -pinene and less concentration of carene.

Significant differences in composition of total sesqui terpenoids was recorded among Appemidi genotypes. Sesquiterpenoids are major active molecules present in mango leaves and reported an anticancer activity in the leaf oil for the first time by

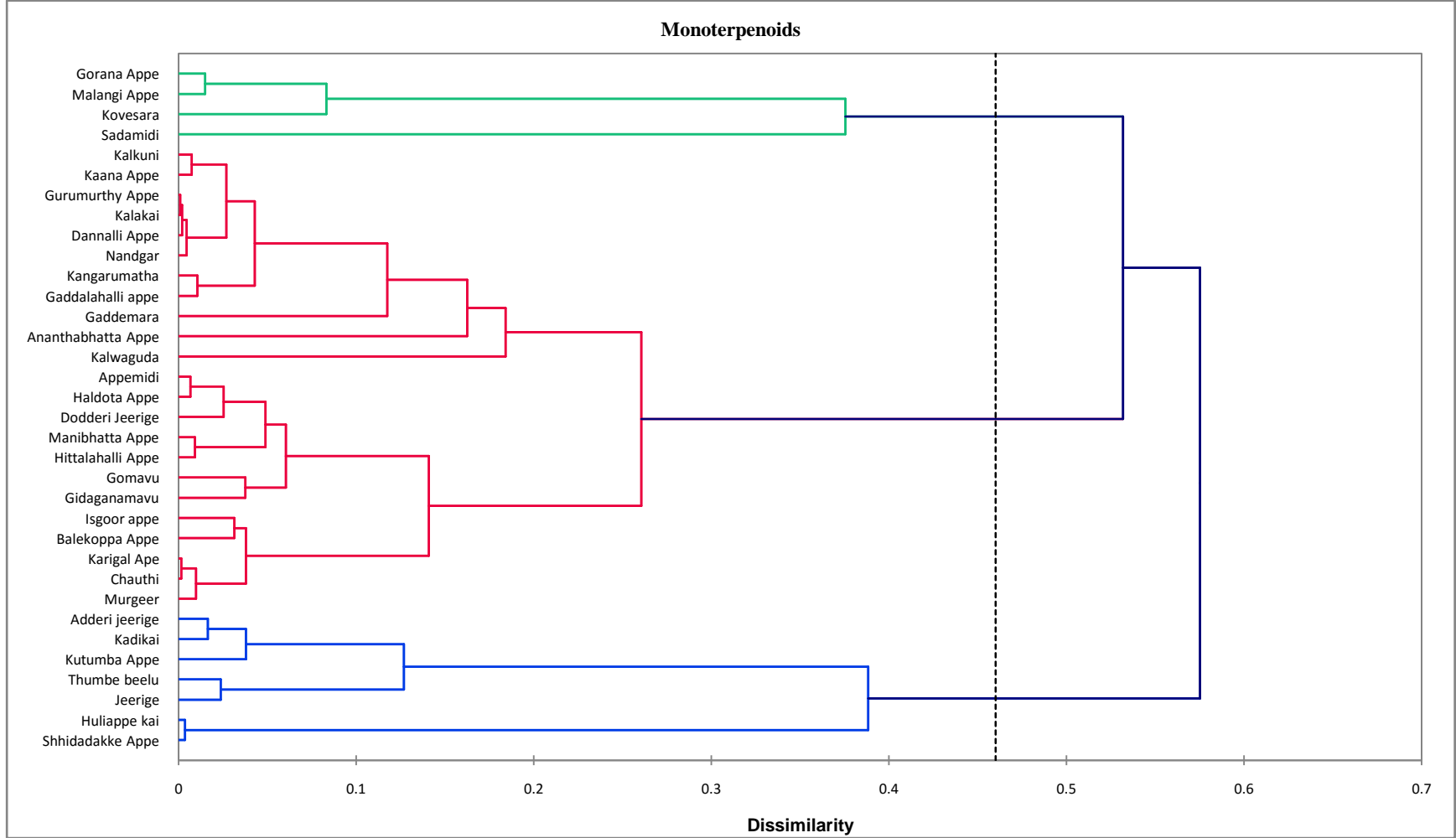


Fig. 20: Dendrogram with average linkage of Appemidi genotypes constructed on the basis of the Euclidean distance matrix for monoterpenoids composition

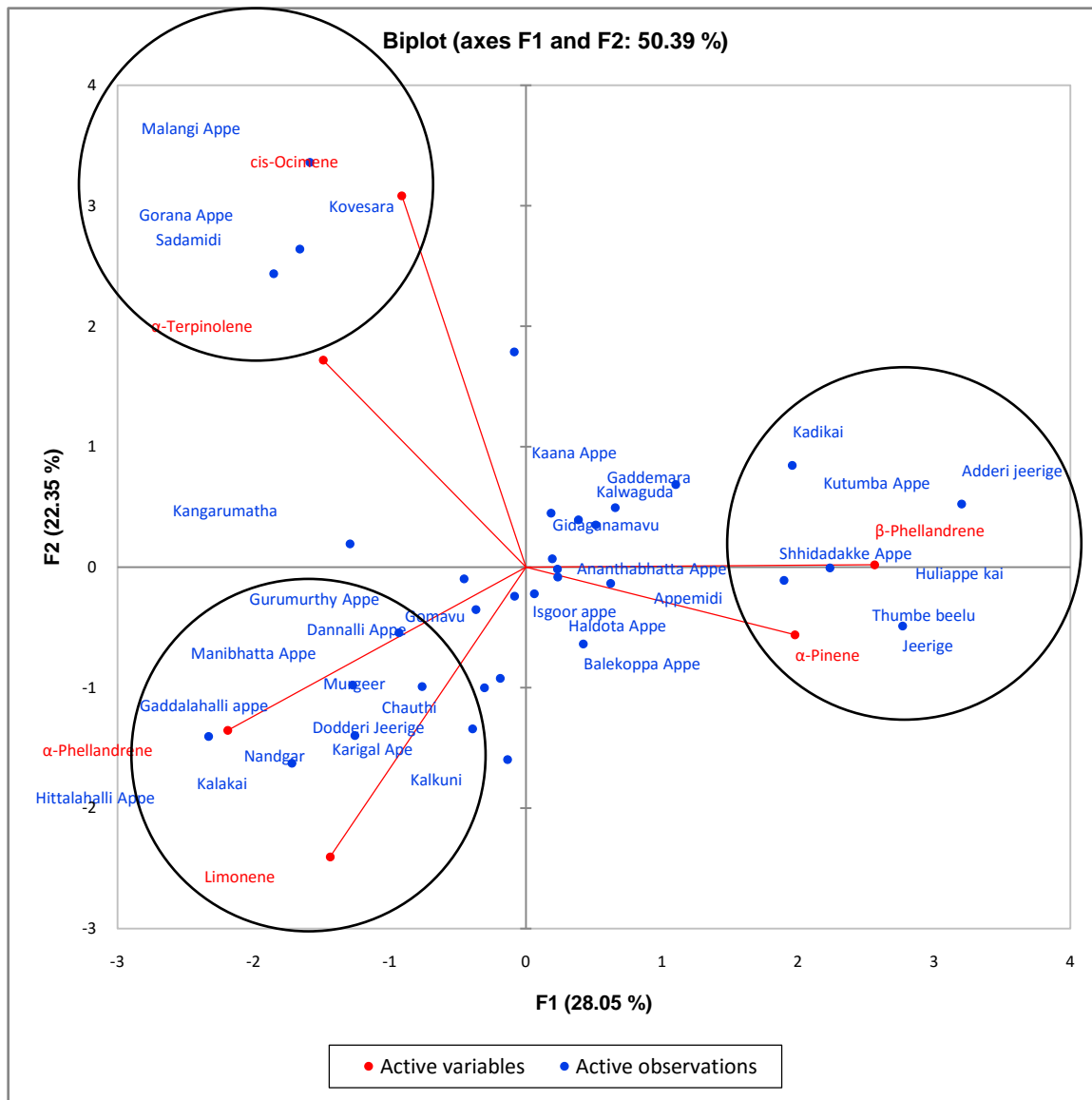


Fig. 21: Scatter diagram of Appemidi genotypes monoterpenoids along with 6 principal components.

Simionatto *et al.*, (2010). Sesquiterpene hydrocarbons form the second largest group of aroma volatiles in mango (Pandit *et al.*, 2009 and Pujari *et al.*, 2009). The highest per cent of sesqui terpenoids composition was observed in genotype Gaddemara (90.39%) followed by Kalwaguda (78.73%). The least content was noticed in genotype Kalkuni (22.82%) followed by Appemidi (23.35%). Among sesqui terpenoids caryophyllene and α -humulene were the major compounds in all the genotypes studied (Fig 22). This was also supported by the investigation carried out by Gebra *et al.*, (2011), in *M. indica* var. coquinho mature leaves were rich in E-caryophyllene, Cedrene and a-humulene. Zhang *et al.*, (2006) also studied the aroma profile of Chinese mango cultivars.

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 the similar results and indicated that, terpenes were the main aroma constituents among the genotypes which include monoterpenes like α -pinene, β -phellandrene, limonene, cis-ocimene and sesquiterpene like α -humulene, α -selinene, caryophyllene and terpinolene. Similarly, significant differences were detected in terpenes compositions and proportions among 34 genotypes (Table 4 & Fig 25, 26). Liu *et al.* (2013) reported that, selinene, eremophilene, and aromadendrene were majorly detected in 'JinHwang' limonene in 'Irwin' and α -caryophyllene in 'Keitt' cultivars. However, the large differences in the terpenes compositions and proportions in the present study may be attributed due to differences in the cultivation practices by the growers in the surveyed regions of western ghat, maturity levels, as well as different genetic composition of cultivars. In these ways, differences in aroma and flavour are generated in leaves and fruits.

Aldehyde occurs at a low level, but plays a key role in mango flavour. This observation was supported by the earlier finding by Macleod *et al.* (1982) that the aldehydes content of mangoes was 0.03%-14.36%. In the present study, only few cultivars contained aldehydes. However, Pino *et al.* (2005) considered that the aldehydes in Cuban mango cultivars were associated with the sweet herbal flavor of their fruit. Alcohols occur in small amounts in mango leaves and contribute slightly to their aroma

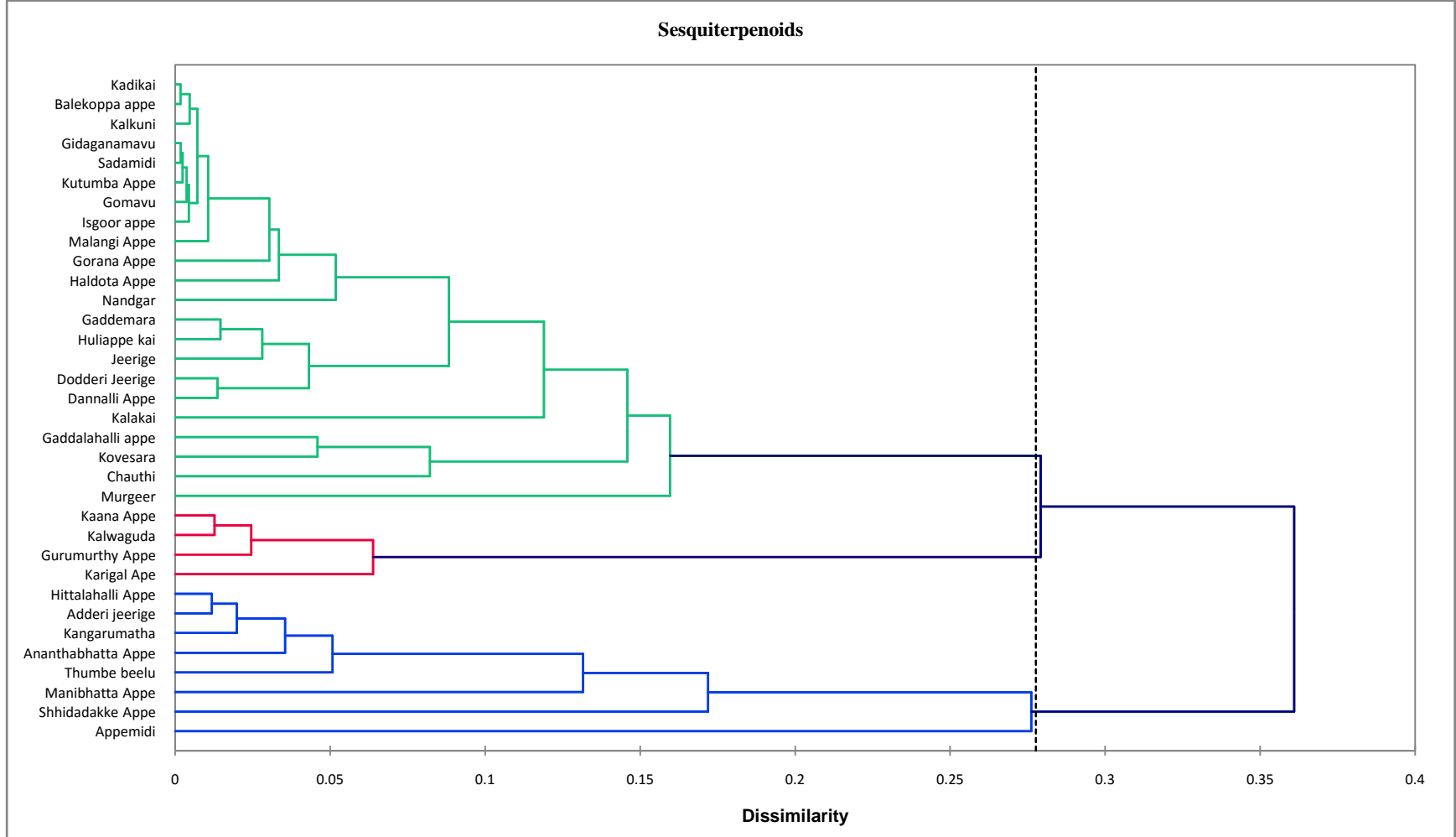


Fig. 22: Dendrogram with average linkage of Appemidi genotypes constructed on the basis of the Euclidean distance matrix for sesquiterpenoids composition.

and flavor (Engel and Tressl, 1983). In this study, cultivars contained less per cent of alcohols. Cinerone was the only ketone detected among the total flavor volatile compounds in the present study, with its highest concentration in Kadikai (0.045%). The total volatile profiling grouped 34 Appemidi genotypes into monoterpenoids rich and sesquiterpenoids rich genotypes (Fig 24).

5.3.1.2 Sap volatile aroma composition

Plant Secondary metabolites, foliar flavonoids have gained reputation as being excellent phyto-chemical markers for cultivar identification (Bhargava *et al.*, 2011). The distribution flavour combination is specific for each cultivar which can be used for varietal identification. This was also in confirmation when we compared the per cent volatile composition between sap and leaf mainly monoterpenoids.

In fruit sap, monoterpenoids occupied the highest proportion. The concentration of aroma compounds was highest in Ananthabhata Appe. α -Phellandrene, β -Phellandrene, α -Pinene β -Pinene 3-Carene Limonene, β -Terpinene and trans-Ocimene are the major compounds identified in fruit sap of 22 Appemidi genotypes. Therefore this gives the typical aroma to Appemidi and these may contribute for longer shelf life and higher preference by the consumer. It gives the clear idea of volatile compounds in pickling type mango which are rich in terpenoids as well as completely different combination of monoterpenoids for their different aroma.

Further these per cent monoterpenoids composition of fruit sap was compared with leaf monoterpenoids to study the relationship between fruit sap and leaf.

5.3.1.3 Per cent similarity between sap and leaf monoterpenoids and correlation analysis of leaf and fruit sap volatiles

Leaf and fruit sap flavour volatiles were analysed independently and compared with each other. This study observed the relationship of per cent volatile composition between sap and leaf mainly monoterpenoids. In genotype Balekoppa Appe the content of β -pinene (8.21 and 36.40%), α -phellandrene (3.33

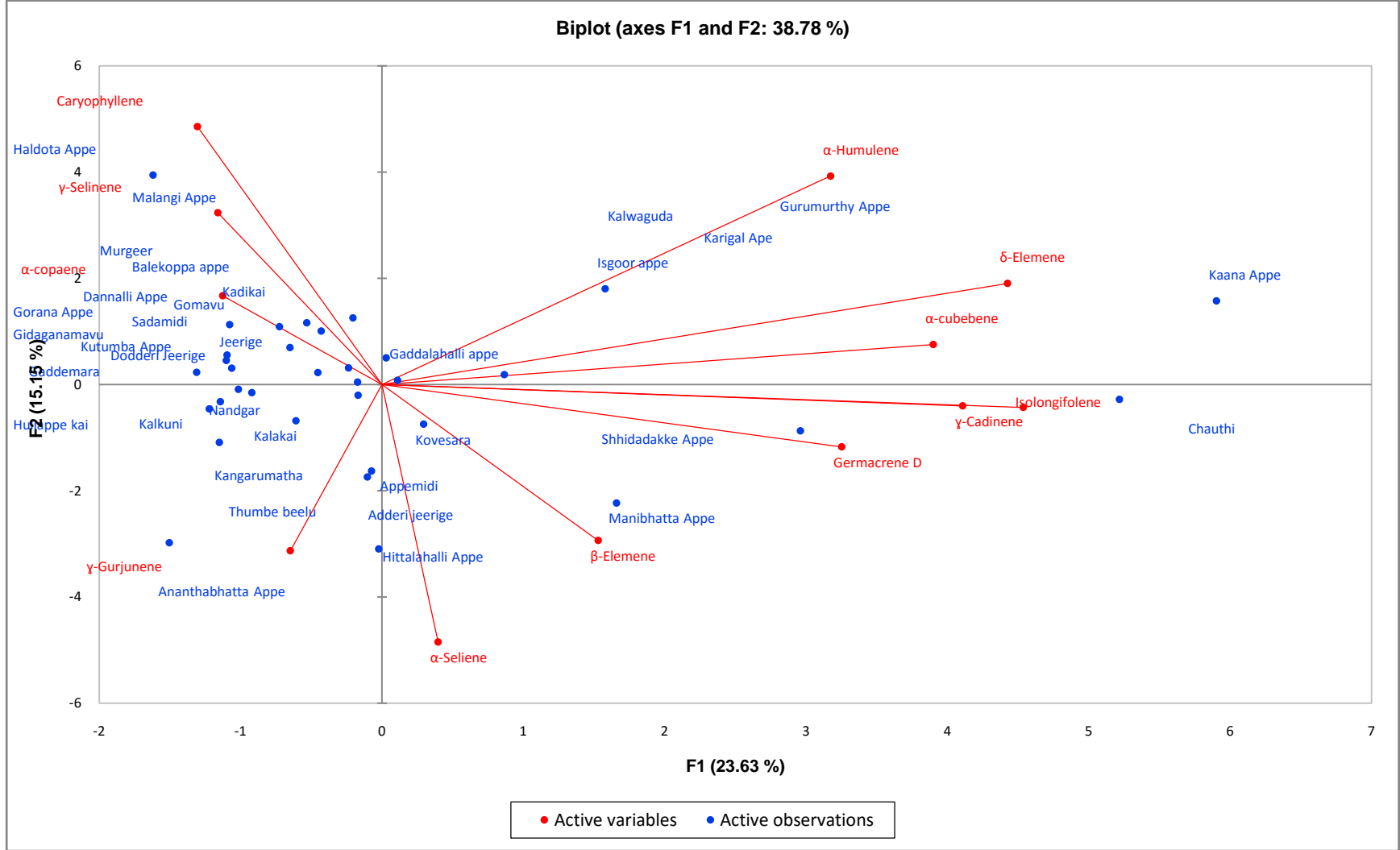


Fig. 23: Scatter diagram of Appemidi genotypes sesquiterpenoids along with 12 principal components

and 48.97%) per cent contribution from leaf and sap followed the same trend. In genotype Kalakai also α -phellandrene (3.33 and 48.97%) contributes the highest per cent among the total volatiles composition both in leaf and sap.

The results indicated that significant positive correlation was established with respect to some of the major monoterpenoids among leaf and fruit sap (Table 38). Such correlation could be used as an alternative approach to characterize genotypes based on the differences found in the volatiles composition and proportion of different plant parts. Among the monoterpenoids subjected for correlation analysis leaf β -phellandrene was having the highest correlation index of 0.899 with fruit sap and *vice versa*.

One of the successful pre-selection indices developed in mango breeding was that "Leaf flavour was reported to be directly correlated with fruit flavour" according to Majumder *et al* (1972). As leaf volatiles are represented in the fruit and account for the characteristic flavour of cultivars this technique offers the opportunity to select for flavour from seedling populations before fruiting or field planting as a screening technique which will shorten the breeding cycle in perennials (Whiley *et al.*, 1993). Therefore the per cent comparison of the present study follows the similar pattern of contribution which will serve as a biochemical marker for cultivar identification as a fingerprint. Both the fruit sap and leaf volatiles follow the similar trend of per cent contributions from major monoterpenes. This was also confirmed by the correlation study. Therefore it can be used as a preselection index for α -phellandrene, β -pinene, β -phellandrene and limonene in breeding programme for selection of cultivars or to reduce the juvenile period in hybridization programme of mango.

5.3.1.4 Principal component analysis and cluster analysis of volatile components

In order to analyse the variance the correlation matrix for monoterpenoids shows the variables that mostly define six principal components: F1 was strongly correlated with β -phellandrene (0.73), F2 with *cis*-ocimene (0.79), F3 with α -terpinolene (0.70), F4 with α -pinene (0.63), F5 with β -phellandrene (0.54) and F6 with limonene (0.38). In case of sesquiterpenoids also the variance correlation matrix showed the variables that mostly define 12 principal components; the factor 1 is contributing more towards total variability having a highest eigen value of 2.835 and 23.62% variability.

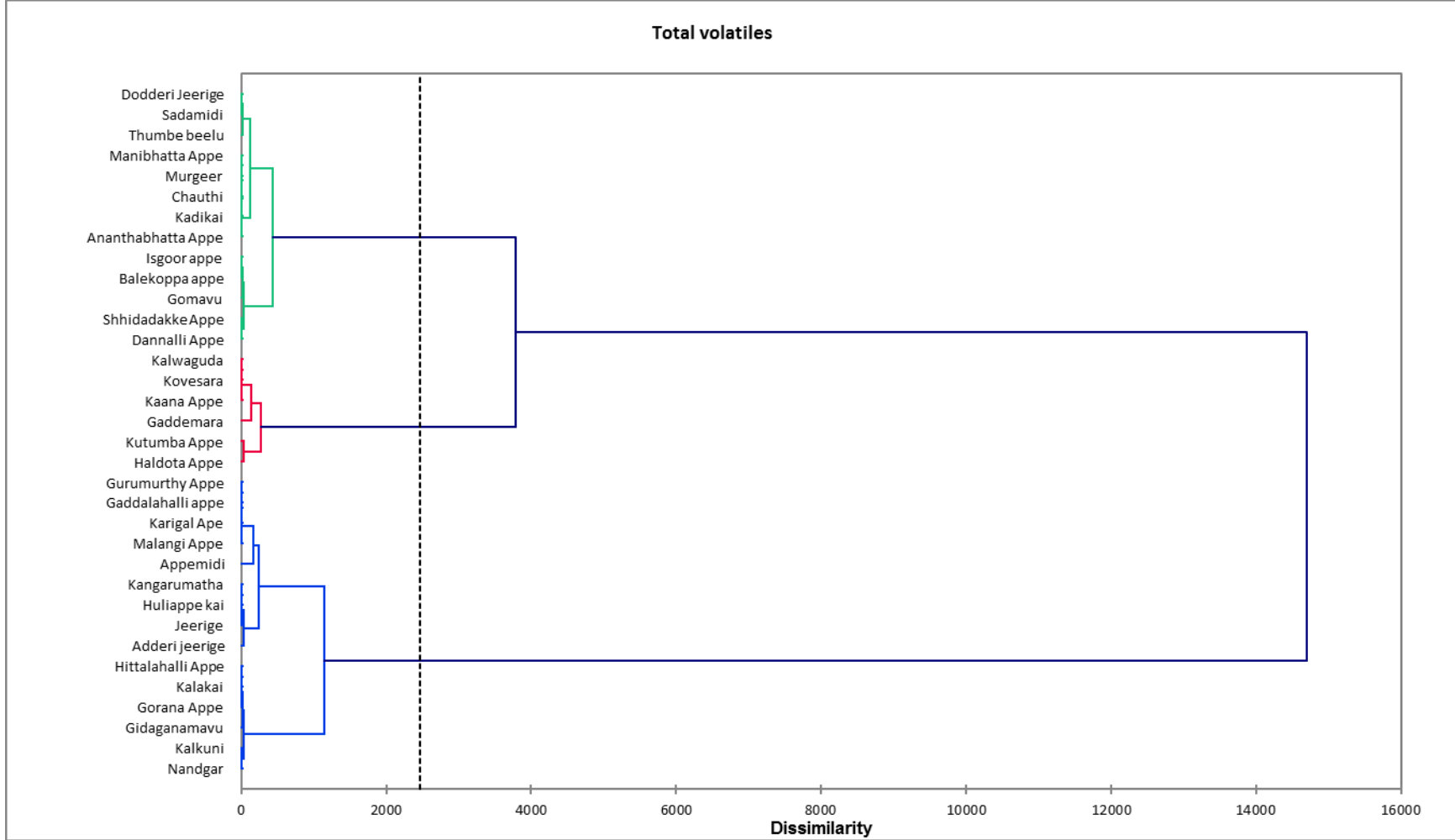


Fig. 24: Dendrogram with average linkage of Appemidi genotypes constructed on the basis of the Euclidean distance matrix for total volatile composition.

The cluster analysis result showed that, genotype Adderi jeerige, Kadikai, Kutumba Appe, Thumbeelu, Jeerige, Huliappekai and Shidadakke Appe were clustered together with diverse proportion of β -phellandrene, α -pinene and limonene as major composition. Genotype Gorana Appe, Malange, Kovesara and Sadamidi were clustered separately because this group is rich in cis-ocimene, whereas cluster 1 grouped with 21 genotypes were analogous in their volatile composition and proportions, with diverse volatile composition and containing mainly limonene and β -phellandrene. The cluster 2 consists of genotypes rich in Cis-ocimene, Cluster 2 with genotypes rich in α -pinene and β -phellandrene and cluster 3 with limonene and β -phellandrene rich genotypes.

The cluster analysis was conducted for the sesqui terpenoids composition of 34 genotypes separately and was grouped into three different clusters based on the diverse proportion of sesquiterpene molecules in them. The cluster 1 consists of 21 genotypes which are caryophyllene rich in nature, the cluster 2 consists of α -selinene rich genotypes viz., Hittlahalli Appe, Adderi Jeerige, Kangaramatha, Thumbeelu, Manibhatta Appe, Shidadakke Appe and Appemidi. The cluster 3 grouped with α -Humulene rich genotypes viz., Kaana Appe, Kalwaguda, Gurumurthy Appe and Karigal Appe.

5.3.2 Profiling of major fat soluble, water soluble vitamins and organic acids in fresh, brined fruits and brine solution of 15 appemidi genotypes

The biochemical parameters like vitamin C, acidity, total phenols, total flavonoids, total antioxidant activity, profiling of major fat soluble and water soluble vitamins as well as major organic acids were done in this experiment to know the important nutritional constituents in our Appemidi pickle. As per the ITK followed by the local people, an experiment set up was made to study the nutritional composition in fresh fruit and salt treated fruits with respect to its food value.

5.3.2.1 Morphological characterization of fruits before and after salt treatment after 30 days

Salt is one of the most widely used additives in food industries because of its low cost and varied properties. It has a preservative and antimicrobial effect as a direct consequence of the capacity of sodium chloride to reduce the water activity values (William *et al.*, 2011).

The maximum fruit weight was recorded in Dannali Appe (27.02g), while the mean fruit weight was 24.19g. Non-significant differences were obtained for salt cured fruit weight. Among fifteen genotypes the maximum fruit weight of 9.28g was obtained in genotype Ananthabhatta Appe after 30 days of salt treatment. There is a significant per cent weight loss observed among fresh and brined fruits. 81.79 per cent of fruit weight loss was observed in genotype Appemidi. It is due to the osmotic dehydration the multicomponent diffusion process, the water flow from fruit to solution and along with some components reduces the fruit weight (Yadav and Singh, 2014).

Fruit length was measured before and after salt treatment, the maximum per cent reduction in fruit length was noticed in Appemidi (46.06%) followed by Holekoppada Appe (37.03%). The highest per cent reduction in fruit width was also noticed in cultivar Appemidi (69.11%) and the least was recorded in Gorana Appe (13.42%), is due to the osmosis the gradual loss of water from the fruit tissue shrunken the fruit which in turn reduces its length and width.

Thickness of the fruits was also measured before and after salt treatment. The maximum fruit thickness was observed in fresh fruits of Appemidi (2.98 cm) followed by Karpooora Jeerige (2.86cm), where as in case of salt cured fruits non-significant differences were noticed with a mean of 1.06 cm. The highest brined fruit thickness of 1.34 cm was recorded by genotype Jeerige. The per cent reduction in fruit thickness was 57.15%.

Firmness is one of the most critical quality attributes influencing the consumer appeal and marketing (Ne Smith *et al.*, 2002). The average fruit firmness recorded for fresh form was 6.50 kg/cm², the maximum firmness was observed in the cultivar Gorana Appe (8.21 kg/cm²), followed by Appemidi (6.83 kg/cm²). The maximum firmness of salt cured fruits was noticed in Appemidi (14.82 kg/cm²). In case of per cent variation, there was an increase in fruit firmness considerably. The average increase in the per cent fruit



Plate 26: Experimental set up of salt treatment in Appemidi consisting of fresh fruit, brined fruit and brine solution obtained after 30 days

firmness was observed to be 94.85 per cent. This is due to the hypothesis of a causal relationship between the moisture loss and postharvest firmness.

The fruit moisture loss plays a major role in determining the firmness responses of blueberries during postharvest (Paniagua *et al.*, 2013). The critical variables such as turgor, skin toughness and cell wall modifications may elucidate the mechanism for this relationship between moisture loss and firmness. This increased 'skin toughness' hypothesis is plausible as the mechanical resistance of the epidermis is known to affect overall fruit firmness (Jackman and Stanley, 1995). Allan-Wojtas *et al.* (2001) associated firming of blueberries with microscopically observed thickening of the cell wall of parenchyma cells of 'Burlington' blueberries during 6 week storage at 0 °C. Chiabrando *et al.* (2009).

5.3.2.2 Biochemical characterization of 15 Appemidi genotypes before and after salt treatment

5.3.2.2.1 Vitamin C (mg/100g)

The Vitamin C content ranged from 20.66 to 263.00 mg/100g, with a mean of 111.57 mg in fresh fruits. The highest Vitamin C content was found in genotype Appemidi (263.00 mg/100g). The Vitamin C content in brined fruits ranged from 4.47 mg to 39.68 mg per 100 gram pulp (Fig 27). Whereas the brine solution obtained after 30 days was also containing some amount of vitamin C, the mean Vitamin C content in brine solution was 8.34 ml.

The highest vitamin C content was observed in fresh fruits, but rapid dropping after salt treatment resulted in lower amount of vitamin C content in salt cured fruits as well as brine solution due to the destruction of cellular structure due to salting. This process is determined by release and activation of ascorbinase from the destroyed cellular structure of fruit and availability of oxygen. The presence of vitamin C in salt cured fruit and brine solution can be accounted for by water solubility of this vitamin (Agnieszka *et al.*, 2013).

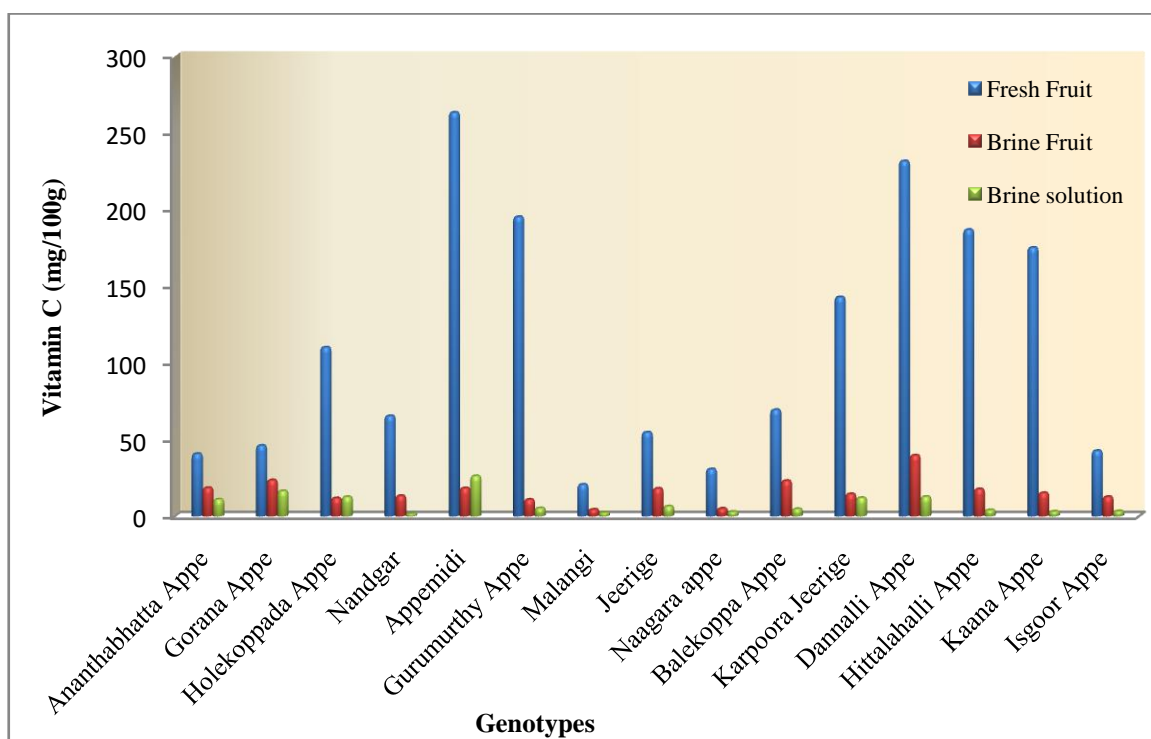


Fig. 27: Vitamin C concentration among 15 Appemidi genotypes at three different forms

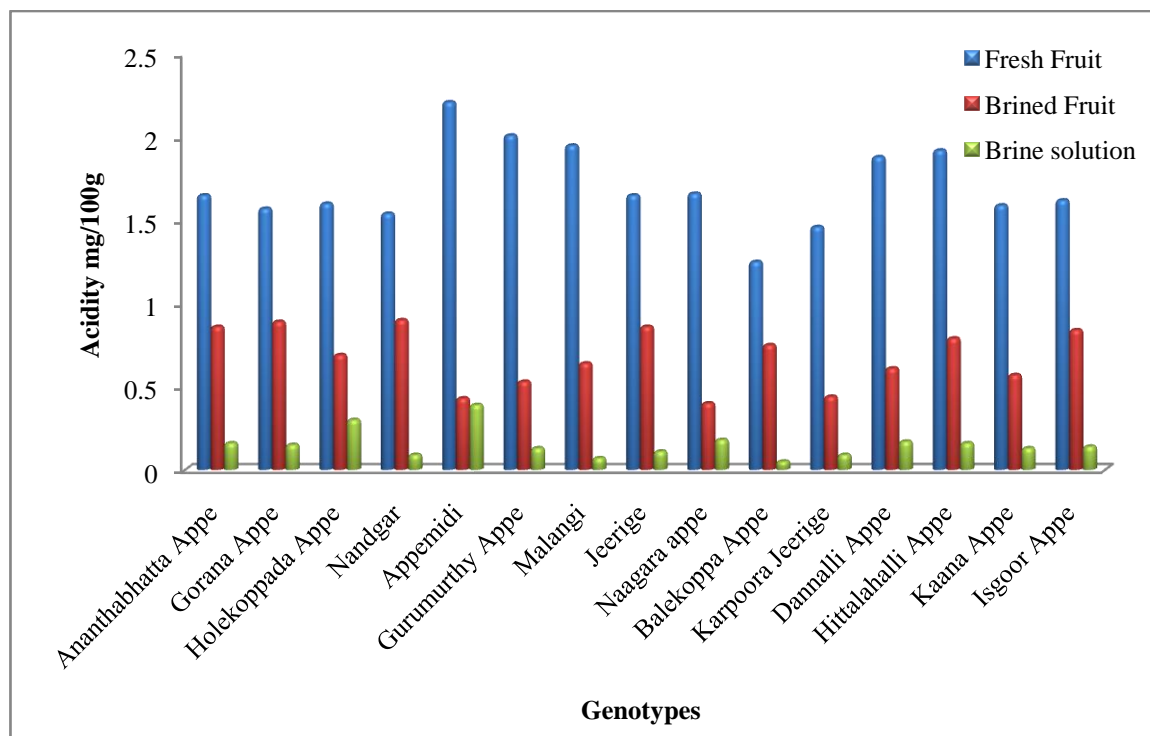


Fig. 28: Acidity concentration among 15 Appemidi genotypes at three forms

5.3.2.3 Acidity (mg/100g)

In fresh fruits the acidity ranged from 1.25 to 2.21 mg/100g, the genotype Appemidi was having the highest acidity in fresh fruit form (2.21mg/100g). In case of brined fruits the acidity ranged from 0.40 to 0.90 mg/100g (Fig 28). The brine solution obtained was also containing some amount of acidity which ranged from 0.05 to 0.39 ml. In this study it was noticed that there was a loss of acidity. Due to the reduced respiration rate, acids were not get converted into other forms (Mehta and Tomar 1979).

Even though nonsignificant differences were obtained for the factors and genotypes, this result showed that over a period of time in curing of Appemidi fruit with a layer of salt due to osmotic pressure the fruit has lost its acidity and vitamin C content and it was found in brine solution to some extent. Because these vitamins are water soluble in nature therefore it has transferred from fruit to brine solution to some extent.

The per cent loss in vitamin C and acidity among the 15 genotypes were varied significantly depending upon their genotypic character (Fig 29).

5.3.2.4 Total phenols (g GAE/100g)

It has been observed that the antioxidant activities of plant products are mainly due to phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Li *et al.*, 2004). These compounds are the intermediates in the phenylpropanoid pathway and play important roles in flavonoid production and lignin biosynthesis. The polyphenol synthesis and their accumulation are generally stimulated in response to biotic/abiotic stresses such as salinity (Ksouri *et al.*, 2007).

The average total phenols content was 0.97 g GAE/100g in fresh fruit form of appemidi genotypes. The fresh fruit form has showed the maximum total phenol content in genotype Balekoppa Appe (1.64 g GAE/100g). The brined fruits also recorded significant amount of total phenol content with an average value 0.70 g GAE/100g, whereas the average total phenols recorded in brine solution was 0.27 g GAE/100g (Fig 30).

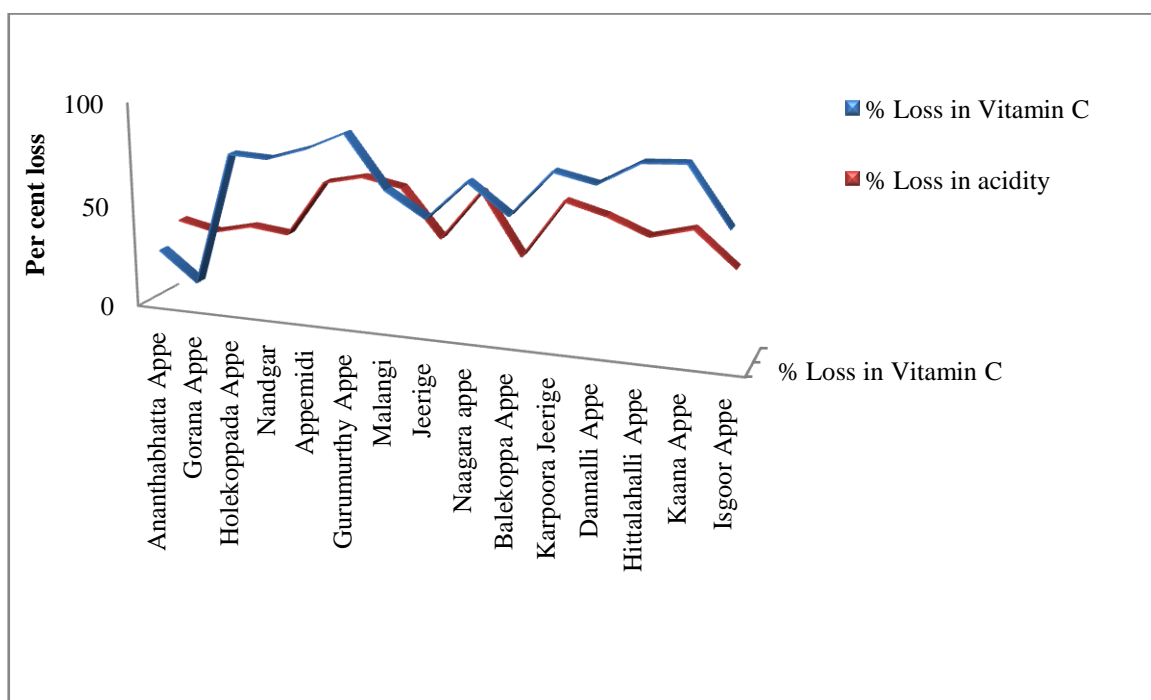


Fig. 29: Per cent loss in vitamin C and acidity among 15 appemidi genotypes after salt treatment

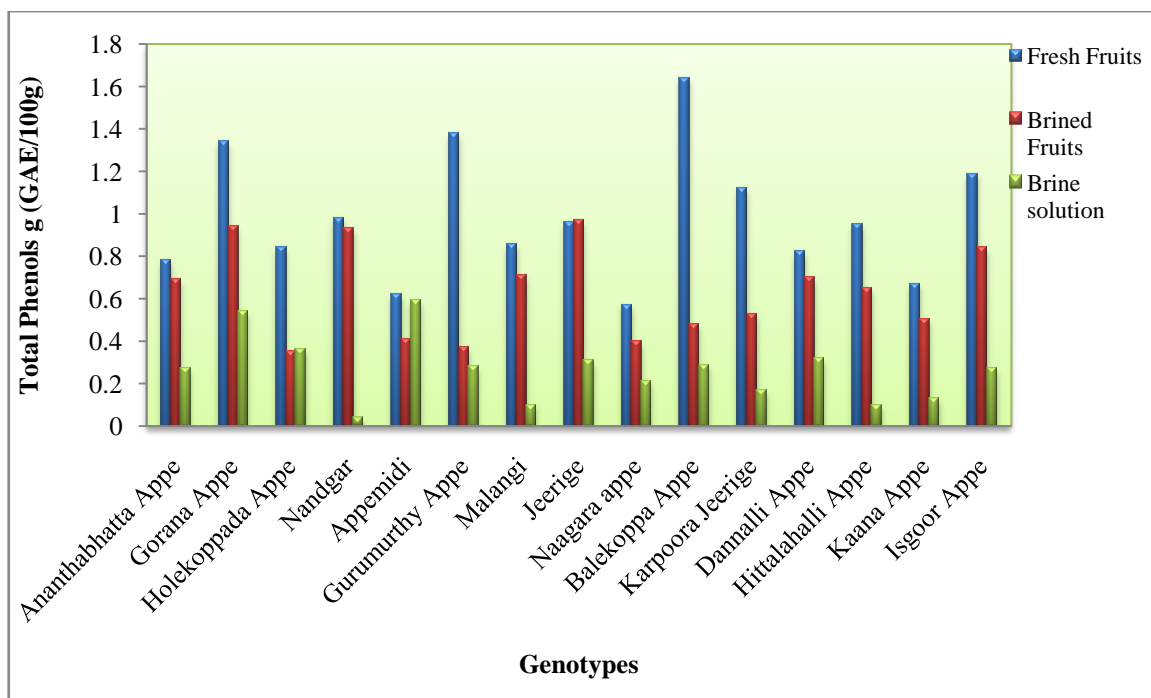


Fig. 30: Total phenols concentration among 15 Appemidi genotypes at three different forms

There was a loss in total phenols concentration from fresh fruits to brined fruit (Fig. 33) because of cellular oxidative damage when it was exposed to salt stress which stimulate the biosynthesis of phenols and some amount was lost in brine solution until the tissue becomes dead the process of leaching out of nutrients will occur later at some point after it has reached the equilibrium. Similar results were obtained by Valifard *et al.*, (2014) in case of *S. mirzayanii* tissues when exposed to moderate NaCl salinity results show concomitant stimulations in both phenolic biosynthesis and antioxidant activity.

5.3.2.5 Total flavonoids (g catechin equivalent/100g)

Significant differences were obtained in total flavonoids content in three different forms among fifteen appemidi genotypes. The fresh form fruit showed an average of 0.05 g of total flavonoids content and it ranges from 0.033 to 0.072 g catechin equivalent/ 100g. The brined fruits mean total flavonoids content was 0.036 g catechin equivalent/ 100g. The brine solution also showed significant amount of total flavonoids content, the average total flavonoids found was 0.22 g. On an average the per cent loss observed in total flavonoids content was 28.50 per cent because of osmosis majority of the water soluble compositions will come out from fresh fruits (Fig 31 & 33).

5.3.2.6 Total antioxidants activity (g AEAC/100g)

The total antioxidant activity varied from 0.38 g to 1.21 g AEAC/100g in fresh fruits whereas the range of total antioxidant activity was from 0.11 g to 0.42 g AEAC/100g in brined fruit. The brine solution also showed differences in total antioxidant activity with an average content of 0.35 g AEAC/100g (Fig 32). The degree of cellular oxidative damage in plants exposed to abiotic stress is controlled by the capacity of the plants to produce antioxidant agents. Therefore, salt tolerance seems to be favoured by the increase in plant antioxidant levels to detoxify the reactive oxygen species produced under these conditions. Our findings support the hypothesis that plants exhibiting tolerance to salt stress are useful systems for the production of secondary metabolites serving both as food stuff and medicinal products (Valifard *et al.*, 2014).

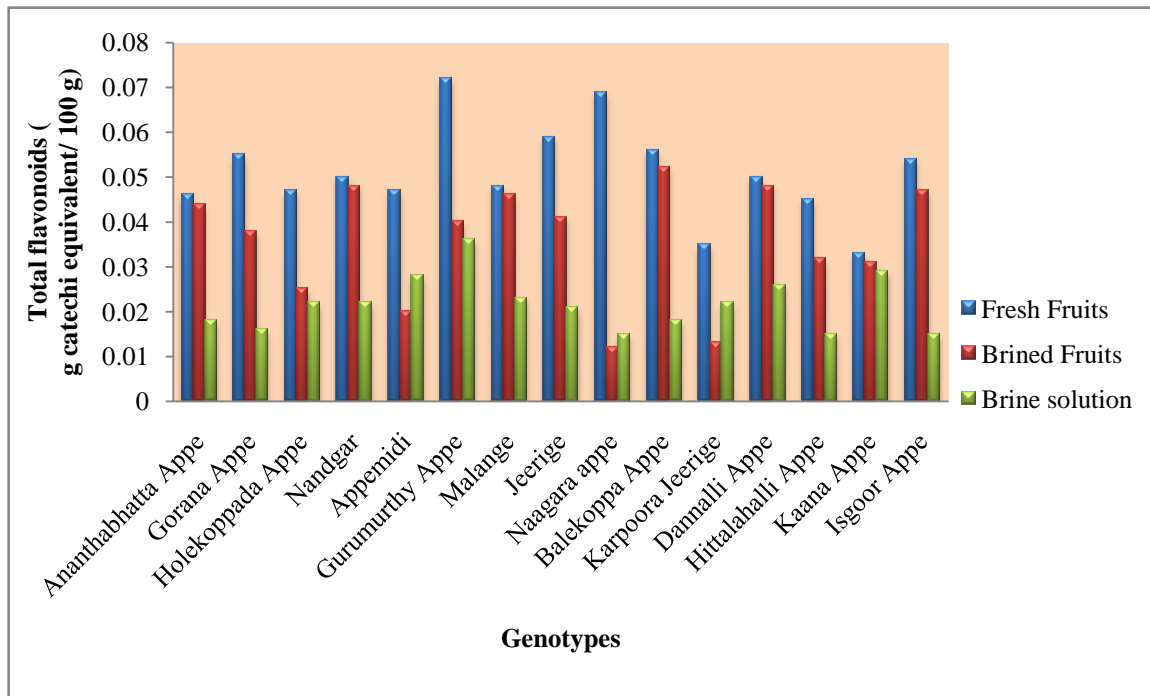


Fig. 31: Total flavonoids concentration among 15 Appemidi genotypes at three different forms

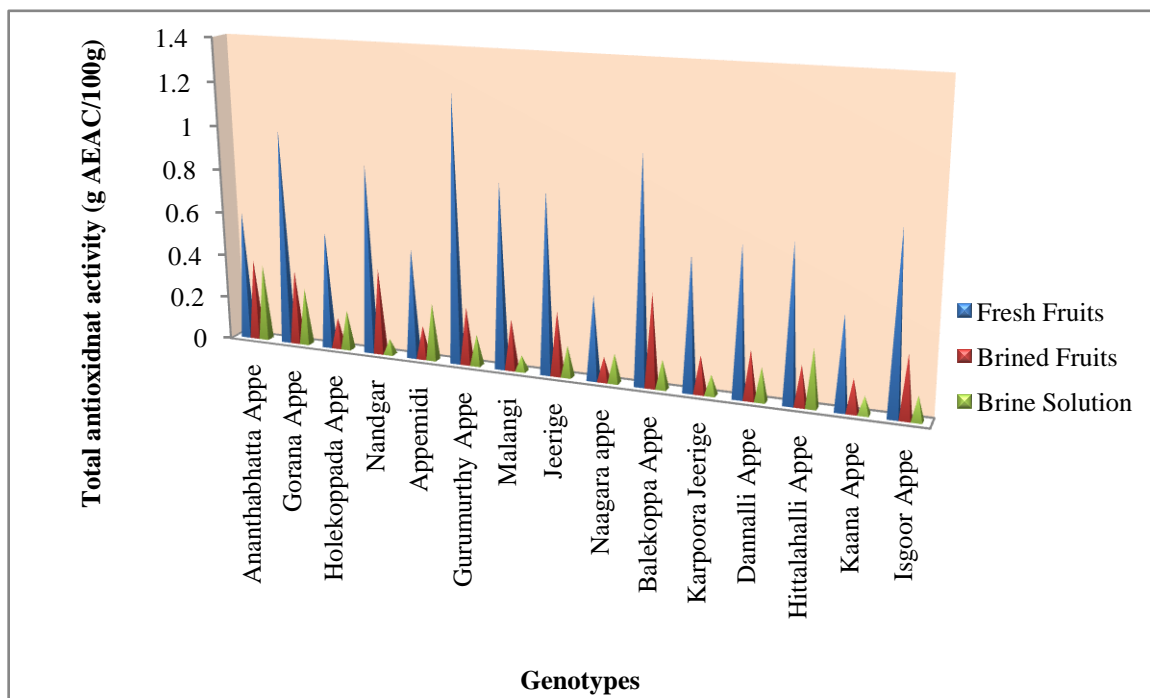


Fig. 32: Total antioxidant activity among 15 Appemidi genotypes at three different forms

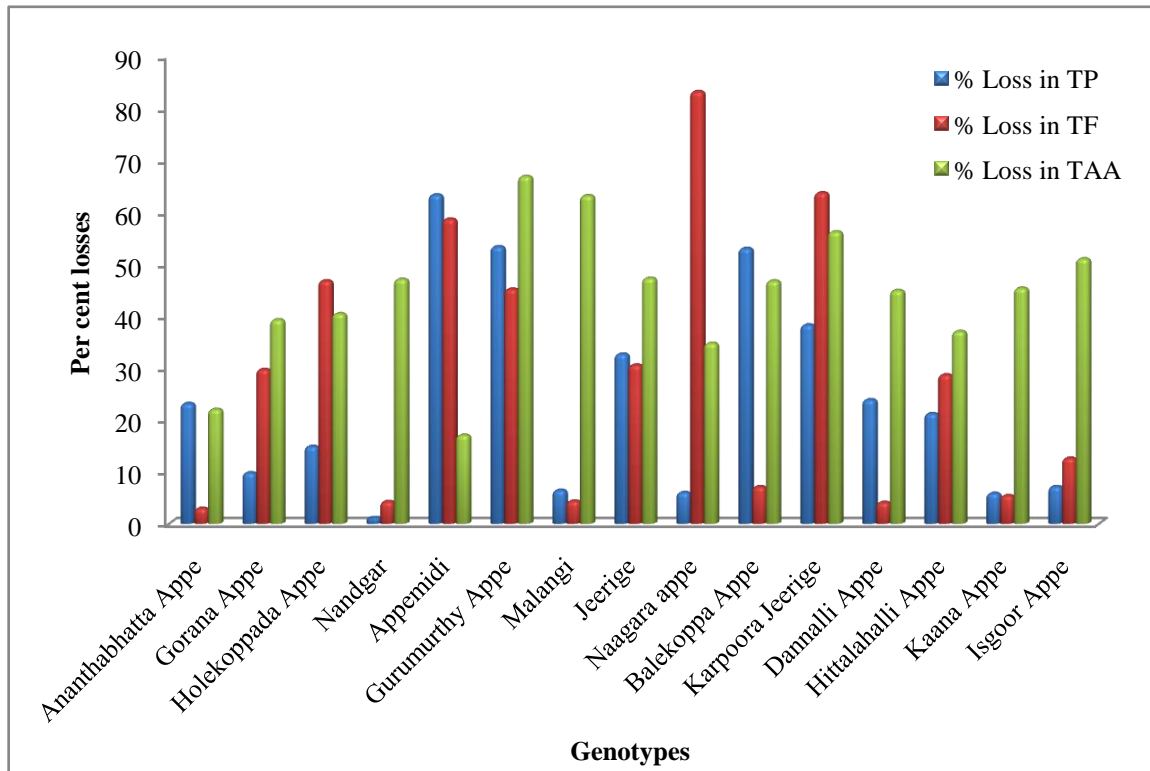


Fig. 33: Per cent loss in total phenols, total flavonoids and total antioxidant activity in 15 appemidi genotypes

5.3.2.7 Water soluble vitamins (ng/g DW)

Vitamins are among the most common substances introduced into food products in order to increase their functionality. Due to low stability of vitamins, it is important to take account of changes they undergo after processing. In this study also we have analysed major vitamins in fresh as well as after salt treatment, to notice the changes in vitamins content. A high salt concentration generates changes in cellular metabolism because of its osmotic effect, which reduce the nutritional value particularly vitamins and minerals (Luck and Pager 2000). Vitamins are compounds without energetic value that are mandatory for human health as these molecules are co-factors of numerous enzymes involved in all types of metabolism contributing to organs function and structure.

Mainly eight water soluble vitamins were profiled and quantified *viz.*, Thiamine, Niacin, Pyridoxine, Pantothenic acid, Biotin, Riboflavin, Folic acid and Cyanocobalamin. Among fresh fruits the maximum concentration of thiamine was found in cultivar Isgoor Appe (185.78 ng/g dry weight). The thiamine concentration was ranged from 30.33 ng to 185.78 ng/g dry weight. The amount of thiamine in brined fruits ranged from 14.91 ng to 63.11 ng /g dry weight. The amount of thiamine in brine solution varied significantly from 0.20 ng to 12.23 ng/g dry weight (Fig 35).

The average niacin content in fresh fruit of appemidi was 67.47 ng/g dry weight. Significant variabilities were observed in niacin concentration in brined fruits of all fifteen Appemidi genotypes. The average niacin content in brined fruit of Appemidi genotypes was 13.26 ng /g dry weight and the average niacin content in brine solution was 103.20 ng/g dry weight.

The concentration of pyridoxine varied significantly among fresh fruits of all fifteen genotypes studied, the concentration varied from 1.82 ng to 47.33 ng/g dry weight. The maximum concentration of pyridoxine was found in cultivar Malange (47.33 ng/g dry weight). The average pyridoxine content in salt cured fruit was 7.98 ng/g dry weight, whereas the amount of pyridoxine found in brine solution was varied from 0.02 ng to 25.78 ng/ ml dry weight.

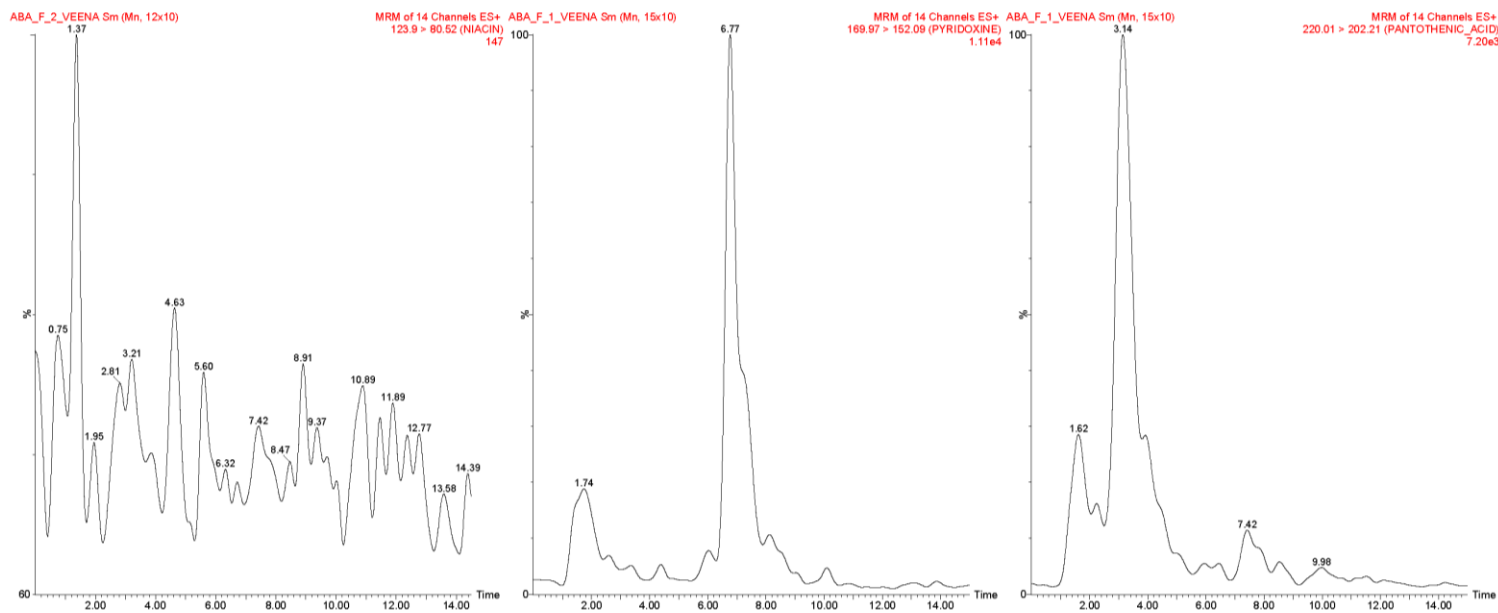


Plate 27: Chromatograms of Niacin, Pyridoxine and Pantothenic acid obtained by LCMS in Appemidi

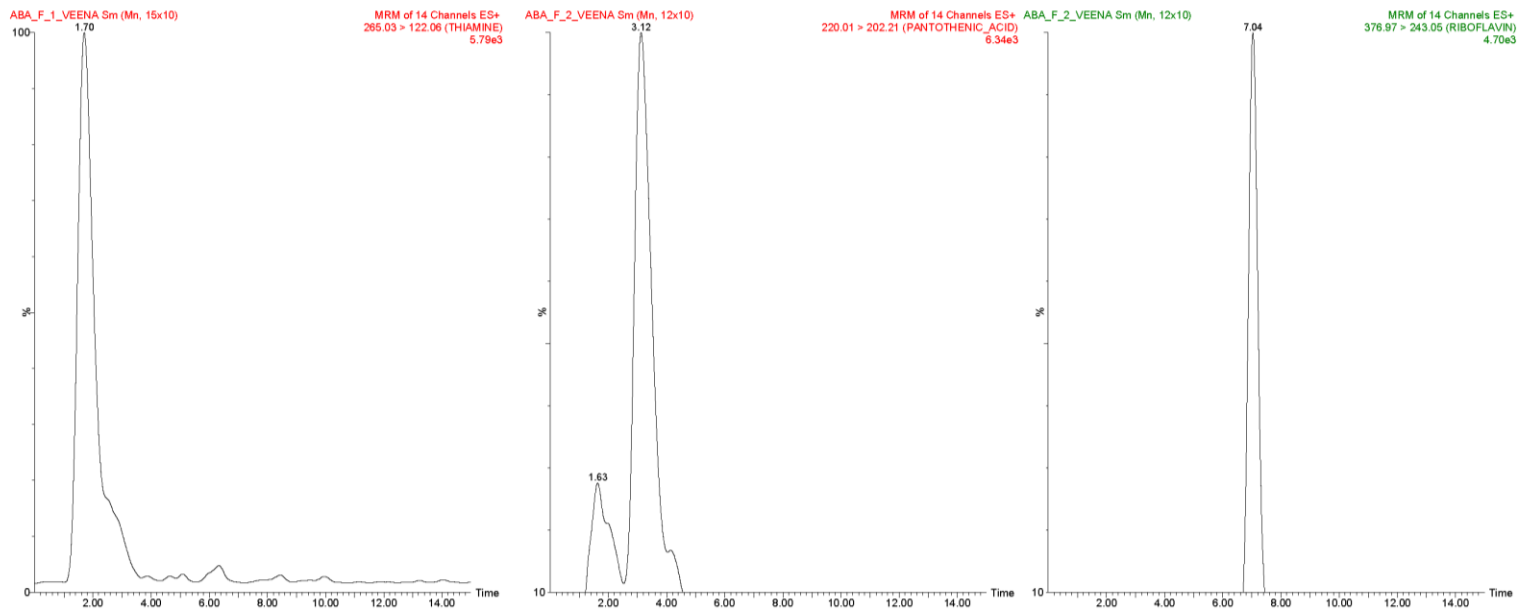


Plate 28: Chromatogram of Thiamine, Pantothenic acid and Riboflavin obtained by LCMS in Appemidi

The average pantothenic acid found in fresh fruits of Appemidi was 55.49 ng/g dry weight. The concentration of pantothenic acid in brined fruit was ranged from 0.45 ng to 10.93 ng/g dry weight and in brine solution it varied from 0.20 to 1.46 ng/ml dry weight.

The highest amount of biotin content in fresh fruit was found in Dannalli Appe (8.84 ng/g of dry weight), whereas in brined fruit form the amount of pantothenic acid was varied from 0.33 ng to 2.42 ng/g. The brine solution also showed significant amount of biotin content with an average concentration of 1.57 ng/ml.

Riboflavin one of the important water soluble vitamin showed a significant difference among the fifteen genotypes studied. The fresh fruit contains an average amount of riboflavin of 15.45 ng/g dry weight. The average content found in brined fruits was 4.07 ng/g dry weight. The maximum amount of riboflavin was found in brine solution (2.09) ng/ml.

The maximum amount of folic acid was found in cultivar Gurumurthy Appe (7.47 ng/g dry weight), whereas the range of folic acid varied from 0.21 ng/g dry weight (Kaana Appe) to 2.36 ng/g dry weight (Karpooora Jeerige) of brined fruits. The brine solution contains around 5.45 ng/ml of folic acid content.

The concentration of cyanocobalamin varied from 0.60 ng to 1.86 ng/g dry weight among fresh fruits of Appemidi. The content of cyanocobalamin in brine solution varied from 0.07 ng/ml to 1.34 ng/ml.

5.3.2.8 Fat soluble vitamins (FSVT) (ng/g)

The average Vitamin D1 content found in fresh fruit was 1.31 ng/g, the average vitamin D1 observed in brined fruit was 0.20 ng/g.

The maximum Vitamin D2 content was observed in Jeerige (90.25 ng/g) and the average vitamin D2 content in brined fruits was 30.79 ng/g (Fig 34).

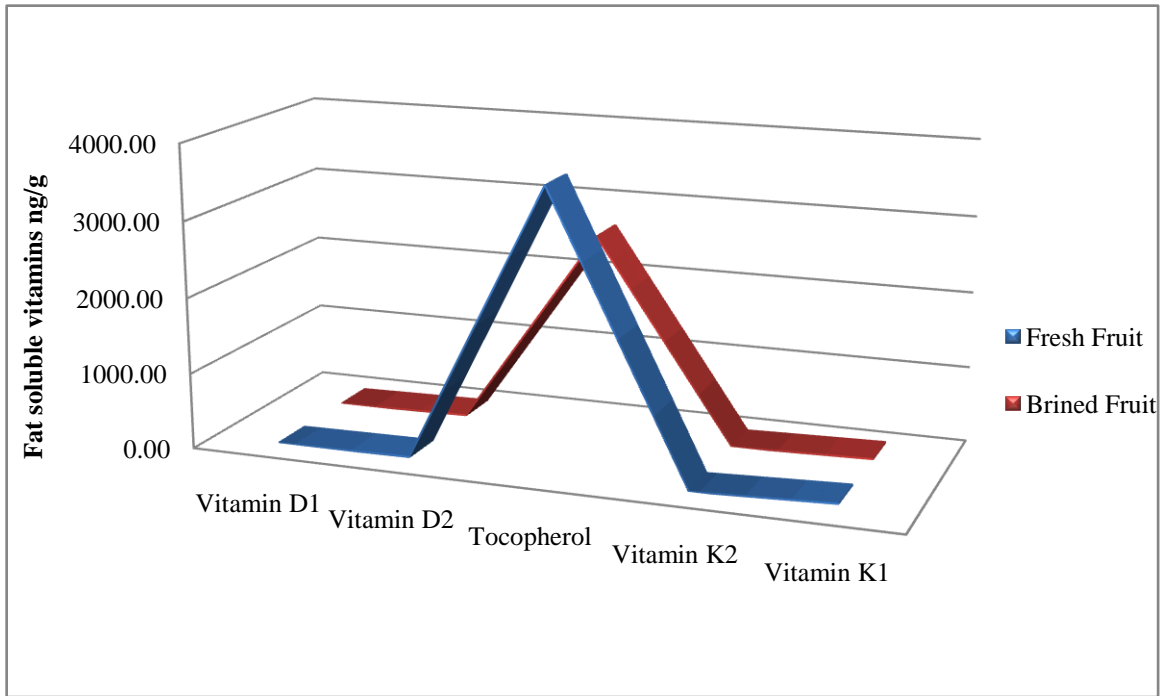


Fig. 34: Fat soluble vitamins profile of Appemidi genotypes in fresh and brined fruit forms

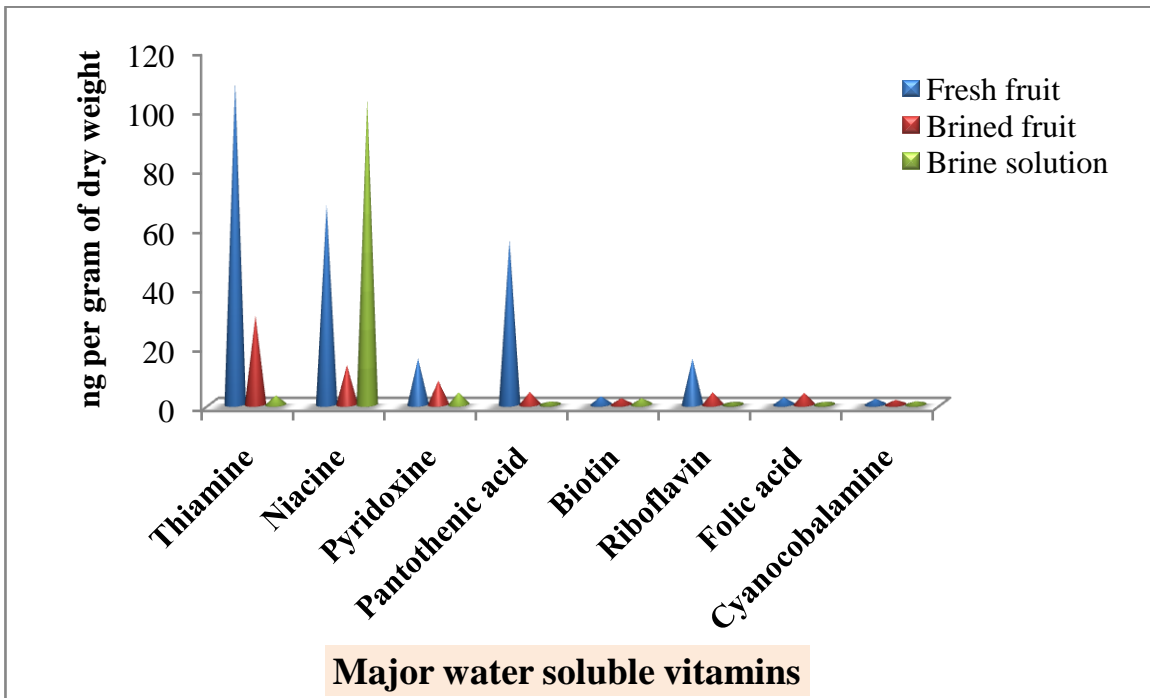


Fig. 35: Water soluble vitamins profile in fresh fruit, brined fruit and brine solution of Appemidi

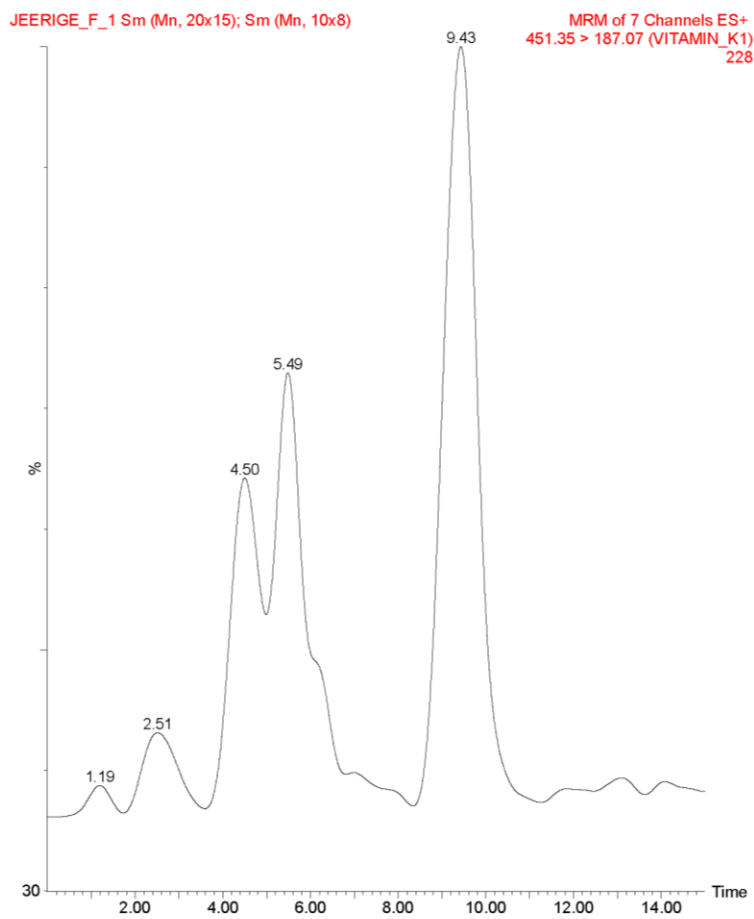
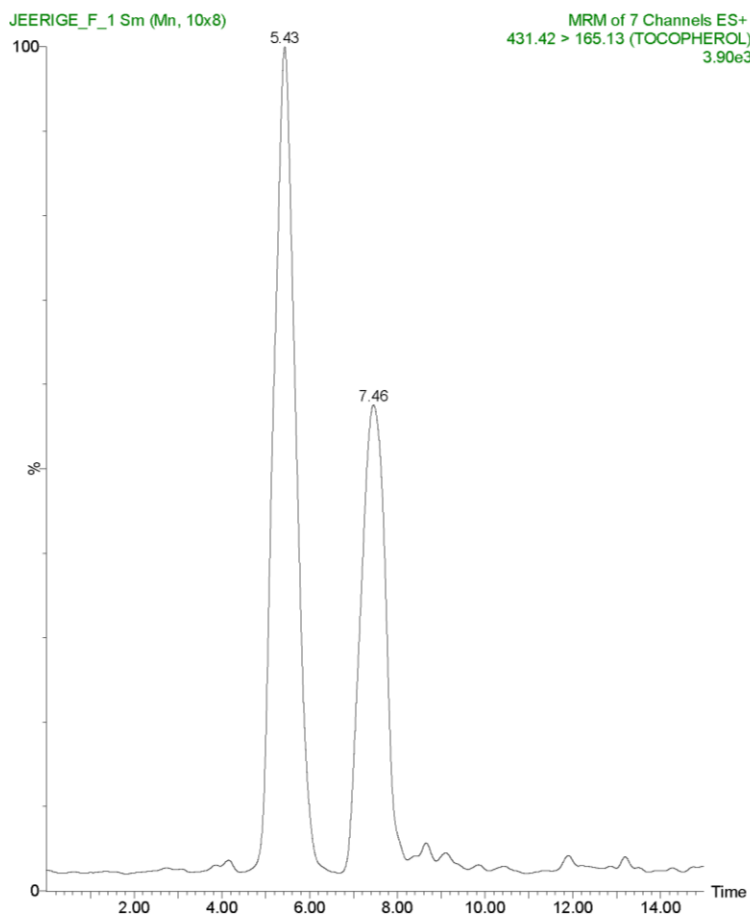


Plate 29: Chromatograms of Tocopherol and Vitamin K1 obtained by LCMS in Appendi

Fresh fruits content of tocopherol ranged from 1.72 μg to 6.50 $\mu\text{g}/100\text{g}$, whereas brined fruit from 1.36 μg to 5.41 $\mu\text{g}/100\text{g}$ with average value of 2.65 μg .

Vitamin K2 ranged from 1.00 to 5.21 ng/g among the fresh fruits, whereas the average Vitamin K2 content in brined fruit was 2.97 ng/g . The brined fruit vitamin K2 content was varying significantly with an average value of 1.67 ng/g .

Vitamin K1 also showed a varied concentration among the cultivars studied with an average content of 71.18 ng/g in fresh fruits. It ranges from 5.40 to 61.42 ng/g with an average concentration of 45.54 ng/g in brined fruits of Appemidi.

The increase of nutrient density by salt treatment is mostly due to decrease of sugar content. This mainly includes vitamins of the B group as well as others such as vitamin K. This is the first report on Appemidi vitamins profile in three different forms.

5.3.2.9 Organic acids (micro gram/ gram)

Mainly Citric acid, Fumaric acid, Maleic acid, Malic acid, Malonic acid, Pyruvic acid, Shikimic acid, Succinic acid and Tartaric acid were profiled in all fifteen genotypes at three different levels (Fig 36).

The range of citric acid concentration in fresh fruits varied from 59.90 $\mu\text{g}/\text{g}$ to 301.75 $\mu\text{g}/\text{g}$. The concentration of citric acid in brined fruits also showed significant differences with an average value of 42.49 $\mu\text{g}/\text{g}$ and the average amount of citric acid found in brine solution of Appemidi genotypes was 4.31 $\mu\text{g}/\text{g}$.

The concentration of fumaric acid varied from 10.20 $\mu\text{g}/\text{g}$ to 60.77 $\mu\text{g}/\text{g}$. The maximum concentration was recorded in cultivar Holekoppada Appe (60.77 $\mu\text{g}/\text{g}$), the highest concentration of fumaric acid was observed (19.46 $\mu\text{g}/\text{g}$) in cultivar Balekoppa Appe brined fruit. Fumaric acid in brine solution was also varied significantly from 0.31 $\mu\text{g}/\text{g}$ (Kaana Appe) to 1.31 $\mu\text{g}/\text{g}$ (Malange).

The concentration of Maleic acid varied from 4.19 $\mu\text{g}/\text{g}$ to 45.35 $\mu\text{g}/\text{g}$ with an average content of 18.21 $\mu\text{g}/\text{g}$. The average maleic acid concentration found in brined fruit was 6.38 $\mu\text{g}/\text{g}$ and the range of maleic acid found in brine solution was 0.26 $\mu\text{g}/\text{g}$ (Kaana Appe) to 0.94 $\mu\text{g}/\text{g}$ (Gurumurthy Appe).

The concentration of Malic acid in fresh immature fruits of all fifteen genotypes showed significant differences with an average value of 82.87 $\mu\text{g}/\text{g}$, in brined fruit 33.77 $\mu\text{g}/\text{g}$ and in brine solution it varied from 0.47 $\mu\text{g}/\text{g}$ (Ananthabhatta Appe) to 14.13 $\mu\text{g}/\text{g}$ (Gurumurthy Appe).

The average malonic acid content of fresh fruit was 65.33 $\mu\text{g}/\text{g}$, whereas it ranged from 13.76 $\mu\text{g}/\text{g}$ to 186.94 $\mu\text{g}/\text{g}$. In case of brined fruit the average malonic acid content was 30.85 $\mu\text{g}/\text{g}$ and in brine solution it varied from 0.95 $\mu\text{g}/\text{g}$ to 6.48 $\mu\text{g}/\text{g}$.

The concentration of pyruvic acid varied significantly in fresh immature fruits, brined fruits as well as in brine solution. The fresh fruits recorded an average content of 23.25 $\mu\text{g}/\text{g}$ and the brined fruits concentration of pyruvic acid varied from 4.68 $\mu\text{g}/\text{g}$ to 18.98 $\mu\text{g}/\text{g}$ and in brine solution it varied from 0.36 to 2.04 $\mu\text{g}/\text{g}$.

Significant differences were observed in shikimic acid concentration, whereas it varied from 227.92 $\mu\text{g}/\text{g}$ to 4555.78 $\mu\text{g}/\text{g}$ in fresh fruit form, 37.91 to 2485.81 $\mu\text{g}/\text{g}$ in brined fruits. The brine solution also contains an average value of 146.13 $\mu\text{g}/\text{g}$. when the plant parts exposed to stress condition the shikimic acid concentration get increased upto certain extent. Shikimic acid is a stress responsive phenolic acid therefore in this investigation also due to salt stress fruits has expressed or retained maximum amount of shikimic acid.

Succinic acid concentration among fresh fruit varied between 2.35 $\mu\text{g/g}$ to 16.08 $\mu\text{g/g}$, 0.11 to 11.42 $\mu\text{g/g}$ in brined fruit and 0.27 $\mu\text{g/g}$ to 2.73 $\mu\text{g/g}$ in brine solution.

Among fresh fruits the tartaric acid content was around 0.32 $\mu\text{g/g}$ and it ranged from 0.11 $\mu\text{g/g}$ to 0.70 $\mu\text{g/g}$ whereas in case of brined fruit also significant amount of tartaric acid was noticed with an average content of 0.14 $\mu\text{g/g}$. The amount of tartaric acid content in brine solution of Appemidi genotypes varied from 0.01 $\mu\text{g/g}$ to 0.04 $\mu\text{g/g}$.

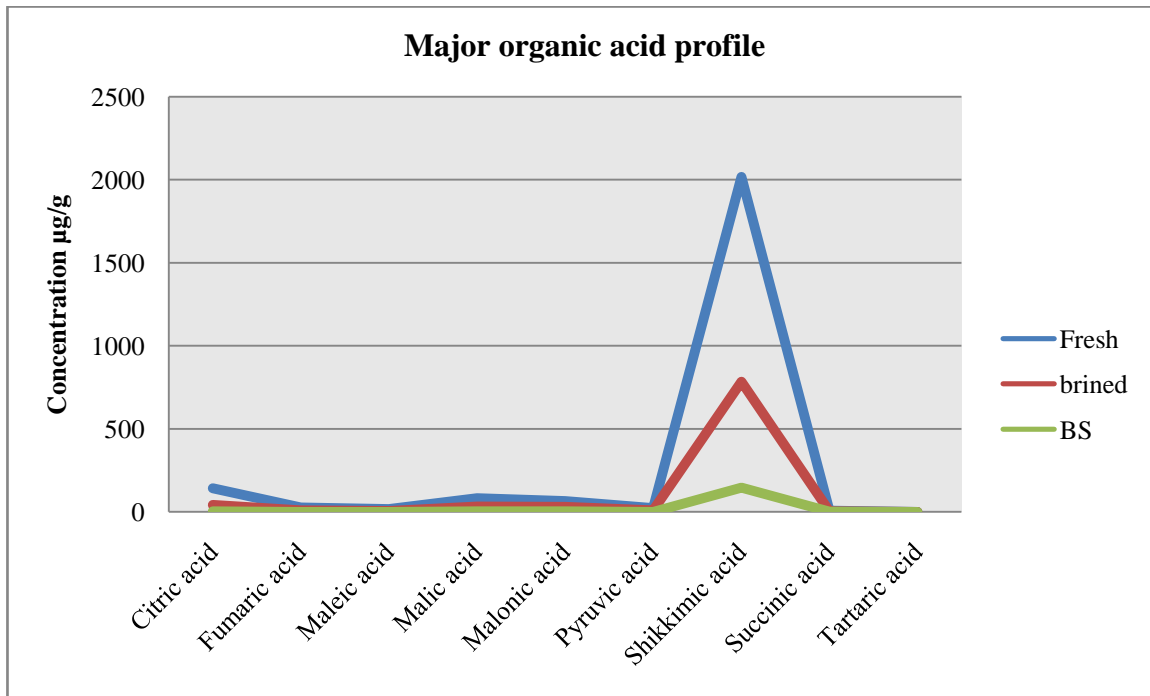


Fig. 36: Organic acid profile in fresh fruit, brined fruit and brine solution of Appemidi

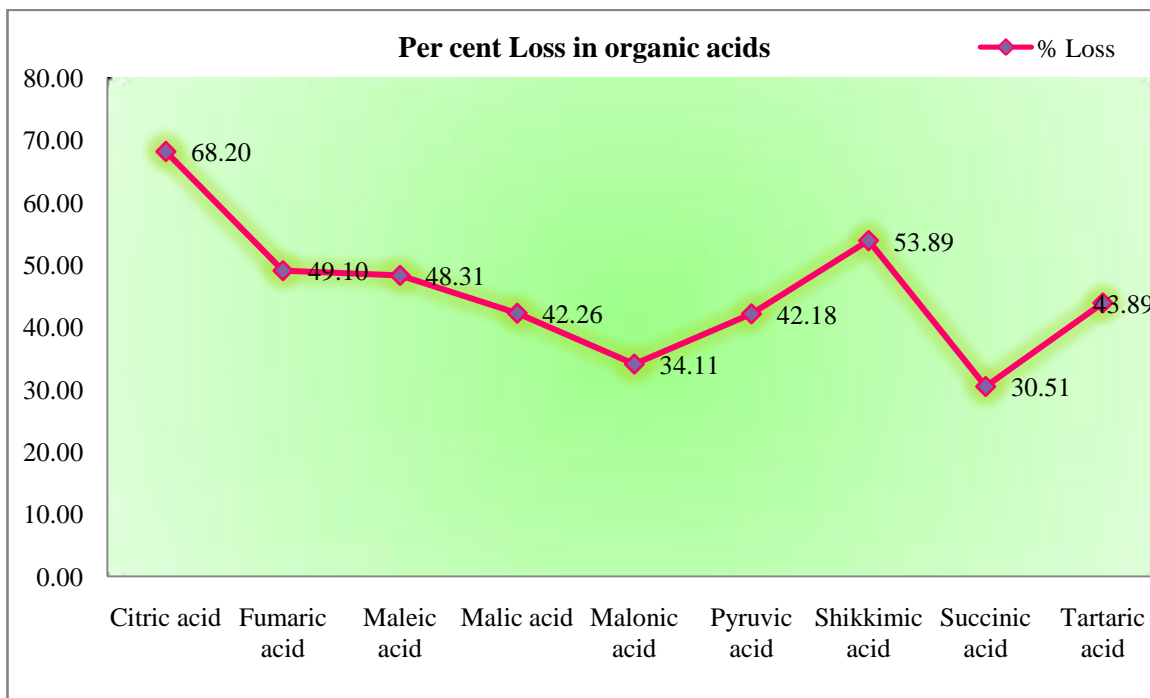


Fig. 37: The percent loss in organic acid among 15 Appemidi genotypes from fresh fruit to brined fruit

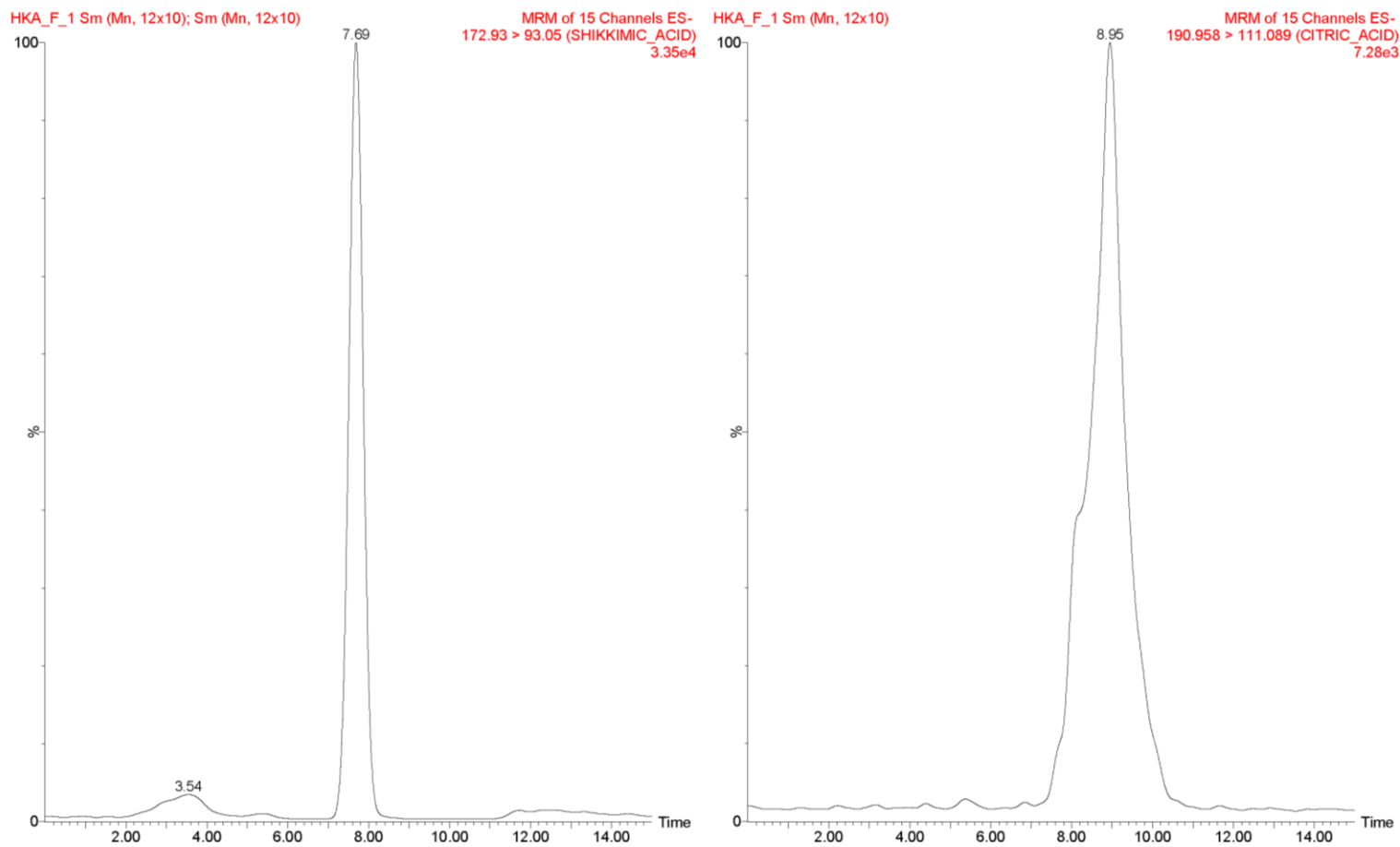


Plate 30: Chromatograms of Shikimic acid and Citric acid obtained by LCMS in Appemidi

The variations could be due to the induction of specific enzymes involved in the biosynthesis of organic acids at brine treatment (Fig 37). There was stimulation for the synthesis of secondary metabolites due to salt stress in plant body in *S. mirzayanii* leaf tissue at moderate salinity (Vilifard, *et al.*, 2014). These results were in assumption with the studies carried out by Burbott and Loomis (1969).

5.4 Indigenous traditional knowledge documentation

Indigenous knowledge is the knowledge of the people living together in a certain area, generated by their own and their ancestors experience including knowledge originating from elsewhere which has been internalized by the local people.

ITK associated with Appemidi land races of the region is predominantly /exclusively related to preparation of different products. In all the surveyed areas Appemidi fruits at different stages of maturity find use in many culinary items and preparation of processed products such as midi uppinakayi/ Jeerige uppinakayi (tender mango pickle). In this line, present observations are also in consonance with Renisha, (2012) who also attempted traditional knowledge documentation with respect to the processing of mango in few villages of Thrissur and Palakkad districts of Kerala and noted a typical varietal specificity for each and every product prepared by the housewives of these regions and the simple traditional skills employed by them for making the products unique.

ITK with respect to selection of fruit for whole tender mango pickle was also documented. The fruits with more sap content, the fruits with thicker peduncle and the fruits which are having higher sap burning time were selected as best midis for pickle preparation because these Appemidi can be preserved for longer period. The sap content of the fruit plays an important role in the long time preservation of these pickles which acts as a preservative. Therefore those midis were subjected for volatile profiling to identify the components mainly responsible for its unique flavour as well its longer shelf life. There was a significant contribution of major monoterpenoids towards its aroma in surveyed Appemidi genotypes. The genotype Doobla has showed maximum per cent of monoterpenoids as well as it is also having longer sap burning period (25 seconds). This indicated that pickle types have a large quantity of terpenoids as well as a completely different combination of monoterpenes for their typical aroma. These may contribute to longer shelf life and higher preference by consumers (Vasugi *et al.*, 2012). The findings were also supported by Brackmann *et al* (1993) that the volatile compounds are responsible for odour and contribute to overall flavour of fresh and processed fruit.

Compared to glass containers the ceramic jars (Bharani or Jaadi) were used for long time preservation of pickle by the local people.

Conservation:

As a part of *insitu* conservation of our genetic diversity ten unique genotypes scions were collected and grafted. These plants were maintained at germplasm block of IIHR. The surveyed pollen samples were also conserved at -196°C *invitro* cryobank of IIHR.



Plate 31: Scions of unique genotypes collected from survey were grafted and conserved insitu



Plate 32: Key farmers of surveyed area

VI. SUMMARY

An investigation entitled “**Studies on Diversity of Indigenous Mango Genotypes**” was carried out at division of Fruit crops, ICAR-IIHR, Bengaluru, during 2013-2014 and 2016-18.

An attempt was made to study the diversity of indigenous mango genotypes of Western Ghat regions of Chikmagalur District in Karnataka state, by conducting a survey and also using molecular markers such as SSRs in order to complement the morphological data and to throw more light on the genetic diversity of Appemidi.

The pollen morphometry was also studied using scanning electron microscopy to study the variability present in pollen grain morphology and to know the evolutionary relationship.

Biochemical profiling of major volatile compounds which are responsible for unique flavour/ aroma was done to identify those aroma fragments present in leaves and were compared with sap volatile fragments.

The major nutraceuticals and organic acids present in Appemidi fruits in fresh and brined fruit forms were also attempted to study the loss of nutrients during the process of brining.

The salient features of experimental findings are summarized below in following heads

6.1 To study the variability in various morphological characters of indigenous mango

Various morphological characterizations were carried out systematically for the entire 40 genotypes of Appemidi collected from survey using standard descriptor for mango by IPGRI (2006) comprising of leaf, inflorescence, fruit and other parameters.

❖ Leaf characterization

The leaf length ranged from 18.77cm to 31.31 cm, the maximum leaf length was recorded in Kanathi-3 (31.31 cm). The leaf width was differed significantly with a mean of 5.59 cm while, the leaf width ranged from 3.90 to 7.57 cm. The petiole length ranged from 1.73 to 4.97 cm. The majority of accessions showed petioles smaller than 4 cm, the mean leaf area recorded was 139.32 cm².

❖ Inflorescence characterization

The maximum Inflorescence length was recoded in Agrahara (22.78 cm) and it ranged from 12.70 cm to 22.78 cm, the maximum inflorescence width was recorded in genotype Shantipura (15.82 cm) and the flower diameter in genotype Agrahara (0.80 cm).

The maximum peduncle length was observed in the cultivar Kumrumane-1 (6.60 cm) and the maximum peduncle width was observed in the cultivar Agrahara and Gonibeedu-1 (0.73 cm).

❖ Fruit Characterization

The maximum fruit length was recorded in Chimankudige-3 (5.65 cm), the fruit diameter in Arenuru-1 (3.92 cm) and maximum fruit thickness was recorded in Agrahara (2.95 cm) whereas the highest fruit weight was recorded in cultivar Arenuru-2 (21.12 g).

❖ Sap burning time (seconds)

The maximum sap burning time was recorded in the cultivar Kudige (28.25 seconds) followed by Arenuru-2.

❖ Pollen morphology and germination studies in Appemidi

Pollen grains of all investigated were symmetric, isopolar, monad, trizonocolpate (three marginal colpi are present) and small in size. The maximum pollen length was observed in genotype Arenuru-2 (59.92 μm) and maximum equatorial diameter was recorded in Marebilu-3 (27.70 μm). Majority of the surveyed genotypes recorded P/E ratio more than two, having perprolate pollen shape and the mean size of the pollen grain was observed to be higher in cultivar Arenuru-2 with striate to reticulate type exine sculpture with perforations.

❖ Genetic variability and genetic divergence study

In the present study, the traits with moderate to high PCV, GCV and genetic advance and high value of heritability was observed in inflorescence width, peduncle length and fruit thickness which revealed the low influence of environment suggesting that fairly rapid genetic gain could be expected for these traits. 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.

Among all the morphological characters studied, fruit diameter had a maximum contribution (13.0%) to the total diversity followed by fruit weight and inflorescence diameter. Among the pollen characters studied colpi per cent showed the maximum per cent contribution towards total divergence followed by equatorial diameter and colpi length.

The genotypes were grouped into different clusters irrespective of its geographical origin they are located or place of collection. The clustering pattern of the genotypes revealed that the genotypes collected from the same region did not fall in a single cluster which indicated the variability among the genotypes irrespective of their place of collection.

PCA confirms the group constellations obtained by D^2 analysis. The characters which contributed more towards divergence in D^2 analysis were loaded in PC-I except leaf length, leaf width, leaf area which showed significant high values in PC-I.

6.2 To study the diversity within the population through molecular characterization

Fourteen selected primers were used to analyse inter and intra cultivar diversity in 40 Appemidi genotypes. High level of polymorphism was observed with MillHR 18 primer. The expected heterozygosity (H_e) values ranged from 0.976 to 0.985, polymorphic information content values ranged from 0.964 to 0.973 with a mean of 0.969 and the majority of them showed high PIC values (0.700) that the markers selected were highly informative. Three major clusters divided into six important sub clusters based on the genetic dissimilarity were obtained.

The morphological, palynological and molecular diversity analysis showed that the grouping of genotypes into three different main clusters and six important sub clusters irrespective of their place of collection.

6.3 To study the chemical profile of fruit sap and leaf

- The genotype Ananthabhata Appe leaf had the highest area of volatiles and 55+ volatile components were detected. The genotype Kalkuni had the highest per cent of monoterpenoids proportion (71.41%) followed by Nandgar (71.18%). Appemidi leaves were rich in β -phellandrene, α -pinene, limonene and cis-ocimene monoterpenoids and among sesquiterpenoids caryophyllene and α -humulene were the major.
- Cluster analysis and principal component analysis of leaf volatiles grouped the genotypes mainly into monoterpenoids rich and sesquiterpenoids rich cultivars separately. The cluster analysis result showed that, genotype Adderi Jeerige, Kadikai, Kutumba Appe, Thumbeelu, Jeerige, Huliappekai and Shidadakke Appe were clustered together with diverse proportion of β -Phellandrene, α -Pinene and Limonene as their major composition.

- In fruit sap, monoterpenoids occupies highest proportion. The concentration of aroma compounds was highest in Ananthabhattacha Appe. α -phellandrene, β -phellandrene, α -pinene, β -pinene, 3-carene, limonene, β -terpinene and trans-ocimene are the major compounds identified in fruit sap. The genotype Ananthabhattacha Appe had the maximum per cent of monoterpenoids compared to other genotypes.
- The per cent contribution from leaf and sap was followed the same trend. In genotype Kalakai α -phellandrene (3.33 and 48.97%) contributed the highest per cent among the total volatiles composition both in leaf as well as sap. Therefore it was proved that the leaf flavour was directly correlated with the fruit sap flavour.
- α -Phellandrene, β -pinene, β -phellandrene and limonene can be used as a pre selection index in breeding programme for selection of aroma rich cultivars.

Profiling of major fat soluble, water soluble vitamins and organic acids in fresh, brined fruits and brine solution of 15 Appemidi genotypes

The maximum fruit weight was recorded in Dannali Appe (27.02g), while the mean fruit weight was 24.19g. Non-significant differences were obtained for salt cured fruit weight. Fruit length was measured before and after salt treatment, the maximum per cent reduction in fruit length was noticed in Appemidi (46.06%) and the per cent reduction in fruit thickness was (57.15%) noticed, whereas an increase in fruit firmness considerably was noticed after salt treatment.

- The highest vitamin C content was found in fresh fruits of genotype Appemidi (263.00 mg/100g). Vitamin C content in brined fruits ranged from 14.16 mg to 141.83 mg per 100 gram of pulp whereas brine solution also showed some amount of vitamin C content in it.
- In fresh fruits the acidity was more (1.25 to 3.99 mg/100g) compared to salt cured fruits and brine solution.
- The maximum total phenol content was recorded in salt cured fruits (2.47 g GAE/100g) because of cellular oxidative damage when it was exposed to salt stress which stimulates the biosynthesis of phenols.
- There was an increase in trend among salt cured fruits composition of flavonoids and antioxidant activity compared to fresh fruits. The total antioxidant activity varied from 0.37 g to 1.21 g AEAC/100g in fresh fruits whereas the range of total antioxidant activity was from 0.49 g to 1.39 g AEAC/100g in brined fruit.
- Mainly eight water soluble vitamins were profiled and quantified viz., Thiamine, Niacin, Pyridoxine, Pantothenic acid, Biotin, Riboflavin, Folic acid and Cyanocobalamin. Five fat soluble vitamins viz., Vitamin D1, Vitamin D2, Tocopherol, Vitamin K2 and Vitamin K1 were profiled.
- The brined fruits showed maximum fat soluble vitamin content compared to fresh fruits. This increase of nutrient density by salt treatment is mostly due to decrease of sugar content. This mainly includes vitamins of the B group as well as others such as vitamin K.
- Citric acid, Fumaric acid, Maleic acid, Malic acid, Malonic acid, Pyruvic acid, Shikimic acid, Succinic acid and Tartaric acid were profiled in all fifteen genotypes at three different levels.
- The range of organic acid concentration in fresh fruits were, Citric acid content varied from 3.33 μ g/g to 470.09 μ g/g, the concentration of Fumaric acid from 1.82 μ g/g to 60.77 μ g/g. Maleic acid from 1.85 μ g/g to 45.35 μ g/g, Malic acid from 2.07 to 760.27 μ g/g, malonic acid from 4.96 μ g/g to 186.94 μ g/g, pyruvic acid from 7.42 μ g/g to 53.99 μ g/g, Shikimic acid from 161.70 μ g/g to 4555.78 μ g/g, Succinic acid from 1.46 μ g/g to 16.08 μ g/g and tartaric acid from 0.11 μ g/g to 0.70 μ g/g. Significant higher concentration of organic acid trend was noticed in salt cured fruits. The concentration varies with respect to genotypes as well as brine treatment.
- Indigenous traditional knowledge associated with these surveyed genotypes was documented.

6.4 Indigenous traditional knowledge documentation

ITK associated with Appemidi landraces of the region is predominantly /exclusively related to preparation of different products. In all the surveyed areas appemidi fruits at different stages of maturity find use in many culinary items.

ITK with respect to selection of fruit for whole tender mango pickle was also documented. The fruits with more sap content, the fruits with thicker peduncle and the fruits which are having higher sap burning time were selected as best midis for pickle preparation because these Appemidi pickles can be preserved for longer period.

Compared to glass containers the ceramic jars (Bharani or Jaadi) were used for long time preservation of pickle by the local people.

As a part of *insitu* conservation ten unique types scions were collected and grafted and the surveyed pollen samples were also conserved at -196°C *invitro* cryobank of IIHR.

Future line of work

- ❖ Appemidi is our indigenous traditional wealth. Therefore conservation and characterization of these unique types will help to identify the superior ones.
- ❖ Aroma is one of the important traits in fruit crop improvement with respect to consumer acceptance. Utilization of these types will help to develop a commercial cultivar with high aroma.
- ❖ Leaf flavour was directly correlated with fruit sap flavour, it can be used as a pre-selection criterion for selection of cultivars to minimize the juvenile period.
- ❖ Conservation of our genetic diversity plays an important role, therefore unique accessions were collected, characterized and conserved *insitu* as well as *exsitu*.
- ❖ Further extensional work is needed popularize these genotypes.
- ❖ Modifications are needed in the process of pickle preparation because there is a loss of important nutritional components with respect to food value.
- ❖ More research is needed to know the scientific reason for long term preservation of these pickles.

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APPENDIX I

Meteorological data during the investigation period at ICAR-Indian Institute of Horticultural Research, Hessaraghatta, Bangalore- 89, 2013- 2015

Month	Temperature (°C)		Relative Humidity (%)		Mean wind speed (km/h)	Total Rainfall (mm)
	Max.	Min.	7.30hrs	14.00hrs		
2013						
December	27.16	15.90	77.32	51.06	3.33	5.00
2014						
January	27.35	16.12	76.41	48.80	3.96	0.00
February	27.89	17.46	75.07	44.5	4.26	0.00
March	29.29	19.93	74.48	47.51	4.44	10.10
April	29.76	20.56	76.46	38.23	4.21	0.00
May	30.03	20.90	74.03	47.51	4.72	137.40
June	30.53	21.33	69.1	45.46	6.70	56.00
July	26.16	19.51	64.83	42.38	8.51	162.30
August	28.64	20.19	67.12	53.93	6.81	149.00
September	28.96	20.96	69.73	47.23	5.47	185.50
October	28.67	21.03	70.25	45.74	3.43	395.00
November	27.8	20.9	71.06	44.3	3.15	26.00
December	27.35	20.80	71.03	52.16	3.66	0.00

APPENDIX II

LIST OF ABBREVIATIONS USED

Abbreviations	Explanations
%	Per cent
Viz.,	Namely
0C	Degree Celsius
*	Significant
Min	Minutes
cm	Centimetre
kg	Kilogram
g	Gram
mg	Milligram
µg	Microgram
µl	Micro litre
ml	Mili litre
µm	Micro meter
NS	Non significant
IU	International unit
Fig	Figure
TSS	Total soluble solids
SSR	Simple sequence repeat
PCR	Polymerase chain reaction
µ	Micro
β	Beta
α	alpha
δ	Delta
γ	Gamma
i.e.	That is
PIC	Polymorphic information content
ITS	Internal transcribed spacer
ng	nanogram