STUDIES ON THE DIVERSITY IN MORPHOLOGICAL, BIO-CHEMICAL AND MOLECULAR CHARACTERIZATION IN SAPOTA (*Manilkara achras* (Mill). Fosberg) GENOTYPES.

BY

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DECLARATION

I, Mr. A. HARSHAVARDHAN, hereby declare that the thesis entitled **"STUDIES ON THE DIVERSITY IN MORPHOLOGICAL, BIO-CHEMICAL AND MOLECULAR CHARACTERIZATION IN SAPOTA (*Manilkara achras* (Mill.) Fosberg) GENOTYPES**" submitted to the Dr. Y.S.R. Horticultural University, Venkataramannagudem, for the degree of Doctor of Philosophy in Horticulture (Fruit Science) is the result of original research work done by me. I declare that no material contained in the thesis has been published earlier in any manner.

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CERTIFICATE

Mr. A. HARSHAVARDHAN has satisfactorily prosecuted the course of research and the thesis entitled **"STUDIES ON THE DIVERSITY IN MORPHOLOGICAL, BIO-CHEMICAL AND MOLECULAR CHARACTERIZATION IN SAPOTA (*Manilkara achras* (Mill.) Fosberg) GENOTYPES""** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination.

I certify that neither the thesis nor its part there of has been previously submitted by him for a degree of any university.

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CERTIFICATE

This is to certify that the thesis entitled “**STUDIES ON THE DIVERSITY IN MORPHOLOGICAL, BIO-CHEMICAL AND MOLECULAR CHARACTERIZATION IN SAPOTA (*Manilkara achras* (Mill.) Fosberg) GENOTYPES**”submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Horticulture (Fruit Science) of Dr. Y.S.R. Horticultural University, Venkataramannagudem, is a record of the bonafide research work carried out by **Mr. A. HARSHAVARDHAN** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of investigation have been duly acknowledged by the author of the thesis.

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(Harshavardhan A)

LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Percent
µg	:	Microgram
µl	:	Microlitre
^{0}C	:	Degree Celsius
2D	:	2 Dimensional
AFLP	:	Amplified Fragment Length Polymorphism
bp	:	Base pairs
cm	:	Centimeter
CTRI	:	Central Tobacco Research Institute
CTAB	:	Cetyltrimethyl Ammonium Bromide
dNTP	:	deoxy Nucleotide Tri-Phosphate
Dr.Y.S.R.H.U.	:	Dr. Y S Rajasekhar Reddy Horticultural University
DRR	:	Directorate of Rice Research
DUS	:	Distinctness, Uniformity and Stability
DW	:	Distilled Water
EDTA	:	Ethylene Diamine Tetra Acetic acid
EC	:	Electrical Conductivity
EMSS	:	Error Mean Sum of Squares
ESS	:	Error Sum of Squares
et al.,	:	and other workers
Fig.	:	Figure
g	:	Gram
GCV	:	Genotypic Coefficient of Variation
G	:	Genotypic
ISSR	:	Inter Simple Sequence Repeats
Kg	:	Kilogram
LB	:	Long bold
LS	:	Long Slender
M	:	Molar
MB	:	Mega bases
mg	:	Milli gram

ml : Milliliter

mM : Millimolar

mm : Millimeter

MB : Mediun Bold

MS : Medium Slender

ng : Nanogram

No. : Number

P : Phneotype

PAGE : Poly Acrylamide Gel Electrophoresis

PCR : Polymerase Chain Reaction

PIC : Polymorphic Information Content

PCV : Phenotypic Coefficient of Variation

rp : Phenotypic Correlation

rg : Genotypic Correlation

RAPD : Random Amplified Polymorphic DNA

RBD : Randomized Block Design

RFLP : Restriction Fragment Length Polymorphism

SD : Standard Deviation

Sem : Standard Error of mean

SS : Sum of Squares

SLS : Sodium Lauryl Sulphate

SSR : Simple Sequence Repeats

STMS : Sequence Tagged Microsatellite Sites

Taq : *Thermusaquaticus*

TBE : Tris Borate EDTA

TE : Tris EDTA

Tris. Cl : Tris (hydroxyl methyl) amino methane hydro chloride

UPGMA : Unweighted Pair Group Method with Arithmetic averages

UV : Ultra violet

VRG : Venkataramannagudem

viz; : Namely

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ABSTRACT

The present investigation, “Studies on the diversity in morphological, bio-chemical and molecular characterization in sapota genotypes**”,** was carried out during 2013-2014 at Horticultural Research Station, Venkataramannagudem, Andhra Pradesh. Genetic diversity was evaluated in thirty three genotypes of sapotaby tree morphological, fruit bio-chemical and molecular markers. Twenty four RAPD and sixteen SSR markers were employed to analyze the molecular diversity among the genotypes.

The analysis of variance for the twenty three quantitative traits revealed significant differences for all the characters studied thus indicating wide variation among the genotypes.The genotype Kirthibarthi recorded maximum plant height, while the genotype Cricket Ball recorded maximum leaf length, leaf width, leaf area and seed weight. The mean fruit length was highest in PKM-4 and maximum fruit width, fruit weight and pulp to seed ratio was recorded in Columbian Sapota. The genotype Tagarampudi recorded highest per cent fruit set and yield/tree. Further, the genotype PKM-4 recorded maximum TSS and total sugars, while maximum reducing sugars was reported in Kalipatti and highest non-reducing sugars was recorded in the genotype CO-2. Highest amount of ascorbic acid content was recorded in CO-1, while maximum titrable acidity and pectin was reported in Mirandi. Further, the genotypeKrishna Rao recorded highest total phenols.

The characters, namely, fruit weight, seed number, seed weight, pulp to seed ratio, per cent fruit set, yield per tree, non-reducing sugars, ascorbic acid, TSS to acid ratio and pectins had recorded higher estimates for phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance as per cent mean.

Correlation coefficient and Path coefficient analysis revealed that the association of plant height, leaf width, leaf area, fruit width, fruit weight, seed weight, pulp to seed ratio and per cent fruit set with yield/tree and among themselves was positive and highly significant and these traits were identified as fruit yield components and exerted high positive direct influence on yield per tree. Similarly the association of TSS, total sugars, reducing sugars, non-reducing sugars, ascorbic acid and TSS to Acid ratio with yield per tree among themselves was positive and highly significant. This indicated that direct selection of yield improvement through these traits would be rewarding.

In D^2 analysis, the characters *viz.*, leaf width, leaf area, fruit length, per cent fruit set, TSS, total sugars, non-reducing sugars, ascorbic acid and TSS to Acid ratio contributed more for the divergence. In PCA, the characters *viz.*, yield per tree, total sugars, TSS, ascorbic acid, titrable acidity, TSS to Acid ratio, phenols and per cent fruit set in PC_1 contributing more towards variability.

The RAPD analysis with twenty four primers produced 204 polymorphic bands with an average of 8.2 polymorphic bands per primer. Out of total 204 polymorphic bands, thirteen bands were unique to particular genotype viz., Gutti (OPC-2), Tagarampudi and PKM-4 (OPG-3), Calcutta Round, Columbian Sapota and Cricket Ball (OPG-4), Pakala Oval (OPG-7), DHS-2 (OPV-9), DHS-1 (OPV-10), Columbian Sapota (OPX-3), Cricket Ball (OPX-11), Kalipatti and Pakala Round (OPX-12) which could be exploited for DNA fingerprinting of these accessions by converting RAPD markers into STS markers and this was highly useful for detecting mixes between genotypes.

Eighty eight alleles were produced by examining the 33 sapota genotypes with sixteen microsatellite loci with an average number of 2.66 alleles per locus. High level of polymorphism was observed with primers SSR S-4 and SSR Mh-12. The PCR product size obtaind by amplification of SSR primers was ranged from 135 to 555 bp. Out of the total 88 polymorphic alleles, twenty one fragments were unique to particular genotypes viz., Columbian Sapota, DHS-1 and DHS-2 (SSR S-1), CO-3 (SSR S-2), Pakala Round (SSR S-3), Seedless (SSR S-4), PKM-2 (SSR S-5), DHS-1 and DHS-2 (SSR S-7), Cricket Ball, Calcutta Round and CO-2 (SSR S-8), Gavarayya (SSR S-9), Singapore, DHS-1 and PKM-2 (SSR S-10), CO-2 (SSR Mh-26), Bombay (SSR Mh-20), Kalipatti and Pakala Round (SSR Mh-12) and Pala (SSR Mh-17) which could be exploited for DNA fingerprinting of these genotypes.Based on outcome of the present investigation, it can be concluded that molecular markers for fingerprinting as well as estimation of genetic diversity and genetic relatedness in sapota genotypes is effective, precise and more efficient than morphological markers.

CHAPTER I

INTRODUCTION

Sapota(*Manilkaraachras* (Mill.) Fosberg)is one of the delicious fruits of humid tropical and subtropical regions, belonging to the family Sapotaceae. It is a native of tropical America and has now spread to almost all tropical countries of the world. It is also called by other names, such as chikku, sapota plum and sapodilla. The pulp of sapota when ripe is soft, granular and very sweet. Sapota fruits may be eaten fresh or used as an ingredient in various milk products. Sapota is an energy rich fruit with high total soluble solids (20-22%) and good source of digestible sugar (14-18%)and has an appreciable amount of protein, fat, fiber and minerals like calcium, phosphorous, iron (Shanmugavelu and Srinivasan, 1973). In fruits, peel can also be eaten along with pulp since it is rich in nutritive value than the pulp alone (Gupta *et al.*, 1981). Latex from sapota tree is used in the manufacture of chewing gum in tropical America. The gum processed from sapota also finds use in dental surgery. The bark of tree contains extractable quantities of tannins which have many industrial uses.

The cultivation of sapota has attracted many farmers on account of its hardy nature and better adaptability to diverse soil and climatic conditions. Though the area under sapota is increasing day by day, the increase is not up to the expected level. One among the several causes attributed for this is the absence of number of good choice varieties. Sapota is a hardy crop and fairly less susceptible to pest and disease which may be due to the presence of endogenous phenolic substances (Lakshminarayana and Subramanyam, 1966). India is a leading producer of sapota with an area and production of 1,58,000 ha and 13,46,000 metric tonnes respectively (Anon., 2010). It is commercially grown in Maharashtra, Gujarat, Karnataka, Andra Pradesh, Tamil Nadu and West Bengal states. In Andhra Pradesh, it occupies an area of 12,208 ha in Guntur, Prakasam, West Godavari, Kurnool and Medak districts with an annual production of 1.22 lakh tonnes (NHB Database, 2013).

Domestication and improvement programmes are enhanced by knowledge of the genetic structure of the existing cultivars in order to capture high level of diversity in breeding programmes. In perennial crops like sapota, guava *etc.*, the important way to develop a new high yielding variety with specific desirable characteristics is selection of superior genotypes based on the phenotype from among the existing variation and use them directly or in future breeding programmes. It is essential to identify and effectively conserve the existing genetic resources with an utmost certainty.

Since, sapota is an open pollinated crop, a great deal of variability was thrown up in the population. A few of the cultivars have been selected based on local preferences for different fruit characteristics and later cultivated by farmers through vegetative propagation. Therefore, the high genetic variability in Indian sapota cultivars might have originated through seedling segregation, inter crossing among cultivars or because of a large number of cultivars or genotypes were introduced. Despite its wide cultivation throughout the world, the genetic diversity of the crop has not been studied using molecular markers. Genetic variability forms the basis for crop improvement programmes which are designed to breed improved hybrids/varieties. In sapota, most of the work on variability has been carried out using morphological characteristics such as tree shape, structure, leaf colour, shape, fruit size and shape (oval and round). Cultivars have been grouped based on the growth habit of the trees , shape and colour of the foliage into four major groups; (a)Trees with erect growth habit, (b)Trees with spreading habit I (leaves green, broad and oval shape), (c)Trees with spreading habit II (leaves light green, narrow elliptical shape), (d)Trees with drooping habit (Bal, 1997). This classification appears to be very arbitrary because morphological characteristics are influenced by agro-climatic conditions. So far, no studies were carried out to estimate the extent of genetic variability in sapota cultivars using either isozymes or DNA markers.

Usually the maintenance and evaluation of germplasm is based on phenotypic features such as morphological, physiological or horticultural descriptions. These descriptions have limited value, as the plants are grown at different locations, as only specific developmental stages are suitable for screening and detection of hybridization and pedigree determination. This is because the genetic control of any such characters is complex, sometimes exhibiting delayed gene expression and demands higher cost is to develop a core collection (Frankel and Brown, 1984), which represents the genetic diversity of a crop species and its relatives with minimum repetitions. The main purpose of core collection is to characterize the germplasm that is preserved and to eliminate duplicates and it should represent the diversity at hand. In any case, the immediate priority is to catalogue the available germplasm and identify duplicates in the germplasm collection maintained at various centers in India. Problems like influence of environment, human (visual) judgments and less genome coverage limit the use of phenotypic markers for this purpose. Such limitations can be largely avoided by using DNA profiling methods. It is now possible to look at differences precisely at the molecular levels using DNA based markers that are seldom influenced by environmental factors.

The studies on the genetic polymorphisms and phenotypic relationships can provide a scientific basis for the utilization of these genotypes for the efficient crop improvement and also could provide information on the origin and evaluation of cultivated sapota and its hybrids. Hence, characterization of varieties at phenotypic levels based on morphological characters supplemented with molecular characterization at genetic level is first step towards efficient conservation and maintenance of the existing genetic diversity.

In the world market, there is an increasing need for molecular methods to characterize, to define genotypes to control quality and to find out genuinely for the prevention of fraudulent commerce. Identification of varieties through utmost certainty is a prerequisite to patent and claiming plant varietals rights. In this respect molecular markers are becoming more popular for DNA fingerprinting of varieties.

DNA can be typified by wide range assortment of fingerprinting techniques. Generally they can be classified into restriction (Restriction), amplification (Randomly Polymorphic DNA) or both restriction and amplification based (Amplified

Fragment Length Polymorphism) marker. Among different types of molecular markers available RAPD (Randomly Amplified Polymorphic DNA) and SSR (Simple Sequence Repeats) markers are attractive because of their simplicity, versatility, modest cost and ability to detect even the relatively small amount of variation (Ragot and Hoisington, 1993). Such precise and refined techniques appear to have not been utilized in the genus *Achras.*

In the light of above information, an investigation entitled "Studies on the diversity in morphological, bio-chemical and molecular characterization in sapota(*Manilkaraachras* (Mill.) Fosberg)genotypes" was planned with the following objectives:

1. To study the fruit morphological and bio-chemical characters of sapota genotypes.
2. To assess the extent of genetic variability, heritability and genetic advance in fruit morphological and bio-chemical characters of sapota genotypes.
3. To assess the extent of morphological diversity among the sapota genotypes by using D^2 analysis.
4. To assess the extent of molecular diversity among the sapota genotypes by using RAPD and SSR markers.
5. To study and compare the clustering pattern of the genotypes based on morphological and molecular characterization by using UPGMA.

CHAPTER - II

REVIEW OF LITERATURE

The cultivation of sapota has attracted many farmers on account of its hardy nature and better adaptability to diverse soil and climatic conditions. Though the area under sapota is increasing day by day, the increase is not up to the expected level may be due to absence of number of superior varieties. At present, a few varieties are being grown commercially and they lack in one or other good characters. Therefore, there is a need to identify good cultivars with higher yield and quality fruits. In this chapter, an attempt has been made to present the literature pertaining to this important aspect of the crop. But the information available on this aspect in sapota is meager. Hence, in addition to reviewing the available information on sapota, the literature available in related fruit crops has also been reviewed under following headings.

2.1 Morphological characters

2.2 Biochemical characters

2.3 Correlation and Path Coefficient analysis

2.4 Coefficient of variation, Heritability and Genetic Advance

2.5 Genetic Divergence

2.6 Molecular characterization

2.1 MORPHOLOGICAL CHARACTERS

2.1.1 Plant height

Under Periyakulam conditions, the tree height differed significantly among the 12 sapota varieties evaluated. Cultivar CO-1 recorded the highest plant height (14.10 cm) in 17 years old trees (Anon., 1997).

Hegde (1997) noticed that the hybrid 3/13, DHS-1 (10/25) and Cricket Ball exhibited the orthotropic nature (5.06 m, 4.76 m and 4.65 m respectively), while

Kalipatti showed plagiotropic nature by recording the lowest tree height (3.06 m) among the varieties and hybrids evaluated under Dharwad conditions.

Gunuki (1998) observed that the cultivar DHS-1 and Cricket Ball exhibited orthotropic nature (5.18 m and 4.78 m, respectively), while *Kalipatti* and DHS-1 showed plagiotropic nature due to lower tree height (9.3 m and 3.52 m respectively**)** under Dharwad conditions.

Under Gandevi conditions, the maximum plant height (4.8 m) was recorded in sapota cv. Bhuripatti in nine years old trees. Among the 14 cultivars of sapota evaluated under Arabhavi conditions, the cultivar CO-1 recorded significantly maximum plant height (3.65 m), which was on par with DHS-1 (3.63 m) and DHS-2 (3.60 m) in nine years old trees (Anon., 2002).

Under Kovvur conditions, the cv. Kalipatti recorded the higher plant height (3.37 m) followed by PKM-3 (3.27 m) and DHS-1 (3.26 m) in seven years old trees of sapota (Anon., 2004). Siddaramayya (2005) reported that plant height was maximum in cv. CO-1 (6.15 m) followed by cv. Long Oval (4.91 m) and cv. DHS-1 (5.85 m) and least in cv. Kirthbarthi (3.06 m).

Shirol *et al.* (2006) reported that maximum plant height was obtained in cv. CO-1 (5.15 m) followed by cv. Gavarayya (4.63 m) and cv.DHS-1 (4.57 m) and minimum was in cv. Tagarampudi (2.50 m). While, Saraswathi *et al*. (2010) evaluated sapota genotypes for growth and yield and they recorded highest tree height (11.20 m) in the hybrid CO-1.

Suhasini *et al.* (2012) studied performance of sapota varieties and hybrids under ghataprabha command area and they reported that highest plant height (6.80 m) was observed in cv. CO-1. Further, Varu Devashi (2012) studied growth characters of sapota cv. Kalipatti and he recorded maximum plant height of 7.26 m.

2.1.2 Fruit length (cm)

Gunuki (1998) noted that, the cv. DHS-2 recorded maximum fruit length (7.25 cm) followed by cvs. Kalipatti (7.10 cm), DHS-1 (6.69 cm) and Cricket Ball (5.79 cm).

Mathew *et al.* (2001) reported that fruit length was maximum in hybrid 7/1 and minimum in cv. Gutti. In a study on growth and development of sapota fruit Dhua *et al.* (2006) reported that the fruit length of cv. Cricket Ball increased more or less uniformly with fruit growth.

Shirol *et al.* (2006) studied the performance of sapota cultivars under northern Karnataka conditions and reported that higher fruit length was noticed in Singapore (6.50 cm), while Rajasekhar (2009) worked on fruit morphological characters of sapota genotypes and stated that mean fruit length among different genotypes was 5.24 cm and highest fruit length (7.75 cm) was recorded in cv. PKM-4. Further, Kamaraj *et al.* (2010) observed maximum fruit length in CO-1 (6.82 cm) and less in Singapore.

Rekha *et al.* (2011) studied genetic correlation and cluster analysis in sapota and they stated that maximum fruit length (7.23 cm) was observed in cv. CO-3 followed by 7.19 cm in Hybrid.

Suhasini *et al.* (2012) studied performance of sapota varieties and hybrids under ghataprabha command area and they reported that highest fruit length (7.57 cm) was observed in cv. PKM-2 followed by 6.57 cm in PKM-1. In anothet study, Radha (2013) reported that highest fruit length in PKM-3 (7.05 cm), followed by Cricket Ball (6.91cm) and DHS-2 (6.11cm).

2.1.3 Fruit width (cm)

A continuous increase in width of the fruit from fruit set till maturity of cv. Kalipatti was reported by Ingle *et al.* (1982). Similar observations have also been made by Paralkar *et al.* (1987) and Raut (1999).

Gunuki (1998) stated that maximum fruit diameter was observed in cv. Cricket Ball and minimum in cv. Guthi. In another study, maximum fruit girth was recorded in cv. DHS-2 (5.63 cm) and cv. DHS-1 (5.53 cm) where as least was recorded in cv. PKM-1 (3.87 cm) (Mathew *et al.*, 2001).

In a study on growth and development of sapota fruit, Dhua *et al.* (2006) reported that the fruit width of cv. Cricket Ball increased more or less uniformly with fruit growth, which remained more or less equal to the length of the fruit indicating the spherical shape of the fruit. Rajasekhar (2009) stated that mean fruit width among different genotypes was 4.69 cm and highest fruit width (6.79 cm) was observed in cv. Cricket Ball.

Pawar *et al.* (2011) observed that the physico-chemical parameters of sapota fruits at different maturity stages and found that the width of fruit showed decreasing trend from mature stage to over ripe stage.

Rekha *et al.* (2011) studied genetic correlation and cluster analysis in sapota and they stated that maximum fruit girth (5.65 cm) was observed in cv. Bombay.

Suhasini *et al.* (2012) studied performance of sapota varieties and hybrids under ghataprabha command area and they reported that highest fruit diameter (5.96 cm) was observed in cv. CO-2. In anothet study, Radha (2013) reported that highest fruit width 7.60 cm was observed in cv. Cricket Ball.

2.1.4 Fruit weight (g)

Chundawat and Bhuva (1982) observed maximum fruit weight in cv. Cricket Ball (142.20 g) followed by cvs. Mohangootee (101.43 g), Kalipatti (98.23 g), Pillipatti (82.40 g) and Zumakhia (57.47 g).

Weight of fruit is one of the indices that can be employed for judging the fruit maturity. In case of sapota cv. Kalipatti, the weight of the fruit increased continuously from 0.129 g at fruit set to 73.969 g at fruit maturity (Paralkar, 1987 and Raut, 1999). However a rapid increase in weight was noted after 90 days at maturity in cv. Kalipatti (Ingle *et al.*, 1982).

Dhua *et al.* (2006) studied growth and development of sapota fruit and reported that the cv. Cricket Ball followed a sigmoid growth curve in terms of fruit weight.

Maximum weight of the fruit was found at 240 days after fruit set and 40-50% increase in weight occurred between 180 and 195 days of fruit growth.

Mathew *et al.* (2001) observed wide variation among the different cultivars with respect to fruit weight. Cricket Ball recorded highest fruit weight while Local (Narikele) weighed lowest.

Rajasehkar (2009) stated that mean fruit weight of different sapota genotypes under study was 96.63g and highest fruit weight (198.74g) was observed in cv. Cricket Ball. In another study, Dhanashree Patil *et al.* (2010) reported that the average weight of the sapota fruits was 4g at fruit set and it increased up to 100.1 g in 9th month from the fruit set.

Saraswathy *et al.* (2010) evaluated sapota genotypes for growth and yield and reported that Cricket Ball registered highest mean fruit weight of 135.00g.

The fruits of cv. Cricket Ball (123.20 g) were superior to those of cv. DHS-1 (122.40 g) and cv. DHS-2 (120.70 g), while lowest fruit weight was recorded in cv. Pala (32.09 g) (Narendra Kumar, 2011).

Rekha *et al.* (2011) studied genetic correlation and cluster analysis in sapota and they stated that maximum fruit weight (143.95 g) was observed in cv. CO-1. Further, Suhasini *et al.* (2012) studied performance of sapota varieties and hybrids under Ghataprabha command area and they reported that highest fruit weight (133.41g) was observed in cv. CO-1.

Varu Devashi (2012) studied growth of sapota cv. Kalipatti and he reported that highest fruit weight of 63.65g among the treatments. Further, Radha (2013) stated that highest fruit weight 125.30g was observed in cv. Cricket Ball.

2.1.5 Colour of pulp

Rokhade *et al.* (1989) observed that cvs. Kalipatti, Hybrid 2/7, hybrid 2/13, hybrid 7/17, hybrid 10/25 and hybrid 12/8 had light orange coloured pulp. The cv. Cricket Ball exhibited medium brown coloured pulp. He further reported that Calcutta

Round, hybrid 3/13 and hybrid 5/17 produced brown coloured pulp, where as, Oval, hybrid 10/17, hybrid 11/13 and hybrid 12/24 had orange coloured pulp.

Mathew *et al.* (2001) observed that cv. Cricket Ball has light brown colour and the cvs Hybrid 7/1, Jhargram Local and Gutti have deep potato colour and the cv. PKM-1 have potato coloured pulp.

Rajasekhar (2009) studied fruit morphological characters in different sapota genotypes and reported that flesh color varied from brown to reddish brown. Brown color was registered in Badami, Dwarapudi, Gede Sapota, Gutti, Kalipatti, Kirthibarthi, Pakala Round, Singapore, CO-3, DHS-1, PKM-1, PKM-2 and PKM-4, while, Yellowish brown color was observed in Cricket Ball, Ulvapadu Selection, CO-1, CO-2, and PKM-3. While, Calcutta Round, Krishna Rao, Tagarampudi and DHS-2 were reddish brown.

Suhasini *et al.* (2012) reported that color of the pulp varied from orange brown to buff color. Orange brown was registered in (DHS-1, Long Oval, Oval) and Light brown in (PKM-1, PKM-2, Cricket Ball, Kalipatti and PKM-4), and Brown in (CO-2, Kirthibarthi, Tagarampudi and Virudnagar), and buff color in (PKM-3, Gutti and Mohangoote).

Radha (2013) studied physico-chemical changes in sapota varieties and reported that the colour of the flesh corresponding to PKM-3 and Cricket Ball was reddish green at 180th and 210th day and became reddish brown at later stages.

2.1.6 Number of seeds

A noticable seed formation was observed from 135 days after fruit set in sapota which continued to increase upto 210 days and thereafter remained constant (Ingle *et al.*, 1982). Similar observations have also been reported by Lakshminarayana and Subrahmanyam (1966).

Sulladamath *et al.* (1978) reported that round fruits had higher number of seeds (3.99 per fruit) than oval fruits (1.52 per fruit), conversely the shape index was higher for oval fruits than round fruits. They also noticed that seeds were uniformly distributed in round fruits than in oval ones. Mone (1989) reported that when the number of seeds were more, they were arranged in a radial manner which may govern the shape of fruit. He also observed that fruits with round shape (variety Kirthbarathi and CO-2) had more number of seeds than oval shaped ones, such as Kalipatti, CO-1, Gavarayya and Tagarampudi.

Chundawat and Bhuva (1982) observed more number of seed per fruit in cv. Pilipatti (4.67) followed by cvs. Zumakhia (3.10), Mohangooti (2.70), Cricket Ball (2.63) and Kalipatti (1.72). Avaiah and Singh (1991) reported that, among the different cultivars, cv. Cricket Ball showed more number of seed (5.146), while**,** cv. Pilipatti (1.626) recorded lowest number of seeds. Further, Rokhade *et al.* (1989) reported that the number of seeds varied between 2 to 6 per fruit in different sapota varieties and hybrids.

Gunuki (1998) noticed that the number of seeds per fruit varied among the cultivars. Cvs. DHS-2 and Cricket Ball recorded the highest number of seeds (4.50 and 4.00, respectively), while**,** cv. DHS-1 recorded the lowest number of seeds (1.50) followed by Kalipatti (2.50).

Mathew *et al.* (2001) reported that more number of seeds was found in cv. Cricket Ball than in Local (Narikeli). Shirol *et al.* (2005) recorded less number of seeds in Kalipatti (1.67) and cv. Oval (1.94), whereas, more number of seeds was observed in cv. Gavarayya (4.03).

Highest seed number of (8) was reported in Brazilian sapota and lowest number (2) in Ulavapudu selection (Rajasekhar 2009). The number of seeds of sapota cv. Kalipatti ranged between 3-4 and average number of seeds were 3.63 (Patil *et al.*, 2010).

Rekha *et al.*, (2011) studied genetic correlation and cluster analysis in sapota and they recorded maximum number of seeds per fruit (6.23) in cv. Gavarayya.

Radha (2013) observed wide variation among sapota varieties with respect to number of seeds per fruit which ranged from 3.27 to 6.07. Maximum number of seeds per fruit was recorded in variety Cricket Ball (6.07) followed by DHS-2

(4.51), while the varieties Pala (3.66) recorded minimum number of seeds per fruit.

2.1.7 Pulp to Seed ratio

Ingle *et al.* (1982) reported that a gradual increase in pulp to seed ratio throughout the development of the fruit in cv. Kalipatti till maturity. This is in confirmity with the results obtained by Lakshminarayana and Subrahmanyam (1966) in sapota. Pawar *et al.* (2011) studied the physico-chemical parameters of sapota fruits at different maturity stages and found that pulp to seed ratio showed decreasing trend from mature stage to over ripe stage.

Rajasekhar (2009) stated that mean pulp to seed ratio among different sapota genotypes was 34.28 with the highest of 100.08 was observed in cv. CO-2. Further, Radha (2013) reported that pulp to seed ratio attained maximum in DHS-2 on 285th day (30.61), followed by (23.71) in PKM-3 on 270th day.

2.1.8 Seed weight (g)

Rajasekhar (2009) studied the fruit morphological characters of different sapota cultivars and reported that mean seed weight was 3.13g and highest seed weight of 7.78 g was observed in cv. Brazilian Sapota. In another study, the seed weight observed in cv. Cricket Ball (5.818g) was maximum followed by the seed weight of cv. DHS-2 (5.615g). Lowest seed weight (2.113g) was recorded in cv. Pala (Narendra Kumar 2011).

Radha (2013) stated that maximum seed weight was recorded in cv. Cricket Ball (5.818 g) followed by the cv. DHS-2 (5.615 g) and lowest seed weight was recorded in cv. Pala (2.113 g).

2.1.9 Season of flowering

Hays (1960) observed three main seasons of flowering in sapota with a maximum during June to August and minimum during March under South Indian conditions**.** Lakshminarayana and Subramanyam (1970) noticed more flowering during July and sparse flowering during other seasons.

Farooqi and Rao (1976) noticed main flowering flush during November-December under Dharwad conditions. Nalawadi *et al.* (1977) reported three main seasons of flowering at Dharwad *viz.,* June to August, October to December and March. They also noticed that flowering was maximum during June to August and minimum during March. Mahadevaiah (1981) also made similar observations under Dharwad conditions.

Similar studies on flowering and fruit set in four sapota cultivars (DHS-1, DHS-2, Kalipatti and Cricket Ball) were carried out under Dharwad conditions. Both the cultivars and hybrids exhibited highest flowering during November-January flush, followed by July-August flush while the lowest flowering was observed in the march flush (Patnaik 1982).

Mone (1989) reported that in sapota there were three main flowering seasons *viz.,* November-January, April-May and July-August with a trace flowering in February-March at Dharwad. Relekar *et al.* (1991) noticed maximum number of sapota flowers opened during November-December.

Two distinct peak flowering periods were observed in five sapota cultivars by Lenka *et al.* (1996). The percentage of flower buds, which developed into flowers ranged from 50.00 in Cricket Ball to 72.72 in Kalipatti. Fruit weight was greatest in cv. CO-2.

2.1.10 Per cent fruit set

Madhavarao and Khader (1961) observed that the percentage of fruit set under natural pollination conditions ranged from 0.0 to 11.00 % while it was 2.0 to 10.0 per cent under selfed conditions. Farooqi *et al.* (1973) reported fruit set in Kalipatti, Cricket Ball, Calcutta Round and oval in the order of 11.4, 23.7, 11.9 and 1.07 per cent respectively under open pollination condition.

Nalawadi *et al.* (1977) reported that maximum per cent fruit set was observed in cv. Kalipatti (100 %) followed by cvs. Local (95 %), Cricket Ball (85 %) and Calcutta Round (60 %) under natural condition.

Farooqi and Rao (1976) reported that main flowering flush was noticed during November-December under Dharawad conditions. Mahadevaiah (1981) also made similar observations under Dharawad conditions and noticed that fruit set was higher in the flushes of November and May but lowest during February-March. Minhas and Sandhu (1985) noticed no fruit set in all the cultivars of Sapota studied during June flowering under Ludhiana conditions. They observed a very low fruit set during November flowering under open and self-pollination. These were three main flowering seasons, *viz.*, November-January, April-May and July-August with a trace flowering in February-March. Mone (1989) noted that among the cultivars studied cv. Kalipatti recorded maximum fruit set in July and November (34.5 % and 34.2 % respectively) under Dharwad conditions. Mulla (1990) also reported that Kalipatti recorded the highest percentage of fruit set among the varieties studied under Rahuri (Maharashtra) conditions.

Relekar *et al.* (1991) noticed that maximum number of sapota flowers opened during November-December and maximum fruit set (22.7 %) in the month of February followed by the months of January (20.92 %), May (15.31 %) and July (15.31 %)**.**

Patnaik (1982) studied fruit set in four sapota cultivars (DHS-1, DHS-2, Kalipatti and Cricket Ball) under Dharwad conditions and observed that fruit set in all the cultivars and hybrids was highest during July-August (17.42 – 29.24%) followed by November-January (13.2 – 15.1%). Fruit set was highest in DHS-1 followed by DHS-2.

Nagargoje *et al.* (2007) investigated floral biology of sapota cv. Kalipatti and reported that in natural pollination, 27 to 34 per cent fruit set was observed in different floweing seasons. Further, Suhasini *et al.* (2012) studied performance of sapota varieties and hybrids under ghataprabha command area and they reported that highest per cent of fruit set (29.50 %) was observed in cv.PKM-1, followed by 26.20% in CO-2.

2.1.11 Yield/tree

Relekar *et al.* (1991) reported an average yield of 80.92 kg per tree per annum in 13 years old sapota trees of cv. Kalipatti. Mone (1989) reported the highest yield in cv. Kalipatti (210.84 kg/tree/year) followed by cv. Gavarayya (134.67 kg/tree/year) under Dharwad conditions. He also observed that the number of fruits per tree was highest in cv. Kalipatti (3442.25) followed by cv. Gavarayya (1218.75).

The hybrid 12/24 excelled all other hybrids and varieties with respect to number of fruits per tree (1381.29) and yield (116.10 kg/trre/year) followed by cv. DHS-1(845.37 kg/tree/year). Hegde (1997) noticed highest fruit yield in cv. DHS-1 (73.46 kg/tree/year).

Gunuki (1998) reported that among all the cultivars cv. DHS-1 recorded higher number of fruits per tree (864.36) and yield (104.97 kg/tree/year) followed by cv.DHS-2 (565.87 and 78.72 kg/tree/year)

Shirol *et al.* (2005) reported that more number of fruits per tree was obtained in cv. DHS-1 (470.00) followed by cv. CO-2 (441.00) and less fruit per tree was obtained in cv. Tagarampudi (22.00).

Saraswathy *et al.* (2010) evaluated sapota genotypes for growth and yield and they reported that PKM-4 registered highest yield per tree (139 kg/tree), followed by virudhunagar (135 kg/tree).

Suhasini *et al.* (2012) studied performance of sapota varieties and hybrids under Ghataprabha command area and reported that highest yield per tree was observed in cv. DHS-1 (169.20 kg/tree). Further, Varu Devashi (2012) studied growth characters of sapota cv. Kalipatti and recorded maximum yield per tree (163.30 kg/tree) among the treatments.

2.2 BIO-CHEMICAL CHARACTERS

2.2.1 Total Soluble Solids (^{0}Brix)

The total soluble solids in fruits of sapota show a regular trend in their contents with the advancement of maturity. The increase of sugars is mainly due to conversion of starch into sugars, thereby resulting in the increase of total soluble solids content. Large variation exists in the total soluble solids content of the fruit of sapota varieties during growth and development. The contents of total soluble solids and sugar have often been employed in many fruits to decide the stage of harvest. During growth, increasing trend was observed for total soluble solids by Ingle *et al.* (1982) in sapota fruits of cv. Kalipatti. Similar type of increase was also reported by Sulladmath (1975).

Madhavarao *et al.* (1971) had studied total soluble solids of sapota genotype oval and reported that TSS was 19^{O}B at ripe stage, while Suryanarayana and Goud (1984) stated that the TSS of genotype oval to be 13.92 ^{O}B at ripe stage. Further, Shanmugavelu and Srinivasan (1973) reported that the TSS of sapota genotypes varied from 24^{O}B in Gutti to 13 ^{O}B in Dwarapudi, while Lakshminarayana (1980) had reported that the TSS of certain Mexican varieties ranged from 19.7 ^{O}B in SCH-08 to 25 ^{O}B in SCH-02.

Ponnuswamy and Irulappan (1987) stated that the TSS of sapota genotypes ranged from 15.02 ^{O}B in CO-1 to 26.28 ^{O}B in PKM-2.

Paralkar *et al.* (1987) reported that TSS of sapota genotype Kalipatti was 21.6 ^{O}B at maturity, while Shanmugavelu *et al.* (1987) reported that the TSS varied from 18 ^{O}B in Cricket Ball to 25 ^{O}B in Long Oval genotype. Similar observation were reported by Waskar and Nikam (1996), who reported that the TSS ranged between 18 ^{O}B to 25 ^{O}B in sapota fruits. Further, Mone *et al.* (1990) reported that the TSS varied from 17 ^{O}B in Tagarampudi to 24^{O}B in Kalipatti.

According to Gautham (1989) there was an increase in TSS of sapota fruits from 18.5° Brix to 27° Brix during ripening. Shinde (1993) observed that TSS content of cv. Kalipatti at maturity and at ripe stage ranged between 21.5-22.4° Brix and 23.8-24.16° Brix respectively.

Mathew *et al.* (2001) reported that the TSS varied from 21 ^{O}B in Gutti to 18^{O}B in Local (Narkeli), while Brito and Narain (2002) had reported that the TSS of Brazilian sapota variety Itapirema-31 was 23.20 ^{O}B.

Dhua *et al.* (2006) studied the chemical changes in the developing fruit of cv. Kalipatti and observed that the total soluble solids contents of fruit increased gradually during fruit development.

Rajasekhar (2009) stated that TSS varied from 20.20 ^{O}B in Cricket Ball to 24.30^{O}B in Pala, while Dhanashree Patil *et al.* (2010) stated that the total soluble solids content of sapota fruit was 19.9^{0}B in 7th month harvested fruits and increased gradually upto 23.9^{0}B as per the advancement of maturity.

Rekha *et al.* (2011) repoted that TSS varied from 15.02 ^{O}B in Guruvayya to 20.39 ^{O}B in Pilapatti. Saraswathy *et al.* (2010) evaluated sapota genotypes for growth and yield and reported that Virudhunagar registered highest TSS contnet 25.50 ^{O}B.

Suhasini *et al.* (2012) studied performance of sapota varieties and hybrids under ghataprabha command area and they reported that highest TSS (23.90 ^{O}B) was observed in cv. DHS-1 followed by 23.00 ^{O}B in cv. DHS-2. Further, Radha (2013) stated that PKM-3 recorded the maximum TSS of (22.54 ^{0}B) on 270th day followed by DHS-2 (22.07 ^{0}B) on 285th day.

2.2.2 Titrable Acidity (%)

At the time of maturity, fruits posses higher amount of acidity, as they advance towards ripening, acid content decreases.

Lakshminarayana and Subramanyam (1966) reported that fruits of sapota cv. Calcutta Round recorded 0.22 per cent acidity at maturity which decreased to 0.11 per cent at edible ripe stage.

It was reported that the titrable acidity content of sapota genotype Oval was 0.1 per cent at ripen stage (Madhava Rao *et al.*, 1971), while Suryanarayana and Goud (1984) observed 0.38 per cent acidity in mature sapota fruits at harvest, which decreased to 0.18 per cent at ripe stage in cv. Oval.

Ingle *et al.* (1981) observed a decrease in acidity during ripening of sapota fruits. It decreased from 0.31 per cent at harvest to 0.20 per cent at edible ripe stage.

Kumbhar and Desai (1986) reported that percentage of acidity decreased from 0.27 to 0.14 during ripening.

The acid content of sapota fruits declined with increase in storage period (Ingle *et al.*, 1982, Banik *et al.*, 1988, Waskar and Nikam, 1996). Rajasekhar (2009) observed titrable acidity of sapota genotypes ranged from 0.01% in DHS-1 to 0.07% in Gavarayya.

Dhanashree Patil *et al.* (2010) observed the titrable acidity content of sapota fruits reduced from 0.27 % in 7th month harvested fruits to 0.23 % in 9th month harvested fruits.

Suhasini *et al.*, (2012) reported that cv. CO-2 registerd highest (0.198%) titrable acidity. While, Radha (2013) stated that among different varieties of sapota, Cricket Ball (0.51 %) showed maximum titrable acidity which was significantly superior over other varieties followed by Pala (0.34 %) and PKM-3 (0.31 %) whereas significantly lowest acidity was recorded by Kirthibarthi (0.21 %) followed by PKM-1 (0.22 %).

2.2.3 TSS to Acid Ratio

Sugar-acid ratio has been often followed for assessing the right stage of maturity in almost all the acid origin fruits such as citrus, mango, pineapple, grapes and in many instances it is proved to be one of the most reliable indices of maturity (Pantastico, 1975).

This is the better measurement of palatability than sugar content or acidity alone. Ponnuswamy and Irulappan (1987) have reported that the sugar to acid ratio of sapota genotypes ranged from 103.58 in Cricket Ball to 659.17 in cross No. 2/2 between Gutti and Kalipatti.

Kariyanna and Reddy (1990) reported that the TSS-Acid ratio in sapota cv. Kalipatti at eating ripe stage ranged between 85.89 to 240.03. However, Rajasekhar (2009) revealed that TSS to Acid ratio in sapota cultivars ranged from 142.01 in Gavarayya to 582.08 in DHS-1.

Among the varieties of sapota, PKM-1 recorded highest TSS to Acid ratio (130.90) where as Cricket Ball (79.42) recorded lowest TSS-acid ratio (Mythri 2012) Further, Radha (2013) stated that the varieties Cricket Ball (29.51) recorded lowest TSS: acid ratio followed by Pala (53.09)c, while DHS -1 recorded maximum TSS: acid ratio (77.35).

2.2.4 Total Sugars (%)

As the fruit advances towards ripening, starch, hemicelluloses and organic acids get converted into various forms of sugars. These changes are largely dependent upon the conditions of storage such as temperature, time and on the physiological status of fruits. Generally, the total sugar content increases from harvest till ripening and declines as senescence approaches. Once the fruit ripens, the sugars undergo metabolic transformations both quantitatively and qualitatively (Pantastico, 1975).

The total sugars content of sapota genotype Oval was reported to be 14.00% at ripen stage by Rao *et al.* (1971) and 12.60% by Suryanarayana and Goud (1984). Further, Shanmugavelu and Srinivasan (1973) found a considerable variation in total sugars (7-12.3 per cent) in different cultivars of sapota.

Gautam and Chundawat (1990) while studying postharvest changes in sapota cv. Kalipatti observed that levels of reducing sugars accelerated throughout the period of ripening. Similarly, Sanjay (1996) reported that an increasing trend was observed with respect to reducing, non-reducing and total sugar content with advancement of storage periods in sapota cv. Kalipatti under ambient storage. In general, sapota fruit contains 12-14 per cent sugars (Roy and Joshi, 1997).

The total sugar content was observed to range from 11.00% in Cricket Ball to 7.70% in Local (Narkeli) by Mathew *et al.* (2001), While Brito and Narain (2002) reported that the total sugar content of variety Itapirema-31 was 14.8%.

Rajasekhar (2009) reported that the total sugars varied from 8.21% in Badami to 11.93% in Pala. However, saraswathy *et al.* (2010) stated that among different cultivars of sapota highest total sugars was recorded in Virudnagar (10.90%) and lowest in Cricket Ball (7.03%). Further, Mythri (2012) revealed that PKM-1 (11.61%)

showed highest total sugars which was on par with Virudhnagar (11.49%) where as the lowest total sugars was observed in Cricket Ball (8.36%).

Devashi (2012) studied yield and quality aspects of sapota in cv. Kalipatti and reported that maximum total sugars (7.10%) was observed among the treatments.

Among different varieties of sapota, Virudnagar (5.12 %) recorded the maximum total sugars followed by PKM-3 (5.05 %) and Kalipatti (4.87 %), while lowest total sugars (3.61 %) was observed in the variety Cricket Ball (Radha. 2013).

2.2.5 Reducing Sugars (%)

The reducing sugars content of sapota genotype Oval was reported to be 3.61% at ripen stage by Madhavarao *et al.* (1971) and 5.83% by Suryanarayana and Goud (1984). According to Shanmugavelu and Srinivasan (1973) found a considerable variation in reducing sugars (7-12.3 per cent) in different cultivars of sapota.

Gautam and Chundawat (1990) while studying postharvest changes in sapota cv. Kalipatti observed that levels of reducing sugars accelerated throughout the period of ripening. Similarly, Sanjay (1996) reported that an increasing trend was observed with respect to reducing, non-reducing and total sugar content with advancement of storage periods in sapota cv. Kalipatti under ambient storage. In general, sapota fruit contains 12-14 per cent sugars (Roy and Joshi, 1997).

Selvaraj and Pal (1984) have reported that the reducing sugar content of sapota genotype Cricket Ball was 11.95% and that of oval to be 11.96%. Further, Paralkar *et al.* (1987) reported that the reducing sugars in cultivar Kalipatti were 8.92% at maturity.

The reducing sugar content was observed to range from 3.60% in PKM-3 to 5.04% in PKM-1 by Mathew *et al.* (2001), While Brito and Narain (2002) reported that the reducing sugar content of variety Itapirema-31 was 9.30%.

Rajasekhar (2009) reported that the reducing sugars varied from 5.88% in Dwarapudi to 10.65% in Kalipatti. However, Varu Devashi (2012) studied yield and quality aspects of sapota in cv. Kalipatti and reported that maximum reducing sugars content (5.02%) was observed among the treatments.

Radha (2013) stated that among different varieties of sapota Virudnagar recorded maximum reducing sugars (3.15 %) followed by PKM-3 (2.94 %), where as lowest reducing sugars were observed in Cricket Ball (2.19 %) and Singapore (2.37 %).

2.2.6 Non-Reducing Sugars (%)

The non reducing sugar content of sapota genotypes ranged from 1% in Baramasi to 4.9% in Cricket Ball (Shanmugavelu and srinivasan, 1973), while Suryanarayana and Goud (1984) have reported that the non-reducing sugar content of sapota variety Oval was 6.77%. However, the non-reducing sugar content of certain Mexican sapota varieties was reported to vary from 4.81% (SCH-08) to 7.35% (SCH-28) by Lakshminarayana (1980). Further Selvaraj and Pal (1984) have reported that the non-reducing sugar content of sapota genotype Cricket Ball to be 2.12%, while that of oval to be 3.10%.

Paralkar *et al.* (1987) reported that the non-reducing sugars in cultivar Kalipatti were 6.48% at maturity, while Mathew *et al.* (2001) reported that the non-reducing sugars varied from 2.60% in Local (Narkeli) to 6.46% in Cricket Ball. In a study conducted in Brazil, the non-reducing sugars content of variety Itapirema-31 was reported to be 5.6% (Brito and Narain, 2002).

Rajasekhar (2009) reported that the non-reducing sugars varied from 1.01% in Krishna Rao to 4.21% in Dwarapudi. While, Radha (2013) stated that among different varieties, PKM-1 (2.23 %) showed significantly highest non-reducing sugars which was followed by PKM-3 (2.16 %) where as significantly lowest non-reducing sugars was observed in Cricket Ball (1.40 %) followed by DHS-2 variety (1.55 %).

2.2.7 Ascorbic Acid (mg/100g)

Lakshminarayana and Subramanyam (1966) reported that ascorbic acid content in mature sapota fruit was 27.4 mg 100 g^{-1}, but at the eating ripe stage it reduced to 2.9 mg 100 g^{-1} fresh weight. Shanmugavelu and Srinivasan (1973) recorded a range in vitamin C content from 0.1 to 11.9 mg 100 g^{-1} at ripe stage in ten cultivars of sapota. Selvaraj and Pal (1984) observed a decline in ascorbic acid content in sapota cv. Cricket Ball. Suryanarayana and Goud (1984) noted 33.0 mg 100 g^{-1} ascorbic acid on the day of harvest which reduced to 18.1 mg 100 g^{-1} by the 10th day of ripening in cv. Oval. Guatam in 1989 recorded decline in ascorbic acid from 29.4 mg 100 g^{-1} to 4 mg100 g^{-1} in cv.Kalipatti.

Das and Mahapatra (1976), Banik *et al.* (1988) and Bandhyopadhyay and Sen (1994) also noticed a decrease in ascorbic acid content during ripening of sapota. In contrast, Broughton and Wong (1979) reported that ascorbic acid content increased with ripening of sapota fuits. Ingle *et al.* (1982) observed that ascorbic acid content of sapota fruits had an initial increase up to 43.65 mg 100 g^{-1} and continuously decreased thereafter to 20.34 mg 100 g^{-1} fresh weight during development.

Paralkar *et al.* (1987) reported that the ascorbic acid content of cultivar Kalipatti was 24.70 mg/100g at maturity, while Ponnuswamy and Irulappan (1987) reported that the ascorbic acid content in sapota cultivars ranged from 1.65 mg/100g in Oval to 3.99 mg/100g in cross 2/2 between Gutti and Kirthibarthi. Further, the ascorbic acid content of variety Itapirema-31 was observed to be 1.80 mg/100g (Brito and Narain, 2002).

Rajasekhar (2009) reported that that ascorbic acid content in sapota cultivars ranged from 5.12 mg/100g in Dwarapudi to 11.37 mg/100g in Kalipatti. In another study, Dhanashree Patil *et al.* (2010) reported that the ascorbic acid content of sapota fruit increased towards maturity from 20.24 mg/100g in 7th month of fruit set to 25.12 mg/100g in 9th month of fruit set.

Saraswathy *et al.* (2010) reported that ascorbic acid content in sapota cultivars ranged from 1.85 mg/100g in Oval to 3.55 mg/100g in Virudnagar. While, Radha (2013) revealed that the ascorbic acid content in DHS-1 reached to 10.14 mg/100g followed by DHS-2 10.01 mg/100g and PKM-3 9.42 mg/100g on 270th day.

2.2.8 Total Phenols

The astringency in sapota fruits is due to the presence of large quantities of polyphenols. The astringency is high when the fruit is unripe and decreases slightly at mature stage.

Lakshminarayana and Subramanyam (1970) reported that the phenolic content of mature sapota fruit at eight month of age was 4.82g, Further, Selvaraj and Pal (1984) reported that the tannins content was 0.10% and 0.20% respectively in sapota genotypes Cricket Ball and Oval, While Paralkar *et al.*(1987) recorded 0.67% of tannins at maturity in the genotype Kalipatti. However, Brito and Narain (2002) observed 0.3% tannin in the Brazilian variety Itapirema-31.

Anand *et al.* (2007) emphasized that sapota juice was found to contain moderate amount of sugar and protein, and it was also found to be a good source of ascorbic acid, carotenoids and phenolics (134 mg/100g).

Rajasekhar (2009) reported that the phenols varied from 0.23 mg/100g in Pala to 0.78 mg/100g in Dwarapudi. Further, Mythri (2012) observed that among different varieties, DHS-2 (1.64 mg/100g) recorded the highest content of total phenols which was on par with PKM-3 (1.59 mg/100g), where as Kirthibharthi recorded the lowest total phenolics (1.42 mg/100g) which was on par with Kalipatti (1.43 mg/100g).

Total phenolic content decreased during storage of peach in both hot water and moist hot air treated fruits (Prashant and Masoodi, 2010).

2.2.9 Pectins

Fisher and Bennett (1991) reported that solubilisation of pectins is the fundamental and important aspect of fruit ripening. Textural changes of fruits during ripening were the consequences of modifications of cell wall polysaccharides, which, resulted in disassembly of primary cell wall and middle lamella structures (Jackman and Stanley, 1995). Fruit softening during ripening was associated with changes in primary cell wall, which consisted of three co-extensive polymer networks: the pectins, cellulosexyloglucan framework, and structural glycoproteins (Cosgrove *et al.*, 1997).

Varanyanond *et al.* (1999) conducted experiment on changes in pectin content during maturation in mango. Soluble pectins during maturation were as follows: mature- 4.11 per cent, half-ripe-3.08 per cent, ripe-2.24 per cent and overripe- 2.15

per cent which demonstrates that soluble pectin content of ripe and overripe fruits was approximately half that of mature fruit.

Pectin content is mainly responsible for the firmness and texture of fruits and vegetables, and hence it became important in food industry as gel forming and thickening agent (Brummell, 2006; Mafra, 2006). In food industry, pectic substances are widely used in the preparation of jams, jellies, marmalades, and fruit preserves of all kinds, especially the natural pectin content of fruits, like orange peel (Zykwinska, 2009).

Jin *et al.* (2006) experimented on changes in cell wall polysaccharides of harvested peach fruit during storage. Galacturonic acid, which made up the main chain of pectins, degraded more quickly in the four pectic fractions during warm storage than during cool storage. This suggests that the rupture of main pectic chains contributes significantly to peach fruit ripening. Pectin content decreased during storage of peach in both hot water and moist hot air treated fruits (Prashant and Masoodi, 2010).

Mythri (2012) stated that highest pectin content was observed in Virudhnagar (5.33%), and lowest pectin content was recorded by Pala (4.53%).

2.3 CHARACTER ASSOCIATION AND PATH COEFFICIENT ANALYSIS

Singh *et al.* (2001) reported that the phenotypic coefficient of variation (ranging from 4.73 for to 38.37) was higher than the genotypic coefficient of variation in papaya. Path analysis revealed that fruit length (0.718), number of fruits per plant (0.134) and peel weight (0.945) showed the greatest positive effects on crop yield.

Genetic variability and character association studies were conducted in papaya and observed Wide range of variability in yield (l.46 to 17.50 kg/plant), number of nodes to first flower (29.66 to 70.66), leaf length (31.00 to 53.66 cm), plant height (81.66 to 191.0 cm), east-west spread (126.6 to 206.66), fruit length (12.36 to 21.56 cm), fruit width (8.33 to 16.30 cm), number of fruits per plant (2.66 to 19.33) and total sugar (6.20 to 12.8). The coefficient of variation ranged from 8.78 to 27.99 in pulp thickness and acidity, respectively. High heritability of 94.8% was found for number of fruits per plant. The yield was significantly correlated with fruit length (0.415), fruit width (0.530), number of fruits per plant (0.585) and fruit weight (0.475). Path analysis revealed that total sugar content, fruit width and number of fruits per plant had high positive direct effect on yield per plant at the genotypic level, which suggests the significance of these characters during selection in a pawpaw breeding programme (Jana *et al.*, 2006).

Correlation and path analysis of 21 morphological characters of 21 banana genotypes were carried out and reported that bunch weight was strongly correlated with the bunch characters, followed by the harvest index, number of days before harvesting and plant weight (Kulkarni *et al.,* 2006). All the fruit characters except for fruit density were significantly correlated to each other. Path analysis indicate that bunch weight may be improved by directly selecting for pulp weight. Bunch weight exhibited high direct effects on harvest index, plant weight, number of hands, peel to pulp ratio and peel weight.

Hazarika (2009) reported that there was a wide variation among aonla accessions. Individual fruit weight ranged from 3.24 to 10.18 g; pulp weight from 2.83 to 9.41 g; seed weight 0.37 to 1.66 g; and pulp to seed ratio from 3.21 to 14.00. There was wide variation in chemical characters also. Total soluble solids varied from 12.0 to 19.0%; titrable acidity 2.29 to 4.61%; ascorbic acid 400 to 850 mg/100 g, reducing sugars 3.76 to 10.98%. Wide variation in physico-chemical analysis of genotypes indicated the scope of individual plant selection based on these characters for the genetic improvement of aonla.

Kavitha *et al.* (2008) studied correlation coefficient components of 19 hybrids in banana to identify desirable genotypes for crop improvement programmes. Based on fruit quality characters along with more number of fruits/hand, bunch weight and early crop duration, the hybrids NPH-02-01 and NPH-03-19 gave high potential. A very strong positive and significant correlation was recorded between bunch weight and number of fruits/hand (0.752) and number of hands/bunch (0.632) indicating that effective improvement through these characters could be achieved in banana.

A total of one hundred and fifty seedling progenies of 'Apple Colour' guava were used to study the character association between yield and yield attributing parameters Kumar *et al.* (2009). Plant height, stem diameter and number of fruits per plant were found to be significantly and positively associated with fruit yield per plant. Path analysis of yield and its components revealed that number of fruits per plant; fruit weight and plant height had direct positive effect on fruit yield. When both correlation and path analysis were taken together, it was found that yield improvement in guava can be brought about by selecting genotypes for number of fruits per plant, plant height, stem diameter and fruit weight.

Maiti (2010) revealed that genotypic and phenotypic correlation in jackfruit were positively significant between fruit weight of edible part (0.980 and 0.977), fruit and rind weight (0.976 and 0.971), and number of stones and flakes (0.999 and 0.999). The path coefficient analysis indicated that weight of edible part had positive direct effect on fruit weight both at genotypic (0.459) and phenotypic (0.451) levels. Hence, at the time of selection of jackfruit genotypes, one has to put emphasis on the characters like weight of edible part, rind weight, flake and stone numbers.

Saraswathy *et al.* (2010) studied correlation coefficient analysis among the sapota genotypes and reported that there was a positive correlation between tree height and canopy spread. The attributes like number of fruits per tree and canopy spread had positive correlation with fruit yield per tree.

High variability in sapota accessions were observed for fresh-fruit weight, fruit length, fruit girth, fruit weight at ripening, pulp weight, peel weight, number of seeds and TSS. Correlation studies among fruit parameters indicated positive relationship between all the parameters studied, except TSS which had negative relationship with the rest of the fruit parameters. Selection of distantly placed cultivars in breeding programs stands to result in better progeny for further evaluation (Rekha *et al.* 2011).

Majumder *et al.* (2012) recorded a positive correlation with inflorescence per shoot, percent perfect flower, percent initial fruit set, number of fruits per plant and fruit weight both at phenotypic and genotypic levels in mango. The residual effects of genetic and phenotypic path analysis were 0.209 and 0.385, respectively, revealed higher genetic variability and also proved lower percent of environmental influence on the selected ten characters. In genotypic path analysis, number of fruits per plant had the highest positive direct effect (0.899) on yield. Higher positive direct effects were also observed for the characters inflorescence per shoot (0.539), percent perfect flower (0.816), and percent initial fruit set (0.292), and fruit weight (0.324). Leaf area, percent flowering shoot, number of fruits per plant, and fruit length showed negative direct effects towards yield. In phenotypic path analysis, except percent flowering, shoot per plant and fruit length and other characters also exhibited similar trend on yield as genotypic path coefficient.

A correlation study was conducted on aonla fruit length, fruit width, fruit weight, fruit volume, specific gravity, total soluble solid (TSS), acidity, ascorbic acid, stone length and stone width, stone weight, pulp:stone ratio and fruit pulp. Correlation analysis study showed a high positive and statistically significant ($P<0.01$) correlation between fruit pulp and fruit weight (0.999). Fruit pulp also had positive and significant correlation with fruit volume (0.874) and fruit width (0.730). Fruit volume indicated negative correlation with specific gravity. Therefore, information on different physico-chemical characters of fruits and fruit pulp yield may be of great importance to a breeder in selecting a desirable genotype (Ganesh Shukla *et al.* 2012).

2.4 COEFFICIENT OF VARIATION, HERITABILITY AND GENETIC ADVANCE

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 improvement. The estimates of heritability and the genetic advance expected after selection indicate the feasibility and the extent to which the improvement is possible.

Genetic variability is determined with the help of certain genetic parameters such as coefficient of variation, heritability and genetic advance. Heritability is the heritable portion of total phenotypic variance and it is 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 is the ratio of genotypic variance to phenotypic variance. Its estimation is important, because it determines the additive effect of genes. If the heritability of a character is 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.*1955).

In citrus, study of highly divergent collection of 40 varieties, revealed 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).

High genotypic coefficient of variation was found for fruit weight, fruit volume and contents of ascorbic acid and reducing sugars in acid lime varieties (Prasad, 1987). Characters like TSS, ascorbic acid, reducing sugar, pH, fruit weight and volume showed high heritability along with high genetic gain. A positive and significant correlation was observed for number of fruits, fruit length and breadth with TSS. Fruit weight was also correlated with yield during both the years of study.

Iyer *et al.* (1989) assessed heritability of various vegetative and fruit characters in forty-two cultivars of banana. They found high heritability (above 95%) for most of the economic characters including components of yield. TSS was also found to have extremely high heritability (99.83%). Heritability was also high for contents of ascorbic acid, reducing sugars and fruit weight.

Variability studies conducted by Kokadwar *et al.* (1992) in forty superior local types of mango in Parbhani area indicated that variability was the highest in total sugar content followed by edible to non edible ratio, pulp weight, fruit size, percentage of acidity, stone: pulp ratio and TSS, where as least variation was observed in reducing sugars percentage. Based on these studies KMN-11, KMN-5 and KMN-23 were found promising for most of the characters. The highest quantity of ascorbic acid was observed in Langra.

Yadav *et al.* (1995) evaluated 25 mango varieties and observed that fruit weight was significantly and positively associated with fruit length, fruit width and fruit pulp. They also noted that total sugar, non-reducing sugar and TSS had significant negative association with fruit weight of mango. They also reported moderate to high estimates of variability and high heritability coupled with high genetic advance for fruit weight, fruit length, reducing sugars, acidity and peel percent in mango at Rewa, Madhya Pradesh.

Lavi *et al.* (1998) estimated phenotypic and genetic variance for thirteen traits in citrus progeny derived from both controlled crosses and open pollination. In all thirteen traits analyzed, the additive genetic variance components were not statistically significant, while in eight of the traits, viz. fruit weight, harvest duration, firmness, taste, appearance, quality and final assessment, the dominant genetic variance was significant. Since the offspring performance is quite unpredictable, parents should not be chosen on the basis of phenotype.

Bisla and Daulta (1988) evaluated 30 *Ziziphus mauritiana* cultivars and observed higher variability in fruit weight (97.2%), fruit size (87.9g), pulp to stone ratio (87.5) and seed weight (84.6). These characters also showed high genetic advance.

Praveen and Patil (1998) observed that genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were highest for stomatal frequency and lowest for leaf area in ber. High values of heritability and genetic gain were observed for stomatal index. Significant and positive correlations were observed between yield per plant and stomatal frequency, leaf area and stomatal index.

A significant difference was recorded in nineteen genotypes of mango for different physico-chemical characters (Attri *et al.,* 1999). The highest range of variation was shown by fruit weight, pulp and carotenoids, whereas the lowest by acidity. They reported that all the characters under study including fruit length, breadth, weight, specific gravity and pulp percent, TSS, acidity, sugars, ascorbic acid and carotenoids had shown high estimates of broad sense heritability ranging from 85.3% for specific gravity of fruits to 99.9% for ascorbic acid and carotenoids. Moreover, they mentioned that the genetic advance could be 324.95, 269.03, 266.23 and 77.36% for carotenoids, fruit weight, its volume and ascorbic acid, respectively with the 5% selection intensity.

Samanta *et al.* (1999) reported significant difference for all morphological characters studied in twenty-five genotypes of mango in West Bengal and observed low differences between the phenotypic coefficient of variation and genotypic coefficient of variation for fruit weight and pulp weight, indicating a small influence of environment; while high heritability along with a high genetic advance was found for the same characters.

Rai and Mishra (2000) conducted experiment to estimate genetic variance in thirty three mango varieties under East Indian conditions and reported high GCV for pulp weight (35.06%), stone weight (32.2%) and skin weight (31.42%). The PCV was generally higher than GCV for all the characters and it was high for pulp weight and skin weight. They observed high heritability estimates for fruit weight and pulp weight.

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

Vashishtha *et al.* (2003) evaluated 12 genetically diverse strains of pomegranate to determine their inter-relationship among different characteristics and their effects on fruit weight. The genotypic and phenotypic correlation coefficient values revealed that the genotypic correlation coefficients were higher on their corresponding phenotypic coefficients.

Singh *et al.* (2004) studied genetic variability for 3 traits (fruit weight, total soluble solids (TSS), acidity) in 43 genotypes of mango in Bihar. A wide range of variability was observed for all characters, except for TSS. The magnitude of phenotypic coefficient of variation was higher than that of genotypic coefficient of variation.

Singh *et al.* (2005) assessed 20 genotypes of mango and found high phenotypic coefficient of variation along with high genotypic coefficient of variation for peel color, fruit weight and ascorbic acid content. High hertitability along with high expected genetic advance was noticed for fruit weight and ascorbic acid content.

A variability study was conducted on 24 indigenous and exotic genotypes of pomegranate (*Punica granatum*) and they reported that high variability, genotypic coefficient of variation, heritability and genetic advance were significantly higher for characters like fruit weight, seed content, weight of 100 arils, juice acidity and weight of 100 seeds (Meena and Roop Singh, 2006). Thus these traits may be effectively used as selection criterion for screening potential genotypes in a breeding programme.

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

Baruah *et al.* (2007) investigated growth and yield performance of twenty banana cultivars and revealed that local cultivars exhibited superiority in terms of growth parameters, while commercial cultivar was found most promising in terms of yield and yield attributing parameters. The highest GCV and corresponding PCV were observed in leaf area among growth parameters and in finger weight among yield attributing parameters indicating vast potential for genetic improvement of these two traits. Additive genetic control for the characters leaf area, LAI and pulp to peel ratio was recorded as these parameters registered higher GA, heritability and GCV. This indicates the suitability of these parameters for simple directional selection.

Kavitha *et al.* (2008) estimated genetic variance, heritability and genetic advance and in banana were studied in 19 hybrids to identify desirable genotypes for crop improvement programmes. Based on fruit quality characters along with more number of fruits/hand, bunch weight and early crop duration, the hybrids NPH-02-01 and NPH-03-19 gave high potential. The estimates of GCV and PCV were high for bunch weight, moderate GCV and PCV for number of hands/bunch and pseudostem height indicated better scope of improvement through selection. The genetic advance as percentage of mean ranged from 35.86 to 96.88. High estimates of heritability values accompanied with high genetic gain were observed for bunch weight, number of fruits/hand, days taken from planting to shoot development, number of leaves at shooting and crop duration.

Bhupendra Singh Uniyal *et al.* (2012) revealed that morpho-chemical characters of *Phyllanthus embilica* exhibited considerable genetic variability in fruit weight, which has high genotypic variation, higher heritability and greater potential for genetic gain. Vitamin C had moderate genetic variance, moderate heritability but greater genetic gain. Selection can therefore be effective based on these two characters and their phenotypic expression should be an indication of their high genotypic potential.

2.5 GENETIC DIVERGENCE STUDIES

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

Intra-specific variability for 11 fruit characteristics (fruit weight, fruit length, fruit diameter, fruit volume, seed weight, seed length, seed diameter, pulp weight, pulp percentage, pulp to seed ratio and total soluble solids) was evaluated, using non-hierarchical Euclidean cluster analysis, in 61 seedling trees of aonla (Sharma 2003). Minimum and maximum values of coefficients of variability were recorded for pulp percentage and pulp weight, respectively. The genotypes were grouped into seven clusters, which showed non-parallelism between geographic and genetic diversity. The genotypes of clusters 6 and 7 were highly diverse from each other, having inter-cluster distance of 14.062. The mean value of most of the fruit characters was highest in cluster 6.

Singh *et al.* (2004) assessed 20 genotypes of mango at IARI, New Delhi for cluster analysis and dendrogram study which resulted in the identification of six clusters and region specific separation of genotypes for North Indian, South Indian and Exotic Cultivars.

Kumar *et al.* (2006) evaluated physico-chemical properties of the fruits of 31 mango genotypes from North Kerala which formed four homogenous clusters. Large sized table fruit varieties like Mulgoa, Phirangiladua and Banganpalli formed a single group. Conversely the low quality large fruited varieties like Bangalora, Gudad and Chotta Jehangir deviated considerably from other mango cultivars. Also Phirangiladua exhibited a close linkage with Banganpalli and Alampur Baneshan.

Sharma *et al.* (2007) assessed genetic diversity by Mahalanobis D^2 statistics in 35 banana genotypes. These genotypes were distributed in seven different clusters and the genotypes grouped in each cluster showed different behavior 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 42 mango cultivars to assess the heritability and divergence in fruit characters. Using group constellation, cultivars were grouped into three distinct clusters. Cultivars with higher mean for fruit weight and peel weight, fruit length, fruit width and fruit and stone thickness, fruit length to width ratio (FLWR) and pulp to 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.

Rajamanickam and Rajmohan (2010) observed twenty eight genotypes of banana, which were grouped into six clusters following the Tocher's method of clustering analysis utilizing data on a set of 23 characters related to yield, vegetative and quality characters. Koonoor Ethan (cluster VI), Vellapalayankodan (cluster V) and Quintal banana (cluster IV) formed individual clusters and had the maximum genetic divergence. Cluster II had the maximum of 14 genotypes while cluster I and III had 9 and 2 genotypes respectively. The highest mean performance was recorded by cluster VI for 13 characters whereas cluster V and IV recorded the same for 7 and 3 characters. The intra and inter cluster genetic distance values ranged from 96 (cluster I) to 150 (cluster III). The maximum inter cluster distance was observed between cluster IV and cluster V (803) followed by cluster VI and cluster I (762). Contribution of individual characters towards divergence revealed that maximum contribution to total divergence was recorded by fingers per hand, shelf life of the fruit, fingers per bunch and finger girth whereas the characters which were contributing minimum towards divergence were leaf width, TSS, pseudostem girth, leaves per plant, suckers per plant, hand weight, pulp/peel ratio, fruit volume and acidity.

Rajasekhar *et al.* (2011) studied diversity and degree of relationship among 25 genotypes of sapota through principle component analysis and reported that fruit characters were sorted out into three major principle components with eigen value more than one which contributed for 89.79% of the total variability. PCI accounted for 59.07% of variability and was characterized by high and positive loadings for fruit surface texture (0.27) and titrable acidity (0.27), coupled with negative loadings for reducing sugars (-0.27) and titrable acidity (-0.27) which indicated that the PC I represented the fruit quality components. PC II explained for 18.93% of variability with high positive loading for total sugars (0.47) and negative loading for total phenols (-0.46), indicating that the PC II reflected sugar and phenol content. PC III described 11.78% of variability and showed high positive correlation for fruit shape (0.52). The dendrogram resulted in grouping of genotypes into six clusters, with cluster I having maximum number of genotypes (9). Inter cluster distance were maximum between cluster IV and cluster VI followed by cluster IV and V. Cluster means were high in cluster VI for fruit weight, ascorbic acid and pulp to seed ratio and for fruit yield/tree in cluster II.

With respect to biochemical characters, the highest TSS was recorded in Himayuddin and the lowest TSS was recorded in Neelum (Barhate *et al.,* 2012). The highest acidity and ascorbic acid content were recorded in Kalepad and the lowest in Baneshan and Mulgoa, respectively. By the application of clustering technique, the twelve genotypes were grouped into four clusters. Among the four clusters, cluster I was the biggest one, consisting of six genotypes and cluster II contained four genotypes, while cluster three and four had one genotype each. Among sixteen traits studied, number of fruits per tree and yield in kg showed higher degree of phenotypic and genotypic coefficient of variation.

Verma *et al.* (2012) studied the overall degree of polymorphism and similarities among pomegranate genotypes by PCA. Correlation studies showed considerable relation among different traits. PCA and cluster analysis showed a significant phenotypic and genetic diversity in the existing cultivars. All the cultivars could be grouped in to four major clusters. Majority of them fall under cluster-III. Local cultivar 'Kashmiri Local' showing maximum dissimilarity. The greater part of variance was accounted by traits such as duration of flowering, fruit set, fruit weight, fruit volume, fruit calyx length, calyx diameter, rind weight, rind thickness, number of seed+aril per fruit and juice percentage.

Majumder *et al.* (2012) assessed the genetic divergence in 60 mango genotypes through D^2 statistics and principal component analysis. The genotypes under study were grouped into eight clusters and the diversity was influenced by the morphological characters, not by the geographical distribution of the genotypes. The clustering pattern revealed that the genotypes collected from the same region did not fall in the single cluster. The maximum inter cluster distance was noticed between cluster II and cluster VIII, and the lowest between clusters VII and cluster VIII. From the cluster means, cluster I was high yielding and ranked first in terms of number of secondary branches per inflorescence, percent fruit set per inflorescence, and yield per plant. Cluster VIII had only one genotype which produced the highest percentage of flowering shoots, % perfect flowers, number of fruits per plant, and % TSS. The genotypes of cluster VII produced the biggest sized fruits. The first nine characters of the principal component axes with eigen values above unity accounted for 88.3% of the total variation among the fifteen characters. Weight of harvested fruits per plant (0.990 and 0.181), number of fruits per plant (0.101 and 0.607) and individual fruit weight (0.027 and 0.107) for both the vectors were positive across two axes indicating the important components of genetic divergence. The genotypes belonging to cluster I, VII and VIII with high to moderate genetic distances might be recommended for use in crossing programs to produce new recombinants with desired traits.

Singh *et al* . (2013) assessed variability in strawberry by using Principal Component Analysis (PCA) and genotypes were classified into four cluster groups for similarity by PCA. The highest inter cluster distance was observed between cluster II and V (129.39), followed by IV and V (114.082) and the lowest between II and IV. The highest intra-cluster distance was observed for cluster III and the lowest for the cluster VI and V. PCA showed that all the four principal component axes had Eigen values greater than one and altogether accounted for 77.34% of the total variation. The major contributing traits in PC1 was number of flowers, number of leaves and number of fruit/plant of leaflets per plant.

2.6 MOLECULAR CHARACTERIZATION

In this chapter efforts have been made to review the existing literature on the importance, origin and distribution, taxonomy, genetic diversity, genetics and inheritance pattern, cytology and morphological markers in sapota and other fruit crops. Since limited work has been reported on the use of molecular markers in sapota in India or elsewhere, exhaustive literature on use of RAPD and SSR markers in discriminating cultivars, genetic diversity studies, phylogenetic studies, and crop improvement pertaining to horticultural crops and DNA extraction protocols have been cited.

2.6.1 GENETICS AND INHERITANCE PATTERN

Comparison of yielding capacity of different Sapota cultivars have been made at different research stations (Chaudhary *et al.*, 1995, Ponnuswamy and Irulappan, 1989; Lenka *et al.*, 1996). Some attempts to produce better yielding hybrids have also been successful, but genetic studies indicating mode of inheritance of desirable quantitative traits (fruit size and shape, skin thickness/leatheriness, flesh, quality, seed size and number, flavor *etc.*) are lacking. However, all these quantitative factors are to be assumed polygenetically controlled. There exists non-significant correlation co-efficient between skin thickness and coarse leathery outer texture. Large X large fruited crosses yield progenies with more number of large fruited seedlings than large X medium or large X small fruited crosses. In many crosses, there is no difference in mean progeny yield scores, which leads to the conclusion that large fruit size is not linked with low yield.

2.6.2 MOLECULAR MARKERS

With the advent of molecular techniques new generations of markers have been introduced over the last two decades, which has revolutionized the entire scenario of biological sciences. Molecular markers include biochemical constituents (*eg.* secondary metabolites in plants) and macro molecules, *viz.*, proteins and deoxyribonucleic acid (DNA). Analysis of secondary metabolites is however, restricted to those plants that produce a suitable range of metabolites which can be easily analyzed and distinguish varieties (Ragot and Hoisingotn, 1993). An ideal marker should be unaffected by environmental effects or management practices. Hence, among the molecular markers used, DNA markers are more suitable and ubiquitous of the living organisms (Joshi *et al.,* 1999).

Waltan (1993) reported that molecular technology is indirectly improving the efficiency of plant breeding programmes. A molecular marker is a measurable chemical or molecular characteristic that is inherited in a simple mendelian fashion. Molecular markers play two main roles in plant breeding programmes, firstly as a source of genetic fingerprints and secondly as a selectable marker linked to phenotypic traits of interest to breeders.

2.6.3 DNA ISOLATION

Extraction of high quality genomic DNA from plant tissues is difficult due in part to their rigid cell wall composed of large amount of polysaccharides (Hottori *et al.,* 1987). The application of molecular biology techniques to the analysis of complex genomes depends on the ability of any protocol to prepare pure, high molecular weight DNA (Ausubel *et al.,* 1989).

Several factors affecting the DNA preparation which inhibit polymerase activity as a negative polymorphic bands have been reported by Gelfand and White (1990) in PCR fingerprinting. This was as a result of combination by polysaccharides and other DNA binding substances, which may confound the interpretation of genetic differences between individuals. Demeke and Adams (1992) reported contamination by polysaccharides is a common problem affecting plant DNA purity that reduces the activity of DNA polymerases, ligases and restriction endonucleases.

Negative consequences of unstable DNA protocols have been described in plants by Rogers and Bandhic (1994). They are i) relatively low yields, ii) consolation of DNA serving quinonic compounds (brown pigments) associated with activity of polyphenols and other secondary compounds, which affects different enzymes used and iii) premature DNA degradation. The large number of samples that are required in breeding programmes demand purification methods that provide high quality DNA, rapidly, simply and inexpensively (Weising *et al.,* 1995).

Intact high molecular weight DNA from plant tissue can be isolated in a number of ways. All methods involves the basic steps of removal of cell wall and nuclear membrane from around the DNA and the separation of DNA from other cell components such as cell wall debris, proteins, lipids or RNA without affecting the integrity of DNA. To obtain DNA of high molecular weight and sufficient purity, two factors affecting are size and smear. To reduce the shearing forces the lysate should be treated gently. In order to reduce nucleases activity (plant cell being rich in nucleases), the tissues should be quickly frozen in liquid nitrogen and after grinding to open the cells in liquid nitrogen, the tissue should not be thawed, only in the presence of extraction buffer containing detergent and a high concentration of EDTA (Shantha *et al.,*1998).

2.6.4 DNA BASED MARKERS

Molecular genetic techniques have been applied to plant cultivar identification in the past decade by developing molecular markers that detect differences in DNA sequences between cultivars. Highly specific marker profiles commonly known as DNA fingerprints, can be developed for each cultivar and used for its identification. Compared to isozyme markers, DNA markers have many advantages. They are detectable in all tissues at all ages of the plant, thereby, enabling early identification. They are virtually insensitive to epistatic and pleotropic effects (Williams *et al.,* 1992). They are developmentally stable and not affected by the environment. Furthermore, the number of DNA markers available for examination is unlimited because genomes are composed of base pairs and many different types of molecular marker systems are available to compare these differences.

Joshi *et al.* (1999) reported about the properties for ideal DNA markers. They should be highly polymorphic in nature, co-dominantly inherited, frequently occur in the genome, selectively neutral behavior, highly reproducible and the data be easily exchangeable between laboratories. Genetic polymorphism is classically defined as the simultaneous occurrence of a trait in the same population of two or more discontinuous variants or genotypes.

Higher plants have a considerable portion of DNA as repetitive non- coding DNA that is not transcribed. Species with large genomes have more repeated DNA and higher proportion of repeated DNA to single copy DNA (Tanksely and Pichersky, 1988). Thus, only small fraction of the total genetic variation at a DNA nucleotide sequence level reveals itself as a distinct trait, showing Mendelian inheritance. The degeneracy of the genetic code ensures that one in three nucleotide changes will not affect the amino acid sequence of protein produced. Thus, the great bulk of genetic variation at the nucleotide level may not have any detectable expression at phenotypic level. It is this genetic variation that is expressed as DNA- based polymorphism.

DNA based markers can be classified into:

a. Restriction based (RFLP)

b. PCR based (RAPD and SSR)

c. Both restriction and PCR based (AFLP)

RFLP and RAPD are the two types of DNA based markers which are widely used and the literature on them is cited below. Since RAPD approach is being adopted in the present study much of the literature reviewed pertains to use of this technique.

2.6.4.1 RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

This method involves digestion of the DNA of the subjected genome with cleaving restriction endonucleases (Grodzicker *et al.*, 1974), fractionating the fragments electrophoretically, and then preferentially visualizing fragments containing particular homologus sequences by hybridizing them to specific DNA probe.

RFLP analysis also has been used in DNA fingerprinting and estimation of diversity in grapes (Bowers *et al.*, 1993), in apple for paternity analysis (Nybom and Schaal, 1990) and in the construction of genetic linkage maps in many crops like tomato (Tanksley *et al.*, 1998, Peterson *et al.*, 1998).

RFLP is a co-dominantly inherited marker system (it can distinguish heterozygote from the two parents). It is relatively reliable and reveals high allelic diversity, but it is slow, more costly, labor intensive and involves use of radioactive chemicals, which are harmful to the users. It is slow because it needs more time to construction of probes, processing the plant materials for high quantity and quality (µg) DNA. Most RFLP probes are single copy probes that reveal one DNA fragment per probe. While recently evolved PCR based molecular markers like RAPD and AFLP reveal several bands per reaction. RFLP cannot be used in crops with very low level of restriction fragments or for which RFLP probe sets are not available. It is also not the best method for applications involving more individuals such as screening a breeding population with marker linked with disease resistance (Walton 1993).

2.6.4.2 AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP)

AFLP combines the reliability of RFLP with power PCR technique. It allows the reliable identification of over 50 loci in a single reaction. It can be applied for DNAs of any origin and complexity. Fingerprints can be produced without prior knowledge using limited sets of generic primers. It is robust and reliable because stringent reaction conditions are used for primer annealing (Vos *et al.*, 1995).

It is very recent technique based on the detection of genomic restriction fragments by PCR amplification. It involves digestion of DNA with two restriction enzymes (one frequent cutter and a rare cutter), enzyme specific oligonucleotide adopters of known sequence are ligated to each end. Restriction fragments are then amplified using enzyme adopter specific primers that are complimentary to adopter sequence and remains of the restriction site plus upto usually one to four random nucleotides at 3' end. The number of restriction fragments amplified will be determined by the complexity of the template and it can also be tuned by selection of specific primer sets with selective extension. It involves two amplifications, pre-amplification and selective amplification. Pre-amplification will be carried out using a primer set with one selective extension. Pre-amplification DNA will be used as template for second selective amplification, which will be carried out with primer sets with three to four nuclieotide extensions. (Hill *et al.*, 1996).

It has been used in assessing genetic diversity of many crops like soyabean (Maughan *et al.,* 1996), tea (Paul *et al.,* 1997), gooseberries (Lanham and Brennan, 1999), neem (Singh *et al.*, 1999).

For AFLP additional purification steps are necessary, as it needs high quality and quantity DNA. It is somewhat lengthy process as it includes two amplifications. It is costly because it involves use of restriction endonucleases, enzyme specific adopters, enzyme adopter specific primers with selective extensions, ligators and use of polyacrylamide gel for electrophoresis (Vos *et al.,* 1995).

2.6.4.3 RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD)

The discovery of Polymerase Chain Reaction (PCR) (Mullis, *et al.,* 1986: Mullis and Faloona, 1987) has led to the development of another genetic marker system for detecting DNA polymorphisms (Welsh and Mc Clelland, 1990; Williams *et al.,* 1990). Randomly amplified polymorphic DNA (RAPD) involves PCR amplification of total genomic DNA using single random primer of about ten bases and separating amplified fragments by agarose gel electrophoresis.

The major advantage of this assay over RFLP method is that there is no prior requirement for DNA sequence information of the genome. The protocol is also relatively quick and easy to perform and uses fluorescence in lieu of radioactivity (Williams *et al.,* 1992). Because the RAPD technique is amplification-based assay, only nanogram quantities of DNA are required and automation is feasible.

This marker is simple and dominant in nature and procedure for this technique is also very simple. The procedure involves extraction of DNA, by any of the standard protocol and amplifying the DNA by PCR using random primers. The presence and absence of the fragment in a segregating population is scored directly from the gel. Running agarose gel, stained with ethidium bromide fractionalizes amplified DNA. Lastly involves visualization and analysis of markers on the gel through photography of the gel.

The PCR program used for RAPD analysis is a typical PCR program except the stringency during primer annealing is much lower than usual because of the primer used in the RAPD procedure is relatively short and has an arbitory sequence. For random 10 decamer primer, annealing at 36° C works well for many organisms, including plants. The limitation of the use of RAPD markers is that they are dominant. This can be overcome by using more than one closely linked marker (Williams *et al.,* 1990).

2.6.5 APPLICATION OF RAPD AND SSR MARKERS

2.6.5.1 RAPD MARKERS

RAPD markers were used for assessment of phylogenetic relationships by various workers in mango (Schnell *et al.*, 1993)**,** grape (Gehrig *et al.*, 1997) and citrus (Federici *et al.,* 1998).

Identification of avocado cultivars with RAPD markers was done by (Lewis, 1992). Preliminary tests were carried out on cultivars ‘Hass’, ‘Fuerte’ and ‘Edranol’ using three arbitrary synthetic DNA primer sequences. The study showed ‘Fuerte’ and ‘Edranol’ were found to be closely related.

Identification of apple cultivars was done using RAPD markers by Koller *et al.* (1993). Eleven apple cultivars were differentiated using RAPD markers. The variability of the technique and the origin of the DNA extract were analyzed. A set of bands consistent in their presence or absence was chosen to create a differentiation banding pattern.

Schnell *et al.* (1993) estimated genetic relationships among *Mangifera* spp. based on RAPD markers. Phylogenetic relationships among nine *Mangifera* species were studied using RAPDs. Analysis was conducted using average taxonomic distance, UPGMA and principal component analysis. Ten selected primers produced 109 usable bands. RAPD- generated cluster did not always agree with taxonomic classification based on the morphological traits. When the two sub-section of the genus (Sub- genera *Mangifera* and *Limus*) were analysed separately, the classification more closely agreed with traditional taxonomic relationships.

Novy *et al.* (1994) identified varietal misclassification and regional divergence in cranberry (*Vaccinium macrocarpon* (Ait.) Pursh) using RAPDs. RAPD technology was used to characterize 22 cranberry varieties. Twenty-two decamer primers amplified 162 scorable DNA fragments, of which 66 (41%) were polymorphic. On the basis of these 66 silver-stained RAPD (ssRAPDs), 17 unique profiles were identified rather than the expected 22. Fourteen varieties had unique ssRAPD profiles, while the remaining eight were represented by three ssRAPD profiles. Permutational analyses of the data suggested that the observed ssRAPD profile duplications were examples of varietal misclassification. Further analyses identified two ssRAPD markers that were found only in Eastern varieties (from Massachusetts and New Jersey) and not in Wisconsin varieties. The varieties differing on an average by 22 bands, RAPDs were shown to be useful in varietal identification and the assessment of genetic diversity in cranberry.

Yae *et al.* (1995 a**)** classified *Malus domestica* and *M. pumila* cultivars using RAPD markers. Of the 139 clear and reproducible bands, 106 were polymorphic to divide the cultivars into 6 groups by cluster analysis. Group I contained ‘Rall’s Janet’, ‘Fuji’ and the bud mutations of ‘Fuji’; group II contained ‘Sikaiichi’, ‘EarliBlaze’, ‘Delicious’ and its strains formed group IV; ‘Jonathan’, ‘Jonared ‘, ‘Kogetsu’ and ‘Mollies Delicious’ formed group V; and group VI contained only ‘Spur Golden Delicious’.

Cheng *et al.* (1996) identified co-dominant RAPD markers tightly linked to fruit skin clolour in apple. Bulked segregate analysis identified one 10-mer base oligomer that generated different fragments in each of the bulks. After testing the primer in four populations, two fragments were associated with red skin colour and another two fragments associated with yellow skin colour. Three of the fragments (1160, 1180 and 1230 bp) were partly sequenced and found to share high sequence homology, suggesting that they generated from the same locus. A pair of universal primers was designed to amplify the fragments. In the cross ‘Rome Beauty’ X ‘White

Angel' population, two fragments were associated with red skin colour: one fragment designated as A1 (1160bp) was from 'Rome Beauty' and another fragment A2 (1180bp) was from 'White Angel'. Progeny possessing both fragments, and one of them, had red fruits. Both parents displayed an alternate fragment, a1 (1230 bp) was associated with yellow-skinned fruit. In three other crosses tested, only fragment A1 co-segregated with red skin colour and two fragments, A1 and A2 (1230 and 1320 bp, respectively) were associated with yellow skin colour.

Damasco *et al.* (1996) detected dwarf off-types in micro propagated Cavendish (*Musa* spp. AAA) bananas using RAPD markers. A marker specific to the dwarf off-type from micro propagation of Cavendish group cultivars 'New Guinea Cavendish' and 'Williams' was identified following an analysis of 57 normal and 59 dwarf plants generated from several micropropagation events. Of 66 random decamer primers used in the initial screen, 28.8 per cent revealed polymorphisms between normal and dwarf plants. Primer OPJ – 4 (5'-CCGAACACGG-3') amplified a 1.5 kb band which was consistently present in normal but absent in all dwarf plants of both cultivars. Reliable detection of dwarf plants was achieved using the marker, providing a suitable means of *in vitro* detection. However, micropropagation- induced RAPD polymorphisms were not associated with the dwarf trait. The effectiveness of RAPD analysis for cultivar identification of persimmons (*Diospyros kaki*) was evaluated by using 10 base primers. Among 20 primers, two primers (OPA-6 and OPA-8) were most effective for cultivar identification and fifteen cultivars tested were completely distinguished from these primers, two bud mutants of cv. 'Hiratenenashi', *i.e.*' Tonewase' and 'Sugitawase', showed different DNA patterns with a few additional minor bands using the OPA-6 primer.

Machado *et al.* (1996) detected genetic relationships among 39 Mediterranean mandarins (*Citrus deliciosa* Tenora) using RAPD markers. About 111 amplification products were identified using 21 random primers. An average of 2.2 RAPD markers was obtained for each primer. UPGMA cluster analysis revealed the low level of genetic variation between accessions of Mediterranean mandarins, whereas their hybrids with other *Citrus* species showed grater genetic dissimilarity. Twenty accessions yielded very similar patterns, suggesting that either they could be a single clone, or that the technique was not able to detect genomic variation.

Graham, M.J. (1996) estimated genetic diversity in eight strawberry cultivars using RAPD markers. Ten random primers successfully amplified DNA fragments from each cultivar and specific fingerprints were generated from the molecular marker data. The cultivars were traced back to founding clones and the relationships between the cultivars were examined from both the molecular and the pedigree data.

Xiang *et al.* (1996) analyzed peach rootstock cultivars using RAPD markers. Eighteen peach rootstock cultivars, most of *prunus persica*, were screened for diagnostic RAPD markers using synthetic decamer oligonucleotide primers. Based on combined banding patterns all 18 rootstocks using 40 markers produced a dendrogram of genetic relatedness which is in good agreement with their putative pedigrees. The first and major bifurcation in the dendrogram divided these rootstock cultivars into two groups according to their resistance or susceptibility to root-knot nematodes.

Bartolozzi *et al.* (1998) estimated the genetic relatedness among 17 almond genotypes and one peach genotype was estimated using 37 RAPD markers. Genetic diversity within almond was found to be limited despite its need for obligate outcrossing. A similarity index based on the proportion of shared fragments showed relatively high levels of 0.75 or greater within the almond germplasm.

Hormaza (1998) suggested the combination of *in vitro* embryo culture with the use of molecular markers to reduce the associated with breeding and to accelerate the breeding process in Cherry.

Ye *et al.* (1998) reported on DNA fingerprinting utilizing RAPD polymorphisms to investigate the relationship among 16 grapevine cultivars and sports thought to have arisen from these cultivars. From 53 primers, a total of 464 bands were generated of which 29 per cent were common to all genotypes tested. Cluster analysis classified all tested cultivars into two main groups (*Vitis vinifera* L. and *Vitis* X *labrusca* Bailey) as expected. No polymorphism was detected among known clones of 'Chardonnay' or 'Pinot Noir'.

RAPD markers were used in cashew to assess the diversity among the varieties and hybrids released in India by Murali (1999). Of the one hundred and twenty random primers screened, ten were chosen to score for 157 bands. The results indicated that the diversity among cashew cultivars and hybrids was moderate.

Hemanthkumar *et al.* (2001) reported that fifty mango cultivars were screened using Randomly Amplified Polymorphic DNA (RAPD) markers with decamer primers of arbitrary sequence. Out of 80 primers screened, ten were selected which gave 139 clear and bright fragments. A dendrogram based on Jaccard's Co-efficient of similarity implied a moderate degree of genetic diversity among the cultivars. The hybrids which had one parent in common were placed together. In the cluster, alternate bearers and regular bearers formed separate groups. Another analysis based on Pearsons's co-efficient of similarity revealed a high degree of genetic diversity. In both the analyses, 'Mulgoa' was found to be very distinct.

Phylogenetic relationships within the *Actinidia* were investigated (Huang *et al.* 2002) by using randomly amplified polymorphic DNA (RAPD) markers. DNAs from 40 taxa, including 31 species encompassing all four sections and four series of the traditional subdivisions within the genus, were amplified using 22 preselected 10-mer oligonucleotide primers. A total 204 DNA bands were scored across the 40 taxa, of which 188 (92%) were polymorphic. A wide range of genetic similarity was observed among the taxa (0.13 to 0.61). The average similarity between varieties of the same species was 0.54, and between different species was 0.28, respectively. The UPGMA phenogram showed that the majority of the species clustered into geographic subgroups in accordance with their natural distribution (the Yangtzi River, southeastern China, southern China and southwestern China).

Meghala *et al.* (2005) examined the genetic relatedness and assessed the diversity among Indian sapota cultivars using random amplification of polymorphic DNA (RAPD) markers. Twenty sapota cultivars widely grown in different regions of India were used. Twenty decamer random primers amplified 232 RAPD markers of which 184 were polymorphic. Both cluster analysis and principal components analysis of the RAPD data indicated wide genetic diversity among the cultivars.

Salhihannach *et al.* (2006) assesed the genetic diversity of Thirty five Tunisian fig *(Ficus carica* L.) using forty four RAPD markers. A total of nine primers were screened for their ability to generate consistently amplified band patterns and to assess polymorphism in the tested varieties. Among these primers, only six (OPA01, OPA02, OPA05, OPA11, OPA16 and OPA18) have revealed unambiguously scorable polymorphic bands. These mentioned primers generated multiple banding profiles with six to nine polymorphic amplified DNA bands ranging in size from 100 to 2500 bp. A total of 44 out of 63 were polymorphic (72.23%) with a mean of 7.33 bands per primer. The dendrogram showed two main groups composed of cultivars with similar geographic origin. Moreover, the male accessions (caprifigs) were clustered indistinctively within the female ones, suggesting a narrow genetic diversity among these accessions.

Jain *et al.* (2007) reported that the genetic diversity of banana varieties (Grand Naine, Red Banana, Nendran and Rasthali) using Random amplified polymorphic DNAs (RAPDs) fingerprinting by three primers (OPA-19, OPB-18, OPD-16). Among the selected primers OPB-18 produced maximum number of polymorphic 4 bands followed by OPA-19 and OPD-16. 43.47 per cent, whereas 30.43 per cent were unique, but only 26.08 per cent revealed the relationship between these genotypes. The dissimilarity matrix of Squard Euclidean Distance (SED) ranges from 2.82 to 3.6 per cent. The highest dissimilarity 3.6 per cent was detected between genotypes Red banana and Rasthali and the least 2.23 per cent between genotypes Nendran and Rashali.

Rahman *et al.* (2007) analyzed 28 mango genotypes using RAPD markers and revealed that out of 20 primers screened, four were 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% 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).

Sharma *et al.* (2007) investigated the diversity among 22 guava cultivars and 2 wild species, *i.e. P. cattleianum* and *P. friedrichsthalianum,* using RAPD markers. Out of a total of 376 clear and reproducible bands, 347 were polymorphic accounting to 92.29% polymorphism. A genetic similarity matrix was constructed and the values ranged between 0.33-0.94. The highest genetic similarity (0.94) was noticed between Hybrid Red Supreme and Super Max Ruby, whereas Hybrid Red Supreme and Spear Acid were found to be genetically most diverse (0.33).

The PCR based Random Amplified Polymorphic DNA (RAPD) and Directed Amplification of Minisatellite DNA (DAMD) markers were used to study the genetic diversity and relatedness among 22 guava accessions comprising commercial cultivars, breeding lines and unimproved cultivars (Bajpai *et al.* 2008). DNA isolated by CTAB method was used for amplification and genetic distance matrix based on Jaccard’s Co-efficient revealed maximum distance between Purple Guava and Allahabad Safed (43%), and a minimum distance of as low as 5.4 per cent between two breeding lines HPSI-20 and HPSI-26. Interestingly half-sib progenies CISH-G-1 to CISH-G-6 had slightly more distance ranging from 1.8 to 24.0 per cent. The clustering revealed that most of the cultivars/accessions originated from Indo-Gangetic plains are grouped together.

Random Amplified Polymorphic DNA (RAPD) markers were used to evaluate genetic similarity and interrelationship among 18 citrus cultivars, including 13 species and 5 hybrids. Out of 40 decamer primers screened, 25 were selected which produced 250 markers; of which 231 were polymorphic and some are species or cultivar specific RAPD markers. The Jaccard’s Co-efficient of similarity using UPGMA cluster analysis clearly separated Jatti-Khatti from all major clusters at a similarity coefficient of 0.61. The average genetic similarity value observed across all the genotypes was 0.63, with the 2 sweet orange cultivars, Jaffa and Blood red, showing maximum similarity (82%). The Jatti-Khatti and King Mandarin were found to be genetically most diverse. The genetic variation between cultivars was quite high and revealed their different origins (Baig *et al.*, 2008).

Bajpai *et al.* (2008) revealed that forty six mango cultivars were screened using RAPD markers. Nine decamer oligonucleotides yielded 110 discrete fragments. RAPD primers yielded 14 monomorphic bands and 96 displayed polymorphism. Per cent polymorphism generated by these primers was 87.3 per cent. OPA 19, OPA 20 and OPC 6 were highly polymorphic primers. UPGMA tree constructed on RAPD data on the basis of Jaccard’s Co-efficient clustered the accessions into 3 groups, one comprising majority of the north Indian varieties and other having eastern Indian and third cluster comprising accessions from both the regions.

A study was carried out to determine genetic relationships among 23 apricot cultivars from Turkey by using randomly amplified polymorphic DNA (RAPD) technology. Among the 40 decamer random primers used for PCR reactions, 12 primers showed reproducible polymorphic patterns (Ercisli *et al.*, 2009). These primers produced 121 bands, out of which 118 were polymorphic. UPGMA (Unweighted Pair Group Method using Arithmetic average) derived dendrogram, grouped the cultivars into one cluster and one outlier. The main cluster further divided into three sub clusters. The highest dissimilarities was found between Hacihaliloglu and Kabaasi cultivars (0.108).

Hussain *et al.* (2009) revealed that 42 genotypes identified through the morphological and physical characters were subjected to the DNA analysis to determine the level of genetic diversity among the local loquat genotypes. Out of 48 amplification products scored, 47 bands (97.92%) were found to be polymorphic. According to the dandrogram, two main groups of the loquat genotypes were identified. All genotypes belonging to a certain location came under any one of the two groups.

Mishra (2009) reported that the phylogenetic relationships of 36 locally grown apricot (*Prunus armeniaca* L.) genotypes using 31 PCR markers (20 RAPDs). RAPD analysis yielded 139 fragments, of which 136 were polymorphic, with an average of

6.8 polymorphic fragments per primer. Clustering of genotypes within groups was not similar when RAPD derived dendrogram were compared, whereas the pattern of clustering of the genotypes remained more or less the same in RAPD.

Anburaj and Sudarmani (2010) revealed that the genetic diversity in five high yielding jackfruits accessions using D19 primer (OPD19 – CTGGGGACTT). Good quality of DNAwas extracted from five different varieties of young tender leaves of *Artocarpus heterophyllus* by CTAB method with minor modification of high detergent concentration of 2 percent PVP and 3 per cent CTAB. The quality of DNA was checked by Agarose Gel Electrophoresis. The isolated DNA was quantified using UV spectrophotometer at 260nm and 280nm. And Quantified DNAwas subjected for PCR amplification. 67 unambiguous, readable and reproducible RAPD markers were produced using the selected primer (OPD19 – CTGGGGACTT). The average number of bands obtained was of 13.4, on using single primer, with the sizes ranging from 250bp to 10000bp. Of the 67 bands, 18 (21.1%) were polymorphic and shared between five individuals, while 45 (52.9%) were monomorphic in five individuals.

Cerqueirasilva *et al.* (2010) evaluated the genetic diversity by RAPD markers in 18 genotypes of *Passiflora trintae*. The 15 primers generated 112 markers, 84% of which were polymorphic. The amplification reactions carried out produced a total number of 112 RAPD bands and an average number of 7.46 bands per primer, with extreme values oscillating from 4 to 12 among the 15 primers used. The number of polymorphic bands observed was 94 (84%), while the number of monomorphic bands observed was 18 (16%). The genetic distance estimated by the complement of the Dice index (average dissimilarity = 0.30) and genotype grouping based on the UPGMA algorithm showed low variability among genotypes.

Hasnaoui *et al.* (2010) reported that the genetic diversity among Tunisian pomegranate using universal primers, the random amplified polymorphic DNA (RAPD) method was used to generate banding profiles from a set of twelve cultivars. Among the 12 primers tested, only four generated unambiguously reproducible bands after two PCR Runs. These are: OPA-04, OPA-19, OPH-08 and OPH-09. The remaining ones all yielded non reproducible bands. A total of 29 bands were generated with a mean of 7.25 per primer. The number of bands generated varied from 3 to 11, with 0.5 to 3 kb size range. OPH-07 and OPA-19 primers generated nine and eight polymorphic bands, respectively, while amplification with OPH-08 and OPA-04 gave only four and threepolymorphic bands, respectively. The total number of polymorphic bands produced using four RAPD primers was 24 out of 29. Data was then computed with appropriate programs to construct a dendrogram illustrating the relationships between the studied cultivars. The cluster analysis has exhibited a parsimonious tree branching independent from the geographic origin of the cultivars.

Yildirim *et al.* (2010) examined the genetic variability of 11 *D. lotus* genotypes sampled from Coruh Valley in Turkey. One hundred and twenty-eight DNA markers were generated by 12 random primers. The highest polymorphism ratio was observed with the primer OPA-01 (71%) while the lowest was with OPY-01 (36%). The band size was between 350 and 2500 bp for these primers. The percentage of polymorphic bands was 58%, which demonstrated the efficiency of these primers. The similarity between genotypes ranged from 0.48 to 0.76.

Ercisli *et al.* (2011) reported that genetic variability in 23 wild-grown pomegranate genotypes using eighty-six RAPD primers. Among which 12 gave reliable polymorphic patterns. These primers generated 145 RAPD bands of which 91% were polymorphic. The highest polymorphism ratio was observed with primers OPY-06, OPY-13, OPBA-03, OPB03, OPB-07, and OPB-08 (100%), while the lowest was with OPB-09 and OPB-10 (75%). The band size ranged between 250 and 2400 bp. There were five main clusters in the dendrogram, the highest genetic similarity was 0.24 and the lowest was 0.08.

Anusha and Shanmukhiya (2011) evaluated the morphological and molecular characteristics of 12 citrus varieties cultivated in Mauritius and compared their genetic relatedness by using the RAPD technique making use of 30 decamer Operon primers. 77.1% polymorphism was obtained with Operon primer OPD-2 being the least discriminatory in contrast to OPA-9 and OPL-14, which were most polymorphic. An Un-weighted Pair Group Method with Arithmetic Mean dendogram of cophenetic ratio 0.9732 was constructed with distance values of 0.222 to 0.667.

Hassam *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% 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 those indigenous to the Delta of Egypt cultivars and the second consisting of other indigenous to the Suez Canal and Sinai region.

2.6.5.2 SSR MARKERS

Azevedo *et al.* (2005) investigated the patterns of spatial distribution, genetic structure, and mating system using 7 microsatellites of the *Manilkara huberi* species. Among the 12 polymorphic loci available from the development study, seven were selected for the analysis based on higher information content and robustness of the analysis with the multiple tailed primer method. The mean number of alleles/loci (A) in the adult generation varied from 15 (Mh22) to 20 (Mh17, Mh20), with a mean of 17.7, totaling 124 alleles. In the progeny population, the mean number of alleles/loci varied from 14 (Mh04, Mh22) to 24 (Mh06), with mean of 18.1, totaling 127 alleles. High levels of multiallelism were observed at all 7 loci analyzed in both generations. However, by comparing the frequency of the more frequent alleles between the two generations, no significant differences were observed.

In a study by Shiva Lal Singh *et al* . (2008) genetic diversity was estimated in 32 accessions from different ecogeographical regions of India representing *Vitis vinifera* and *V. champini*. Twenty pre-selected SSR primers generated a total of 64 scorable amplification products and out of which 60 were (93.7%) polymorphic. The cluster analysis indicated that grape germplasm in India has broad genetic base with the values of genetic similarity co-efficient ranging from 0.48 to 0.86. UPGMA analysis resulted in the formation of two main clusters, consisting mainly of commercial cultivars (*Vitis vinifera*) and rootstock (*Vitis champini*). The *Vitis vinifera* group was more heterogenous and it further divided in sub clusters. Two varieties belonging to *Vitis vinifera* grouped with *Vitis champini.* All other varieties were differentiated by unique pattern with 20 SSR markers. The high discriminating power of the loci suggests that SSR markers may be able to differentiate commercial cultivars, rootstocks or hybrids.

Viji *et al.* (2010) characterized the diversity of guava germplasm by using seven PCR primers amplifying fourteen previously developed microsatellite loci were used to identify and characterize 13 guava accessions. Allelic variation was observed in each of the cultivars irrespective of geographic origin. A total of seven alleles were detected ranging from a minimum of two (mPgCIR01 and mPgCIR07) to a maximum of seven (mPgCIR16 and mPgCIR21). Expected heterozygosity values for individual loci ranged from 0.14 to 0.80 with an average of 0.61

In a study by Dacumos *et al.* (2011) Simple Sequence Repeats (SSRs) were used to determine the genetic variability and relationships among sixty-one genotypes of Philippine bananas. Ten out of twelve primer pairs tested were found to be polymorphic and generated 43 alleles ranging from 3 to 13 alleles per primer. Seven out of the ten primers gave unique bands for some of the genotypes tested showing their potential use in cultivar identification and fingerprinting. The resolving power of molecular markers measured as the polymorphic information content (PIC) ranged from 0.68 to 0.94. Cluster analysis indicated a wide genetic variation existing within the different Musa genotypes. Cluster analysis separated the genotypes of *Musa acuminata* from other genotypes with A and B genomes.

Latha *et al.* (2012) characterized nine guava cultivars using 23 microsatellite markers. The number of alleles ranged from 3 to 12 with a mean of 6.39 alleles per locus. The polymorphic information content of the markers ranged from 0.340 to 0.900 with a mean of 0.749. The expected heterozygosity ranged from 0.392 to 0.961 with a mean of 0.824. The probability of identity ranged from 0.031 to 0.487 with a mean of 0.143. The clustering pattern distinctly grouped the pink flesh varieties and the white flesh varieties into two separate groups.

Ravishankar *et al.* (2012) assessed the genetic diversity among mango (*Mangifera indica*) cultivars to test their amplification in closely related species.

Thirty-six microsatellite (Simple Sequence Repeats) 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. 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.

Twelve simple sequence repeat (SSR) loci were used to determine the genetic diversity of 62 acid lime landraces collected from high hills, mid hills and terai agro-ecological zones of eastern Nepal (Shrestha *et al.* 2011) and they reported that average number of allele was detected 2.91 in terai accessions followed by 2.82 and 2.73 in high hill and mid hill accessions respectively. The average polymorphic information content (PIC) value was observed to be higher in terai (0.53) as compared to mid hills (0.50) and high hills (0.49). UPGMA clustering based on Jaccard's similarity matrix grouped 62 accessions into five main clusters. The average genetic similarity index among the acid lime accessions was observed to be 0.80, with the lowest being 0.77 observed in terai and highest of 0.82 in the mid hills.

The genetic variability and the relationship between and within four main groups of the *Citrus* genus (lemon, mandarin, grapefruit and sweet orange) were investigated by using 11 SSR primer pairs (El-Mouei *et al.,* 2012). Specific markers allowed the distinction between and within four *Citrus* groups. The highest value of genetic diversity was detected in Mandarin group (0.513) while the lowest was in the Grapefruit group (0.074).

Vasugi *et al.* (2012) investigated the mango germplasm to identify duplicates in the germplasm using molecular methods (SSR) which 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.

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.

Six landraces viz., DM Acc-3, 4,7,15,17 and 18 were characterized for their genetic distinctiveness and relationships with the choicest juicy cultivars of mango in Andhra Pradesh (‘Peddarasam, Chinnarasam, Cherukurasam, Panchadarakalasa and Suvarnarekha) at the molecular level, using 109 mango-specific microsatellite markers (SSRs). Jaccard’s similarity coefficient ranged from 0.40 to 0.69 signifying wide variability in the landraces of mango to an extent of 31-60%, offering ample scope for selection. Of the 109 SSR markers validated, 57 were polymorphic, of which 10 were highly polymorphic. Microsatellite SSR-84 was able to differentiate and/or identify all of the 11 genotypes under study. UPGMA cluster analysis revealed that the six elite landraces viz., DM Acc-3, 4, 7, 15, 17 and 18 clustered according to their geographical origin. Being horticulturally superior and genetically distinct, these six land races can be clonally selected for further testing and multiplication for commercial exploitation. (Hameedunnisa *et al.*, 2013).

CHAPTER III

MATERIAL AND METHODS

The present investigation entitled "**Studies on the diversity in morphological, bio-chemical and molecular characterization in sapota (*Manilkara achras* (Mill.) Fosberg) genotypes**" was conducted at Horticultural Research Station, Venkatramannagudem, Andhra Pradesh during the period from 2013 to 2014. The materials used and methods followed are presented in this chapter.

3.1 LOCATION OF THE EXPERIMENTAL SITE

The experimental site is located at Horticultural Research Station, Venkataramannagudem, West Godavari District. The location falls under the Agro-climatic zone no.10, East Coast Plain and Hills (Krishna-Godavari zone) at an altitude of 34 m (112 feet) above mean sea level with its geographical position is 16.83^0 N latitude and 81.5°E longitude.

3.2 AGRO-CLIMATIC CONDITIONS

It experiences hot humid summer and mild winters with an average rainfall of 950 mm per annum and temperatures range from 18^{O}C during winter to about 49^{O}C during summer. The meteorological data pertaining to mean maximum and mean minimum temperatures, relative humidity and rainfall recorded during the period of investigation at Horticultural Research Station Venkataramannagudem is presented in Appendix 1.

3.3 SOIL CHARACTERISTICS

The soils are sandy to red sandy loams with a pH of 7.2, electrical conductivity of 0.22 dSm^{-1} with good texture. The available nutrient status of the soil is 158.80 Kg, 28.5 Kg and 250 Kg of N, P and K per ha respectively.

3.4 EXPERIMENTAL CODITIONS

The trees were planted during 1996 at 10 m X 10 m spacing and provided with drip irrigation. Fertilizers were applied at the rate of 400 g Nitrogen, 200 g Phosphorous and 450 g Potash per tree per annum. For maintaining the trees in healthy condition timely intercultural operations were adopted along with proper plant protection measures.

3.5 GERMPLASM ACCESSIONS

The following genotypes of sapota were used in the present study

1 Badami
2 Bombay
3 Brazilian Sapota
4 Calcutta Round
5 Columbian Sapota
6 Cricket Ball

18 Pala
19 Seedless
20 Simhoudi
21 Singapore
22 Tagarampudi
23 Ulvapadu selection

7	Dwarapudi	24	Virudnagar
8	Gavarayya	25	CO-1
9	Gutti	26	CO-2
10	Hybrid	27	CO-3
11	Kalipatti	28	DHS-1
12	Kirthibarthi	29	DHS-2
13	Krishna Rao	30	PKM-1
14	Mirandi	31	PKM-2
15	Pakala	32	PKM-3
16	Pakala Oval	33	PKM-4
17	Pakala Round		

3.6 SAILENT FEATURES OF THE GENOTYPES

Sapota is a medium sized, spreading, evergreen tree growing to a height of 20 m. Stem short, branching from lower part forming umbrella shaped crown. Flowering occurs throughout the year in tropics and the salient features of the varieties selected for the present study are given here under and shown in plate-1.

3.6.1 Badami

It is medium growing tree. The foliage is dark green in colour. The fruits are medium size with oval to round shape.

3.6.2 Bombay

It is medium sized tree with small leaves, and fruits appear in bunches. The fruit skin has buff color and flesh color is light brown with a golden tinge towards the cavity.

3.6.3 Brazilizn Sapota

It bears medium to large, oval shaped fruits with ridges running from base to upwards. The apex is round, pulp is good colored with a tinge of muskmelon. In taste fruits are medium in taste.

3.6.4 Calcutta Round

It is grown in West Bengal, Karnataka and other states. The foliage is light green in colour. Fruits are large but the flesh is gritty and of moderate quality. It is susceptible to leaf spot disease.

3.6.5 Columbian Sapota

It has thick foliage but the leaves are medium sized and bearing is medium. Fruits are very large in size, pulp is sweet, but not of any distinctive flavour.

3.6.6 Cricket Ball

It is grown in Tamil Nadu, Karnataka, Maharashtra, West Bengal and Andhra Pradesh. It bears large sized fruits which are round in shape. The pulp is very sweet, but not of any distinctive flavour and is gritty and granular.

3.6.7 Dwarapudi

It is popular in Andhra Pradesh. Fruits resemble Cricket Ball, but smaller in size. Fruits have a sweet pulp and are in great demand.

3.6.8 Gavarayya

It is grown in Andhra Pradesh and Tamil Nadu. Small sized fruits with one shoulder dropping and the other raised; eight to ten marked ridges, medium to large in size with ovate shape having soft and melting pulp.

3.6.9 Gutti

It is grown in Andhra Pradesh and Tamil Nadu. Tree medium in height, round in shape, leaves elliptic lanceolate, slightly folded and wavy. Fruit elliptic, small, brown, flesh soft, juicy, gritty and brown in colour.

3.6.10 Hybrid

Dwarf growing plants with medium sized round fruits mainly popular in Gujarath and Maharashtra.

3.6.11 Kalipatti

It is the leading popular variety of Maharashtra, Gujarat and North Karnataka. Leaves are broad, thick and green colour. Fruits borne in single, oval shaped, less seeded with sweet mellow pulp of excellent quality.

3.6.12 Kirthibarthi

It is grown in Andhra Pradesh and Tamil Nadu. Fruits are small egg shaped with four to six ridges on the rind, peel is rough, medium thick and has a buff colour, taste very sweet when fully ripe.

3.6.13 Krishna Rao

It is grown in Andhra Prdesh. Fruits are medium sized, ovate with a depression near the stalk end. The fruit skin has buff color, pulp is firm, cream color and sweet in taste.

3.6.14 Mirandi

It is medium growing tree. The foliage is dark green in colour. The fruits are medium sized with oval to round shape.

3.6.15 Pakala

It is grown in Andhra Pradesh and Tamil Nadu. The fruits are small to medium size and oval (or) egg shaped. Pulp is a fine grained and very sweet. It is a shy bearing cultivar.

3.6.16 Pakala Oval

This is also grown in Tamil Nadu. Trees are tall in height, dome shaped, leaves elliptic, lanceolate, strongly folded, slightly waxy, fruits are long oval shaped and medium to large. Pulp of the fruit is cinnamon brown colour, flesh soft, brown in colour, juicy, gritty, medium aroma and flavour, tastes very sweet and seeds few.

3.6.17 Pakala Round

It is grown in Andhra Pradesh and Tamil Nadu. Fruits are small to medium in size and round shaped. The pulp of the fruit is brown in color, flesh soft and very sweet.

3.6.18 Pala

It is a popular variety of Andhra Pradesh and Tamil Nadu. The fruits are small to medium sized and oval or egg shaped with apex broadly pointed and are very sweet. The bearing is heavy and fruits are borne in clusters, the fruit has thin skin and good flavour.

3.6.19 Seedless

Tree is medium in height, conical in shape, leaves oval lanceolate, slightly folded, entire, acute, fruit oval, medium sized, snuff brown, flesh soft, tastes very sweet. It contains low number of seeds.

3.6.20 Simhoudi

Tree bears medium sized, round or oval shaped fruits. Fruit skin is thin and rough without any ridge. The flesh is buff coloured, streaked, melting and juicy. It has a very sweet taste.

3.6.21 Singapore

It is grown in Andhra Pradesh. It is a medium stature plant, fruits are oval in shape, medium sized.

3.6.22 Tagarampudi

It is also grown in Andhra Pradesh and Tamil Nadu. It bears medium sized, round or oval shaped fruit with a flat base. Fruit skin is thin and rough without any ridge. The flesh is buff coloured, streaked, melting and juicy. It has a very sweet taste and good for export.

3.6.23 Ulvapadu Selection

It is grown in Andhra Pradesh. Leaves are broad thick and green colour. Fruits borne in single, oval shaped, with sweet mellow pulp of good quality.

3.6.24 Virudnagar

It is grown in Tamil Nadu. It is a dwarf stature plant suitable for high density planting. Fruits are oval in shape.

3.6.25 CO-1 (Coimbatore-1)

This is a hybrid between Cricket Ball and Oval developed by TNAU. The combination was designed to bring together, the large fruit size of the female parent and superior taste of the male parent. The hybrid comes to bearing four years after planting. Fruits are bigger in size and are oval in shape with reddish brown flesh. It is grown in Tamil Nadu.

3.6.26 CO-2 (Coimbatore-2)

It is a selection from Baramasi grown in Tamil Nadu. Trees are medium in height with conical shape. Leaves elliptic, lanceolate, slightly folded and waxy, fruits ovate to round, medium sized cinnamon brown, flesh soft, juicy, gritty light brown in colour, aroma slight, tastes medium sweet.

3.6.27 CO-3 (Coimbatore-3)

It is a medium growing tree. Tree is compact, foliage is dark green. It is grown in Tamil Nadu. It is a cross between Cricket Ball x Vavivalasa.

3.6.28 DHS-1 (Dharwad Hybrid Sapota-1)

This hybrid was released by University of Agricultural Sciences, Dharwad during 1991 for growing in Karnataka. It is a cross between Kalipatti x Cricket Ball. It gives about 30 per cent higher yield than its parents. The fruits are oval in shape and pulp is very sweet with light orange soft and mellow. Fruits are large size and also having good shelf life.

3.6.29 DHS-2 (Dharwad Hybrid Sapota-2)

This hybrid was released by University of Agricultural Sciences, Dharwad during 1991 for growing in Karnataka. It is also a cross between Kalipatti x Cricket

Ball. It gives about 30 to 35 per cent higher yield than its parents. The fruits are round shaped and pulp texture is granular soft and mellow having light orange colour.

3.6.30 PKM-1 (Periyakulam-1)

It is a popular variety developed by Tamil Nadu Agricultural University, which is selection from Gutti. Trees are short stature with dense crown, fruits are medium in size, elliptic to oblong in shape with thin skin, rich in sugars, profuse bearing habit.

3.6.31 PKM-2 (Periyakulam-2)

It is leading variety of Tamil Nadu. It is a hybrid clone of Gutti and Kirthbharti. The fruits are medium to large size with oval shape.

3.6.32 PKM-3 (Periyakulam-3)

This is a popular variety of Tamil Nadu. It is a hybrid clone of the cross between Gutti and Cricket Ball. Trees are tall, erect growing fruits are large and oval in shape and cluster bearing in habit.

3.6.33 PKM-4 (Periyakulam-4)

It is an open-pollinated clone of PKM-1 with compact tree canopy, which is highly suited for high density planting. It bears fruits in clusters, and the spindle shaped fruits of 11 to 13 cm length are suitable for production of dry flakes. Fruits have two or three seeds.

3.7 EXPERIMENTAL DETAILS

The research work was carried out by studying the tree morphological and fruit bio-chemical characters and to assess the extent of variability and genetic diversity by using RAPD and SSR markers. The investigation was carried out in two broad sequential experiments *viz.,*

1. Genetic diversity studies of sapota genotypes through morphological and biochemical characters.

2. Genetic diversity stidies of sapota genotypes by using molecular markers *viz.*, Randomly Amplified Polymorphic DNA and Simple Sequence Repeats.

EXPERIMENT NO.1

GENETIC DIVERSITY STUDIES ON THE SAPOTA GENOTYPES THROUGH MORPHOLOGICAL AND BIO-CHEMICAL CHARACTERS.

3.8 OBSERVATIONS RECORDED

Periodical observations were recorded in the field for various morphological characters. All morphological observations were taken as per the descriptor for sapota developed at IIHR, Bangalore (Vasugi and Rekha, 2005). The observations recorded and the methodology adopted for bio-chemical and molecular analysis and units of measurement are presented hereunder.

3.8.1 TREE MORPHOLOGICAL CHARACTERS

3.8.1.1 Growth habit of tree

The growth habit of tree was recorded based on four point score

Growth habit	Score
Drooping	1
Spreading	2
Semi-erect	3
Erect/Upright	4

3.8.1.2 Shape of the tree

The shape of the tree was recorded based on seven point score

Tree Shape	**Score**
Irregular	1
Elliptical	2
Oblong	3
Semi-circular	4
Spherical	5
Broadly Pyramidal	6
Pyramidal	7

3.8.1.3 Branching pattern

It was recorded based on five point score

Branching Pattern	**Score**
Irregular	1
Horizontal	2
Verticillate	3
Erect	4
Opposite	5

3.8.1.4 Branching Density

It was recoded on three point score

Branching Density	**Score**
Sparse	1

Medium dense	2
Dense	3

3.8.1.5 Leaf length (cm)

Average length of five randomly selected leaves was measured from tip to base of leaf blade and expressed in centimeters.

3.8.1.6 Leaf width (cm)

Average width of five randomly selected leaves was measured at the widest portion leaf blade and expressed in centimeters.

3.8.1.7 Leaf area (cm^2)

Average leaf area of five randomly selected leaves were recorded by graph paper method and expressed in sq.cm.

3.8.1.8 Ovary size (mm)

Ovaries of unopened flower buds was measured with vernier callipers. The average was computated and expressed in millimeters.

3.8.1.9 Fruit clustering habit

It was recoded on two point score

Fruit clustering	Score
Solitary	1
Cluster	2

3.8.1.10 Per cent fruit set (%)

This was recorded by confirming enlargement of ovary, drying up of stigmatic surface and non-essential parts of the flower. The number of fruits set was counted and expressed as percentage.

3.8.1.11 Fruit availability

This was recorded based on the main harvesting period and availability of fruits in the local market.

3.8.2 FRUIT CHARACTERS

3.8.2.1 Fruit length (cm)

Length of five randomly selected fruits was measured with vernier callipers. The measurement of the length was made in the polar axis of the fruit, i.e., between apex and stem, the average was computated and expressed in centimeters.

3.8.2.2 Fruit width (cm)

Diameter of five randomly selected fruits was measured with vernier calliper at the point of maximum width/girth in the direction perpendicular to the axis, the average was computated and expressed in centimeters.

3.8.2.3 Fruit weight (g)

Five randomly selected fruits were weighed and the average weight of the fruits is taken as weight per fruit in gram.

3.8.2.4 Fruit Shape

Fruit shape was measured on seven point score.

Fruit Shape	Score
Turbinate	1
Acron	2
Globose	3
Sub-globose	4
Elliptical	5
Oblong	6
Oval	7

3.8.2.5 Fruit surface texture

It was recoded on three point score

Fruit surface texture	**Score**
Scurfy/Rough	1

Smooth	2
Web pattern on the surface	3

3.8.2.6 Fruit yield per tree (kg)

Fruits are harvested when they were fully matured. Fruit weight in kilogram was recorded at every harvest. The total yield was calculated by adding the values obtained in different harvests of the year and expressed in kilogram per tree per year.

3.8.2.7 Peel color

It was recorded on afive point score

Peel color	**Score**
Surfy brown	1
Brown with streaks	2
Greenish brown	3
Brown	4
Yellowish brown	5

3.8.2.8 Pulp color

It was recorded on six point score

Pulp color	**score**
Reddish green	1
Pale green	2
Greenish brown	3
Brown	4
Yellowish brown	5
Reddish brown	6

3.8.2.9 Pulp texture

It was recorded on four point score

Pulp texture	**Score**
Sand	1
Firm	2

Soft	3
Melting	4

3.8.2.10 Pulp taste

It was recorded on four point score

Pulp taste	**Score**
Astringent	1
Bland	2
Starchy	3
Sweet	4

3.8.2.11 Seed number per fruit

The number of seeds in five random fruits was counted and the average was expressed as seed number/fruit.

3.8.2.12 Seed weight (g)

Weight of a seed in a fruit was recorded in grams for five randomly selected fruits and their average weights were taken as seed weight/fruit.

3.8.2.13 Pulp to Seed ratio

Fruit pulp and seeds were weighed seperately for five randomly selected fruits and their average weights were recorded in grams. Ratio of their average weights was recorded as pulp to seed ratio. The ratio of fruit pulp to seed was recorded as given below.

$$\text{Pulp to seed ratio} = \frac{\text{Weight of pulp (g)}}{\text{Weight of seed (g)}}$$

3.8.3 BIOCHEMICAL PARAMETERS

3.8.3.1 Total soluble solids (^{0}Brix)

The total soluble solids were determined by using ERMA hand refractometer and expressed as °Brix (Ranganna, 1986).

3.8.3.2 Titrable acidity (%)

Ten grams of sample was taken, ground well and transferred to volumetric flask and volume was made up to 100 ml with distilled water. The contents were filtered through Whatman No.1 filter paper. An aliquot of 10 ml was taken into conical flask to which 2-3 drops of phenolphthalein indicator was added and titrated against 0.1 N NaOH till pink colour was obtained as the end point which persisted atleast for 15 seconds, (Ranganna, 1986).

$$\text{Titrable acidity (\%)} = \frac{\text{Eq. wt. of acid} \times \text{Normality of NaOH} \times \text{titer value} \times 1000}{10 \times \text{weight of sample}}$$

3.8.3.3 TSS to Acid ratio

TSS to Acid ratio was calculated as follows.

$$\text{TSS to Acid ratio} = \frac{\text{TSS}}{\text{Titrable acidity}}$$

3.8.3.4 Reducing sugars (%)

Reducing sugars were determined by the method described by Lane and Eyon (AOAC, 1965).Ten grams of fruit pulp was taken, ground well and transferred to 250 ml volumetric flask and 100 ml of distilled water was added to it. Two ml of lead acetate solution (45 %) was added to the flask for precipitation of colloidal matter and the excess amount was precipitated by 2 ml of potassium oxalate (22 %). The volume was then made up to 250 ml using distilled water.

The contents of the flask were filtered through Whatman No. 1 filter paper after testing a little of filtrate for its freedom from lead by adding a drop of potassium oxalate. The lead free solution was taken in a burette and titrated against 10 ml of standard Fehling's solution (mixture of A and B 1: 1) using methylene blue as an indicator till brick red precipitate was formed and the end point was noted. The titration was carried out in boiling Fehling’s solution on the heating mantle.

$$\text{Reducing sugars (\%)} = \frac{\text{Factor} \times \text{dilution} \times 100}{\text{Titer value} \times \text{weight of sample}}$$

3.8.3.5 Total sugars (%)

Total sugars were determined by the method given by Lane and Eyon (AOAC, 1965). 50 ml lead free filtrate was taken in a 100 ml volumetric flask, 5 ml of concentrated HCl was added to it, mixed well and then kept for 24 hours at room temperature. The acid was then neutralized with NaOH using a drop of phenolphthalein as an indicator till the pink colour persisted for at least few seconds. The volume was then made up to 100 ml. Total sugars was estimated by titrating this solution against standard Fehling’s solution A and B (1:1) mixture using methylene blue as an indicator till brick red colour was formed and noted as the end point.

$$\text{Total sugars (\%)} = \frac{\text{Factor} \times \text{dilution} \times 100}{\text{Titer Value} \times \text{weight of sample}}$$

3.8.3.6 Non-Reducing sugars (%)

Non-reducing sugars in the sample was calculated by subtracting reducing sugars from total sugars.

$$\text{Non-reducing sugars (\%)} = \text{Total sugars} - \text{Reducing sugars}$$

3.8.3.7 Ascorbic acid (mg/100g)

Ten grams of freshly ground sample was blended with 3 per cent metaphosphoric acid and made up to 100 ml with 3 per cent metaphosphoric acid (HPO_3). The contents were filtered through Whatman No.1 filter paper. 10 ml of the HPO_3 extract was taken and titrated against standard 2,6-Dichloro phenol indophenol dye to a pink end point (Ranganna, 1986).

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titer Value} \times \text{dye equivalent} \times \text{dilution} \times 100}{\text{weight of sample}}$$

3.8.3.8 Total phenols (mg of gallic acid /100g)

Phenols were estimated by using Folin-Ciocalteau’s reagent according to the procedure given by Ranganna (1986). One gram of sample was extracted with 10 ml of 80 per cent methanol. The homogenated extracts were centrifuged at 10,000 rpm for 20 min by using “5430 R eppendrof” centrifuge and the supernatant saved was evaporated in a water bath. The residue was dissoloved in 5 ml of distilled water and added with 0.5 ml of Folin -Ciocalteau's reagent (1N). To that, 2 ml of sodium carbonate (20%) solution was added and after mixing thoroughly the tubes were placed in boiling water for one minute, cooled and the absorbance was measured at 650 nm using “Evolution 201” (UV visible) spectrophotometer.

Standard curve was drawn using gallic acid as standard. Different concentrations of gallic acid were prepared and O.D was read at 650 nm. The concentration of the samples was calculated based on the standard curve.

$$\text{mg gallic acid equivalence per 100g} = \frac{\text{O.D} \times \text{Factor} \times \text{volume made up} \times 100}{\text{Aliquot taken} \times \text{weight of the sample} \times 1000}$$

3.8.3.9 Pectin (%)

Fifty grams of sample was taken into a 1000 ml beaker to which 400 ml of water was added and boiled for one hour. The evaporated water was replaced and then cooled and filtered. 300 ml of distilled water was added to 100ml of filtrate followed by 10 ml 1N NaOH solution and kept overnight. 25 ml 1N $CaCl_2$ is added and kept for an hour and filtered thorough Whatman No. 4 filter paper. Filter paper was dried in oven and weighed and noted as calcium pectate.

$$\text{Pectin (\%)} = \frac{\text{Wt of calcium pectate X 100}}{\text{wt of sample}}$$

3.9 STATISTICAL ANALYSIS OF DIVERSITY STUDIES ON SAPOTA ACCESSIONS

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 and Path-coefficient analysis were performed on the data obtained as detailed here under.

3.9.1 Analysis of variance

Analysis of variance for each character was carried out by using the Randomized Block Design method. The significance test was carried out by referring to 'F' table value given by Fisher and Yates (1963).

$$Y_{ij} = \mu + \varepsilon_i + b_j + e_{ij}$$

Where,

Y_{ij} = Performance of j^{th} genotype in i^{th} block

μ = general mean

ε_i = effect of i^{th} block

b_j = effect of j^{th} genotype

e_{ij} = random error associates with i^{th} block and j^{th} genotype.

The ANOVA model is as indicated below:

Source of variation	**d.f**	**SS**	**MSS**	**F.ratio**
Replications	r-1	RSS	Mr = σ^2e+ σ^2r	Mr/Me
Treatments (genotypes)	t-1	TrSS	Mt= σ^2e+ σ^2g	Mt/Me
Error	(r-1)(t-1)	ESS	Me= σ^2e	
Total	**(rt-1)**	**TSS**		

Where, r = Number of replications

t = Number of genotypes

df = Degrees of freedom

SS = Sum of squares

MSS = Mean sum of squares

σ^2e = Error variance

σ^2g = Variance due to genotypes

σ^2r = Variance due to replications

M r = Mean squares due to replications

Mt = Mean squares due to treatments

Me = Mean squares due to error

The test of significance was carried out using ‘F’ table values given by Fisher and Yates (1963).

3.9.2 Estimation of genetic parameters

3.9.2.1. Coefficient of Variation

The genotypic and phenotypic variance was calculated as per the formulae of Burton, (1952).

$$\text{Genotypic variance } (\sigma^2_g) = \frac{(\text{Mean sum of squares due to treatments} - \text{Mean sum of squares due to error})}{\text{Number of replications}}$$

$$\text{Phenotypic variance } (\sigma^2_p) = (\sigma^2_g) + (\sigma^2_e)$$

$$(\sigma^2_e) = \text{Error variance}$$

The genotypic and phenotypic coefficients of variation were calculated according to the formula given by Falconer (1981).

$$\text{Phenotypic Co- efficient of variation (PCV \%)} = \frac{\sqrt{\text{Phenotypic variance}}}{\text{Grand mean}} \times 100$$

$$\text{Genotypic Co- efficient of variation (GCV \%)} = \frac{\sqrt{\text{Genotypic variance}}}{\text{Grand mean}} \times 100$$

As suggested by Siva Subramanian and Menon (1973), GCV and PCV were categorised,

Low = Less than 10%

Moderate = 10-20%

High = More than 20%

3.9.2.2. Heritability in broad sense [h^2 (b)] (per cent)

Heritability in broad sense in per cent was estimated as per Lush (1949).

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

As suggested by Johnson *et al*. (1955), h^2 (b) estimates were categorized

Low = 0 – 30%

Moderate = 31-60%

High = 61% and above

3.9.2.3. Genetic advance as per cent of mean (GAM)

Genetic advance refers to the expected gain or improvement in the next generation by selecting superior individuals under certain amount of selection pressure. From the heritability estimates the genetic advance was estimated by the following formula given by Burton (1952).

$$GA = K.\ h^2 (b).\ \sigma_p$$

Where

GA = Expected genetic advance

K = Selection differential, the value of which is 2.06 at 5% selection intensity

σ_p = phenotypic standard deviation

h^2 (b) = heritability value in broad sense

In order to visualize the relative utility of genetic advance among the characters, genetic advance as per cent for mean was computed as follows.

$$\text{GAM} = \frac{\text{GA}}{\text{Grand mean } (\overline{X})} \times 100$$

The range of genetic advance as per cent of mean was classified as suggested by Johnson *et al.* (1955).

Low = Less than 10%

Moderate = 10-20%

High = More than 20%

3.9.3. Correlation Analysis

Analysis of Co-Variance

Analysis of Co-Variance was computed the by following procedure.

$$Y_{ij} = M + t_i + b_j + B\,(X_{ij} - X) + e_{ij}$$

Where,

Y_{ij} = Performance of i^{th} genotype in the j^{th} replication

M = general mean

t_i = true effect of i^{th} genotype

b_j = true effect of j^{th} block

b_{yx} = regression coefficient of y on x

$X_{ij} - X$ = Covariated

e_{ij} = random error

The structure of analysis of covariance is as follows

Source	**df**	**SSY**	**SSX**	**SPXY**	**MSSY**	**MSSX**	**MSPXY**
Replications	(r-1)	RY	RX	RXY	ry	rx	rxy
Genotypes	(t-1)	TY	TX	TXY	ty	tx	txy
Error	(r-1)(t-1)	EY	EX	EXY	ey	ex	exy

Where,

df = Degrees of freedom

SS = Sum of squares

SP = Sum of products

MSS = Mean sum of squares

MSP = Mean sum of products

r = Number of replications

t = Number of genotypes

Genotypic Covariance $(X_i.X_j)g$ = (MSPt – MSPe) / r

$(X_i.X_j)g$ = Genotypic Covariance between i^{th} and j^{th} characters

MSPr = Mean sum of products of genotypes

MSPe = Error mean sum of products

Phenotypic Covariance $(X_i.X_j)p$ = $(X_i.X_j)g + e_ie_j$

$(X_i.X_j)p$ = Phenotypic Covariance between i^{th} and j^{th} characters

$(X_i.X_j)g$ = Genotypic Covariance between i^{th} and j^{th} characters

e_ie_j = environmental covariance between i^{th} and j^{th} characters

Phenotypic and genotypic correlations were worked out by using the formulae suggested by Falconer (1981).

Phenotypic coefficient of correlation (r_p)

$$Cov(x_i.x_j)p$$

$r(x_i.x_j)p$ = -----------------------

$$\sqrt{V(x_i)p.V(x_j)p}$$

Where,

$r(X_i.X_j)p$ = Phenotypic correlation between i^{th} and j^{th}character

COV $(X_i.X_j)p$ = Phenotypic covariance between $i^{th}$and j^{th} character

$V(X_i)p$ = Phenotypic variance of i^{th} character

$V(X_j)p$ = Phenotypic variance of j^{th} character

Genotypic coefficient of correlation (r_g)

$$r(x_i.x_j)g = \frac{Cov(x_i.x_j)g}{\sqrt{V(x_i)g.V(x_j)g}}$$

Where,

$r(X_i.X_j)g$ = Genotypic correlation between i^{th} and j^{th} characters

COV $(X_i.X_j)g$ = Genotypic covariance between i^{th} and j^{th}characters

$V(X_i)g$ = Genotypic variance of i^{th} character

$V(X_j)g$ = Genotypic variance of j^{th} character

3.9.3.1 Test of significance

Significance of correlation coefficients was tested by comparing phenotypic correlation coefficients with the table values (Fisher and Yates, 1963) at (n-2) degrees of freedom at 5% and 1% level where ‘n’ denotes the number of paired observations used in the calculation.

3.9.4 Path coefficient analysis

Path coefficient analysis, suggested by Wright (1921) and elaborated to Dewey & Lu (1959) was used to calculate the direct and indirect contribution of various traits to yield.

For estimation of various direct and indirect effects, a set of simultaneous equations were formed:

$$r_{1y} = P_{1y} + r_{12}\,P_{2y} + r_{13}\,P_{3y} + \ldots + r_{1k}\,P_{ky}$$

$$r_{2y} = r_{21}\,P_{1y} + P_{2y} + r_{23}\,P_{3y} + \ldots + r_{2k}\,P_{ky}$$

$$r_{iy} = r_{i1}\,P_{1y} + r_{i2}\,P_{2y} + r_{i3}\,P_{3y} + \ldots + r_{ik}\,P_{ky}$$

$$r_{ky} = r_{k1}\,P_{1y} + r_{k2}\,P_{2y} + r_{k3}\,P_{3y} + \ldots + r_{kk}\,P_{ky}$$

Where,

r_{1y} to r_{ky} = Coefficient of correlations between causal factors 1 to K and dependent character Y.

r_{12} to r_{k-1},k = Coefficient of correlations among causal factors.

P_{1y} to P_{ky} = Direct effects of characters 1 to k on character y

The above equations were written in a matrix form as under:

$$\begin{array}{ccc}
\textbf{A} & & \textbf{C} & \textbf{B} \\
\begin{bmatrix} r_{1y} \\ r_{2y} \\ \cdot \\ \cdot \\ r_{ky} \end{bmatrix} & = & \begin{bmatrix} 1 & r_{12} & r_{13} & \cdot & \cdot & \cdot & r_{1k} \\ r_{21} & 1 & r_{23} & \cdot & \cdot & \cdot & r_{2k} \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ r_{k1} & r_{k2} & r_{k3} & \cdot & \cdot & \cdot & 1 \end{bmatrix} & \begin{bmatrix} P_{1y} \\ P_{2y} \\ \cdot \\ \cdot \\ P_{ky} \end{bmatrix}
\end{array}$$

or

$A=BC$

$C = B^{-1} A$ inverse of matrix 'B' obtained by elimination procedure or through Adjoint method.

$$\text{Then } (B)^{-1} = \begin{bmatrix} C_{11} & C_{12} & \cdot & \cdot & \cdot & \cdot & C_{1k} \\ C_{21} & C_{22} & \cdot & \cdot & \cdot & \cdot & C_{2k} \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ C_{k1} & C_{k2} & \cdot & \cdot & \cdot & \cdot & C_{kk} \end{bmatrix}$$

Then direct effects were calculated as follows,

$$P_{1y} = \sum_{i=1} C_{1i.}\, r_{iy}$$

$$P_{2y} = \sum_{i=1} C_{2i.}\, r_{iy}$$

$$P_{ky} = \sum_{i=1} C_{ki.}\, r_{iy}$$

Lenka and Mishra (1973) have suggested a scale for the importance of direct and indirect effects values as given below:

1.00 and above = very high
0.30 to 0.99 = high
0.20 to 0.29 = moderate
0.10 to 0.19 = low
Below 0.10 = negligible

3.9.5 Genetic Divergence

3.9.5.1 Mahalanobis' D^2 analysis

The data collected on different characters was analyzed using Mahalanobis' D^2 analysis to determine the genetic divergence among the genotypes.

3.6.5.1.1 Test of significance

Variances were calculated for all the characters investigated and test of significance was done. Analysis of covariance for the character pairs was estimated on the basis of mean values (Panse and Sukhatme, 1957). After testing the difference between genotypes for each of the characters, a simultaneous test of significance for differences in the mean values of a number of correlated variables with regard to the pooled effect of characters was carried out using 'V' statistic, which in turn utilizes Wilk's criterion. The sum of squares and sum of products of error and error + variety, variance – covariance matrix were used for this purpose. The estimation of Wilk's criterion was done using the following relationship.

$$\wedge = \frac{\mathbf{(E)}}{\mathbf{(E+V)}}$$

Where,

^ = Wilk's criterion

(E) = Determinant of error matrix and

(E+V) = Determinant of error + variety matrix

$$V(\text{Stat}) = -m \log_e{}^{\wedge} = -\left(n - \frac{P+Q+1}{2}\right) \log_e{}^{\wedge}$$

Where,

m = n-(P+Q+1)/2

n = Degrees of freedom for error + varieties (p x q-1)

$\log_e{}^{\wedge} = 2.3026 \log_{10}{}^{\wedge}$

P = Number of variables or characters (11)

Q = Number of variaties – 1(or d.f for varieties) (59)

V (Stat) is distributed as χ^2 with PQ (649 = 11 x 59) degrees of freedom.

Transformation of correlated variables

In the present model, computation of D^2 values were reduced to simple summation of the differences in the mean values of various characters of the two genotypes i.e. $\sum d^2_i$. Therefore, transformation of the correlated variables into uncorrelated ones was done before working out the D^2 values. Transformation was done using pivotal condensation method.

3.9.5.1.2 Computation of D^2 values

For the given combination of i and j genotype, the mean deviation *i.e.*, $Y_t^i - Y_t^j$ for t=1,2…p variables are computed and the D^2 values were calculated as

$$D^2_{ij} = \sum_{t=1} (Y_t^i - Y_t^j)^2$$

Where,

Y_t^i is uncorrelated mean value of ith genotype for character 't'

Y_t^j is uncorrelated mean value of jth genotype for character 't'

D^2_{ij} is D^2 value between ith and jth genotype.

3.9.5.1.3 Testing the significance of D^2 values

The D^2 value obtained for a pair of population is taken as calculated value of χ^2 and is tested against the tabulated value of χ^2 for P (10) degrees of freedom where P (10) is the number of characters studied.

3.9.5.1.4 Contribution of individual characters towards divergence

In all combinations, each character was ranked on the basis of their contribution towards divergence between two entries ($d_i = Y_t^i - Y_t^j$). Rank 1 is given to the highest mean difference and the rank P to the lowest difference, where, P is the total number of characters. Percentage contribution towards genetic divergence was calculated using the following formula.

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

Where,

N = Number of genotype combinations where the character was ranked first.

M = All possible combinations of number of genotypes considered.

3.9.5.1.5 Grouping of genotypes into various clusters

The grouping of genotypes into different clusters was done using the Tocher's method as described by Rao (1952). The criterion was that the two varieties belonging to the same cluster at least on an average show a smaller D^2 value than those belonging to different clusters. For this purpose, D^2 values of all combinations of each genotype were arranged in ascending order of magnitude in a tabular form as described by Singh and Chaudhary (1977).

To start with, two populations having the closest distance from each other were considered, to which the third population having the smallest D^2 value from the first two populations was added.

Similarly, the next nearest fourth population was considered and this procedure was continued. At certain stage, when it was felt that after adding a particular population there was an abrupt increase in the average D^2, that population was not considered for including in that cluster.

The genotypes of the first cluster were then eliminated and the rest were treated in a similar way. This procedure was continued till all the genotypes were included into one or the other cluster.

3.9.5.1.6 Average intra cluster distance

For the measurement of intra cluster distances, the formula used was $\Sigma D^2_i/n$ where, D^2_i was the sum of distances between all possible combinations (n) of the populations included in a cluster.

3.9.5.1.7 Average inter cluster distance

Clusters were taken one by one and the distances from other clusters were calculated.

The distance between two clusters was the sum of D^2 values between the members of one cluster to each of the members of the other clusters divided by the product of number of genotypes in both the clusters under consideration.

$$\text{Average inter cluster distance} = \frac{\sum D^2}{(n_1 \text{x } n_2)}$$

Where,

n_1 and n_2 are number of genotypes in two clusters.

3.9.5.2 Principal component analysis and cluster analysis

Principal component analysis was carried according to procedure described by Banfield (1978). PCA can be performed on two types of data matrices *viz.,* variance – covariance matrix and correlation matrix. With characters of different scale a correlation matrix standardizing the original data set is preferred. If the characters are of same scale, a variance – covariance matrix can be used. In the present study, PCA was performed on the correlation matrix of traits, thereby removing the effects of scale (Jackson, 1991).

3.9.5.2.1 Eigen values and eigen vectors

The eigen values and eigen vectors were computed from data matrix. Eigen values define the amount of total variation that is displayed on principal components. The proportion of variation accounted for each principal component (PC) is expressed as the eigen value divided by the sum of the eigen values.

Eigen value (PC1)

Per cent variance explained for PC1 = -------------------------

Sum of eigen values

The eigen vector (loading) defines the correlation of each variable with the principal components.

The principal components were identified by following procedure.

The j^{th} principal component (Y_i) of the observations X is the linear combination given as follow :

$$Y_j = A_{1j}X_1 + \ldots\ldots\ldots\ldots + A_{pj} X_p$$

Where,

A_{ij} are found such that Y_j is uncorrelated $Y_1, Y_2, \ldots\ldots\ldots Y_j\text{-}_1$ the j^{th} largest variance. The A_{ij} are the elements of the normalized eigen vector associated with largest j^{th} eigen value. The variance of the j^{th} principal component of the λ_j and the total system variance trace (S) = $\lambda_1+\lambda_2 + \ldots + \lambda_{p.}$ The importance of the j^{th} principal component is given by

$$\frac{\lambda_j}{\text{Trace (S)}}$$

This is informative about the proportion of total variation that can be accounted for the i^{th} principal component. The correlation between the i^{th} original variable X_i and the j^{th} principal component Y_j is given by

$$\rho(X_i, Y_j) = \frac{A_{ij}}{} \frac{\sqrt{\lambda_j}}{\sqrt{S_i}}$$

Where S_i is the standard deviation of $X_{i.}$

Thus, a principal component is linear function of the test variables given as follows

$$\text{Principal component} = ax_1 + bx_2 + \ldots\ldots\ldots + hx_8$$

Where, a,b,…. are coefficients and x_1, x_2 ….. etc., are the variables in such a way that the principal component has a unit variance as reported by Ehrenberg (1985).

PCA scores for each genotype under concerned PCs were computed and utilized to derive a 2D or 3D (dimensional) scatter plot of individuals.

3.9.5.2.2 Grouping of genotypes into various clusters

Agglomerative hierarchical clustering technique was followed as given by Anderberg (1993).

1. Obtaining data matrix

PCA scores for 60 genotypes were used as input for clustering because principal component analysis provides variable independence and balanced weighting of traits, which leads to an effective contribution of different characters on the basis of respective variation.

2. Computing the resemblance matrix

A resemblance coefficient, which measures the over all resemblance (the degree of similarity or distance) between a pair of genotypes was computed. Here 60 genotypes were taken in data matrix therefore resemblance coefficient was computed for all combinations i.e ${}^{60}C_2$ ways.

The data matrix was transformed to distance matrix (resemblance matrix) based on the dissimilarity coefficients using squared Euclidean distance method.

$$\text{Squared Euclidean distance } [d_{ij}] = \sum_{K=1}^{p} (X_{ik} - X_{jk})^2$$

Where,

P = number of genotypes *i.e* 60

X_{ik} = value of i th genotype for k PCA scores

X_{jk} = value of j th genotype for k PCA scores

3. Execution of the clustering method

Distance matrix was converted into dendrogram by using Ward’s method where the distance between two clusters is the sum of squares between two clusters summed over all variables. At each stage in the clustering procedure, the within cluster sum of squares is minimized over all partitions obtained by combining 2 clusters from previous stage.

3.9.5.2.3 Complete linkage diagram

This is one of the hierarchical methods as classified by Everitt (1974), starts with the computation of the ‘distance’ or similarities of each individual with every other individual. A comparison of such similarity coefficients among the pairs of individuals or objects finally leads to a tree diagram, referred as ‘Dendrogram’. For the dendrogram, the clusters of homogeneous units can be identified.

Sorenson (1948) first developed the method of complete linkage dendrogram. This method was based on the distance matrix D. Computation of a similarity measurement between all possible pairs of D^2 values would result in an ‘n’ x symmetrical matrix (where n is the number of genotypes). Similarity measurement was nothing but correlation coefficient between the variables. Any coefficient C_{ij} in the matrix gives the resemblance between i and j. The next step is to arrange the objects into a hierarchy. So objects (genotypes) were associated with other groups which they most closely resemble and so on until all the objects have been placed into a complete classification scheme (Sneath and Sokal, 1973).

The essential features of this particular method of cluster analysis could be summarized as,

1. The correlation coefficient was used as a similarity measure.

2. Highest similarities were clustered or linked first.

3. Two objects (genotypes) could be connected only if they had mutually highest correlation with each other.

4. After two objects (genotypes) were clustered, their correlations with all other objects were averaged.

EXPERIMENT NO. 2

GENETIC DIVERSITY STUDIES OF SAPOTA GENOTYPES BY USING MOLECULAR MARKERS RANDOMLY AMPLIFIED POLY- MORPHIC DNA AND SIMPLE SEQUENCE REPEATS.

Detection of molecular polymorphism among the germplasm accessions in different clusters of sapota was performed by RAPD and SSR analysis.

The following genotypes 33 accessions were used for molecular study

1	Badami
2	Bombay
3	Brazilian Sapota
4	Calcutta Round
5	Columbian
6	Cricket Ball
7	Dwarapudi
8	Gavarayya
9	Guthi
10	Hybrid
11	Kalipathi
12	Kirthibarthi
13	Krishna Rao
17	Pakala Round
18	Pala
19	Seedless
20	Simhoudi
21	Singapore
22	Thagarampudi
23	Ulvapadu selection
24	Virudnagar
25	CO-1
26	CO-2
27	CO-3
28	DHS-1
29	DHS-2

14	Mirandi	30	PKM-1
15	Pakala	31	PKM-2
16	Pakala Oval	32	PKM-3
		33	PKM-4

In total 33 sapota genotypes have been analysed in the present investigation available at Horticultural Research Station, Venkataramannagudem, Dr.Y.S.R. Horticultural University. Fresh matured leaves free from pests or disease and available throughout the year were used for isolation of DNA. Leaf samples were collected in brown paper covers and stored in poly bags at -20 °C.

3.10.1 Processing of sample

Approximately 150-200 mg of leaf samples were ground in effendorf tube by micro pestle for 5-10 minutes using extraction buffer. The ground product was used for DNA isolation.

3.10.1.2 DNA isolation protocol

Stock solutions

CTAB :	5 Percent	RNA'se :	10mg/ml
Nacl :	5M	Isopropanol	
Tris-Base :	1M	Sodium acetate :	3M
EDTA :	0.5 M	PVP :	2 Per cent
Chloroform: Isoamylalcohol	24:1 (V/V)	β-Mercaptoethanol :	2 Per cent

Extraction buffer (EB): 5 % CTAB, 100 mM Tris pH 8.0, 25 mM EDTA pH 8.0, 2 M NaCl, 2 % PVP and 2 % β-Mercaptoethanol.

Isolation of total genomic DNA of sapota was carried out according to Porebski *et al.* (1997) using CTAB with some modifications. The protocol standardized was as follows.

a) 200 mg leaf samples were grinded with 750 µl extraction buffer and incubated on water bath at 65 °C for one hour.

b) The mixture was cooled to room temperature and 200 µl of cold Chloroform: Isoamyl alcohol (24:1 v/v) was added and the contents were gently vortexed and spun at 6000 rpm at 16 °C for 15 minutes.

c) The supernatant was carefully decanted and 500 µl Chloroform: Isoamyl alcohol (24:1 v/v) was added and the contents were mixed and spun at 6000 rpm at 10°C for 15 minutes. The supernatant was carefully decanted. This step was again repeated for one more time if the supernatant was not clear.

d) To the supernatant, 0.6 ml volume of Isopropanal was added and gently vertexed and kept the eppedorf tube was kept at -20°C for one hour for precipitation.

e) It was vortexed for 2-3min and spun at 6000 rpm at 16 °C for 15 minutes and the supernatant was discarded.

f) The pellet was washed with 70 % ethanol and dried at room temperature and it was spun at 6000 rpm at 16 °C for 15 minutes.

g) The ethanol was discarded and DNA pellet was dried untill evaporated.

h) The pellet was dissolved in 125 µl TE buffer and incubated with 3 µl RNA'se on a water bath at 37 °C for one hour.

i) To this 150 µl Phenol and 150 µl Chloroform: Isoamyl alcohol (24:1) was added and centrifuged at 14,000 rpm for 10 minutes at 4^0 C.

j) To the supernatant collected, equal volume of Chloroform: Isoamyl alcohol (24:1) was added and centrifuged at 14,000 rpm for 10 minutes at 4^0 C.

k) Supernatant was collected in a separate tube to which equal volume of 95 per cent ethanol and 1/3rd volume of sodium acetate (pH 5.2) was added and left at -20^0 C overnight for precipitation of DNA.

l) After incubation, the tubes were spun at 14,000 rpm for 10-15 minutes. Later, the pellet obtained was washed with 70 per cent ethanol (100 µl) and centrifuged at 14,000 rpm for 5 minutes.

m) The pellet obtained was air dried and was dissolved in TE buffer and stored at -40^0 C for further use.

3.10.2 DNA quantification

The purity and concentration of the isolated genomic DNA samples was estimated by UV-absorption spectrophotometer (Beckman DU 650 model) as per the procedure described by Sambrook *et al.* (1989). The ratio of absorbance at 260 nm and 280 nm was used as an indicator of DNA purity. A ratio between 1.4 and 1.8 was considered as relatively pure DNA samples as it did not show any effect on PCR reaction.

Genomic DNA concentration in µg/µl= OD 260 nm x 50 µg/µl x dilution factor.

Quantification of DNA was also done by analyzing the purified DNA on 0.8% agarose gel with diluted ladder DNA as standard. The concentration and quality of genomic DNA in individual samples was determined based on the intensity and thickness of the bands in comparision with λ DNA.

3.11 Standardization of protocol for PCR conditions for amplification of sapota DNA

3.11.1 PCR ingredients (Stock solutions)

Template DNA **:** 20 ng/µl

dNTPs **:** 1 mM (2.5 mM each)

10X PCR buffer **:** 10 mM Tris-HCL, pH 8.8; 500 mM KCl; 15 mM $MgCl_2$; 0.1 per cent Gelatin; 0.05 per cent Tween-20 and 0.05 per cent NP 40

Primers **:** 3 µM (Operon Technologies Ltd.)

***Taq* DNA polymerase :** 3units/µl

The PCR procedure described by Williams *et al.* (1990) was followed with minor modifications. A single decamer primer of arbitrary sequence was used in each reaction. PCR conditions were optimized to achieve informative, consistent and reproducible fingerprinting profiles of sapota. Amplification reaction was performed in a final volume 20 µl reaction mixture containing template DNA (20 ng/µl), primer (0.3 µM), dNTPs (100 µM), *Taq* polymerase (1 unit/µl), 10X Buffer.

3.11.2 PCR programme

PCR was carried out in Thermo cycler [Thermo Electro Corporation Company (USA)] using programme profile consisting of an initial hot start at $94^{O}C$ for 5 minutes, followed by 38 cycles of one minute denaturation at $94^{O}C$, one minute annealing at $37^{O}C$ (RAPD), $44\text{-}55^{O}C$ (SSR) and 1.5 minutes extension at $72^{O}C$, with final extension step of ten minutes at $72^{O}C$ and $4^{O}C$ hold.

To avoid contamination in PCR reaction autoclaved tips, tubes, pipettes and reagents were used.

3.11.3 Agarose gel electrophoresis for resolution of RAPD and SSR markers

Amplification products were resolved on high resolution 3% agarose gels (3g agarose/100ml of 1 X TAE buffer) for SSR's and 1.4% (1.4g agarose/100ml of 1X TAE buffer) for RAPD's by using a horizontal gel electrophoresis unit (CBS Scientific, USA). The agarose was weighed and transferred to a reagent bottle containing 100 ml of 1X TAE buffer and mixed well. The contents were then boiled gently in a microwave oven with intermittent mixing. The process was followed until complete melting of agarose was achieved and the solution became crystal clear. The gel-casting tray was washed with water and wiped with ethanol. When the boiled agarose had cooled down at 55^0C substantially, 2 µl of ethidium bromide (10mg/ml) was added to the melted agarose, mixed thoroughly and poured in to the gel cast tray and the combs were arranged in the slots on the gel-casting tray and allowed to solidify at room temperature for 20-30 minutes. Care was taken so that no air bubble was present. Later the gel was transferred to Submarine Horizontal Electrophoresis Unit containing 1X TAE buffer. Before loading on to the gel, PCR amplified products were mixed with 1/6th volume of gel loading dye (40% sucrose and 0.25% bromophenol blue) and loaded into the wells. Then the electrodes were connected to power pack. The samples were run at 100 V for 2 to 2½ hours. Ladder (100 bp for SSR, 1000bp for RAPD) was added in one well for each primer pair to determine the size of amplified fragments. The DNA fragments were then visualized under UV-transilluminator and documented using ALPHA IMAGER gel documentation system (Alpha innotech, USA). The images were stored for further scoring and permanent records.

3.12.1 Data analysis

Fragments amplified by the primers used were scored for their presence or absence and a matrix of different RAPD phenotypes was assembled. Further, a fragment was counted only if it was intense, clear and strong. Diffused and/or very weak fragments were not scored as such fragments have been reported to possess the greatest propensity for poor reproducibility. The band sizes were estimated by using a 1000 bp ladder marker, which was run along with the amplified products. Only the clear and unambiguous bands of SSR markers were scored. The size of the amplified fragments was estimated with the help of Alpha image software using 100 bp DNA ladders (NEB) as size standards. Markers were scored for the presence (1) or absence (0) of the corresponding band among the genotypes. A data matrix comprising of ‘1’ and ‘0’ was formed depending upon the character which was subjected to further analysis.

3.12.2 Statistical analysis and estimating genetic distances

The scored band data was subjected to statistical analysis using the computer programme 'NTSYS-2.0i' package (Rohlf, 1998). The resultant similarity matrix was used to generate a tree by UPGMA (Unweighted Pair Group Method with Arithmetical averages). Jaccard's Co-efficient (S_J) was computed as;

$$(S_J) = n_{AB}/n_A + n_B$$

n_{AB} is the number of bands common for samples A and B, n_A is the total number of bands in sample A and n_B is the total number of bands in sample B.

To measure the informativeness of the markers, the polymorphism information content (PIC) (http://www.agri.huji.ac.il/-Weller/Hayim/parent/PIC) for each SSR marker was calculated according to the formula (Botstein *et al.*, 1980):

$$PIC=1-\Sigma Pi^2 - \Sigma\Sigma Pi^2 Pj^2$$

where 'i' is the total number of alleles detected for SSR marker and 'Pi' is the frequency of the i^{th} allele in the set of 88 genotypes investigated and j = i+1. This formula gives us an indicator of how many alleles a certain marker has, and how much these alleles divide evenly.

To determine the genetic diversity in 33 genotypes studied, binary data matrix was subjected to cluster analysis. The excel file containing the binary data was imported into NT Edit in NTSYS-pc 2.02J. The 0/1 matrix was used to calculate similarity as Jaccard's coefficient using SIMQUAL subroutine in SIMILARITY routine. The resultant similarity matrix was employed to construct dendogram using Sequential Agglomerative Hierarchical Non-overlapping (SAHN) based Unweighted Pair Group Method with Arithmetic means (UPGMA) to infer genetic relationships.

3.12.3 Cluster analysis

The agglomerative method of clustering using UPGMA (Unweighted Pair Group Method with Arithmetical averages) for developing dendrogram was adopted. This calculates the congruence between assays of values typically densitometric assays. As it compares curves as a whole, it is independent of band definitions and is thus ideally suited for a quick comparison of pattern without first having to edit the bands. It is largely insensitive to relative concentrations, but is sensitive to differences in background. Jaccard's Co-efficient considers only the presence of band as similarity and hence, is more conservative in declaring genetic diversity.

CHAPTER IV

RESULTS AND DISCUSSION

Sapota**(***Manilkara achras* (Mill.)Fosberg) is one of the delicious fruits of humid tropical and subtropical regions, belonging to the family Sapotaceae. Domestication and improvement programmes are enhanced by knowledge of the genetic structure of the existing cultivars in order to capture high level of diversity in breeding programmes. In perennial crops like sapota, mango, guava,*etc.*, an important method to develop a new variety with specific desirable characteristics is selection of superior genotypes based on the phenotype from among the existing variation and use them directly or in future breeding programmes. It is essential to identify and effectively conserve the existing genetic resources with an utmost certainty.

Since, sapota is an open pollinated crop, a great deal of variability was thrown up in the population. A few of the cultivars have been selected based on local preferences for different fruit characteristics and later cultivated by farmers through vegetative propagation.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 to ensure the effectiveness of sapota improvement programmes. Current knowledge on genetic variability and divergence in sapota genotypes are meager.

Hence, an attempt was made to study the diversity among sapota germplasm using molecular markers such as RAPD and SSR in order to complement morphological data and to throw more light on the genetic diversity of sapota.The present investigation,**"Studies on the diversity in morphological, bio-chemical and molecular characterization in sapota (*Manilkara achras* (Mill.)Fosberg)genotypes",** was conducted at Horticultural Research Station, Venkataramannagudem during 2013 to 2014. The data collected was statistically analyzed and the results obtained were discussed under the following heads.

4.1 Morphological characters
4.2 Fruit biochemical characters
4.3 Genetic variability
4.4 Character association studies
4.5Path coefficient analysis
4.6 Genetic divergence
4.7 Molecular characterization

4.1 TREE AND FRUIT MORPHOLOGICAL CHARACTERS

4.1.1 Growth habit of the tree

The genotypes under study differed in growth habit,which varied fromspreading to erect (Table-3). Spreading growth habit was noticed for Kalipatti, Kirthibarthi, Pala, Singapore,Ulavapadu Selection, Virudnagar,CO-2 and PKM-1. Erect growth habit was recorded for the genotype CO-1, Columbian Sapota and Mirandi, while, Badami, Bombay, Brazilian Sapota, Calcutta Round, Cricket Ball, Dwarapudi, Gavarayya, Gutti, Krishna Rao, Pakala, Pakala Round, Pakala Oval, Simhoudi, Seedless, Tagarampudi, CO-3, DHS-1, DHS-2, PKM-2, PKM-3, and PKM-4 recorded semi-erect type of growth. Similar results were reported by Mathew *et al.* (2001) and Rajasekhar (2009) in sapota genotypes.

4.1.2 Shape of the tree

The genotypes differed in tree shape (Table-3) which ranged from irregular to broadly pyramidal. Majority of genotypes recorded broadly pyramidal tree shape (Badami, Calcutta Round, Columbian Sapota, Cricket Ball, Kalipatti, Kirthibarthi, Krishna Rao, Pakala, Pakala Round, Pakala Oval, Pala, Singapore, Tagarampudi, PKM-1, PKM-2, PKM-3 and PKM-4), While the genotypes, Brazilian Sapota, Gavarayya, Gutti, Simhoudi and DHS-2 recorded spherical shape of the tree. Further, the genotypes Bombay, Seedless and DHS-1 recorded semi-circular shape and the genotypes, namely, Dwarapudi, Ulvapadu Selection and CO-1 recorded elliptical shape, while, Mirandi, CO-2 and CO-3 recorded irregular shape. These reports are in close agreement with the findings of Sulladamath (1975), Mone (1989), Mathew *et al.* (2001) and Rajasekhar*et al.* (2010) in sapota genotypes.

4.1.3 Branching pattern

The differences in branching pattern among the sapota genotypes were provided in Table-3. Majority of the genotypes recorded Verticillate branching pattern (Badami, Bombay, Brazilian Sapota, Calcutta Round, Columbian Sapota, Dwarapudi, Gutti, Krishna Rao, Pakala, Pakala Round, Simhoudi, Ulvapadu Selection, Virudnagar, CO-1, CO-2, C0-3, DHS-1, DHS-2, PKM-1, PKM-2, PKM-3 and PKM-4), while, the genotypes Cricket Ball, Kalipatti, Kirthibarthi, Pakala Oval, Pala, Seedless, Singapore, Tagarampudirecorded horizontal branching pattern. Further, irregular branching pattern was recorded in Gavarayya andMirandi. These observations are in close conformity with the findings of Siddaramayya (2005) and Rajasekhar *et al.* (2010) in sapota genotypes.

4.1.4 Branching density

The differences in branching density (Table-3) among the genotypes ranged from sparse (1.00), medium dense (2.00) to dense (3.00).The genotypes, namely, Bombay, Dwarapudi, Gutti, Kalipatti, Simhoudi, Singapore, Tagarampudi, Virudnagar, DHS-2, PKM-1 and PKM-2 had recorded dense branching, while Badami, Brazilian Sapota, Calcutta Round, Cricket Ball, Gavarayya, Kirthibarthi, Krishna Rao, Pakala, Pakala Round, Pakala Oval, Pala, Seedless, Ulvapadu Selection, DHS-1, PKM-3 and PKM-4 genotypes recorded medium dense branching. However, sparse branching was noticed in Columbian Sapota, Mirandi, CO-1, CO-2, and CO-3 genotypes.

4.1.5 Plant height (m)

The genotypes differed significantly in plant height (Table-4) from a minimum of 2.83 m to a maximum of 7.74 m for the various genotypes studied. The mean plant height for the genotypes studied was 5.61 m. Significantlyhighest plant height of 7.74 m was recorded in Kirthibarthi followed by DHS-2 (7.14 m) and DHS-1 (6.90 m), while lowest plant height of 2.84 m was recorded in Bombay followed by Ulvapadu Selection (3.27 m) and Krishna Rao (3.51 m). The other genotypes were in intermediary range.Present investigation is in confirmity with the earlier repots of Hegde (1997), Gunuki (1998), Shirol *et al.* (2006), Saraswathi *et al.* (2010) and Suhasini *et al.* (2012) in sapota genotypes.

4.1.6 Leaf length (cm)

There were significant differences in the leaf length among the genotypes which ranged from a minimum of 7.20 cm to maximum of 13.81 cm and mean leaf length of 10.09 cm (Table-4 and Fig-1). Significantly highest leaf length of 13.81 cm was recorded in Cricket Ball followed by Seedless (12.37 cm) and PKM-3 (12.17 cm), while lowest leaf length of 7.20 cm was recorded in Pakala followed by Pakala Oval (8.33 cm), Ulvapadu Selection (8.63 cm) and Calcutta Round (8.77 cm). The other genotypes were in intermediary range.

4.1.7 Leaf width (cm)

Leaf width differed significantly for the various genotypes (Table-4 and Fig-1) studied with a mean of 3.77 cm and it ranged from a minimum of 2.30 cm to maximum of 5.86 cm. Significantly highest leaf width was found in Cricket Ball (5.87 cm), while lowest leaf width of 2.30 cm was recorded in Pakala followed by Ulvapadu Selection (2.70 cm), Mirandi (2.80 cm), Pakala Round (3.13 cm) and Pakala Oval (3.40 cm) The other genotypes were in intermediary range.

4.1.8 Leaf area (cm^2)

Significant differences were revealed among the genotypes with respect to leaf area (Table-4 and Fig-1). The average leaf area was observed to be 27.41 cm^2, while the leaf area ranged from a minimum of 17.00 cm^2 to maximum of 44.42cm^2. The highest leaf area of 44.42 cm^2 was recorded in Cricket Ball, followed by PKM-3 (40.17 cm^2) and CO-2 (36.17 cm^2), while lowest leaf area of 17.00 cm^2 was recoded in Ulvapadu Selection followed by Pakala (17.83 cm^2), Pakala Round (19.75 cm^2) and Mirandi (20.67 cm^2).

4.1.9 Fruit length (cm)

The differences in fruit length among the various genotypes were found to be significant. The mean fruit length was 6.40 cm, while it ranged from 8.39 cm to 4.8 cm (Table-5). Maximum fruit length was recorded for PKM-4 (8.39 cm) followed by Columbian Sapota (7.91 cm) and CO-1 (7.81 cm). However, the minimum fruit length was recorded for Pakala Round (4.84 cm) genotype followed by Bombay (5.07 cm), Cricket Ball (5.32 cm), Calcutta Round (5.37 cm), Pakala (5.39 cm) and Dwarapudi (5.45 cm). These findings are in agreement with the results of Gunuki *et al.* (1998), Mathew *et al.*(2001), Shirol *et al.* (2006), Kamraj *et al.* (2010), Rekha*et al.* (2011), Suhasini *et al.* (2012) and Radha (2013) in sapota genotypes.

4.1.10 Fruit width (cm)

Significant differences were observed among genotypes (Table-5) in respect of fruit width and they recorded a mean fruit width of 5.48 cm, while, the fruit width ranged from 5.09 cm to 8.20 cm. Maximum fruit width was recorded for Columbian

Sapota (8.20 cm) followed by CO-3 (7.63 cm), Cricket Ball (7.34 cm) and DHS-1 (7.02 cm), while minimum fruit width was recorded in Gutti (5.09 cm) followed by Hybrid (5.18 cm), Pala (5.37 cm), Badami (5.49 cm), Krishna Rao (5.51 cm), Pakala Oval (5.77 cm), Tagarampudi (5.80 cm) and Virudnagar (5.87 cm). Present investigation is in confirmity with the earlier reports of Ingel *et al*. (1982), Paralkar *et al*. (1987), Mathew*et al.* (2001), Raut (1999), Dhua *et al*. (2006), Rajasekhar (2009), Pawar *et al.*(2011), Suhasini *et al*. (2012) and Radha (2013) in sapota genotypes.

4.1.11 Fruit weight (g)

The data on the fruit weight (Table-5) showed significant differences among different accessions with a mean weight of 158.43 g while the fruit weight ranged from 73.60 g to 540.27 g. The genotype Columbian Sapota recorded the highest fruit weight (540.27g) followed by Cricket Ball (259.70g), C0-2 (239.18g), CO-1 (222.33g) and Brazilian sapota (221.08g). Minimum fruit weight of 73.60g was recorded in Krishna Rao followed by Pakala Round (75.61g), Gutti(78.95 g), Badami (83.80g), Tagarampudi (89.25g) and Pala (94.25g). These reports were in close agreements with the findings of Chundawat and Bhuva (1982), Paralkar *et al*. (1987), Raut (1999), Mathew*et al.* (2001), Dhua *et al*. (2006), Rajasekhar (2009), Dhanashree Patil *et al*. (2010), Narendra Kumar (2011), Rekha*et al*. (2011), Suhasini *et al*. (2012), Varun Devashi (2012) and Radha (2013) in different sapota genotypes.

4.1.12 Fruit shape

The differences in fruit shape among the sapota genotypes (Table-6 and Plate-2) ranged from turbinate to oval. In the genotypes CO-1, CO-2 the fruit shape was turbinate (1.00), while fruit shape was acorn (2.00) in Dwarapudi, Kalipatti, Krishna Rao, Singapore, Virudnagar, CO-3, DHS-1 and PKM-2. Further, Badami, Bombay, Calcutta Round, Columbian Sapota, Cricket Ball, Kirthibarthi, Pala, Pakala Round, Tagarampudi and DHS-2 recorded globose (3.00) fruit shape, while in the genotypes, PKM-1 and PKM-4, the fruit shape was elliptic (5.00) and in the genotypes Gavarayya, Gutti, Hybrid, Mirandi, Pakala, Seedless and Simhoudi it was oblong (6.00). However, the genotypes Brazilian Sapota, Pakala Oval, Ulvapadu Selection and in PKM-3 recorded Oval (7.00) fruit shape. Similar results in sapota genotypes with regard to fruit shape were reported by Rajasekhar (2009) and Radha (2013).

4.1.13 Fruit clustering habit

The genotypes differed in the score points given for fruit clustering habit (Table-6) and it was recorded on two score solitary (1) and clustering (2). Majority of genotypes *viz.,* Badami, Bombay, Brazilian Sapota, Columbian Sapota, Calcutta Round, Cricket Ball, Dwarapudi, Gavarayya, Hybrid, Kalipatti, Kirthibarthi, Krishna Rao, Mirandi, Pakala, Pakala Round, Pakala Oval, Pala, Seedless, Simhoudi, Singapore, Tagarampudi, Virudnagar, Ulvapadu Selection, CO-1, CO-2, CO-3, DHS-1, DHS-2, recorded solitary (1.00). Further, the genotypes PKM-1, PKM-2, PKM-3, PKM-4 and Gutti had clustering habit (2.00).

4.1.14 Fruit surface texture

The differences in fruit surface texture among the genotypes (Table-6) ranged from scurfy (1.00), smooth (2.00) to web pattern on the surface (3.00). The genotypes, *viz.,*Badami, Bombay, Brazilian Sapota, Calcutta Round, Cricket Ball, Gavarayya, Hybrid, Krishna Rao, Pakala, Pakala Oval, Pakala Round, Pala, Tagarampudi, Virudnagar, CO-2, DHS-1 and DHS-2 had recorded rough (1.00) fruit surface texture, while it was smooth (3.00) in Colmbian Sapota, Dwarapudi, Gutti, Kalipatti, Kirthibarthi, Mirandi, Simhoudi, Seedless, Singapore, Ulvapadu Selection, CO-1, CO-3, PKM-1, PKM-2, PKM-3 and PKM-4. These results were in accordance with Mathew *et al*. (2001), Rajasekhar (2009), Suhasini *et al*. (2012) and Radha (2013) in sapota.

4.1.15 Peel color

The genotypes differed in the peel color and it was observed that majority of genotypes (Table-6) recorded brown peel color (4.00) in Badami, Calcutta Round, Dwarapudi, Gavarayya, Gutti, Krishna Rao, Kirthibarthi, Pakala, Pakala Round, Pakala Oval, Simhoudi, Pala, Singapore, Tagarampudi, Ulvapadu Selection, Virudnagar, CO-2, CO-3, DHS-1, DHS-2, PKM-1, PKM-2, PKM-3 and PKM-4, while it was greenish brown (3.00) in Brazilian Sapota, Columbian Sapota, Mirandi genotypes. Further, the genotypes Bombay, Cricket Ball, Hybrid, Kalipatti,Kirthibarthi, Seedless and CO-1 recorded yellowish brown (5.00) peel color. Similar results in sapota genotypes with regard to peel color were reported by Rajasekhar (2009) and Radha (2013).

4.1.16 Flesh colour

Flesh colour among the sapota genotypes (Table-6)under studyvaried from greenish brown to reddish brown. It was greenish brown (3.00) in Brazilian Sapota, Columbian Sapota and Mirandi ; brown (4.00) in Badami, Dwarapudi, Gavarayya, Gutti, Kalipatti, Kirthibarthi, Pakala, Pakala Round, Pakala Oval, Pala, Simhoudi, Singapore, Virudnagar, CO-3, PKM-1 and PKM-2 ; yellowish brown (5.00) in Cricket Ball, Seedless, Ulvapadu Selection, CO-1, CO-2, PKM-3 and PKM-4 ; reddish brown (6.00) in Bombay, Hybrid, Calcutta Round, Krishna Rao, Tagarampudi, DHS-1 and DHS-2. These reports were in close agreement with the findings of Rokhade et *al*. (1989), Mathew *et al*. (2001), Rajasekhar (2009), Dhanashree and Patil *et al*. (2010), Narendra Kumar (2011), Rekha*et al*. (2011), Suhasini *et al*. (2012) and Radha (2013) in different sapota genotypes.

4.1.17 Flesh texture

The flesh texture of the genotypes under studyranged from sandy to soft (Table-6). Bombay, Brazilian Sapota, Columbian Sapota, Cricket Ball, CO-1, CO-2, CO-3 possessed sandy (1.00) flesh texture, while firm (2.00) flesh texture was observed in the genotypes Calcutta Round, Dwarapudi, Gavarayya, Kalipatti, Krishna Rao, Mirandi, Seedless, Singapore, Tagarampudi, DHS-2, PKM-2 and PKM-3. Further, the genotypes, Badami, Hybrid, Gutti, Kirthibarthi, Pakala, Pakala Round, Pakala Oval, Pala, Simhoudi, Ulvapadu Selection, Virudhnagar, DHS-1, PKM-1 and PKM-4 were observed to possess soft flesh texture (3.00). These findings were in agreement with the results of Rajasekhar (2009) and Radha (2013) in sapota genotypes.

4.1.18 Flesh taste

The genotypes differed in flesh taste which ranged from starchy to sweet (Table-6). Majority of genotypes *viz.*, Badami, Cricket Ball, Dwarapudi, Hybrid, Kalipatti, Kirthibarthi, Krishna Rao, Pakala, Pakala Round, Pakala Oval, Pala, Seedless, Singapore, Virudnagar, Ulvapadu Selection, CO-1, CO-2, CO-3, DHS-1, DHS-2, PKM-1, PKM-2, PKM-3 and PKM-4 recorded sweet flesh (4.00), while, the genotypes Bombay, Brazilian Sapota, Columbian Sapota, Calcutta Round, Gavarayya, Gutti, Mirandi, Simhoudi and Tagarampudi were starchy (3.00).

4.1.19 Ovary size (mm)

The results on ovary size revealed that there were significant differences among the various genotypes as presented in table-7. The average ovary size was 3.97 mm, while it ranged from 3.12mm to 4.79mm. Maximum ovary size was recorded in Singapore (4.79 mm) followed by Dwarapudi and Ulvapadu Selection (4.49 mm), Brazilian Sapota (4.47 mm), DHS-1 (4.34 mm). Further, minimum ovary size was recorded in Pala (3.13 mm) followed by Badami (3.37mm) and Gutti (3.61 mm).

4.1.20 Per cent fruit set (%)

Percent fruit set differed significantly among the genotypes (Table-7) studied which ranged from 5.43% to 32.00% and average per cent fruit set recorded was 14.48 %. In the present study, significantly maximum percent fruit set was recorded in Tagarampudi(32.00%) followed by PKM-1 (28.47%) and Virudnagar (27.00%), While lowest per cent fruit set was recorded in Calcutta Round (5.43%) followed by Badami (6.00%), Bombay (6.80%) and Brazilian Sapota (7.13%). Present investigation is in confirmity with the earlier reports of Madhavarao and Khader (1961), Farooqi *et al*. (1973), Nalwadi *et al*. (1977), Mahadevaiah (1981), Relekar *et al*. (1991), Patnaik (1982), Nagargoje *et al*. (2007) and Suhasini *et al*. (2012) in different sapota genotypes.

4.1.21 Yield/tree (kg)

The data on yield/tree showed significant differences among sapota accessions with a mean yield/tree of 74.27 kg, while the yield/tree ranged from 14.67 kg to

187.14 kg (Table-7). The genotype Tagarampudi recorded the highest yield/tree 187.14 kg followed by Virudnagar (184.57 kg), PKM-1 (151.83 kg) and PKM-4 (111.83 kg). Minimum yield/tree of 14.67 kg was recorded in Badami followed by Calcutta Round (16.41kg), Bombay (17.63 kg) and Brazilian Sapota (20.49 kg). Identical results in respect of yield/tree were reported earlier by Mone (1989), Relekar *et al.* (1991), Hegde (1997), Gunuki (1998), Shirol *et al.* (2005), Saraswathi*et al.* (2010), Suhasini *et al.* (2012) and Varu Devashi (2012) in different sapota genotypes.

4.1.22 Seed number

The results presented in table-5 revealed an average seed number per fruit to be 4.60, while it ranged from 2.33 to 9.33. Maximum seed number per fruit was recorded in Krishna Rao (9) followed by Mirandi (6.67), Cricket Ball and Dwarapudi (6.33), while lowest seed number per fruit was recorded in the genotype Seedless (2.33) followed by CO-2 (3), Ulvapadu Selection, DHS-2 and PKM-2 (3.33). Similar results were reported earlier by Lakshminarayana and Subrahmanyam (1966), Sulladamath *et al.* (1978), Mone (1989), Chundawat and Bhuva (1982), Avaiah and Singh (1991), Rokhade *et al.* (1989), Gunuki (1998), Mathew *et al.* (2001), Shirol *et al.* (2005), Rajasekhar (2009), Patil *et al.* (2010), Rekha (2011) and Radha (2013) in different sapota genotypes.

4.1.23 Seed weight (g)

The seed weight revealed significant differences among the genotypesas presented in table-5. The seed weight of the sapota genotypes studied in the present investigation ranged from 2.46 g to 7.81g. Significantly maximum seed weight was recorded in Cricket Ball (7.81g) followed by Columbian Sapota (7.07g), Krishna Rao (6.93g) and Brazilian Sapota (6.22g) while lowest seed weight was recorded in Seedless (2.45g), followed by Ulvapadu Selection (2.58g) Pala (2.84g), Simhoudi (2.93g) and Pakala Oval (2.99g). Similar observations in different sapota genotypes were reported by Rajasehar (2009), Narendra Kumar (2011) and Radha (2013).

4.1.24 Pulp to seed ratio

Significant differences were revealed in pulp to seed ratio as in table 5 which revealed that the mean pulp to seed ratio to be 35.55, while the range of pulp to seed ratio was from 17.27 to 75.67 among the sapota genotypes studied. Maximum pulp to seed ratio was recorded in Columbian Sapota (75.67) followed by Mirandi (61.50), Ulvapadu Selection (50.40), CO-2 (47.82), Brazilian Sapota (43.54) and CO-1 (40.20)while minimum pulp to seed ratio was recorded in Dwarapudi (17.27) followed by Tagarampudi (18.29), Bombay (23.10), Gutti (23.41), Pakala Round (24.19) and Krishna Rao (24.16). Similar variations in the pulp to seed ratio of sapota genotypes was reported earlier by Ingel *et al*. (1982), Rajasekhar (2009), Pawar *et al*. (2011) and Radha (2013).

4.2 FRUIT BIO-CHEMICAL CHARACTERS

4.2.1 Total soluble solids (TSS)

The TSS content in sapota fruits differed significantly. The results presented in table-8 revealed that the total soluble solids ranged from 18.60 ^{O}B to 25.33 ^{O}B among the sapota genotypes studied, while the average TSS recorded was 21.73 ^{O}B in the genotypes. TSS was observed to be the highest with the genotype, PKM-4 (25.33 ^{O}B), while the genotypes namely PKM-3(24.60^{O}B), Pala (24.33^{O}B), PKM-2 (24.03^{O}B), Brazilian Sapota (23.79^{O}B) and Gutti (23.50^{O}B) were found to be on par with PKM-4. Lowest TSS was recorded in the genotype Columbian Sapota (18.60^{O}B). Further, the genotypes Bombay (18.63^{O}B), Mirandi (19.40^{O}B), Hybrid (19.50^{O}B), CO-3 (19.77^{O}B), Cricket Ball (19.90 ^{o}B), Simhoudi (20.37^{O}B), Badami (20.57 ^{O}B), Pakala Oval (20.60^{O}B) and Pakala (20.63^{O}B), recorded total soluble solids on par with Columbian Sapota.Similar trend in TSS content of sapota genotypes was earlier reported by several workers (Lakshminarayana, 1980, Ponnuswamy and Irulappan 1987, Paralkar *et al*., 1987, Shanmugavelu *et al*., 1987, Mone *et al*., 1990, Waskar and Nikam 1996, Mathew *et al*., 2001, Dhua *et al*., 2006, Rajasekhar, 2009, Saraswathy *et al*., 2010, Dhanashree Patil *et al*., 2010, Rekha*et al*., 2011, Suhasini *et al*., 2012 and Radha, 2013) and strengthen these results.

4.2.2 Total sugars

The total sugars content differed significantly among the genotypes (Table-8 and Fig-3) and the mean total sugar content to be 10.12 percent, while the range was 8.21 percent to 11.99 percent. Maximum total sugar content was recorded in PKM-4 (11.99%), while other genotypes namely, Pala (11.84%), Kalipatti (11.47%), PKM-3 (11.19%) and DHS-1 (11.08%) were found to be onpar with PKM-4. Lowest total sugar content was recorded for the genotype Badami (8.21%) followed by Columbian Sapota (8.61%), Cricket Ball (8.97%), Dwarapudi (8.93%) and Mirandi (8.97%). The other genotypes were found to be intermediary. These results were in line with the observations repoted earlier by Madhava Rao *et al.* (1971), Shanmugavelu and Srinivasan (1973), Suryanarayana and Goud (1984), Gautam and Chundawat (1990), Mathew *et al.* (2001), Brito and Narain (2002), Rajasekhar (2009), Saraswathy *et al.* (2010), Mythri (2012), Varu Devashi (2012) and Radha (2013) in different sapota genotypes.

4.2.3 Reducing sugars

The sapota genotypes differed significantly in reducing sugars content (Table-8 and Fig. 3) with a mean of 8.07%, while it ranged from6.60%to 9.23%. Highest amount of reducing sugars was recorded in the genotype, Kalipatti (9.23%) which was on par with Pala (9.13%), Kirthibarthi (9.10%), PKM-1 (9.07%) and PKM-4 (9.00%). Further, lowest amount of reducing sugars was recorded in the genotype Dwarapudi (6.23%). The genotypes Columbian Sapota (6.60%), CO-3 (6.66%), Hybrid (6.69%) and Seedless (6.89%) were found to be on par with Dwarapudi. The other genotypes were found to be intermediary. Similar variations in the reducing sugars content of sapota genotypes were earlier reported by Madhava Rao *et al.* (1971), Shanmugavelu and Srinivasan (1973), Suryanarayana and Goud (1984), Selvaraj and Pal (1984), Paralkar *et al.* (1987), Gautam and Chundawat (1990), Mathew *et al.* (2001), Briton and Narain (2002), Rajasekhar (2009), Varu Devashi (2012) and Radha (2013).

4.2.4 Non-reducing sugars

Significant differences were observed in non-reducing sugars and the result presented in table-8 and fig 3 revealed that the mean non-reducing sugars content to be 2.10%, while the range of non-reducing sugars was 0.90% to 3.66% among the sapota genotypes studied. Maximum non-reducing sugars content was recorded in CO-2 (3.66%) followed by CO-3 (3.14%), CO-1 (3.10%), PKM-2 (3.00%) and PKM-4 (2.98%). Minimum non-reducing sugars content was recorded in Simhoudi (0.90%) followed by Mirandi (0.93%), Bombay (1.03%), Pakala Oval (1.10%) and Krishna Rao (1.14%). The other genotypes were found to be intermediary. Identical results in respect of non-reducing sugars were reported earlier by Madhava Rao *et al.* (1971), Shanmugavelu and Srinivasan (1973), Suryanarayana and Goud (1984), Selvaraj and Pal (1984), Paralkar *et al.* (1987), Gautam and Chundawat (1990), Mathew *et al.*(2001), Briton and Narain (2002), Rajasekhar (2009) and Varu Devashi (2012) in different sapota genotypes.

4.2.5 Ascorbic acid

The sapota genotypes under study differed significantly in the ascorbic acid content (Table-8). The average ascorbic acid content for the sapota genotypes studied in the present investigation was 8.7 mg/100g, while the range was noticed to be 5.15 mg/100g to 12.16 mg/100g. Highest amount of ascorbic acid content was recorded in CO-1 (12.16 mg/100g), which was on par with Kalipatti (12.10 mg/100g), Singapore (11.76 mg/100g), CO-2 (11.27 mg/100g), PKM-3 (11.05 mg/100g) and Virudnagar (10.68 mg/100g). Further lowest amount of ascorbic acid was recorded in the genotype Bombay (5.15 mg/100g), which was on par with Dwarapudi (5.35 mg/100g), Hybrid (6.16 mg/100g), Cricket Ball (6.22 mg/100g) and Badami (6.63 mg/100g). The other genotypes were found to be intermediary. These reults are in conformity with the earlier reports of Lakshminarayana and Subrahmanyam (1966), Shanmugavelu and Srinivasan (1973), Paralkar *et al.* (1987), Banik *et al.* (1988), Bandhyopadhyay and Sen (1994), Briton and Narain (2002), Rajasekhar (2009), Saraswathy *et al.*(2010) and Radha (2013).

4.2.6 Titrable acidity

The titrable acidity differed significantly among the genotypes (Table-8 and Fig 4) and the mean titrable acidity to be 0.220%, while the range was 0.295% to

0.168%. Maximum titrable acidity was recorded in Mirandi (0.295%), which was onpar with Krishna Rao (0.282%), Bombay (0.283%) and Badami (0.266%), while lowest titrable acidity was noticed for the genotype Kirthibarthi (0.168%), followed by PKM-4 (0.169%), PKM-1 (0.177%), Pala (0.181%), Virudnagar (0.184%) and DHS-1 (0.191%). The other genotypes were found to be intermediary.Similar variation in titrable acidity content of sapota genotypes was reported by Madhava Rao *et al*. (1971), Ingel *et al*. (1981), Kumbhar and Desai (1986), Rajasekhar (2009), Suhasini *et al*. (2012) and Radha (2013).

4.2.7 TSS to Acid ratio

The sapota genotypes differed significantly in TSS to acid ratio content with a mean value of 101.99, while it ranged from 149.88 to 65.76 (Table-8 and Fig 4). Maximum TSS to acid ratio was recorded in the genotype, PKM-4 (149.88) which was on par with Kithibarthi (135.89), PKM-1 (135.00), Pala (134.41), Virudhnagar (125.38) and PKM-2 (123.23). Further Minimum TSS to acid ratio was recorded in the genotype Mirandi (65.76). The genotypes Bombay (65.83), Columbian Sapota (75.91), Krishna Rao (75.59), Hybrid (78.62), Cricket Ball (78.96), Simhoudi (80.51), Pakala Round (86.38), Pakala Oval (89.22) and CO-2 (90.50) were found to be on par with Mirandi. The other genotypes were found to be intermediary. These resuluts are in contradictory with the findings of Ponnuswamy and Irulappan (1987), Kariyanna *et al*. (1990), Rajasekhar (2009), Mythri (2012) and Radha (2013) in sapota genotypes.

4.2.8 Total phenols

The data on the total phenols showed significant differences among different accessions with a mean of 0.47 mg/100g (Table-8 and Fig 4), while the total phenols ranged from 0.30 mg/100g to 0.62 mg/100g. The genotype Krishna Rao recorded the highest total phenols 0.62 mg/100g, followed by Mirandi (0.60 mg/100g), Badami (0.61 mg/100g), Pakala (0.558 mg/100g),PKM-4 (0.528 mg/100g) and Kirthibarthi (0.527 mg/100g). Further, lowest amount of total phenols were recorded in the genotype CO-1 (0.300 mg/100g), which was on par with Pala (0.382 mg/100g), PKM-3 (0.383 mg/100g), PKM-1 (0.381 mg/100g),Tagarampudi (0.392 mg/100g) and

DHS-1 (0.393 mg/100g). The other genotypes were found to be intermediary. Similar variations in the reducing sugar content of sapota genotypes was earlier reported by Lakshminarayana and Subramanyam*et al*. (1966), Selvaraj and Pal (1984), Paralkar *et al*. (1987), Briton and Narain (2002), Anand *et al*. (2007), Rajasekhar (2009) and Mythri (2012).

4.2.9 Pectin

The pectin content differed significantly among the genotypes (Table-8) and the mean total pectin content to be 1.94 percent, while the range was 1.17 percent to 2.86 percent. Maximum pectin content was recorded in Mirandi (2.86%), while other genotypes namely, DHS-2 (2.73%), Singapore (2.70%), DHS-1 (2.63%) and Badami (2.55%) were found to be on par with Mirandi. Lowest pectin content was recorded for the genotype Simhoudi (1.17%) followed by CO-3 (1.18%), Columbian Sapota (1.34%), Brazilian Sapota (1.42%) and Seedless (1.44%). The other genotypes were found to be intermediary. These results are in confirmity with the earlier repots of Varanyanond *et al*. (1999) in mango, Jin *et al*. (2006) in peach and Mythri (2012) in sapota.

4.3 GENETIC VARIABILITY STUDIES

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

Besides genetic variability, knowledge on heritability and genetic advance measures the relative degree to which a character is transmitted from one generation to next. High heritability indicates that a major part of phenotypic variability in any character is controlled by additive gene effect, which can be improved by simple selection procedures (Lush, 1949). Heritability also provides information to predict genetic gain obtained by selection. Therefore, for successful improvement of any crop, it is necessary to have a thorough knowledge on the above genetic parameters. The characters with high heritability coupled with high genetic advance further indicate the possibility of making selections in earlier generations. The genetic variability together with the heritability would give a better idea on the amount of genetic advance expected out of selection. The magnitude of heritable variability is the most important aspect of genetic contribution of the breeding material, which has close relationship on its response to selection.

The genetic parameters, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense and genetic advance as percent mean were estimated for all characters under study and the results on variability among 33 sapota genotypes are presented in table-9 and fig-5 and variability present in different characters was discussed under the following heads:

4.3.1 Plant height

The estimates of phenotypic coefficient of variation (18.68%), genotypic coefficient of variation (18.67%) were observed to be medium for this character, while heritability (96.90%) and genetic advance as percent mean (38.43%) recorded high estimate indicated the presence of additive gene action in inheritance of this trait and simple selection will be highly helpful for improving this character. These results are in consonanace with the reports given by Bisla and Daulta (1988) in ber, Rai and Mishra (2000) in pomgranate, Rajasekhar (2009) in sapota, Baruah *et al*. (2007) in banana and Bhupendra Singh Uniyal*et al.* (2012) in aonla.

4.3.2 Leaf length

This trait recorded medium phenotypic coefficient of variation (14.98%), low genotypiccoefficient of variation (11.14%), high heritability (55.3%) and genetic advance as percent mean (17.06%) were found to be high for this character. High heritability coupled with moderate genetic advance as per cent of mean was observed for this trait indicates that this trait could be improved through hybridization followed by selection in later generations. These results are in confirmity with the reports of Gangwar and Tripathy (1973) in citrus, Kokadwar *et al.* (1992), Attri *et al.* (1999) in mango, Kavitha *et al.* (2008) in banana and Rajasekhar (2009) in sapota.

4.3.3 Leaf width

The variability parameters studied for this trait namely, phenotypic coefficient of variation (19.79%), genotypic coefficient of variation (15.61%) were found to be medium, heritability (62.20%) and genetic advance as percent mean (25.36%) were found to be high for this character. High heritability coupled with high genetic advance as per cent mean was observed for this trait indicating the presence of additive gene action in the inheritance and further selection of its improvement is effective. These findings are in accordance with Iyer *et al*. (1989), Kokadwar *et al*. (1992), Attri *et al.* (1999) in mango, , Kavitha *et al.* (2008) in banana and Rajasekhar (2009) in sapota.

4.3.4 Leaf area

Higher values were recorded for phenotypic coefficient of variation (29.04%), genotypic coefficient of variation (19.00%) and genetic advance as per cent mean (25.60%) for this character, while the estimates for heritability (42.80%) were found to be moderate for the trait. The high PCV and GCV of this character indicated the presence of large amount of variation among the genotypes. The results are in consonanace with the reports given by Iyer *et al*. (1989), Kokadwar *et al*. (1992), Attri *et al.* (1999) in mango, Kavitha *et al.* (2008) in banana and Rajasekhar (2009) in sapota.

4.3.5 Ovary size

The estimates for ovary size are low for phenotypic coefficient of variation (10.52%), genotypic coefficient of variation (7.40%) and geneticadvance as per cent mean (10.73%), while the estimates for heritability (49.50%) are moderate for the trait. Medium heritability coupled with low genetic advance as per cent of mean observed for this trait indicates the presence of non-additive gene action in the inheritance and further selection for its improvement was ineffective.

4.3.6 Fruit length

The estimates for fruit length are medium for both phenotypic coefficient of variation (14.84%), and genotypic coefficient of variation (12.47%), while the estimates for heritability (70.50%)and genetic advance as percent mean (21.57%) was found to be high for the trait.

Fruit length recorded medium values of PCV and GCV indicating that variation among the genotypes was low. This trait shows high heritability and moderate genetic advance as per cent of mean. Hence, this trait can be improved by simple selection. These results are in confirmity with the earlier reports of Yadav *et al.* (1995), Lavi *et al.* (1998) Attri *et al.* (1999) in mango, Bisla and Daulta (1988) in ber, Meena and Roop Singh (2006) in pomegranate, Baruah *et al.* (2007), Kavitha *et al.*(2008) in banana and Rajasekhar (2009) in sapota.

4.3.7 Fruit width

Moderate phenotypic coefficient of variation (12.27%), low genotypiccoefficient of variation (9.60%), high heritability (61.10%) and high genetic advance as percent mean (15.46%) were reorded for the trait. The low GCV values indicate that variation among the genotypes was low. High heritability coupled with high genetic advance as per cent mean was observed for this trait indicating the presence of additive gene action in the inheritance and further selection for its improvement is effective. Similar results were reported by Yadav *et al.* (1995), Lavi *et al.* (1998), Attri *et al.* (1999) in mango, Bisla nd Daulta (1998) in ber, Meena and Roop Singh (2006) in pomegranate, Baruah *et al.* (2007), Kavitha *et al* .(2008) in banana and Rajasekhar (2009) in spota.

4.3.8 Fruit weight

All the variability parameters studied, namely, phenotypic coefficient of variation (55.59%), genotypic coefficient of variation (52.77%) along withheritability (90.10%) and genetic advance as percent mean (29.21%) were high for the character studied.The high PCV and GCV of characters indicated the presence of large amount of variation. The higher estimates of heritability for this trait suggested that simple selection would be more efficient on the basis of phenotypic performance of genotypes. The higher estimates of genetic advance indicated that this character was governed by additive genes and selection would be helpful for their improvement.These results are in confirmity with the reports of Attri *et al.* (1999), Singh *et al*. (2004) in mango, Vashishtha *et al.* (2003) in pomegranate, Rajasekhar (2009) in sapota and Bhupendra Singh Uniyal *et al.*(2012) in aonla.

4.3.9 Seed number

The character recorded high estimates for all variability parameters studied, namely, phenotypic coefficient of variation (30.05%), genotypic coefficient of variation (27.77%), heritability (85.40%) and genetic advance as percent mean (52.85%) this indicated the presence of additive gene action in inheritance and simple selection will be highly effective for improving of this character.

4.3.10 Seed weight

The estimates for phenotypic coefficient of variation (32.41%),genotypic coefficient of variation (31.63%), heritability (95.30%) and genetic advance as percent mean (63.54%) were high for thetrait.The higher estimates of heritability for this trait suggested that simple selection would be more efficient on the basis of phenotypic performance of genotypes. The higher estimates of genetic advance indicated that this character was governed by additive genes and selection would be helpful for its improvement.

4.3.11 Pulp to seed ratio

Higher values for phenotypic coefficient of variation (32.54%), genotypic coefficient of variation (33.44%), heritability (98.42%) and genetic advance as percent mean (68.87%) were recorded for the trait.The high PCV and GCV of characters indicated the presence of large amount of variation. The higher estimates of heritability for this trait suggested that simple selection would be more efficient on the basis of phenotypic performance of genotypes. The higher estimates of genetic advance indicated that this character was governed by additive genes and selection would be helpful for their improvement.Similar results in sapota genotypes with regard to pulp to seed ratio were reported by Bisla and Daulta (1988) in ber, Rathod (2007) in mango and Rajasekhar (2009) in sapota.

4.3.12 Per cent fruit set

This character recorded high estimates for all the variability parameters studied namely, phenotypic coefficient of variation (44.53%), genotypic coefficient of variation (44.15%), heritability (98.30%) and genetic advance as percent mean (90.16%). High heritability in conjugation with high genetic advance as per cent of mean observed for this trait indicates the preponderance of additive gene action. Hence this trait could be effectively improved by simple selection procedures.

4.3.13 Yield per tree

The estimates for phenotypic coefficient of variation (80.25%), genotypic coefficient of variation (77.46%), heritability (93.20%) and genetic advance as percent mean (88.01%) werehigh forthe trait. The high values of PCV and GCV indicates that variation among the genotypes was also high. High heritability in conjugation with high genetic advance as per cent of mean observed for this trait indicates the preponderance of additive gene action. Hence this trait could be effectively improved by simple selection procedures. These results are in confirmity with the reports of Attri *et al.* (1999), Singh *et al.* (2004) in mango, Vashishtha *et al.* (2003) in pomegranate, Dinesh (2003) in papaya, Kavitha *et al.* (2008), Baruah *et al.* (2007) in banana and Bhupendra Singh Uniyal *et al.*(2012) in aonla.

4.3.14 TSS

The estimates for phenotypic coefficient of variation (8.40%),genotypic coefficient of variation (6.92%) and genetic advance as per cent mean (11.74%) were low for this trait, while high estimates were registered for heritability (67.80%).

High heritability coupled with low genetic advance as per cent of mean observed for this trait indicates the presence of non-additive gene action in the inheritance and further selection for its improvement was ineffective. The findings are in accordance with Iyer *et al*. (1989), Karibasappa *et al*. (1999) in mango, Praveen and Patil (1998) in ber, Meena and Roop Singh (2006) in pomegranate, Baruah *et al*. (2007) and Kavitha *et al*. (2008) in banana.

4.3.15 Total sugars

The estimates for phenotypic coefficient of variation (9.47%), genotypic coefficient of variation (8.80%) were low for this character, while the estimates for heritability (86.50%) and genetic advance as percent mean (16.87%) were high for the trait. High heritability coupled with low genetic advance as per cent of mean observed for this trait indicates the presence of non-additive gene action in the inheritance and further selection for its improvement was ineffective. Similar results were also reported by Karibasappa *et al*. (1999), Rathod (2007) in mango, Praveen and Patil (1998) in ber, Vashishtha *et al.* (2003) in pomegranate, Baruah *et al*. (2007), Kavitha *et al*. (2008) in banana and Rajasekhar (2009) in sapota.

4.3.16 Reducing sugars

Moderate estimates for phenotypic coefficient of variation (12.01%) and genotypic coefficient of variation (11.67%), and high estimates for heritability (94.60%) and genetic advance as percent mean (23.40%) were registered for the character. The estimates of PCV and GCV were moderate for this trait. High heritability coupled with high genetic advance as per cent of mean observed for this trait indicatesthe preponderance of additive gene action. Hence this trait could be effectively improved by simple selection. Identical results in respect of reducing sugars were reported earlier by Iyer *et al*. (1989), Karibasappa *et al*. (1999) in mango, Praveen and Patil (1998) in ber, Meena and Roop Singh (2006) in pomegranate, Baruah *et al*. (2007), Kavitha *et al*. (2008) in banana and Rajasekhar (2009) in sapota.

4.3.17 Non-reducing sugars

Higher values were recorded for phenotypic coefficient of variation (36.25%), genotypic coefficient of variation (35.89%), heritability (98.10%) and genetic advance as percent mean (73.22%) for thetrait. The high values of PCV and GCV indicates that variation among the genotypes was also high. High heritability in conjugation with high genetic advance as per cent of mean observed for this trait indicates the preponderance of additive gene action. Hence this trait could be effectively improved by simple selection procedures. Similar results were also reported by Karibasappa *et al*. (1999), Rathod (2007) in mango, Praveen and Patil (1998) in ber, Vashishtha *et al.* (2003) in pomegranate, Baruah *et al*. (2007), Kavitha *et al*. (2008) in banana and Rajasekhar (2009) in sapota.

4.3.18 Ascorbic acid

The estimates for phenotypic coefficient of variation (22.01%), genotypic coefficient of variation (21.96%), heritability (99.60%) and genetic advance as percent mean (45.14%) werehigh for the character.The high PCV and GCV of the character indicated the presence of large amount of variation. The higher estimates of heritability for this trait suggested the simple selection would be more efficient on the basis of phenotypic performance of genotypes. The higher estimates of genetic advance indicated that this character was governed by additive genes and selection would be helpful for its improvement.Present investigation is in confirmity with the earlier reports of Praveen and Patil (1998) in ber, Meena and Roop Singh (2006) in pomegranate, Rathod (2007) in mango, Baruah *et al*. (2007), Kavitha *et al*. (2008) in banana, Rajasekhar (2009) in sapota and Bhupendra Singh Uniyal *et al.*(2012) in aonla.

4.3.19 Titrable acidity

Moderate estimates were noticed for phenotypic coefficient of variation (15.34%), genotypic coefficient of variation (15.33%), while high estimates were registered for heritability (99.80%) and genetic advance as percent mean (31.54%) for this trait. High heritability coupled with high genetic advance as per cent of mean observed for this trait indicates the preponderance of additive gene action. Hence this trait could be effectively improved by simple selection. These results are in confirmity with the reports of Singh *et al.* (2004), Rathod (2007) in mango, Praveen and Patil (1998) in ber, Meena and Roop Singh (2006) in pomegranate, Kavitha *et al.* (2008) in banana, Rajasekhar (2009) in sapota and Bhupendra Singh Uniyal *et al.*(2012) in aonla.

4.3.20 TSS to Acid ratio

The estimates for phenotypic coefficient of variation (20.89%) genotypic coefficient of variation (20.19%), heritability (92.44%) and genetic advance as percent mean (43.64%) were high for the trait. High heritability coupled with high genetic advance as per cent of mean observed for this trait indicates the preponderance of additive gene action. Hence this trait could be effectively improved by simple selection. Similar results in sapota genotypes with regard to TSS to Acid ratio were reported by Kavitha *et al*. (2008) in banana, Rajasekhar (2009) in sapota and Bhupendra Singh Uniyal *et al.*(2012) in aonla.

4.3.21 Total phenols

All the variability parameters studied for the trait namely, phenotypic coefficient of variation (21.09%), genotypic coefficient of variation (9.97%), heritability (22.41%) and genetic advance as percent mean (9.72%) were found to be low. The estimates of PCV and GCV were low for this trait. Low heritability coupled with low genetic advance as per cent of mean observed for this trait indicates the presence of non-additive gene action in the inheritance and further selection for its improvement was ineffective.

4.3.22 Pectin

All the variability parameters studied namely, phenotypic coefficient of variation (24.63%), genotypic coefficient of variation (24.13%), heritability (96.00%) and genetic advance as percent mean (48.69%) were found to be high for the character.The high PCV and GCV of character indicated the presence of large amount of variation. The higher estimates of heritability for this trait suggested the simple selection would be more efficient on the basis of phenotypic performance of genotypes. The higher estimates of genetic advance indicated that this character was governed by additive genes and selection would be helpful for their improvement.

From the results presented in the aforesaid paragraphs it is evident that the characters, namely, fruit weight, seed number, seed weight, pulp to seed ratio, per cent fruit set, yield per tree, non-reducing sugars, ascorbic acid, TSS to acid ratio and pectins had recorded higher estimates for phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance as per cent mean.

4.4 CHARACTER ASSOCIATION STUDIES

Crop yield is the end product of the interaction of a number of other, often interrelated attributes. A thorough understanding of the interaction of characters among themselves great use in plant breeding. The efficiency of selection for yield mainly depends on the direction and magnitude of association between yield and its component characters and also among themselves. Character association provides information on the nature and extent of association between pairs of traits and helps in selection for the improvement of the character. The aim of correlation studies is primarily to know the suitability of various characters for indirect selection because selection of any particular trait may bring about undesirable changes in other associated characters. Phenotypic correlation is the association between two variables which can be directly observed. It includes both genotypic and environmental effects and therefore, it differs under different environmental conditions. Genotypic correlation is the inherent or heritable association between two variables. This type of correlation may be either due to pleiotropic action of genes or due to linkage or more likely both. This type of correlation is more stable and is of paramount importance for a plant breeder to bring about genetic improvement in one character by selecting the other character of a pair that is genetically correlated.

Phenotypic and genotypic correlation coefficients were worked out on yield and its component characters in thirty three genotypes of sapota. In general, genotypic correlations were higher than phenotypic correlations,which indicates that though there is strong inherent association between characters studied, its expression is lessened due to influence of environment. The genotypic and phenotypic correlation coefficients among yield and its component characters for morphological characters are presented in table-10 and for biochemical characters are presented in table-11.

4.4.1 Plant height

Plant height registered positive and significant correlation with fruit width (0.2166 P, 0.2815 G), fruit weight (0.1688 P, 0.1791 G), seed weight (0.1865 P, 0.1919 G), positive and non-significant correlation with leaf length (0.0475 P, 0.0648 G), leaf width (0.0926 P, 0.116 G), leaf area (0.0909 P, 0.1376 G), pulp to seed ratio (0.0655 P, 0.0665G) and per cent fruit set (0.0962 P, 0.0973 G). It had non significant negative correlation with ovary size (-0.1428 P, -0.1997 G) and seed number (-0.1092 P, -0.1189 G).

This trait exhibited significant positive association with fruit width, fruit weight and seed weight at both phenotypic and genotypic levels and the value of genotypic correlation coefficient was higher than phenotypic correlation coefficient, hence it is understood that there is a strong genetic association between these characters. The results are in cofirmity with the findings of Singh *et al*. (2001) in papaya, Kulkarni *et* *al.* (2006) in banana, Hazarika (2009) in aonla, Saraswathy *et al.* (2010) and Rekha*et al.* (2011) in sapota.

4.4.2 Leaf length

At both phenotypic and genotypic levels, leaf length had significant and positive correlation with leaf width (0.7806P, 0.9575G), leaf area (0.7504 P, 0.8335 G), seed weight (0.3527 P, 0.5206 G) and per cent fruit set (0.2098P, 0.2843 G). The correlation was positive and non-significant with ovary size (0.0265 P, 0.0641 G), fruit width (0.0588 P, 0.2740 G), fruit weight (0.0618 P, 0.1241 G), Non-significant positive correlation at phenotypic level and negative correlation at genotypic levels was found for characters like seed number (0.0064 P, -0.0396 G), while, non-significant negative correlation at phenotypic level and positive correlation at genotypic levels was found for characters like fruit length (-0.0346 P, 0.0572 G).This trait exhibited significant positive association with leaf width, leaf area, seed weight and per cent of fruit set at both phenotypic and genotypic levels and the value of genotypic correlation coefficient was higher than phenotypic correlation coefficient indicating that there was a strong genetic association between these characters. The results are in accordance with the reports of Maiti (2010) in jackfruit, Praveen and Patil (1998) in ber, Vashishtha *et al.* (2003) in pomegranate and Kumar *et al.* (2009) in guava.

4.4.3 Leaf width

This character exhibited positive and significant correlation with leaf area (0.7874 P, 0.9305 G), seed weight (0.4062 P, 0.5351 G), per cent fruit set (0.2216 P, 0.2979 G) at both phenotypic and genotypic levels. Its relationship with seed number(-0.0324 P, -0.065 G), pulp to seed ratio (-0.2341 P, -0.2965 G) was negative and non significant at both levels.The character fruit length recorded non significant negative correlation at phenotypic level and positive correlation at genotypic level (-0.0176 P, 0.0844 G). Similar results with regard to leaf width was reported earlier by Maiti (2010) in jackfruit, Praveen and Patil (1998) in ber, Vashishtha *et al.* (2003) in pomegranate and Kumar *et al.* (2009) in guava.

4.4.4 Leaf area

Leaf area had positive and significant correlation with seed weight (0.3591 P, 0.5999 G) and per cent fruit set (0.2442 P, 0.372 G) at both phenotypic and genotypic levels. Non-significant and negative correlation was noticed for ovary size (-0.0938 P, -0.0657 G), pulp to seed ratio (-0.1806 P, -0.277 G) at both phenotypic and genotypic levels.

This trait exhibited significant positive association with seed weight and per cent of fruit set at both phenotypic and genotypic levels and the value of genotypic correlation coefficient was higher than phenotypic correlation coefficient, it means that there was a strong genetic association between these characters.

4.4.5 Ovary size

The association of ovary size had positive and significant correlation with fruit weight (0.2185 P, 0.3971 G), seed weight (0.2236P, 0.3120 G), pulp to seed ratio (0.2188 P, 0.3121 G), where as its association was negative and non-significant with per cent fruit set (-0.1460 P, -0.1732 G) at phenotypic and genotypic level. Fruit weight (0.2185P, 0.3971G), seed number (0.0117 P, 0.0472 G) had positive and non-significant association at both levels.

4.4.6 Fruit length

This character recorded significant and positive correlation with fruit width (0.3645 P, 0.3406 G), fruit weight (0.4661 P, 0.5497 G), pulp to seed ratio (0.5419 P, 0.6449 G) and per cent fruit set (0.1857 P, 0.2206G), while, seed number recorded significant negative correlation (-0.2129 P, -0.2626 G) at both phenotypic and genotypic levels. Non-significant and positive correlation was recorded for the character seed weight (0.0538 P, 0.0659 G) at phenotypic and genotypic levels. This character showed significant positive association with fruit width, fruit weight, pulp to seed ratio and per cent fruit set at both phenotypic and genotypic levels and the value of genotypic correlation coefficient was higher than phenotypic correlation coefficient, it means that there was a strong genetic association between these characters. The results were in conformity with research findings of earlier workers, Singh *et al*. (2001) in papaya, Kulkarni *et al.* (2006) in banana, Hazarika (2009) in aonla, Saraswathy *et al*. (2010), Rekha*et al*. (2011) in sapota and Majumder *et al.* (2012) in mango.

4.4.7 Fruit width

The association of fruit width had positive and significant correlation with fruit weight (0.6804 P, 0.8088 G), seed weight (0.5137 P, 0.6864 G), pulp to seed ratio (0.4656 P, 0.5966 G), where as its association was negative and non-significant with seed number (-0.1088 P, -0.1421 G), per cent fruit set (-0.0683 P, -0.0765 G) at both phenotypic and genotypic levels. This trait showed significant positive association with fruit weight, seed weight and pulp to seed ratio at both phenotypic and genotypic levels and the value of genotypic correlation coefficient was higher than phenotypic correlation coefficient, it means that there was a strong genetic association between these characters. The results are in consonanace with the reports given by Singh *et al*. (2001) in papaya, Kavitha *et al.* (2008), Hazarika (2009) in aonla, Saraswathy *et al*. (2010), Rekha*et al*. (2011) in sapota, Majumder *et al.* (2012) in mango and Ganesh Shukla *et al*. (2012) in aonla.

4.4.8 Fruit weight

Fruit weight had positive and significant correlation with seed weight (0.6247 P, 0.6777 G) and pulp to seed ratio (0.7373 P, 0.7774 G) at both phenotypic and genotypic levels. Non-significant and negative correlation (-0.1361 P, -0.1499 G) was noticed for per cent fruit set, while seed number (0.0216 P, -0.0012 G) recorded non significant positive correlation and negative correlation at phenotypic and genotypic level respectively.This trait exhibited significant positive association with seed weight and pulp to seed ratio at both phenotypic and genotypic levels and the value of genotypic correlation coefficient was higher than phenotypic correlation coefficient, it means that there was a strong genetic association between these characters. The results are in accordance with the reports given by Singh *et al*. (2001) in papaya, Kavitha *et al.* (2008), Hazarika (2009) in aonla, Saraswathy *et al*. (2010), Rekha*et al*.

(2011) in sapota, Majumder *et al.* (2012) in mango and Ganesh Shukla *et al.* (2012) in aonla.

4.4.9 Seed number

At both phenotypic and genotypic levels, seed number had significant and positive correlation with seed weight (0.1665P, 0.1619G). The correlation wasnon-significant and negative for pulp to seed ratio (-0.0721 P, -0.0786 G) and percent fruit set (-0.199 P, -0.224 G) at phenotypic and genotypic levels.

4.4.10 Seed weight

Seed weight had positive and non-significant correlation with pulp to seed ratio (0.088 P, 0.0901 G) at both phenotypic and genotypic level and non-significant negative correlation (-0.1012 P, -0.1036 G) was recorded for percent fruit set at phenotypic and genotypic levels.

4.4.11 Pulp to seed ratio

The correlation was non-significant and negative for per cent of fruit set (-0.0628 P, -0.0634 G) at phenotypic and genotypic levels.

4.4.12 TSS

TSS registered positive and significant correlation with total sugars (0.7499 P, 0.9473 G), reducing sugars (0.5192 P, 0.6621 G), non-reducing sugars (0.2975 P, 0.3794 G) ascorbic acid (0.5338 P, 0.6547 G) and TSS to acid ratio (0.7529 P, 0.9142 G) at phenotypic and genotypic levels. Positive and non-significant correlation withpectin (0.0522 P, 0.0684 G) and significantly negative correlation was recorded with titrable acidity (-0.6044P, -0.7375 G) at phenotypic and genotypic levels. Phenols recorded (-0.191 P, -0.6015 G) negatively non-significant at phenotypic and negatively significant at genotypic level. This trait exhibited significant positive association with total sugars, reducing sugars, non-reducing sugars, ascorbic acid and TSS to acid ratio at both phenotypic and genotypic levels and the value of genotypic correlation coefficient was higher than phenotypic correlation coefficient, it means that there was a strong genetic association between these characters. The results are in accordance with the reports given by Jana *et al*. (2006) in papaya and Hazarika (2009) in aonla.

4.4.13 Total sugars

Total sugars had positive and significant correlation with reducing sugars (0.5448 P, 0.6104 G), non-reducing sugars (0.4687 P, 0.4988 G), ascorbic acid (0.6734 P, 0.727 G) and TSS to acid ratio (0.7204 P, 0.7745 G) at both phenotypic and genotypic levels. Non-significant and positive correlation (0.0322 P, 0.0344 G) was noticed for pectin, while titrable acidity (-0.5841 P, -0.6291 G) and phenols (-0.3015 P, -0.7351 G) were recorded significant negative correlation with total sugars at phenotypic and genotypic levels. This character showed significant positive association with reducing sugars, non-reducing sugars, ascorbic acid and TSS to Acid ratio at both phenotypic and genotypic levels and the value of genotypic correlation coefficient was higher than phenotypic correlation coefficient, it means that there was a strong genetic association between these characters. The results are in accordance with the reports given by Jana *et al*. (2006) in papaya, Kulkarni *et al*. (2006) in banana and Saraswathy *et al*. (2010) in sapota.

4.4.14 Reducing sugars

The association of reducing sugars had positive and significant correlation with ascorbic acid (0.5362 P, 0.5513 G), TSS to acid ratio (0.4716 P, 0.4849 G) and pectin (0.2225 P, 0.2320 G), where as its association was negative and significant with non-reducing sugars (-0.3871 P, -0.4038 G), titrable acidity (-0.3276 P, -0.3369 G) and phenols (-0.1207 P, -0.3017 G) at both phenotypic and genotypic levels. These results were in confirmity with the findings of earlier workers, Jana *et al*. (2006) in papaya, Saraswathy *et al*. (2010) in sapota, Mujumder *et al.* (2012) in banana and Ganesh Shukla *et al*. (2012) in aonla.

4.4.15 Non-reducing sugars

Non-reducing sugars registered positive and significant correlation with ascorbic acid (0.2371 P, 0.2399 G) and sugars to acid ratio (0.3976 P, 0.4015 G) at phenotypic and genotypic levels. Negative and non-significant correlation was recorded with titrable acidity (-0.413 P, -0.4186 G)and phenols (-0.2370 P, -0.5189 G) at phenotypic and genotypic levels. Pectins recorded (-0.1927 P, -0.2016 G) negatively non-significant correlation at phenotypic level and negatively significant at genotypic level. Similar results were repored by Jana *et al.* (2006) in papaya, Saraswathy *et al.* (2010) in sapota, Mujumder *et al.* (2012) in banana and Ganesh Shukla *et al.* (2012) in aonla.

4.4.16 Ascorbic acid

Ascorbic acid registered positive and significant correlation with sugars to acid ratio (0.5127 P, 0.5138 G), while positive and non-significant correlation with pectins (0.0783 P, 0.0792 G), at phenotypic and genotypic levels. Negative and significant correlation with titrable acidity (-0.4368 P, -0.4382 G) and phenols (-0.3189 P, -0.6531 G) was recorded at phenotypic and genotypic levels. Similar results were reported by Saraswathy *et al.* (2010) in sapota, Mujumder *et al.* (2012) in banana and Ganesh Shukla *et al.* (2012) in aonla.

4.4.17 Titrable acidity

The association of titrable acidity had positive and significant correlation with phenols(0.3011 P, 0.6433 G), positive and non-significant correlation with pectin (0.0278 P, 0.0279 G), where as its association was negative and significant with sugars to acid ratio (-0.9425 P, -0.9436 G) at both phenotypic and genotypic levels. This trait exhibited significant positive association with phenols at both phenotypic and genotypic levels and the value of genotypic correlation coefficient was higher than phenotypic correlation coefficient, hence can be inferred that there was a strong genetic association between these characters. The results are in accordance with the reports given bySingh *et al.* (2001) in papaya, Maiti *et al.* (2010) in jackfruit andRekha*et al.* (2011) in sapota.

4.4.18 TSS to Acid ratio

This character recorded negative and significant correlation with phenols (-0.2643 P, -0.5584 G), while positive and non-significant correlation with pectins (0.0311 P, 0.0318 G) at phenotypic and genotypic levels. Similar results were earlier reported by Kumar *et al.* (2009) in guava, Majumder *et al.* (2012) in banana and Rekha*et al.* (2011) in sapota.

4.4.19 Phenols

The correlationof phenols with pectin (-0.0019 P, -0.0488 G) was non-significant and negative at phenotypic and non-significant, positive at genotypic level.

Correlation coefficient analysis revealed that the association of plant height, leaf width, leaf area, fruit width, fruit weight, seed weight, pulp to seed ratio and per cent fruit set with yield/tree and among themselves was positive and highly significant and these traits were identified as fruit yield components. Similarly the association of TSS, total sugars, reducing sugars, non-reducing sugars, ascorbic acid and TSS to acid ratio with yield per tree and among themselves was positive and highly significant. This indicated possibility of simultaneous selection of all these characters for yield improvement.

4.5 PATH COEFFICIENT ANALYSIS

Path coefficient analysis takes into account the cause and effect relation between the variable and unique in partitioning the association into direct and indirect effects through other independent variables and it also measures the relative importance of casual factors involved. In order to obtain a clear picture of the inter-relationship between different characters, the direct and indirect effects of the important quantitative characters on the yield by using path coefficient analysis both at genotypic and phenotypic levels are discussed here under which were presented in Table-12 and Fig 6 for morphological characters and in Table-13 and Fig 7 for biochemical characters.

4.5.1 Plant height

The trait had genotypic moderate direct positive effect on yield/tree (0.2663) and correlation between the two traits was positive and significant (0.1225). It had positive and indirect influence on yield/tree via leaf length (0.1958), leaf area (0.0929), ovary size (0.0828), fruit length (0.0040), seed number (0.0623), seed weight (0.4472), pulp to seed ratio (0.110) and percent fruit set (0.0715) and negative, significant, indirect effects through leaf width (-0.4432), fruit width (-0.5101) and fruit weight (-0.2570). These results are in accordance with the earlier workers, Singh *et al.* (2001) in mango, Kulkarni *et al.* (2006) in banana, Maiti (2010) in jackfruit and Ganesh Shukla *et al*. (2012) in aonla.

4.5.2 Leaf length

This trait registered negligible direct positive genotypic effect on yield/tree (0.0217) and correlation between the two traits was positive, significant (0.3141). It exhibited positive indirect influence through plant height (0.0173), leaf area (0.5631), fruit length (0.0049), seed number (0.0208), seed weight (1.2130) and percent fruit set (-0.3755) and negative indirect influence through leaf width (-0.6590), ovary size (-0.0266), fruit width (-0.4965), fruit weight (-0.1781) and pulp to seed ratio (-0.3755). The results are in accordance with the reports of Praveen and Patil (1998) in ber, Vashishtha *et al.* (2003) in pomegranate,Kumar *et al.* (2009)in guavaand Maiti (2010) in jackfruit.

4.5.3 Leaf width

Leaf width exhibited high genotypic negative direct effect on yield/tree (-0.8220) and the correlation between the two traits was positive and significant (0.2982). It showed positive indirect influence on yield per tree through plant height (0.0309), leaf length (0.8932), leaf area (0.6286), ovary size (0.0166), fruit length (0.0072), seed number (0.0341), seed weight (0.247) and per cent fruit set (0.2188) and negative indirect effects via fruit width (-0.3128), fruit weight (-0.1528) and pulp to seed ratio (-0.4906). These findings are in accordance with Iyer *et al*. (1989), Kokadwar *et al*. (1992), Attri*et al.* (1999) in mango and Kavitha *et al.* (2008) in banana.

4.5.4 Leaf area

This character had genotypic high positive direct effect of 0.6756 on yield/tree and the correlation between the two characters was positive and significant (0.4357). It recorded positive indirect effects on yield per tree through plant height (0.0366), leaf length (0.5185), ovary size (0.0273), fruit length (0.0151), seed number (0.0015), seed weight (0.3978) and per cent fruit set (0.2733) and negative indirect effects through leaf width (-0.5532), fruit width (-0.3065), fruit weight (-0.1919) and pulp to seed ratio (-0.4584).

4.5.5 Ovary size

This trait showed genotypic moderate negative direct effect on yield/tree (-0.4147) and the correlation between the two characters was negative and significant (-0.2831). It exhibited positive indirect effets on yield per tree through leaf length (0.1935), leaf width (0.1529), fruit length (0.009), seed weight (0.7269) and pulp to seed ratio (0.5165) and negative indirect effects via plant height (-0.0532), leaf area (-0.0444), fruit width (-0.6481), fruit weight (-0.5698), seed number (-0.0246) and per cent fruit set (-0.1217).

4.5.6 Fruit length

This character had genotypic high positive direct effect of 0.5854 on yield/tree and the correlation between the two characters was positive and significant (0.2264). It had indirect positive influence on yield per tree through plant height (0.0125), leaf length (0.1727), leaf area (0.1196), seed number (0.1376), seed weight (0.1535), pulp to seed ratio (0.6549) and per cent fruit set (0.1621) and negative indirect effects through leaf width (-0.3226), ovary size (-0.0436), fruit width (-0.6172) and fruit weight (-0.7885). The results were in conformity with research findings of earlier workers, Singh *et al*. (2001) in papaya, Kulkarni *et al.* (2006) in banana, Hazarika (2009) in aonla and Majumder *et al.* (2012) in mango.

4.5.7 Fruit width

Fruit width exhibited high genotypic negative direct effect on yield/tree (-0.8122) and the correlation between the two traits was negative and non-significant (-0.1295). It showed positive indirect influence on yield per tree through plant height (0.0751), leaf length (0.8278), leaf area (0.1143), fruit length (0.0291), seed number (0.0744), seed weight (0.5994) and pulp to seed ratio (0.9874) and negative indirect effects via leaf width (-0.6596), ovary size (-0.1483), fruit weight (-0.1606) and per cent fruit set (-0.0562). The results are in consonanace with the reports given by Singh *et al*. (2001) in papaya, Kavitha *et al.* (2008), Hazarika (2009) in aonla, Saraswathy *et al*. (2010), Majumder *et al.* (2012) in mango and Ganesh Shukla *et al*. (2012) in aonla.

4.5.8 Fruit weight

This trait registered moderate positive direct genotypic effect on yield/tree (0.4350) and correlation between the two traits was negative, non-significant (-0.1511). It exhibited positive indirect influence through plant height (0.0477), leaf length (0.375), leaf area (0.0903), fruit length (0.0469), seed number (0.0006), seed weight (0.5843) and pulp to seed ratio (0.2865) and negative indirect influence through leaf width (-0.4069), ovary size (-0.1647) and per cent fruit set (-0.1101). The results are in accordance with the reports given by Singh *et al*. (2001) in papaya, Kavitha *et al.* (2008), Hazarika (2009) in aonla, Majumder *et al.* (2012) in mango and Ganesh Shukla *et al*. (2012) in aonla.

4.5.9 Seed number

Seed number exhibited high genotypic negative direct effect on yield/tree (-0.5239) and the correlation between the two traits was negative and non-significant (-0.1262). It showed positive indirect influence on yield per tree through leaf width (0.2485), fruit width (0.2574), fruit weight (0.0017) and seed weight (0.3773) and negative indirect effects via plant height (-0.0317), leaf length (-0.1197), leaf area (-0.0019), ovary size (-0.0195), fruit length (-0.0224), pulp to seed ratio (-0.1301) and per cent fruit set (-0.1619).

4.5.10 Seed weight

Seed weight exhibited moderate genotypic positive direct effect on yield/tree (0.3302)and the correlation between the two traits was negative and non-significant (-0.0409). It showed positive indirect influence on yield per tree through plant height (0.0511), leaf length (0.573), leaf area (0.4053), fruit length (0.0056) and pulp to seed ratio (0.1489) and negative indirect effects via leaf width (-0.0453), ovary size (-0.1294), fruit width (-0.2438), fruit weight (-0.9756), seed number (-0.0848) and per cent fruit set (-0.0761).

4.5.11 Pulp to seed ratio

This trait registered high positive direct genotypic effect on yield/tree (0.6549) and correlation between the two traits was negative, non-significant (-0.1339). It exhibited positive indirect influence through plant height (0.0177), leaf width (0.1331), fruit length (0.0551), seed number (0.0412), seed weight (0.2096) and pulp to seed ratio (0.6549) and negative indirect influence through leaf length (-0.6856), leaf area (-0.1871), ovary size (-0.1294), fruit width (-0.0813), fruit weight (-0.1155) and per cent fruit set (-0.0466).

4.5.12 Per cent fruit set

This character had genotypic high positive direct effect of 0.7347 on yield/tree and the correlation between the two characters was positive and significant (0.9461). It had indirect positive influence on yield per tree through plant height (0.0259), leaf length (0.8591), leaf area (0.2513), ovary size (0.0718), fruit length (0.0188), fruit width (0.1387), fruit weight (0.2151) and seed number (0.1155) and negative indirect effects through leaf width (-0.1384), seed weight (-0.2414) and pulp to seed ratio (-0.1049).

4.5.13 Total soluble solids

TSS exhibited moderate genotypic positive direct effect on yield/tree (0.2264) and the correlation between the two traits was positve and significant (0.2793). It showed positive indirect influence on yield per tree through total sugars (0.1764), reducing sugars (0.9837), non-reducing sugars (0.1467) and negative indirect effects via ascorbic acid (-0.8058), titrable acidity (-0.4418), TSS to acid ratio (-0.9413), total phenols (-0.4415) and pectins (-0.0634). The results are in accordance with the reports given by Jana *et al.* (2006) in papaya and Hazarika (2009) in aonla.

4.5.14 Total sugars

This trait exhibited moderate genotypic positive direct effect on yield/tree (0.2418) and the correlation between the two traits was positve and significant (0.3320). It showed positive indirect influence on yield per tree through TSS (0.2145), reducing sugars (0.9069), non-reducing sugars (0.1929) and negative indirect effects via ascorbic acid (-0.8948), titrable acidity (-0.3768), TSS to acid ratio (-0.7975), total phenols (-0.5396) and pectins (-0.0319). Similar results were reported by Karibasappa *et al.* (1999), Rathod (2007) in mango, Praveen and Patil (1998) in ber, Vashishtha *et al.* (2003) in pomegranate and Baruah *et al.* (2007) in banana.

4.5.15 Reducing sugars

Reducing sugars exhibited moderate genotypic positive direct effect on yield/tree (0.4858) and the correlation between the two traits was positive and significant (0.2724). It showed positive indirect influence on yield per tree through TSS (0.1499), total sugars (0.758) and negative indirect effects via non-reducing sugars (-0.1562), ascorbic acid (-0.6786), titrable acidity (-0.2018), TSS to acid ratio (-0.4992), total phenols (-0.2214) and pectins (-0.2151). Identical results in respect of reducing sugars were reported earlier by Iyer *et al.* (1989), Karibasappa *et al.* (1999) in mango, Praveen and Patil (1998) in ber and Meena and Roop Singh (2006) in pomegranate.

4.5.16 Non-reducing sugars

This trait exhibited moderate genotypic positive direct effect on yield/tree (0.3867) and the correlation between the two traits was negative and significant (-0.6614). It showed positive indirect influence on yield per tree through TSS (0.0859), total sugars (0.6195), pectin (0.1869) and negative indirect effects via reducing sugars (-0.6000), ascorbic acid (-0.2953), titrable acidity (-0.2508), TSS to acid ratio (-

0.4134) and total phenols (-0.3809). Similar results were reported by Jana *et al*. (2006) in papaya, Majumder *et al*. (2012) in banana and Ganesh Shukla *et al*. (2012) in aonla.

4.5.17 Ascorbic acid

Ascorbic acid exhibited moderate genotypic positive direct effect on yield/tree (0.2308) and the correlation between the two traits was negative and significant (-0.6122). It showed positive indirect influence on yield per tree through TSS (0.1482), total sugars (0.9028), reducing sugars (0.8191), non-reducing sugars (0.0928), and negative indirect effects via titrable acidity (-0.2625), TSS to acid ratio (-0.529), total phenols (-0.4794) and pectins (-0.0734). These resuluts are in contradictory with the findings of Kumar *et al.*(2009) in guava, Maiti (2010) in jackfruitm and Ganesh Shukla *et al.* (2012) in ber.

4.5.18 Titrable acidity

This trait exhibited high genotypic positive direct effect on yield/tree (0.5990) and the correlation between the two traits was positve and significant (0.9454). It showed positive indirect influence on yield per tree through ascorbic acid (0.5393), TSS to acid ratio (0.9715), total phenols (0.4722) and negative indirect effects via TSS (-0.1671), total sugars (-0.7813) reducing sugars (-0.5005), non-reducing sugars (-0.1619) and pectins (-0.0259). The results are in accordance with the reports given by Singh *et al.* (2001) in papaya and Maiti (2010) in jackfruit.

4.5.19 TSS to Acid ratio

TSS to acid ratio showed very low genotypic positive direct effect on yield/tree (0.0296) and the correlation between the two traits was positive and significant (0.3095). It showed positive indirect influence on yield per tree through TSS (0.207), total sugars (0.9618), reducing sugars (0.7204), non-reducing sugars (0.1552), and negative indirect effects via titrable acidity (-0.5652), total phenols (-0.4099) and pectins (-0.0295). Similar results were earlier reported by Kumar *et al.* (2009) in guava and Majumder *et al.* (2012) in banana.

4.5.20 Total phenols

This trait exhibited high genotypic positive direct effect on yield/tree (0.7341) and the correlation between the two traits was negative and significant (-0.5092). It showed positive indirect influence on yield per tree through ascorbic acid (0.8038), titrable acidity (0.3853), TSS to acid ratio (0.5749) and negative indirect effects via TSS (-0.1362), total sugars (-0.9129) reducing sugars (-0.4482), non-reducing sugars (-0.2007) and pectins (-0.0453).

4.5.21 Pectin

This trait exhibited high genotypic negative direct effect on yield/tree (-0.9271) and the correlation between the two traits was negative and non-significant (-0.1409). It showed positive indirect influence on yield per tree through TSS (0.0155), total sugars (0.0427), reducing sugars (0.3447), titrable acidity (0.0167), phenols (0.0359) and negative indirect effects via non-reducing sugars (-0.0782), ascorbic acid (-0.0975) and TSS to acid ratio (-0.0327).

The association of different component characters among themselves and with yield is quite important for devicing an efficient selection criterion for yield. The total correlation between yield and it'scomponent characters may be some times misleading, as it might be an over-estimate or under-estimate because of its association with other characters. Hence, indirect selection by correlated response may not be some times fruitful. When many characters are affecting a given character, splitting the total correlation into direct and indirect effects of cause as deviced by Wright (1921) would give more meaningful interpretation to the cause of association between the dependent variable like yield and independent variables like yield components. This kind of information will be helpful in formulating the selection criteria. The selection for these characters is likely to bring about on overall improvement in single plant yield directly.

4.6 GENETIC DIVERGENCE

For a successful breeding programme, the diversity of parents is of utmost importance, since the crosses made between the parents with maximum genetic divergence are more likely to yield desirable recombinants in the progenies. Hence, it is desirable to select suitable genetically divergent parents based on information about the genetic variability and genetic diversity present in the available germplasm.

The multivariate analysis using Mahalanobis' D^2 statistic provides auseful statistical tool for measuring the genetic diversity in germplasm collections with respect to the characters considered together. It also provides a quantitative measure of association between geographic and genetic diversity based on generalized distance (Mahalanobis, 1936). Further, the problem of selecting diverse parents for hybridization programme can be narrowed, if one can identify the characters responsible for the discrimination between the populations.

The data collected on quantitative characters (both morphological and bio-chemical)*viz.,*plant height, leaf length, leaf width, leaf area, ovary size, fruit length, fruit width, fruit weight, seed number, seed weight, pulp to seed ratio, yield per tree, per cent fruit set, total soluble solids, total sugars, reducing sugars, non-reducing sugars, ascorbic acid, titrable acidity, sugars to acid ratio, phenols and pectin for 33 genotypes of sapota were subjected to multivariate analysis by using Mahalanobis D^2 statistic for quantitative assessment of genetic divergence contributing characters. D^2values were calculated for 528 possible pairs of combinations (n(n-1)/2) from means of 33 genotypes for 22 characters.

4.6.1Mahalanobis D^2 analysis

4.6.1.1 *Wilk's* 'v'criterion test

Significant differences among the genotypes for individual characters were first determined and later the statistical significant differences between the genotypes based on the pooled effects of all the characters were carried out using the *Wilk's criterion* $^{\text{'}\wedge\text{'}}$.

The *Wilk's* criterion thus obtained was used in calculations of 'V' statistic. The statistic 3155.4 was highly significant (more than the tabulated χ^2 value) indicating that genotypes differed significantly when all the characters were considerd simultaneously.

4.6.1.2 Relative contribution of characters towards genetic divergence

The percent contribution of various characters towards genetic divergenceis presented in table-14 and fig-8). The maximum contribution towards genetic divergence is by plant height (38.72%), yield/tree (14.19%), per cent fruit set (7.07%), fruit length (5.46%), fruit width (4.59%), fruit weight (4.28%), seed number (3.83%), leaf length (3.05%), leaf width (2.11%), leaf area (2.08%), seed weight (2.14%) and total soluble solids (2.25%). The characters viz., pulp to seed ratio (1.65%), total sugars (1.87%), reducing sugars (1.23%), non-reducing sugars (1.86%), ovary size (1.07%), ascorbic acid (0.54%), titrable acidity (0.25%), sugars to acid ratio (0.62%), phenols (0.18%) and pectin (0.96%) contributed very less towards genetic divergence in the sapota genotypes under the study.The results are in accordance with the reports given by Singh (2005), Barhate *et al.* (2012) in mango, Sharma *et al.* (2006) in jackfruit and Verma *et al.* (2012) in pomegranate.

4.6.1.3 Grouping of genotypes into various clusters

Thirty three genotypes were grouped into eight clusters based on D^2 values using the Tocher's method (Rao, 1952) such that the genotypes belonging to same cluster had an average smaller D^2 values than those belonging to different clusters. The distribution of genotypes into various clusters is shown in table-15 and Fig.10. Out of eight clusters, cluster III was largest comprising of eight genotypes (Gavarayya, Gutti, Calcutta Round, PKM-3, Singapore, Dwarapudi,Simhoudi, and DHS-1) followed by Cluster II with six genotypes (Pakala Round, CO-1, CO-2, Pakala, CO-3 and Pakala Oval), while cluster I had six genotypes (Cricket Ball, Hybrid, Badami, Krishna Rao, Seedless, Columbian Sapota), Cluster V four genotypes (Brazilian Sapota, DHS-2, Ulvapadu Selection and Tagarampudi) and cluster IV (Pala, PKM-1 and Kirthibarthi) and VII (Virudnagar, PKM-2 and Kalipatti) had three genotypes each, cluster VI with two genotypes (Bombay and

Mirandi) followed by cluster VIII which had a single genotype (PKM-4) indicating high degree of heterogeneity among the genotypes.

This suggests forces such as exchange of breeding material, natural and artificial selection, genetic drift, migration, gene flow and variation in environment may be responsible for this diversity. These findings are in agreement with the reports of Kumar *et al.* (2006), Rajan (2009) and Majumder *et al.* (2012) in mango, Rajamanickam and Rajmohan (2010) in banana and Singh *et al.* (2013) in straw berry

4.6.1.4 Average inter and intra cluster distances

The inter-cluster D^2 values ranged from 42.68 (cluster I and III) to 459.86 (cluster VII and VIII). The maximum inter cluster distance (459.86) was observed between VII and VIII clusters followed by clusters VI and VII (398.84) and cluster VI and VIII (364.33) suggesting that the crosses involving varieties from these clusters would give desirable recombination. While, the minimum inter cluster distance of 42.68 was recorded between cluster I and III, followed by, cluster I and II (70.62), cluster I and IV (70.71) and cluster II and V (94.75) indicating that genotypes of these clusters had maximum number of gene complexes.

The average inter and intra cluster D^2 values are presented in Table-16 and Fig 10. Intra-cluster D^2 values ranged from zero (cluster VIII) to 95.64 (cluster III). Maximum intra cluster distance was observed in cluster III (95.64), followed by cluster V (81.91), cluster VII (78.68), cluster II (32.12), cluster I (27.89), cluster VI (25.82) and cluster IV (20.15) indicating that some divergence still existed among the genotypes. Promising genotypes included in cluster III that had maximum intra cluster distance are Gavarayya, Gutti, Calcutta Round, PKM-3, Singapore, Dwarapudi, Simhoudi and DHS-1 which are highly divergent among themselves. This could be made use in the yield improvement through recombination breeding.

Genetic diversity is the most important tool to select prospective parents in crop improvement programme. The genotypes from the clusters which were separated by high estimated distance could be utilized in hybridization programme for obtaining wide variation among segregants.

It is assumed that maximum amount of heterosis will be manifested in cross combinations involving the parents belonging to most divergent clusters. The greater the distance between two clusters, the wider the genetic diversity between the genotypes. Keeping this in view, it is indicated that wider the genetic diversity between the cluster VII (Virudnagar, PKM-2, Kalipatti) and cluster VIII (PKM-4), cluster VI (Bombay, Mirandi) and cluster VII (Virudnagar, PKM-2, Kalipatti) and cluster VI (Bombay, Mirandi) and cluster VIII (PKM-4) would produce encouraging results. The genotypes of these clusters may be used as parents in the crossing programme to generate breeding material with high diversity.

Genotypes grouped into the same cluster presumably differ little from one another as the aggregate of characters were 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 could be practiced.

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

4.6.1.5 Mean performance of characters in clusters

Cluster means indicate average performance of all varieties clubbed in a cluster. The cluster mean values for all the characters under study are presented in Table-17.

The maximum plant height was recorded in cluster IV (6.50) followed by cluster III (5.92), while minimum plant height was reported in cluster VI (4.12) followed by cluster VIII (4.81) and cluster I (5.45).

The maximum leaf length was observed in cluster III (10.55) followed by cluster I (10.36), cluster V (10.22) and cluster IV (10.11), while minimum leaf length was reported in cluster II (9.39) preceded by cluster VII (9.82).

The maximum leaf width was noticed in cluster VIII (4.30) followed by cluster I (4.02), while minimum leaf widthwas reported in cluster VI (3.23) followed by cluster II (3.49) and cluster VII (3.62).

The maximum leaf area was noticed in cluster VIII (32.42) followed by cluster III (29.03), while minimum leaf area was reported in cluster VI (24.42) followed by cluster VII (25.47) and cluster II (25.97).

The maximum ovary size was observed in cluster V (4.12) followed by cluster III (4.08), while minimum ovary size was noticed incluster IV (3.63) preceded cluster VI (3.82).

The maximum fruit length was observed in cluster VIII (8.39) followed by cluster VII (6.83), while minimum fruit length was reported in cluster IV (5.97) followed by cluster VII (6.27) and cluster I (6.30).

The maximum fruit width was recorded in cluster II (6.76) followed by cluster III (6.38), while minimum fruit width was reported in cluster IV (6.05) followed by cluster VIII (6.09) and cluster VI (6.17).

The maximum fruit weight was observed in cluster I (194.52) followed by cluster VIII (181.64), while minimum fruit weight was reported in cluster IV (129.42) followed by cluster VI (134.32) and cluster III (135.65).

The maximum seed number was noticed in cluster I (5.83) followed by cluster VI (5.5), while minimum seed number was reported in cluster VII, cluster VIII (4.00) followed by cluster II (4.17) and cluster III (4.29).

The maximum seed weight was observed in cluster I (4.46) followed by cluster V (4.35), while minimum seed weight was recorded in cluster VI (2.93) followed by cluster IV (3.39) and cluster II (4.08).

The maximum pulp to seed ratio was observed in cluster VI (42.30) followed by cluster II (38.62), while minimum pulp to seed ratio was reported in cluster III (28.87) followed by cluster IV (34.68).

Highest yield per tree was observed in cluster VII (133.21) followed by cluster VIII (111.81), while lowest yield per tree was noticed in cluster VI (38.55) followed by cluster I (48.13) and cluster III (54.99).

The maximum per cent fruit set was observed in cluster VIII (22.60) followed by cluster VII (21.34), while minimum per cent fruit set was reported in cluster VI (9.71) followed by cluster I (10.79) and cluster III (11.77).

The maximum TSS was recorded in cluster VIII (24.57) followed by cluster IV (23.69), while minimum TSS was reported in cluster VI (19.02) followed by cluster I (20.09) and cluster II (21.08).

The maximum total sugars was noticed in cluster VIII (11.99) followed by cluster IV (10.94), while minimum total sugars was reported in cluster VI (8.60) followed by cluster I (9.21).

The maximum reducing sugars was observed in cluster VII (9.54) followed by cluster VIII (9.00), while minimum reducing sugars was reported in cluster I (7.31) followed by cluster VI (7.61) and cluster III (7.97).

The maximum non-reducing sugars was recorded in cluster VIII (2.98) followed by cluster IV (2.37) and cluster III (2.33), while minimum non-reducing sugars was reported in cluster VI (0.98) followed by cluster I (1.88) and cluster V (2.08).

The maximum ascorbic acid was noticed in cluster VIII, cluster VII both (10.50) followed by cluster IV (9.47), while minimum ascorbic acid was reported in cluster VI (6.16) followed by cluster I (7.22) and cluster V (8.31).

The maximum titrable acidity was observed in cluster VI (0.29) followed by cluster I (0.26) and cluster II (0.23), while minimum titrable acidity was reported in cluster VIII (0.17) followed by cluster IV (0.18) and cluster VII (0.19).

The maximum TSS to acid ratio was observed in cluster VIII (149.88) followed by cluster IV (135.10), cluster VII (122.96), while minimum TSS to acid ratio was reported in cluster VI (65.82) followed by cluster I (77.99) and cluster II (90.37).

The maximum phenols was recorded in cluster VI (0.57) followed by cluster VIII (0.53) and cluster I (0.52), while minimum phenols was reported in cluster V (0.42) followed by cluster IV (0.43) and cluster VII (0.44).

The maximum pectin was noticed in cluster VII (2.34) followed by cluster VI (2.29) and cluster III (2.07), while minimum pectin was reported in cluster VIII (1.66) followed by cluster V (1.75) and cluster I, IV both (1.83).

The clusters means for each of the characters studied are presented in Table-17. From the data it can be seen that considerable differences exist for all the characters under study. Cluster VIII had high mean value for leaf width, leaf area, fruit length, per cent fruit set, TSS, total sugars, non-reducing sugars, ascorbic aid and TSS to acid ratio ; cluster I had high mean value for fruit weight, seed number and seed weight ; cluster VI had high mean value for pulp to seed ratio and phenols and cluster VII had high mean value for yield per tree, reducing sugars and phenols. The result indicates that selection of genotypes having high values for particular trait could be made and used in the hybridization programme for improvement of that character.

The genotypes PKM-4, Cricket Ball, Hybrid, Badami, Krishna Rao, Seedless, Columbian Sapota, Virudnagar, PKM-2, Kalipatti, Bombay and Mirandifrom these clusters have high mean values for the characters listed in table which may be directly used for adaptation or may be used as parents in future hybridization programme.

4.6.2 PRINCIPAL COMPONENT ANALYSIS

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

Inprincipal component analysis, the number of variables are reduced to a linear expression called the principal components and these are accounted for most of the variation produced.

Principal component analysis (PCA) for fruit morphological and bio-chemical characters was performed by adopting correlation matrix method. The character loading values for principal components represented the weights defining the contribution of different characters for the respective principal components. Further, the loading signs(+ / -) indicatethe 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 fruit morphological and bio-chemical characters studied are furnished in table-18. The principal components with eigen values less than one were considered to be non-significant as per the procedure. In the present investigation, six principal components with eigen values more than one contributed to 78.69 per cent of cumulative variability among the 33 accessions of sapota evaluated for 22 morphological and bio-chemical characters.

The first principal component (PC I) contributed highest towards variability (26.74%). The character loading values for principal components represented the weights defining the contribution of different characters for the respective principal components (Table-19). Characters like plant height (0.15), leaf length (0.15), leaf width (0.19), leaf area (0.22), fruit length (0.15), fruit width (0.05), fruit weight (0.03), seed weight (0.07), per cent fruit set (0.23), yield per tree (0.45), TSS (0.35), total sugars (0.36), reducing sugars (0.22), non-reducing sugars (0.18), ascorbic acid (0.33), TSS to acid ratio (0.35) and pectin (0.01) showed positive loadings coupled with negative loadings for ovary size (-0.01), seed number (-0.19), pulp to seed ratio (-0.04), titrable acidity (-0.32) and phenols (-0.29)explained the maximum variance in the first principal component (PC_1). This suggests that this component reflects yield per tree, total sugars, TSS, ascorbic acid, titrable acidity, TSS to acid ratio, phenols, per cent fruit set of each accession.

The second principal component (PC_2), which described 17.15% of the total variance reflected significant loadings of fruit weight (0.45), fruit width (0.43), seed weight (0.42), leaf length (0.26), pulp to seed ratio (0.27) and yield per tree (-0.27) were noted to explain maximum variability. Thissuggests that this component reflects fruit weight, fruit width and seed weight of each accession.

The third principal component (PC_3) contributed 13.68 per cent of total variance and characterized by conspicuously high loadings of pulp to seed ratio (0.43), leaf width (0.41), leaf length (0.40), leaf area (0.36), fruit length (0.31), which were positively correlated. This suggests that this component reflects pulp to seed ratio, leaf width, leaf length, leaf area, fruit length of each accession.

The fourth principal component (PC_4) contributed 8.49 per cent of total variance and characterized by conspicuously high loadings of non-reducing sugars (-0.58), reducing sugars (0.47), pectins (0.40), seed number (0.26), plant height (0.25) were noted to explain the variability and it suggests that this component reflects non-reducing sugars, reducing sugars, pectins of each accession.

The fifth principal component (PC_5) contributed 7.00 per cent of total variance and characterized by conspicuously high loadings of plant height (-0.48), pectins (-0.36), per cent fruit set (0.34), titrable acidity (0.30), seed weight (-0.30) and it suggest that this component reflects plant height, pectins, per cent fruit set, titrable acidity and seed weight of each accession.

The sixth principal component (PC_6) contributed 5.61 per cent of total variance and characterized by conspicuously high loadings of ovary size (0.51), seed number (0.48), plant height (-0.37), TSS (0.36) were noted to explain the variability and it suggest that this component reflects ovary size, seed number, plant height and TSS of each accession.

Uma *et al.* (2000) in banana, Rajasekhar *et al.* (2011) in sapota, Verma *et al.* (2012) in pomegranate, Majumder *et al.* (2012) in mango and Singh *et al.* (2013) in strawberry studied the utilization of principal component analysis in genetic divergence studies in different fruit crops.

4.6.2.1 Cluster analysis

The principal component scores of genotypes were used as input for clustering procedures in order to group the genotypes into various clusters and to confirm the results of principal component analysis.

To group the genotypes into various clusters, hierarchical cluster analysis was followed. Principal component scores were used as variables instead of attributes for clustering procedures, making the results equivalent to those from initially standardized data as the correlation matrix was used for principal component analysis.

Agglomerative hierarchical clustering method *i.e.,* Ward's method was followed to group the entries into different clusters based on Euclidean^2 distance (dissimilarity coefficient).The tree like structure called dendrogram was constructed based on Euclidean^2 distance computed from PCA scores of genotypes by utilizing the Ward's method (Fig.11). Utilization of principal component analysis combined with clustering by Ward's method in genetic diversity studies was reported by Rajasekhar (2009) in sapota. The 33 genotypes were grouped into six clusters and their distribution is presented in Table-20. Clusters II was largest comprising of 10 genotypes(Brazilian Sapota, Kalipatti, Kirthibarthi, Pala, CO-2, CO-3, DHS-1, DHS-2, PKM-3 and PKM-4) followed by cluster I comprising 8 genotypes (Badami, Bombay, Pakala, Pakala Round, Pakala Oval, Simhoudi, Ulvapadu Selection, Seedless), clusterIII consisting 7 genotypes (Calcutta Round, Gavarayya, Gutti, Mirandi, Singapore, Thagarampudi and CO-1),cluster IV having six genotypes (Dwarapudi, Hybrid, Krishna Rao, Virudhnagar, PKM-1 and PKM-2), and cluster V and cluster VI consistingone genotype each Cricket Ball and Columbian Sapota respectively. The pattern of distribution of genotypes into different clusters with different divergence values was at random supporting that geographical diversity is 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. These results are in agreement with the findings of earlier workers, Rajasekhar *et al.* (2011) in sapota, Verma *et al.* (2012) in pomegranate, Majumder *et al.* (2012) in mango, Singh *et al.* (2013) in strawberry and Srivastava *et al.* (2014) in sweet cherry.

4.6.2.2 Average inter and intra cluster Euclidean^2 distance values

The average intra and inter cluster Euclidean^2 distances estimated based on Ward's minimum variance dendrogram (Anderberg, 1993) are presented in the Table-21. The mutual relationships between clusters are represented diagrammatically (Fig-13) by taking average intra and inter cluster Euclidean^2 distances.

The mean inter and intra cluster distances among six clusters were presented in the Table-22 and Fig13.Intra cluster Euclidean^2 distance values ranged from 0.00 to 1523.80. The maximum intra cluster distance (984.02) was observed in cluster IV followed by cluster III (724.99), cluster II (403.44) and cluster I (216.32). The intra cluster distance values were zero in case of cluster V and VI which has single genotype.

The highest inter cluster Euclidean^2 distancevalue was recorded between clusters III and VI (1523.80) while the lowest inter clusterEuclidean^2 distance value was recorded between cluster I and II (456.51). Based on these studies crosses may be made between the genotypes of cluster III (Calcutta Round, Gavarayya, Gutti, Mirandi, Singapore, Tagarampudi and CO-1) and cluster VI (Columbian Sapota) and cluster II (Brazilian Sapota, Kalipatti, Kirthibarthi, Pala, CO-2, CO-3, DHS-1, DHS-2, PKM-3 and PKM-4) and cluster VI (Columbian Sapota). Thus, it can be inferred that genotypes in these clusters are genetically diverse and may give rise to high heterotic response. Hence the crosses between the genotypes falling in cluster III and cluster VI, cluster II and cluster VI could be exploited by hybridization programme for obtaining the desirable segregants with high potential.

The highest intra cluster Euclidean^2 distancevalue was observed in cluster IV (984.02) indicates the presence of wide genetic diversity among the genotypes *viz.*, Dwarapudi, Hybrid, Krishna Rao, Virudnagar, PKM-1 and PKM-2, while the lowest intra cluster D^2 value was observed in cluster I (216.32) with 8 cultivars *viz.*,Badami, Bombay, Pakala, Pakala Round, Pakala Oval, Simhoudi, Ulvapadu Selection and Seedless.

Genetic diversity is the most important tool to select prospective parents for crop improvement programme. The genotypes belonging to the clusters separated by high estimated distance could be utilized in hybridization programme for obtaining wide variation among segregants.

4.6.2.3 Mean performance of characters in clusters

The cluster means in respect of 22 characters and overall character wise score across the 6 clusters are presented in the table-22. Cluster means for 22 characters in the present study revealed the presence of considerable variation among the clusters.

Cluster mean for plant heightranged from 6.56 to 4.92. The maximum plant height was observed in cluster VI (6.56) followed by cluster IV (5.92), while minimum plant height was reported in cluster I (4.92).

With regard to leaf length, cluster means ranged from 13.81 to 9.16. Genotypes of cluster V had maximum leaf lenth with mean 13.81, followed by cluster II (10.38), while the genotypes of cluster I had minimum leaf length with mean 9.16, followed by cluster III(9.43).

Cluster mean values for leaf width raged from 5.87 to 2.80. The genotypes lying in cluster V (5.87) had maximum leaf width, while genotypes in cluster III (2.80) had minimum leaf width followed by cluster I (3.33).

With respect to leaf area, cluster mean values varied from 40.17 to 22.54. The genotypes of cluster VI (40.17) had maximum leaf area, followed by cluster II (29.56), while minimum leaf area was reported in cluster I (22.54) followed by cluster VI (23.35).

For ovary size, the cluster mean values ranged from 4.49 to 3.64. Maximum ovary size was observed in the genotypes of cluster IV (4.49) followed by cluster

VI(4.27), while minimum ovary size was reported in cluster III (3.64) preceded by cluster II (3.93).

Fruit length cluster means ranged from 7.91 to 5.32. The genotypes lying in cluster VI had maximum fruit length 7.91 followed by cluster III (7.47), while minimum fruit length was reported in the genotypes falling under cluster V (5.32) followed by cluster IV (5.45).

In case of fruit width, the cluster means ranged from 8.20 to 5.65. Genotypes of cluster VI showed maximum fruit width of 8.20 followed by cluster V (7.34), where as the genotypes of cluster I exhibited minimum fruit width of 5.65 followed by cluster IV (5.65).

The cluster mean for fruit weight ranged from 540.32 to 105.40. The genotypes lying in cluster VI showed characterstic fruit weight 540.32 followed by cluster IV (221.11), while the genotypes fall under cluster IV exhibited least fruit weight of (92.70) followed by cluster I (105.44).

The cluster meanvalues for seed number varies from 6.67 to 4.20. Maximum seed number was observed in cluster III (6.67) followed by cluster IV and V (6.33), while minimum seed number was reported in cluster II (4.20) followed by cluster I (5.00).

With regard to seed weight, the cluster mean values ranged from 7.81 to 2.02. Maximum seed weight was observed in cluster IV (7.81) followed by cluster VI (7.07), while minimum seed weight was reported in cluster III (2.02) followed by cluster I (3.07) and cluster II (4.24).

Cluster mean values for pulp to seed ratio ranged from 75.67 to 17.27. Maximum pulp to seed ratio was observed in the genotypes of cluster VI (75.67) followed by cluster III (61.50), while minimum pulp to seed ratio was reported in cluster IV (17.27) followed by cluster V (28.81).

For yield per tree, cluster mean values ranged from 91.97 to 22.06. The genotypes lying in cluster V (91.97) had registered highest yield per tree followed by cluster II (90.09), while lowest yield per tree was reported in cluster IV (22.06) followed by cluster I (48.96).

Per cent fruit set mean values ranged from 16.19 to 8.62. Maximum per cent fruit set was observed in the genotypes come under cluster II (16.19) followed by cluster V (16.03), while minimum per cent fruit set was reported in cluster VI (8.62) followed by cluster IV (9.47) and cluster I (11.92).

The cluster mean values for TSS varies from 22.65 to 18.60. The genotypes lying in cluster II had noticed maximum TSS 22.65 followed by cluster IV (22.23), while minimum TSS was reported in the genotypes fall under cluster VI (18.6) followed by cluster V (19.90).

Total sugars cluster mean values ranged from 10.64 to 8.61. Genotypes of cluster II had maximum total sugars with mean value of 10.64, followed by cluster I (9.52), while the genotypes of cluster VI had minimum total sugars with mean value of 8.61, followed by cluster IV (8.93).

With regard to reducing sugars, the cluster mean values ranged from 8.45 to 6.23. Highest mean value for reducing sugars was observed in cluster II (8.45) followed by cluster III (8.04), while lowest mean value for reducing sugars was reported in cluster IV (6.23) followed by cluster VI (6.60) and cluster V (6.87).

For non-reducing sugars, the cluster mean values ranged from 2.77 to 0.93. Highest mean value for non-reducing sugars was observed in cluster IV (2.77) followed by cluster II (2.31), while lowest mean value for non-reducing sugars was reported in cluster III (0.93) followed by cluster I (1.66) and cluster VI (2.01).

Cluster meanvalues for ascorbic acid ranged from 9.89 to 5.35. Maximum ascorbic acid was observed in the genotypes of cluster II (9.89) followed by cluster VI (7.69), while minimum ascorbic acid was reported in cluster IV (5.35) followed by cluster V (6.22).

With respect to titrable acidity, the cluster mean values ranged from 0.295 to 0.200. Maximum titrable acidity was observed in the genotypes come under cluster III

(0.295) followed by cluster V (0.252), while minimum titrable acidity was reported in cluster III (0.200) followed by cluster IV (0.202) and cluster VI (0.245).

The cluster mean values for TSS to acid ratio varies from 114.60 to 65.76. The genotypes lying in cluster II had noticed maximum TSS to acid ratio of 114.60 followed by cluster IV (110.00), while minimum TSS to acid ratio was reported in the genotypes fall under cluster III (65.76) followed by cluster VI (75.91).

In case of phenols the cluster means ranged from 0.611 to 0.425. Genotypes of cluster III showed maximum mean value for phenols 0.611 followed by cluster VI (0.551), where as the genotypes of cluster V exhibited minimum mean value of 0.425 followed by cluster II (0.440).

Pectincontent cluster mean values were ranged from 2.86 to 1.34. Maximum pectin content was observed in the genotypes come under cluster III (2.86) followed by cluster V (2.61), while minimum pectin content mean value was reported in cluster VI (1.34) followed by cluster I (1.83) and cluster II (1.96).

4.6.2.4 Comparative study of D^2analysis, principal component analysis and cluster analysis

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

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 which contributed towards maximum variability of the genotypes. In PCA, standardization of data made attributes to contribute equally towards the divergence studies irrespective of the units taken.

On grouping 33 genotypes, 8 clusters were formed using D^{2}stastic and six clusters were formed using PCA respectively. Regarding clustering pattern, maximum number of genotypes 8 for cluster III, followed by cluster II (6), cluster I (5), cluster V (4), cluster IV, VII (3), cluster VI (2) and single genotype for cluster VIII in case of D^2 statistic. While, Cluster II was largest comprising of 10 genotypes followed by cluster I (8), clusters III (7), cluster IV (6) and single genotype each in cluster V and VI in case of principal component analysis. The lowest number of genotypes were observed in cluster VIII (1) in case of D^2 statistic. While cluster V (1) and VI (1) in case of principal component analysis.

The pattern of distribution of genotypes into different clusters was at random. Furthermore, the two clustering methods grouped 33 genotypes differently and the clustering pattern was observed to be similar. There was one solitary cluster (cluster VIII) formed in D^2 analysis, whereas in Ward's minimum variance method cluster V and VI represented as solitary clusters. Genetic diversity was the outcome of interaction of several factors along with geographical diversity. Hence, the selection for hybridization should be more based on genetic diversity rather than geographic diversity.

The intra-cluster distances was maximum in case principal component analysis, which is 984.2 (cluster IV). When compared to of D^2 statistic *i.e.,*81.91 (cluster V). The same pattern observed in case of inter-cluster distances, which is maximum in between clusters III and VI (1523.2). When compared to D^2 statistic clusters VII and VIII (459.86) the differences in cluster mean values are more or less similar in both D^2 statistic and principal component analysis.

Ward's minimum variance dendrogram (cluster analysis) created subgroup within a cluster, and hence relative position of genotypes within the clusters could be examined by seeing the dendrogram distance. In case of D^2 analysis, one could know only the intra-cluster distance but not relative position of genotypes in the respective cluster.

The principal component analysis sorted out the total 22 characters into three main principal components. The contribution of the main characters for variance is easily identified by the characters loaded on the PC_1 as it explained maximum variance. By PCA, the in-depth analysis for genetic diversity can be made. In D^2 analysis, the characters *viz.*, leaf width, leaf area, fruit length, percent fruit set, TSS, total sugars, non-reducing sugars, ascorbic aid and TSS to Acid ratio are more for the divergence. In PCA, the characters *viz.*, yield per tree, total sugars, TSS, ascorbic acid, titrable acidity, TSS to Acid ratio, phenols and per cent fruit set in PC_1 contributing more towards variability.

4.7 MOLECULAR CHARACTERIZATION

Molecular diversity among 33 genotypes of sapota was studied by using RAPD and SSR markers. Indexing the variability in germplasm accessions and breeding stocks had become increasingly important for research in both genetics and breeding programme. Such information could be obtained by using an array of molecular techniques, even though they differ technically, but possess fewer relative advantages over one another. The information obtained by using molecular markers like RAPD and SSRs offer many benefits for identifying variation and for establishing diversity among the accessions. Molecular markers are used for assessment of genetical similarity among accessions during early development stages which helpful in divergence studies (Nicolosi *et al.*, 2000). In the present study, in addition to morphological and bio-chemical traits, RAPD and SSR markers were also used to estimate the extent of molecular diversity among sapota genotypes. All the 33 sapota genotypes were evaluated for their genetic diversity at molecular level using 24 Random Amplified Polymorphic DNA (RAPD) markers and 22 Simple Sequence Repeat (SSR) markers.

4.7.1 DNA yield and purity assessment

The DNA was isolated from young healthy leaves of 33 sapota genotypes by using the modified CTAB method**.** This protocol resulted in white translucent DNA pellets that were easily solubilized in $T_{10}E_1$ (Tris EDTA) buffer. The DNA resulted by this method was homogeneous and not degradable. The purity of fragment size was verified with Nano-drop spectrophotometer and also by agrose gel electrophorosis.The results indicated that the isolated DNA was of good quality and had high molecular weight (Plate 3).

The genomic DNA was extracted from all the 33 genotypes and the yield of DNA ranged from 352.00 to 2530.00ng/µl. The DNA yield (ng/µl) and the ratio of OD values (260/280) for all the genotypes are presented in Table-23. The highest DNA concentration (2530.00 ng/µl) was observed in PKM-1 where as the lowest DNA concentration (352.00 ng/µl) was observed in Gavarayya.The purity of DNAwasdetermined byratio ofOD values at 260 and 280 nm. The ratio of 1.60 or less indicated the protein contamination, while values exceeding the ratio of 1.80 indicate the presence of RNA (Sambrook *et al.,* 1989). The purity of DNA sapota genotypes used in the present study ranged from of 1.69 to 2.02 which indicate that the DNA obtained from this protocol was free of contaminants.

4.7.2 RAPD analysis

In addition to morphological characterization, RAPD markers have been successfully applied for cultivar identification and genetical analysis in a wide range of plant species. Out of 100 primers screened in this study, the RAPD pattern of 24 primers were produced amplified fragments were scored for analysis (Table 24) and remaining primers did not produce amplified fragments with different genotypes. The molecular size of PCR amplified bands ranged from 350 bp to 3000 bp. This was comparable with results generated by polymorphic bands ranging from 200 bp to 3000 bp (Kumar *et al.* 2001), 190 bp to 2400 bp (Karihaloo *et al.*2003), 105 bp to 1500 bp (Rahman *et al.*2007), 250 bp to 2500 bp (Anuj *et al.*2007), 240 bp to 2100 bp (Ahmad *et al.* 2008), 300 bp to 3500 bp (Hassam*et al.* 2014) in mango, 300 bp to 3500 bp (Graham M.J.1996) in strawberry, 250 bp to 3800 bp (Sharma *et al.* 2007) in guava, 200 bp to 4000 bp (Meghala *et al.* 2005) in sapota, 250 bp to 4050 bp (Ercisli *et al.* 2011) in pomegranate.

The twenty four selected primers(OPB-6, OPC-2,OPG-3, OPG-4, OPG-5, OPG-6, OPG-7, OPG-13, OPV-3, OPV-4, OPV-5, OPV -9, OPV-10, OPV-17, OPV-18, OPX-2, OPX-3, OPX-5, OPX-09, OPX 11, OPX 12, OPX-13, OPX-14, OPX-15 and OPX-20) amplified and produced unambiguous, readable polymorphic bands. A total of 204 alleles were produced by these 24 primers and the number of bands varied from 3-15 with an average of 8.5 bands per primer in the 33 genotypes of sapota. The bands which are more than 100 bp were selected for scoring. The characterstic fragments generated by 24 decamer primers employed as single orbitrary primers are summarized in the table 24 and plate 4. Similar results were reported earlier in different fruit crops by Mishra (2009) in apricot, Anburaj and Sudarmani (2010) in jackfruit, Cerqueirasilva *et al.* 2010 in passiflora, Hasnaoui *et al.* (2010) in pomegranate, Baig *et al.* (2008) in citrus and Meghala *et al.* (2005)in sapota reported that amplification of 6.8, 13.4, 7.46, 7.25, 8.90 and 8.25 bands per primer respectively.

RAPD profiles illustrated the typical level of polymorphism in sapota genotypes. Almost all RAPDs were common to many groups and identification of genotypes for the purpose of finger prints, the most important function of a primer is to descriminate as many genotypes as possible. Polymorphic fragments present in one genotype was alone considered to be unique (Table-25). Out of total 204 polymorphic fragments, sixteen fragments were unique to particular genotype viz., Gutti (OPC-2), Tagarampudi and PKM-4 (OPG-3), Calcutta Round, Columbian Sapota and Cricket Ball (OPG-4), Pakala Oval (OPG-7), DHS-2 (OPV-9), DHS-1 (OPV-10), Columbian Sapota (OPX-3), Cricket Ball (OPX-11), Kalipatti and Pakala Round (OPX-12) which could be exploited for DNA fingerprinting of these accessions by converting RAPD markers into STS (Sequence Tagged Site) markers and this was highly useful for detecting mixes between genotypes (Fernandez *et al.* 2002 and Chimote *et al.* 2007).

The data confirmed RAPD as a useful tool for identification of closely related accessions. RAPD principally helps in simultaneous detection of many randomly distributed loci with in the genome, in a simple, cost-effective manner requiring no previous knowledge of genome sequence. RAPD primers quickly scan the whole genome for detecting the insertion and deletion events (Milbourne *et al.* 1997) hence they most frequently used in genetic divergence studies.

4.7.2.1 Polymorphic information content of RAPD marker analysis

The polymorphic information content values (PIC) were calculated for RAPD markers and presented in table 24. The highest PIC was reported by the RAPD marker OPG-03 (0.90) and the lowest PIC was reported by the RAPD marker OPX-2 (0.55). Higher the PIC value, more is the informative of RAPD marker and hence, OPG-03 was found to be highly informative.

4.7.2.2 Similarity index

Banding profiles obtained with 24 primers for 33 sapota genotypes were analyzed on the basis of presence or absence of the band. Genetical similarity based on Jaccard's coefficient value revealed considerable level of diversity among the genotypes under study. The average genetical similarity among thirty three sapota genotypes was ranged from coefficient value of 0.32 to 0.80 with an average similarity coefficient value of 0.56 among the group of genotypes studied.

4.7.2.3 Cluster analysis

The genetical relationships among sapota genotypes were evaluated by UPGMA cluster analysis on RAPD data set, using minimum variance algorithm (Fig 15). The description of the data by using three dimensional pictorial graph was presented in Fig 15 and Plate- 4.

The dendrogram demonstrated the distribution of genotypes into two main clusters at similarity coefficient value of 0.35, on the basis of their similarity the proportion of shared alleles were calculated. PKM-1 and PKM-2 of cluster I were present at the extreme ends of the dendrogram with a similarity coefficient value of 0.80. This clearly indicated that diversity was mainly because of inherent genetic differences at DNA level. The dendrogram derived from the UPGMA cluster analysis revealed one major cluster with 32 genotypes further it grouped into several sub clusters and cluster II with one genotype (Fig. 15).

Cluster I was further divided into sub cluster IA with 30 genotypes and IB with 2 genotypes (Krishna Rao and Pala), at similarity coefficient value of 0.45 as shown in the dendrogram. Cluster IA was further divided into sub cluster IA_1 containing only one genotype (Badami) and IA_2 with 29 genotypes at similarity coefficient value of 0.53. The sub cluster IA_2 further divided into two clusters IA_2a possessed 25 genotypes and IA_2b comprised of 4 genotypes (Tagarampudi, Pakala Oval, Singapore and PKM-3), while IA_2a was further divided into 2 clusters IA_2a_1 containing 23 genotypes and IA_2a_2 comprising 2 genotypes (CO-1 and CO-2) at similarity coefficient value of 0.58 in the dendrogram. The sub cluster IA_2a_1 further divided into two clusters IA_2a_1x comprising of 17 genotypes and IA_2a_1y containing 6 genotypes (Brazilian Sapota, Calcutta Round, Dwarapudi, Hybrid, Columbian Sapota and Cricket Ball), while IA_2a_1x is further divided into $IA_2a_1x_1$ consisting 16 genotypes and $IA_2a_1x_2$ comprising one genotype (Gutti) at similarity coefficient value of 0.60 as shown in the dendrogaram. Cluster $IA_2a_1x_1$ was further divided into 2 clusters $IA_2a_1x_1y$ consisting of two genotypes (Bombay and Pakala Round) and $IA_2a_1x_1z$ comprising of 14 genotypes) at similarity coefficient value of 0.67 as shown in the dendrogram. Further, $IA_2a_1x_1z$ was divided into two clusters $IA_2a_1x_1z_1$ comprising of 2 genotypes (Kalipatti and Kirthibarthi) and $IA_2a_1x_1z_2$ cluster possessed 12 genotypes. At similarity coefficient value of 0.68 cluster $IA_2a_1x_1z_2$ further divided into two clusters $IA_2a_1x_1z_21$ consisting 7 genotypes (Mirandi, Pakala, Simhoudi, Ulvapadu selection, Virudnagar, Seedless and DHS-2) and cluster $IA_2a_1x_1z_22$ with 5 genotypes (CO-3, DHS-1, PKM-1, PKM-2 and PKM-4).

The dendrogram (Fig 16) based on RAPD analysis showed that most of the sapota genotypes were closely clustered. Although the genotypes have been clustered into different small sub groups, the genetic similarity among 33 genotypes studied was relatively high and it could differentiate the individuals in each group, suggesting that the genetic base of sapota germplasm is quite diverse. Variation in sapota had taken place mainly due to highly heterozygous nature of the crop. Hence, utilization of available local germplasm resources in hybridization programme is advocated to evolve high yielding cultivars of sapota. Similar pattern of high genetic diversity with RAPD markers were observed by Kumar *et al. (*2001) in India, Faleiro *et al.* (2010b) in Brazil, Jena *et al*. (2010) in Orissa, Gajera *et al.* (2011) in Indian Gir forest region, Hassam *et al.* (2014) in Egypt.

4.7.3 SSR analysis

In the present study, in addition to morphological traits and RAPD markers, SSR markers were also used to estimate the extent of molecular diversity among the sapota genotypes. Twenty two SSR primers were used to analyze the inter-cultivar diversity in 33 genotypes of sapota. The annealing temperatures and PCR conditions for these SSRs were first standardised by using a PCR with temperature gradient technique, which indicated that annealing temperatures of 48to 57 °C were optimum for obtaining scorable bands. Stuttering of bands was common with SSRs if annealing temperatures were not optimized.

Out of 22 primers used, 14 primers amplified with varied degree of polymorphism whereas four primers, produced monomorphic bands and remaining four primers did not produce any band in the genotype studied. A total of eighty eight alleles were produced by these 14 microsatellite loci with an average number of 5.50 alleles per locus.This is similar to the findings of Azevedo *et al.* (2005) in sapota, Shiva Lal Singh *et al.* (2008) in grape, Dixit *et al.* (2010) in citrus, Kanupriya Latha *et al.* (2011) in guava, Dacumos *et al.* (2011) in banana, Shrestha *et al.* (2011) in acid lime, Ravishankar *et al.* (2011) in mango who reported as 12.00, 5.58, 6.39, 7.45, 2.91 and 3.38 alleles per primer respectively in the above fruit crops.

The details of SSR primers used for assessing the molecular diversity among 33 genotypes is furnished in table-26. The 18 SSR primers used in the present study produced discrete, scorable and unambiguous bands. High level of polymorphism was observed with primers SSR S-4 and SSR S-1. The PCR product size obtaind by amplification of SSR primers was ranged from 110 to 555 bp. It was highly comparable with the results generated by polymorphic bands ranging from 120 bp to 450 bp (Azevedo *et al.* 2005) in sapota, 150 bp to 450 bp (Shiva Lal Singh *et al.* 2008) in grape, 100 bp to 550 bp (Kanupriya Latha *et al.* 2011) in guava,90 bp to 370 bp (Hammedunnisa *et al.* 2013) in mango. The amplification and banding pattern of the above SSR primers is illustrated in plate-5.

The most important function of a primer is to descriminate as many genotypes as possible. The polymorphic fragments present in only one genotype could be considered as unique fragments. Out of the total 88 polymorphic alleles, seventeen fragments were unique to particular genotype *viz.,* SSR S-1 produce in one genotype Columbian Sapota (235 bp), SSR S-2 produced in CO-3 (190 bp), SSR S-3 in Pakala Round (180 bp), SSR S-4 in Seedless (175 bp), SSR S-8 produced in 3 genotypes, Cricket Ball (220 bp), Calcutta Round (180 bp) and CO-2 (220 bp), SSR S-9 in Gavarayya (135 bp), SSR S-10 produced in 3 genotypes, Singapore (220 bp), DHS-1 (280 bp) and PKM-2 (300 bp), SSR Mh-26 in CO-2 (195 bp), SSR Mh-20 in Bombay (130 bp), SSR Mh-12 produced in 2 genotypes Kalipatti (220 bp) and Calcutta Round (230 bp) and SSR Mh-17 in Pala (180 bp). However, it is interesting to note that, SSR S-7 and SSR S-1 produced common bands (160 bp) in the hybrids DHS-1 and DHS-2, which could be due to common parentage of two hybridscan be exploited for DNA fingerprinting of these genotypes (Table-27).

4.7.3.1 Polymorphic information content (PIC) of SSR markers

The polymorphic information content values calculated for the SSR markers used in this study are presented in table-26. The PIC value ranged from a minimum of 0.55 (SSR Mh-20) to maximum of 0.94 (SSR S-4) indicating the hypervariable nature of these microsatellite markers. The high PIC value of SSR S-4 indicated that this primer is highly informative.

4.7.3.2 Similarity index

The banding pattern of SSR markers scored in the form of binary data was used for computing similarity index values are presented in the form of dendrogram (Fig 17). The similarity coefficient based on 14 SSR markers was ranged from 0.20 to 0.80. Out of 33 genotypes studied, the highest similarity index (0.80) was found between two pairs *i.e.* (Hybrid & Krishna Rao) and (Pakala Oval & PKM-4).

4.7.3.3 Cluster analysis

The dendrogram derived from the UPGMA cluster analysis revealed one major cluster (I) with 25 genotypes further it grouped into several sub clusters and another major cluster (II) with 8 genotypes, at similarity coefficient value of 0.20(Fig 17).

Cluster I was divided into sub cluster IA with 17 genotypes and IB with 8 genotypes, at similarity coefficient value of 0.25 as shown in the dendrogram. Cluster IA was further divided into sub cluster IA_1 containing 15 genotypes and IA_2 with 2 genotypes (Brazilian Sapota and PKM-3) at similarity coefficient value of 0.28. The sub cluster IA_1 further divided into two clusters IA_1a possessing 8 genotypes and IA_1b comprised of 7 genotypes, while IA_1b was further divided into 2 clusters IA_1b_1 containing 5 genotypes (Calcutta Round, Simhoudi, Seedless, Tagarampudi and Ulvapadu Selection) and IA_1b_2 comprising 2 genotypes (CO-2 and PKM-3) at similarity coefficient value of 0.34 in the dendrogram. The sub cluster IA_1a further divided into two clusters IA_1a_1 comprising of 6 genotypes and IA_1a_2 containing 2 genotypes (Bombay and Dwarapudi), while IA_1a_1 is further divided into IA_1a_1x consisting one genotype (Badami) and IA_1a_1y comprising 5 genotypes (Hybrid, Krishna Rao, Mirandi, Pakala Oval and PKM-4) at similarity coefficient value of 0.37 as shown in the dendrogaram. Cluster IA_1a_1x formed as a solitary cluster at similarity coefficient value of 0.37 as shown in the dendrogaram. . Cluster IB was further divided into sub cluster IB_1 containing 4 genotypes (Columbian Sapota, Pakala, Kirthibarthi and Pala) and IB_2 with 4 genotypes (Gavarayya, Virudnagar, DHS-1 and DHS-2) at similarity coefficient value 0.30.

Cluster II was further divided into IIA consisting of 5 genotypes and IIB comprising of 3 genotypes (Singapore, CO-1 and PKM-2) at similarity coefficient value of 0.28 as shown in dendrogram. At similarity coefficient value 0.34 cluster IIA was further divided into 2 sub clusters IIA_1 containing 3 genotypes (Cricket Ball, Gutti and Kalipatti) and IIA_2 comprised 2 genotypes (Pakala Round and CO-3).

The dendrogram of 33 genotypes (Fig 17) of sapota indicated that, all round, elliptic, oblong and oval shaped fruit bearing genotypes are grouped together (Cluster I) are Badami, Bombay, Calcutta Round, Columbian Sapota, Kirthibarthi, Pala, Pakala Round, Tagarampudi and DHS-2 were round shaped fruits, Gavarayya, Gutti, Hybrid and Simhoudi were oblong fruits, Brazilian Sapota, Ulvapadu Selection, PKM-1, PKM-3 and PKM-4 were oval shaped fruits. Pakala Oval and PKM-4 are closely associated to each other may be due to oval shape fruits and broadly pyramidal shape of tree. Similarly, Hybrid and Krishna Rao are closely associated to each other may be due to semierect growth habit of the tree and having acorn shape of fruits. Sub cluster IA_2 included 2 genotypes, Brazilian Sapota and PKM-3 are grouped near due to their oval shape of fruit. Further, sub-cluster IA_1b_1 containing 5 genotypes, Calcutta Round, Simhoudi, Seedless, Tagarampudi and Ulvapadu Selection are grouped near due to their dense branching density habit and sub-cluster IA_1b_2 comprising 2 genotypes CO-2 and PKM-3 are closely associated may be due to spreading type of growth habit and verticillate branching pattern. The sub-cluster IA_1a_2 containing 2 genotypes Bombay and Dwarapudi which are closely associated to each other may be due to dense branching density. Cluster IB was further divided into sub cluster IB_1 containing 4 genotypes, Columbian Sapota, Pakala, Kirthibarthi and Pala were closely grouped in a dendrogram which may due to their broadly pyramidal shape of tree and globose shape of fruit, while in sub-cluster IB_2 comprising 4 genotypes, Virudnagar, DHS-1 and DHS-2 are grouped near due to semierect growth habit of tree and verticillate branching pattern except Gavarayya which is having irregular branching pattern but similar in growth habit.In cluster II sub-cluster IIA consisting of 5 genotypes, Cricket Ball, Gutti, Kalipatti, Pakala Round and CO-3 were grouped together which may be due to horizontal branching pattern and brown flesh color and Singapore, CO-1 and PKM-2 has been placed in subcluster IIB due to solitary fruit bearing habit. Similar discriminative ability of SSR markers in identifying species, cultivars and hybrids have been demonstrated in many other crops like avocado (Koller*et al.,*1993), sapota (Azevedo *et al.,* 2005), grape (Shiva Lal Singh *et al.,* 2008), guava (Kanupriya Latha *et al.,* 2011), banana(Dacumos *et al.,* 2011), acid lime (Shrestha *et al.,* 2011), mango (Ravishankar *et al.,* 2012 and Hameedunnisa *et al.,* 2013).

The correct determination of genetic diversity of sapota genotypes in the germplasm is important for breeding programmes, allowing selection of the desired sapota genotypes for crossing. This will maximize the probability of transgressive segregation and increase the probability that unrelated accessions contribute with positive alleles at different loci. Furthermore, the assessment of genetic diversity of sapota accessions present in the working germplasm collection will help the breeders to formulate crosses by choosing accessions with different genetic backgrounds and will assist in the development of gene-mapping populations with greater marker polymorphism.

4.7.3.4 Variation among hybrids

Dendrogram with Jaccard’s Co-efficient of similarity values has been generated for hybrids and presented in Fig 19. Hybrids are distributed based on fruit shape, growth habit, parentage and bearing habit, which made two distinctive groups. Group-I contains DHS-1, DHS-2, PKM-2, CO-1 and CO-3 which are grouped together because of their acorn and round shaped fruits with spreading and single bearing habit. Within this group DHS-1 and DHS-2 were 100 per cent similar as they are having same parentage (Kalipatti X Cricket Ball). CO-1 and CO-3 are closely linked with DHS-2 due to round shaped fruits. PKM-3 has been placed in separate cluster (group-II), which have oval shaped fruits, vertical growth and cluster bearing.

Microsatellite markers used for characterization of sapota genotypes revealed that 16 polymorphic microsatellite markers were found to be efficient to distinguish genotypes and would certainly be useful for purposes such as for certification of varieties, and for development of superior plants from the crosses of these sapota genotypes.

A few amplification products could be effectively used as markers for differentiation of a part of the sapota genotypes studied. Hence, this could lead to a non-informative or biased analysis. Further a finer molecular analysis of sapota genotypes was required with more number of SSR markers as far as possible in order to detect and identify unique as well as fine resolution of molecular polymorphism between different identical genotypes. The use of larger number of SSRs with greater genome coverage could help to reveal genetic diversity more accurately and also help to differentiate the accessions unambiguously with identical allelic patterns as revealed by the set of primers used in this study. Further, the better assessment of genetic diversity was possibleby collecting more number of genotypes from different locations along with the use of a more number of polymorphic SSR markers.

Identification of markers associated with specific traits of great interest, which could be converted into SCAR markers, needs attention to speed up the crop improvement programme. The degree of genetic variability among sapota genotypes were evaluated through molecular characterization could form the basis for future breeding programme and a finer molecular analysis with additional SSR loci and use of other molecular markers like AFLP and ISSRs is required to differentiate the point mutations involved in identical accessions. Further, research work on QTL mapping for improvement of sapota through marker assisted breeding for selection of elite genotypes and to speed up the breeding programme has to be accomplished. The data generated in the present investigation provides useful information not only for improvement of sapota through selection programmes, but also useful for development of genetical relationships among the sapota genotypes world wide.

Table-3 Tree morphological characters (based on scores) of sapota genotypes

S.No	Genotypes	Growth habit	Shape of tree	Branching Pattern	Branch density
1	Badami	3	6	3	3
2	Bombay	3	4	3	3
3	Brazilian Sapota	3	5	3	2
4	Calcutta Round	3	6	3	2
5	Columbian Sapota	4	6	3	1
6	Cricket Ball	3	6	2	2
7	Dwarapudi	3	2	3	3
8	Gavarayya	3	5	1	2
9	Gutti	3	2	3	2
10	Hybrid	3	2	3	2
11	Kalipatti	2	5	3	3
12	Kirthibarthi	2	6	2	3
13	Krishna Rao	3	6	2	2

14	Mirandi	3	6	3	2
15	Pakala	3	6	3	2
16	Pakala Oval	3	6	2	2
17	Pakala Round	3	6	3	2
18	Pala	2	6	2	2
19	Seedless	3	4	2	2
20	Simhoudi	3	5	3	3
21	Singapore	2	6	2	3
22	Tagarampudi	3	6	2	3
23	Ulvapadu selection	2	2	3	2
24	Virudnagar	2	2	3	3
25	CO-1	4	2	3	1
26	CO-2	2	1	3	1
27	CO-3	3	1	3	1
28	DHS-1	3	4	3	2
29	DHS-2	3	5	3	3
30	PKM-1	2	6	3	3
31	PKM-2	3	6	3	3
32	PKM-3	3	6	3	2
33	PKM-4	3	6	3	2

Table 4 Plant height and leaf morphological characters of sapota genotypes

S.No.	Genotypes	Plant Height (m)	Leaf length (cm)	Leaf width (cm)	Leaf Area (cm^2)
1	Badami	5.11	8.93	3.50	21.67
2	Bombay	2.84	10.33	3.67	28.17
3	Brazilian Sapota	4.92	11.00	4.03	32.92
4	Calcutta Round	6.52	8.77	3.33	23.33

5	Columbian Sapota	6.56	9.83	3.47	23.25
6	Cricket Ball	5.55	13.81	5.87	44.42
7	Dwarapudi	5.92	9.97	3.57	26.58
8	Gavarayya	5.73	11.27	3.77	31.42
9	Gutti	4.69	10.13	4.07	31.58
10	Hybrid	6.03	9.77	3.87	26.15
11	Kalipatti	5.88	10.67	3.77	28.92
12	Kirthibarthi	7.74	9.40	3.53	27.87
13	Krishna Rao	3.51	9.57	3.60	25.25
14	Mirandi	5.40	9.43	2.80	20.67
15	Pakala	5.62	7.20	2.30	17.83
16	Pakala Oval	6.00	8.33	3.40	22.17
17	Pakala Round	5.98	9.43	3.13	19.75
18	Pala	6.29	9.50	3.73	24.25
19	Seedless	5.50	12.37	4.57	26.83
20	Simhoudi	5.95	10.27	3.83	24.92
21	Singapore	5.60	9.97	3.77	22.33
22	Tagarampudi	6.80	11.57	4.17	33.35
23	Ulvapadu Selection	3.27	8.63	2.70	17.00
24	Virudnagar	5.55	9.27	3.83	29.33
25	CO-1	4.99	10.03	3.73	29.25
26	CO-2	6.19	11.20	4.43	36.17
27	CO-3	4.65	10.13	3.97	30.68
28	DHS-1	6.90	9.73	3.37	25.75
29	DHS-2	7.14	9.67	4.13	28.08
30	PKM-1	5.47	11.43	4.37	34.08

31	PKM-2	5.65	9.53	3.27	18.16
32	PKM-3	6.48	12.17	4.80	40.17
33	PKM-4	4.81	9.83	4.30	32.42
	Mean	5.61	10.09	3.77	27.41
	SEm (±)	0.55	1.65	0.75	9.80
	CD (5%)	1.38	3.50	2.22	20.25

Table-5 Fruit morphological characters of sapota genotypes

S.No.	Genotypes	Fruit Length (cm)	Fruit width (cm)	Fruit Weight (g)
1	Badami	6.03	5.49	83.80
2	Bombay	5.07	5.74	100.74
3	Brazilian Sapota	6.36	6.44	221.08
4	Calcutta Round	5.37	6.76	144.93
5	Columbian Sapota	7.91	8.20	540.27
6	Cricket Ball	5.32	7.34	259.70
7	Dwarapudi	5.45	5.65	92.70
8	Gavarayya	6.83	6.04	105.91
9	Gutti	5.83	5.09	78.95
10	Hybrid	6.05	5.18	106.55

11	Kalipatti	6.83	6.52	169.46
12	Kirthibarthi	5.59	6.44	139.52
13	Krishna Rao	5.80	5.51	73.60
14	Mirandi	7.47	6.25	167.91
15	Pakala	5.39	6.58	142.49
16	Pakala Oval	6.88	5.77	120.14
17	Pakala Round	4.84	6.22	75.61
18	Pala	5.64	5.37	94.25
19	Seedless	6.19	6.42	102.15
20	Simhoudi	6.67	6.14	108.76
21	Singapore	6.60	6.67	198.61
22	Tagarampudi	5.81	5.80	89.25
23	Ulvapadu Selection	6.59	5.99	138.56
24	Virudnagar	6.99	5.87	147.03
25	CO-1	7.81	6.88	222.33
26	CO-2	6.28	6.78	239.18
27	CO-3	6.85	7.63	217.86
28	DHS-1	7.19	7.02	205.85
29	DHS-2	6.69	6.59	165.82
30	PKM-1	6.69	6.25	146.60
31	PKM-2	6.67	6.46	152.10
32	PKM-3	7.27	6.38	164.03
33	PKM-4	8.39	6.16	181.64
	Mean	6.40	5.48	158.43
	SEm (±)	0.84	0.79	45.11
	CD (5%)	2.26	1.73	105.70

Table-7 Fruit yield characters of sapota genotypes

S.No.	Genotypes	Ovary Size (mm)	Percent fruit set (%)	Yield/Tree (Kg)
1	Badami	3.37	6.00	14.67
2	Bombay	4.00	6.80	17.63
3	Brazilian Sapota	4.47	7.13	20.49
4	Calcutta Round	3.87	5.43	16.41
5	Columbian Sapota	4.27	8.60	35.01
6	Cricket Ball	4.03	16.03	91.97
7	Dwarapudi	4.49	9.47	22.06
8	Gavarayya	3.73	11.52	79.54
9	Gutti	3.61	14.30	87.20
10	Hybrid	4.16	8.47	33.08
11	Kalipatti	4.02	17.00	76.69
12	Kirthibarthi	3.67	9.70	46.60
13	Krishna Rao	3.93	10.20	50.64
14	Mirandi	3.64	12.60	59.47
15	Pakala	4.08	16.70	71.95
16	Pakala Oval	4.03	15.80	61.43
17	Pakala Round	4.01	16.10	69.70
18	Pala	3.13	17.85	71.92
19	Seedless	3.78	15.20	60.70
20	Simhoudi	4.09	15.43	63.42
21	Singapore	4.79	11.10	34.79

22	Tagarampudi	3.67	32.00	187.14
23	Ulvapadu Selection	4.49	11.80	58.08
24	Virudnagar	3.67	27.00	184.57
25	CO-1	3.66	18.00	91.80
26	CO-2	4.32	17.00	82.18
27	CO-3	3.98	13.97	55.07
28	DHS-1	4.34	9.20	44.41
29	DHS-2	3.83	8.50	32.77
30	PKM-1	4.08	28.47	151.83
31	PKM-2	4.02	20.02	101.13
32	PKM-3	4.00	17.90	94.80
33	PKM-4	3.93	22.60	111.83
	Mean	3.97	14.48	69.12
	SEm (±)	0.48	1.39	25.40

S.No.	**Genotypes**	**Seed Number**	**Seed weight (g)**	**Pulp:Seed ratio**
1	Badami	4.33	3.60	26.90
2	Bombay	4.33	3.85	23.10
3	Brazilian Sapota	6.00	6.22	43.54
4	Calcutta Round	5.00	5.09	26.53
5	Columbian Sapota	5.33	7.07	75.67
6	Cricket Ball	6.33	7.81	28.80
7	Dwarapudi	6.33	5.50	17.27
8	Gavarayya	3.67	3.74	24.32
9	Gutti	4.00	3.20	23.41

10	Hybrid	4.63	3.90	31.03
11	Kalipatti	4.67	4.28	35.03
12	Kirthibarthi	6.00	5.26	33.05
13	Krishna Rao	9.33	6.93	24.16
14	Mirandi	6.67	5.02	61.50
15	Pakala	4.67	3.33	43.95
16	Pakala Oval	4.33	2.99	38.46
17	Pakala Round	5.00	3.10	24.19
18	Pala	3.67	2.84	30.62
19	Seedless	2.33	2.45	30.03
20	Simhoudi	4.00	2.93	41.23
21	Singapore	5.00	4.58	36.46
22	Tagarampudi	5.00	4.70	18.29
23	Ulvapadu Selection	3.33	2.58	50.40
24	Virudnagar	4.00	3.68	35.32
25	CO-1	4.00	5.00	40.20
26	CO-2	3.00	4.83	47.82
27	CO-3	4.00	5.23	37.09
28	DHS-1	3.67	4.35	40.46
29	DHS-2	3.33	4.00	36.75
30	PKM-1	5.00	3.37	40.35
31	PKM-2	3.33	3.70	38.07
32	PKM-3	4.33	4.77	32.49
33	PKM-4	4.00	4.23	36.88
	Mean	4.62	4.37	35.55
	SEm (±)	0.87	0.47	2.45

CD (5%)	2.11	0.95	5.16

Table-6 Fruit morphological characters (based on scores) of sapota genotypes

S.No.	Genotypes	Fruit Shape	Fruit surface texture	Peel color	Flesh color
1	Badami	3	1	4	4
2	Bombay	3	1	3	4
3	Brazilian Sapota	7	1	3	3
4	Calcutta Round	3	1	4	6
5	Columbian Sapota	3	3	3	5
6	Cricket Ball	3	1	5	5
7	Dwarapudi	2	2	4	4
8	Gavarayya	6	1	4	4
9	Gutti	6	2	4	4
10	Hybrid	3	2	5	4
11	Kalipatti	2	2	5	4
12	Kirthibarthi	3	2	5	4
13	Krishna Rao	2	2	4	6
14	Mirandi	6	3	3	3
15	Pakala	6	2	4	4
16	Pakala Oval	7	2	4	4
17	Pakala Round	3	2	4	4
18	Pala	7	1	4	4
19	Seedless	4	2	5	3
20	Simhoudi	6	2	4	4
21	Singapore	2	2	4	4
22	Tagarampudi	3	1	4	6
23	Ulvapadu Selection	7	2	4	5

24	Virudnagar	6	1	4	4
25	CO-1	1	2	5	5
26	CO-2	1	1	4	5
27	CO-3	2	3	5	4
28	DHS-1	2	1	4	5
29	DHS-2	4	1	4	6
30	PKM-1	5	2	4	4
31	PKM-2	2	2	4	4
32	PKM-3	7	2	4	5
33	PKM-4	5	2	4	6

Table 6 cont..

S.No	Genotypes	Flesh texture	Flesh taste	Fruit availability	Fruit clustering habit
1	Badami	3	4	April- June	1
2	Bombay	2		March-June	1
3	Brazilian Sapota	1	3	March-June, October-November	1
4	Calcutta Round	2	3	March-June, October-November	1
5	Columbian Sapota	2	3	March to June	1
6	Cricket Ball	3	3	March- June,October -November	1
7	Dwarapudi	2	4	March-June, October-November	1
8	Gavarayya	2	3	March-June, October-November	1
9	Gutti	3	4	March-June, October-November	2
10	Hybrid	2	4	March-June, October-November	1
11	Kalipatti	2	4	March-June, October-November	1
12	Kirthibarthi	3	4	March-June,October-November	1
13	Krishna Rao	2	4	March-June	1
14	Mirandi	2	3	April-June	1
15	Pakala	3	4	March-June, October-November	1
16	Pakala Oval	2	4	March-June, October-November	2
17	Pakala Round	3	4	March-June, October-November	1
18	Pala	3	4	March-June, October-November	1
19	Seedless	2	4	March-June, October-November	1
20	Simhoudi	3	4	March-June, October-November	1
21	Singapore	2	4	March-June, October-November	1
22	Thagarampudi	1	3	March-June,	1
23	Ulvapadu Selection	3	4	March-June	1

24	Virudnagar	3	4	February-June, October-November	1
25	CO-1	1	4	April-June, October-November	1
26	CO-2	1	4	April-June, October-November	1
27	CO-3	1	4	April-June, October-November	1
28	DHS-1	3	4	March-June, October-November	1
29	DHS-2	2	4	March-June	1
30	PKM-1	3	4	March-June, October-November	2
31	PKM-2	2	4	March-June, October-November	2
32	PKM-3	2	4	March-June, October-November	2
33	PKM-4	3	4	March-June, October-November	2

S.No.	Genotypes	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)
1	Badami	9.30	7.06	2.24
2	Bombay	8.21	7.18	1.03
3	Brazilian Sapota	11.00	8.50	2.51
4	Calcutta Round	10.36	8.76	1.60
5	Columbian Sapota	8.61	6.60	2.01
6	Cricket Ball	8.97	6.87	2.10
7	Dwarapudi	8.93	6.23	2.70
8	Gavarayya	9.87	7.33	2.54
9	Gutti	10.90	8.22	2.68
10	Hybrid	9.57	6.69	2.88
11	Kalipatti	**11.47**	**9.23**	**2.24**
12	Kirthibarthi	10.75	9.13	1.62
13	Krishna Rao	9.47	8.33	1.14
14	Mirandi	8.97	8.04	0.93
15	Pakala	9.72	8.30	1.42
16	Pakala Oval	9.67	8.57	1.10
17	Pakala Round	9.90	7.84	2.06
18	Pala	11.04	9.13	1.91
19	Seedless	9.55	6.89	2.65
20	Simhoudi	9.20	8.30	0.90
21	Singapore	10.55	8.40	2.15
22	Tagarampudi	9.82	8.17	1.65
23	Ulvapadu Selection	10.53	8.33	2.20
24	Virudnagar	10.99	8.48	2.51

25	CO-1	10.71	7.60	3.10
26	CO-2	11.84	8.18	3.66
27	CO-3	9.80	6.66	3.14
28	DHS-1	11.08	8.39	2.69
29	DHS-2	10.01	8.55	1.46
30	PKM-1	10.91	9.07	1.84
31	PKM-2	10.99	7.99	3.00
32	PKM-3	11.19	8.98	2.21
33	PKM-4	11.99	9.00	2.98
	Mean	10.17	8.03	2.15
	SEm (±)	0.203	0.130	0.061
	CD (5%)	0.576	0.368	0.174

Table 8 cont…

S.No.	Genotypes	Ascorbic acid (mg/100g)	Total phenols (mg/100g)	Pectin (%)
1	Badami	6.63	0.610	2.55
2	Bombay	5.15	0.527	1.72
3	Brazilian Sapota	8.62	0.462	1.42
4	Calcutta Round	7.46	0.500	2.24
5	Columbian Sapota	7.69	0.551	1.34
6	Cricket Ball	6.22	0.425	2.09
7	Dwarapudi	5.35	0.478	1.98
8	Gavarayya	8.57	0.466	1.80
9	Gutti	9.14	0.520	1.66
10	Hybrid	6.16	0.433	1.56

11	Kalipatti	12.10	0.410	2.35
12	Kirthibarthi	8.85	0.527	2.13
13	Krishna Rao	8.60	0.620	1.71
14	Mirandi	7.18	0.600	2.86
15	Pakala	6.87	0.558	1.83
16	Pakala Oval	7.20	0.525	2.02
17	Pakala Round	7.43	0.509	2.10
18	Pala	10.32	0.382	1.62
19	Seedless	9.58	0.471	1.44
20	Simhoudi	8.02	0.476	1.17
21	Singapore	11.76	0.479	2.70
22	Tagarampudi	8.46	0.392	1.34
23	Ulvapadu Selection	7.05	0.445	1.51
24	Virudnagar	10.68	0.464	2.30
25	CO-1	12.16	0.300	2.17
26	CO-2	11.27	0.444	1.83
27	CO-3	10.50	0.467	1.18
28	DHS-1	10.00	0.393	2.63
29	DHS-2	9.10	0.398	2.73
30	PKM-1	9.23	0.381	1.74
31	PKM-2	8.50	0.437	2.23
32	PKM-3	11.05	0.383	2.10
33	PKM-4	10.51	0.528	1.66
	Mean	8.70	0.473	1.94
	SEm (±)	0.374	0.051	0.094
	CD (5%)	1.208	0.144	0.249

Table-9Genetic variability in sapota genotypes for morphological and biochemical characters

Characters	Phenotypic coefficient of variation(%)	Genotypic coefficient of variation (%)	Heritability (%)	Genetic advance as percent of mean (%)
Plant height	18.68	18.67	96.90	38.43
Leaflength	14.98	11.14	55.30	17.06
Leaf width	19.79	15.61	62.20	25.36
Leaf area	29.04	19.00	42.80	25.60
Ovarysize	10.52	7.40	49.50	10.73
Fruit length	14.84	12.47	70.50	21.57
Fruit width	12.27	9.60	61.10	15.46
Fruit weigth	55.59	52.77	90.10	29.21
Seed number	30.05	27.77	85.40	52.85
Seed weight	32.41	31.63	95.30	63.59
Pulp to Seed ratio	32.54	33.44	98.42	68.87
Per cent fruit set	44.53	44.15	98.30	90.16
Yield/tree	80.25	77.46	93.20	88.02
TSS	8.40	6.92	67.80	11.74
Totalsugars	9.47	8.81	86.50	16.87
Reducingsugars	12.01	11.68	94.60	23.40
Non-reducing sugars	36.25	35.89	98.10	73.22
Ascorbicacid	22.01	21.96	99.60	45.14

Titrable acidity	15.34	15.33	99.80	31.54
TSS to Acid ratio	20.89	21.19	92.44	43.65
Total Phenols	21.09	9.98	22.40	9.73
Pectins	24.63	24.13	96.00	48.70

Characters		Plant Height	Leaf length	Leaf width	Leaf Area	Ovary Size	Fruit length	Fruit Width	Frit weight	Seed Number	Seed Weight	Pulp: Seed ratio	Per cent fruit set	Yield/Tree
Plant Height	rp	**0.0307**	0.0029	-0.0091	0.002	0.0072	0.0021	-0.0088	0.0106	-0.006	0.0093	-0.0085	0.0869	0.1072
	rg	**0.2663**	0.1958	-0.4432	0.0929	0.0828	0.004	-0.5101	-0.257	0.0623	0.4472	0.1100	0.0715	0.1225
Leaf length	rp	0.0015	**0.0603**	-0.0779	0.0164	-0.0013	-0.0018	-0.0024	0.0039	0.0003	0.0175	0.0216	0.1895	0.2276
	rg	0.0173	**0.0217**	-0.659	0.5631	-0.0266	0.0049	-0.4965	-0.1781	0.0208	1.213	-0.3755	0.2089	0.3140
Leaf width	rp	0.0028	0.0471	**-0.0998**	0.0172	0.0015	-0.0009	-0.0017	0.0036	-0.0018	0.0202	0.0300	0.2001	0.2184
	rg	0.0309	0.8932	**-0.822**	0.6286	0.0166	0.0072	-0.3128	-0.1528	0.0341	0.247	-0.4906	0.2188	0.2982
Leaf Area	rp	0.0028	0.0453	-0.0786	**0.0218**	0.0047	0.0038	-0.0006	0.0044	0.0003	0.0178	0.0232	0.2206	0.2657
	rg	0.0366	0.5185	-0.5532	**0.6756**	0.0273	0.0151	-0.3065	-0.1919	0.0015	0.3978	-0.4584	0.2733	0.4357
Ovary size	rp	-0.0044	0.0016	0.003	-0.002	**-0.0505**	0.0029	-0.0065	0.0137	0.0006	0.0111	-0.0218	-0.1319	-0.1904
	rg	-0.0532	0.1935	0.1529	-0.0444	**-0.4147**	0.009	-0.6481	-0.5698	-0.0246	0.7269	0.5165	-0.1271	-0.2831
Fruit length	rp	0.0013	-0.0021	0.0018	0.0016	-0.0029	**0.5015**	-0.0149	0.0292	-0.0117	0.0027	-0.0695	0.1677	0.1546
	rg	0.0125	0.1727	-0.3226	0.1196	-0.0436	**0.5854**	-0.6172	-0.7885	0.1376	0.1535	0.6549	0.1621	0.1387
Fruit Width	rp	0.0066	0.0035	-0.004	0.0003	-0.008	0.0188	**-0.0408**	0.0426	-0.006	0.0255	-0.0597	-0.0617	-0.0828
	rg	0.075	0.8278	-0.6596	0.1143	-0.1483	0.0291	**-0.8122**	-0.1606	0.0744	0.5994	0.9874	-0.0562	-0.1295
Frit weight	rp	0.0052	0.0037	-0.0058	0.0015	-0.011	0.024	-0.0277	**0.0626**	0.0012	0.031	-0.0946	-0.1229	-0.1329
	rg	0.0477	0.375	-0.4069	0.0903	-0.1647	0.0469	-0.4657	**-0.435**	0.0006	0.5843	0.2865	-0.1101	-0.1511
Seed Number	rp	-0.003	0.0004	0.0032	0.0001	-0.0006	-0.011	0.0044	0.0013	**0.0549**	0.0083	0.0092	-0.1797	-0.1127
	rg	-0.0317	-0.1197	0.2485	-0.0019	-0.0195	-0.0224	0.2574	0.0017	**-0.5239**	0.3773	-0.1301	-0.1619	-0.1262
Seed Weight	rp	0.0057	0.0213	-0.0405	0.0078	-0.0113	0.0028	-0.0209	0.0391	0.0091	**0.0497**	-0.0113	-0.0914	-0.04

	rg	0.0511	0.573	-2.0453	0.4053	-0.1294	0.0056	-0.2438	-0.9756	-0.0848	**0.3302**	0.1489	-0.0761	-0.0401
Pulp: Seed ratio	rp	0.002	-0.0102	0.0234	-0.0039	-0.011	0.0279	-0.019	0.0461	-0.004	0.0044	**-0.1283**	-0.0568	-0.1294
	rg	0.0177	-0.6856	0.1331	-0.1871	-0.1294	0.0551	-0.0813	-0.1155	0.0412	0.2096	**0.6549**	-0.0466	-0.1339
Per cent fruit set	rp	0.0029	0.0127	-0.0221	0.0053	0.0074	0.0096	0.0028	-0.0085	-0.0109	-0.005	0.0081	**0.9032**	0.9053
	rg	0.0259	0.859	-0.1384	0.2513	0.0718	0.0188	0.1387	0.2151	0.1155	-0.2414	-0.1049	**0.7347**	0.9461

Table-12 Phenotypic and genotypic path coefficient analysis of morphological characters in sapota genotypes.

Note-**5% level of significance, *1% levelof significance

Table 13 Phenotypic and genotypic path coefficient analysis of fruit morphological characters in sapota genotypes

Character		**TSS**	**Totalsugars**	**Reducing sugars**	**Non-reducing sugars**	**Ascorbic acid**	**Titrable acidity**	**TSS: Acid ratio**	**Phenols**	**Pectin**	**Yield/Tree**
TSS	rp	**-0.04**	0.0118	0.0228	-0.0052	-0.0314	0.1642	-0.2336	0.0096	0.0021	0.1788
	rg	**0.2264**	0.1764	0.9837	0.1467	-0.8058	-0.4418	-0.9413	-0.4415	-0.0634	0.2793
Total sugars	rp	0.0300	**0.0158**	0.0239	-0.0082	-0.0397	0.1587	-0.2235	0.0152	0.0012	0.2921
	rg	0.2145	**0.2418**	0.9069	0.1929	-0.8948	-0.3768	-0.7975	-0.5396	-0.0319	0.3320
Reducing sugars	rp	-0.0208	0.0086	**0.0439**	0.0068	-0.0316	0.089	-0.1463	0.0061	0.0086	0.2627
	rg	0.1499	0.758	**0.4858**	-0.1562	-0.6786	-0.2018	-0.4992	-0.2214	-0.2151	0.2724
Non-reducing sugars	rp	-0.0119	0.0074	-0.017	**-0.0175**	-0.014	0.1122	-0.1234	0.0119	-0.0074	0.0240
	rg	0.0859	0.6195	-0.600	**0.3867**	-0.2953	-0.2508	-0.4134	-0.3809	0.1869	0.0242

Ascorbic acid	rp	-0.0213	0.0106	0.0235	-0.0042	**-0.0589**	0.1186	-0.1591	0.0161	0.003	0.2882
	rg	0.1482	0.9028	0.8191	0.0928	**-0.2308**	-0.2625	-0.529	-0.4794	-0.0734	0.2917
Titrable acidity	rp	0.0242	-0.0092	-0.0144	0.0072	0.0257	**-0.2716**	0.2925	-0.0152	0.0011	-0.2898
	rg	-0.1671	-0.7813	-0.5005	-0.1619	0.5393	**0.599**	0.9715	0.4722	-0.0259	0.9454
TSS:Acid ratio	rp	-0.0301	0.0114	0.0207	-0.007	-0.0302	0.256	**0.3103**	0.0133	0.0012	0.2988
	rg	0.2070	0.9618	0.7204	0.1552	-0.6324	-0.5652	**-0.0296**	-0.4099	-0.0295	0.3095
Phenols	rp	0.0076	-0.0048	-0.0053	0.0042	0.0188	-0.0818	0.0821	**-0.0504**	-0.0001	-0.2762
	rg	-0.1362	-0.9129	-0.4482	-0.2007	0.8038	0.3853	0.5749	**0.734**	-0.0453	-0.5090
Pectin	rp	-0.0022	0.0005	0.0098	0.0034	-0.0046	-0.0075	-0.0097	0.0001	**0.0385**	-0.1314
	rg	0.0155	0.0427	0.3447	-0.078	-0.0975	0.0167	-0.0327	0.0359	**-0.9270**	-0.1409

Note-**5% level of significance, *1% levelof significance

Table 10 Phenotypic and genotypic correlation coefficents of morphological characters in sapota genotypes

Character		Plant Height	Leaf length	Leaf width	Leaf Area	Ovary Size	Fruit length	Fruit Width	Frit weight	Seed Number	Seed Weight	Pulp: Seed ratio	Per cent fruit set	Yield/Tree
Plant Height	**rp**	1	0.0475	0.0909	0.0926	-0.1428	0.0414	0.2166*	0.1688	-0.1092	0.1865	0.0665	0.0962	0.1072*
	rg	1	0.0648	0.116	0.1376	-0.1997	0.0468	0.2815*	0.1791	-0.1189	0.1919	0.0665	0.0973	0.1225*
Leaf length	**rp**		1	0.7806**	0.7504**	0.0265	-0.0346	0.0588	0.0618	0.0064	0.3527**	0.1684	0.2098**	0.2276**
	rg		1	0.9575**	0.8335**	0.0641	0.0572	0.274*	0.1241	-0.0396	0.5206**	-0.2269	0.2843**	0.3134**
Leaf width	**rp**			1	0.7874**	-0.0298	-0.0176	0.0405	0.0583	-0.0324	0.4062**	-0.2341	0.2216**	0.2184**
	rg			1	0.9305**	-0.0400	0.0844	0.1726*	0.1065	-0.065	0.5351**	-0.2965	0.2979**	0.2981**

Leaf Area	**rp**	1	-0.0938	0.0748	0.0153	0.0706	0.006	0.3591**	-0.1806	0.2442**	0.2657**
	rg	1	-0.0657	0.1771	0.1691*	0.1337	-0.0028	0.5999**	-0.2770	0.372**	0.4326**
Ovary Size	**rp**		1	0.057	0.1588*	0.2185**	0.0117	0.2236**	0.2188**	-0.1460	-0.1904
	rg		1	0.1052	0.3576*	0.3971**	0.0470	0.312**	0.3121**	-0.173	-0.2830
Fruit length	**rp**			1	0.3645**	0.4661**	-0.2129	0.0538	0.5419**	0.1857*	0.1546
	rg			1	0.3406**	0.5497**	-0.2626	0.0659	0.6449**	0.2206*	0.1387
Fruit Width	**rp**				1	0.6804**	-0.1088	0.5137**	0.4656**	-0.0683	-0.0828
	rg				1	0.8088**	-0.1421	0.6864**	0.5966**	-0.0765	-0.1296
Frit weight	**rp**					1	0.0216	0.6247**	0.7373**	-0.1361	-0.1329
	rg					1	-0.0012	0.6777**	0.7774**	-0.1499	-0.1511
Seed Number	**rp**						1	0.1665*	-0.0721	-0.199	-0.1127
	rg						1	0.1619*	-0.0786	-0.2204	-0.1262
Seed Weight	**rp**							1	0.088	-0.1012	-0.04
	rg							1	0.0900	-0.1036	-0.0411
Pulp: Seed ratio	**rp**								1	-0.0628	-0.1294
	rg								1	-0.0634	-0.134
Per cent fruit set	**rp**									1	0.9053
	rg									1	0.9461
Yield/Tree											1

Note-**5% level of significance, *1% levelof significance

Table 11 Phenotypic and genotypic correlation coefficents of fruit biochemical characters in sapota genotypes

Character		TSS	Totalsugars	Reducingsugars	Non-reducing sugars	Ascorbicaid	Titrable acidity	TSS: Acid ratio	Phenols	Pectin	Yield/Tree
TSS	rp	1	0.7499**	0.5192**	0.2975**	0.5338**	-0.6044	0.7529**	-0.191	0.0522	0.1788*
	rg	1	0.9473**	0.6621**	0.3794**	0.6547**	-0.7375	0.9142**	-0.6015	0.0684	0.2793*
Total Sugars	rp		1	0.5448**	0.4687**	0.6734**	-0.584	0.7204**	-0.3015	0.0322	0.292**
	rg		1	0.6104**	0.4988**	0.727**	-0.6291	0.7745**	-0.7351	0.0344	0.332**
Reducing Sugars	rp			1	-0.3871	0.5362**	-0.3276	0.4716**	-0.1207	0.2225*	0.2627**
	rg			1	-0.4038	0.5513**	-0.3369	0.4849**	-0.3017	0.232*	0.2724**
Non-reducing Sugars	rp				1	0.2371*	-0.413	0.3976**	-0.237	-0.1927	0.024
	rg				1	0.2399*	-0.4186	0.4015**	-0.5189	-0.2016	0.0242
Ascorbic Acid	rp					1	-0.4368	0.5127**	-0.3189	0.0783	0.2882**
	rg					1	-0.4382	0.5138**	-0.6531	0.0792	0.2917**
Titrable Acidity	rp						1	-0.9425	0.3011**	0.0278	-0.2898
	rg						1	-0.9436	0.6433**	0.0279	-0.3016
TSS: Acid ratio	rp							1	-0.2643	0.0311	0.2988
	rg							1	-0.5584	0.0318	0.3095
Phenols	rp								1	-0.0019	-0.2762
	rg								1	0.0488	-0.509
Pectin	rp									1	-0.1314
	rg									1	-0.1409

Yield/Tree	1

Note-**5% level of significance, *1% levelof significance

Table 17 Mean values of eight clusters obtained by Tocher's method estimated from genotypes of sapota

No.	Plant height	Leaf length	Leaf width	Leaf area	OVary size	Fruit length	Fruit width	Fruit weight	Seed Number	Seed weight	Pulp Seed ratio	Yield/ tree	Percent fruit set	TSS	Total sugars	Reducing sugars	Non-reducing sugars	Ascorbc acid	Titrable acidity	TSS: Acid ratio	Total Phenols	Pectin
I	5.45	10.36	4.02	26.9	3.98	6.30	6.34	194.52	5.83	4.46	37.97	48.13	10.79	20.09	9.20	7.31	1.88	7.22	0.26	77.99	0.52	1.83
II	5.57	9.39	3.49	25.97	4.01	6.34	6.76	169.27	4.17	4.08	38.62	72.02	16.26	21.08	10.14	8.02	2.12	9.23	0.23	90.37	0.48	1.86
III	5.92	10.55	3.92	29.03	4.08	6.34	6.38	135.65	4.29	4.27	28.87	54.99	11.77	22.56	10.26	7.97	2.33	9.11	0.20	113.01	0.46	2.07
IV	6.5	10.11	3.88	28.73	3.63	5.97	6.05	129.42	4.87	3.39	34.68	101.03	18.67	23.69	10.94	8.79	2.37	9.47	0.18	135.10	0.43	1.83
V	5.53	10.22	3.76	27.84	4.12	6.36	6.34	163.31	4.42	4.35	37.25	99.62	14.86	21.97	10.34	8.26	2.08	8.31	0.22	99.01	0.42	1.75
VI	4.12	9.88	3.23	24.42	3.82	6.27	6.17	134.32	5.50	2.93	42.3	38.55	9.71	19.02	8.6	7.61	0.98	6.16	0.29	65.84	0.57	2.29
VII	5.69	9.82	3.62	25.47	3.90	6.83	6.35	156.20	4.00	3.88	36.14	133.21	21.34	22.77	10.83	9.54	2.15	10.5	0.19	122.96	0.44	2.34
VIII	4.81	9.83	4.31	32.42	3.93	8.39	6.09	181.64	4.00	4.23	36.88	111.81	22.60	24.57	11.99	9.00	2.98	10.5	0.17	149.88	0.53	1.66

Table 18 Eigen values, percent variability and cumulative variability for principal components of tree morphological and fruit biochemical characters in sapota genotypes.

	PC1	PC2	PC3	PC4	PC5	PC6
Eigen Value	5.61	3.6	2.87	1.78	1.46	1.17
Per cent of varience explained	26.74	17.17	13.68	8.49	7.00	5.61
Cumulative varience explained	26.74	43.91	57.59	66.08	73.08	78.69

Table 19 Characters loading of principal components for tree morphological and fruit bio-chemical characters in sapota genotypes

S.No.	Character	PC1	PC2	PC3	PC4	PC5	PC6
1	**Plant height**	0.15	0.06	0.07	0.25	**-0.48**	-0.37
2	**Leaflength**	0.15	0.26	**-0.40**	0.07	0.13	-0.04
3	**Leaf width**	0.19	0.23	**-0.41**	0.08	0.08	-0.01
4	**Leaf area**	0.22	0.21	**-0.36**	0.17	0.14	0.06
5	**Ovarysize**	-0.01	0.19	0.11	-0.21	0.05	**0.51**
6	**Fruit length**	0.15	0.19	0.31	0.02	**0.31**	-0.10
7	**Fruit width**	0.05	0.43	**0.19**	0.05	-0.16	-0.10
8	**Fruit weigth**	0.03	**0.45**	0.26	-0.03	-0.04	0.05
9	**Seed number**	-0.19	0.04	-0.12	0.26	-0.10	**0.48**
10	**Seed weight**	0.07	**0.42**	-0.12	-0.03	-0.30	0.19
11	**Pulp to Seed ratio**	-0.04	0.27	**0.43**	0.04	0.20	-0.06
12	**Per cent fruit set**	**0.23**	-0.06	-0.07	0.08	0.34	-0.26
13	**Yield/tree**	0.45	-0.27	0.05	0.02	-0.06	**0.27**
14	**TSS**	0.35	-0.17	0.03	0.02	-0.05	**0.36**
15	**Totalsugars**	**0.36**	-0.13	0.14	-0.07	0.08	0.19

16	**Reducingsugars**	0.22	-0.15	0.17	**0.47**	0.14	0.18
17	**Non-reducing sugars**	0.18	0.02	-0.02	**-0.58**	-0.14	0.02
18	**Ascorbicacid**	**0.33**	0.05	0.14	0.11	0.18	0.03
19	**Titrable acidity**	**-0.32**	0.13	-0.08	0.13	0.30	0.06
20	**TSS Acid ratio**	**0.35**	-0.17	0.08	-0.06	-0.20	0.09
21	**Phenols**	**-0.29**	-0.09	0.11	0.10	0.02	0.17
22	**Pectins**	0.01	-0.02	0.09	**0.40**	-0.36	0.04

Table 20 Clustering pattern of sapota genotypes by PCA method

Cluster number	Number of genotypes	Name of genotypes
I	8	Badami, Bombay,Pakala, Pakala Round, Pakala Oval, Simhoudi, Ulvapadu Selection and Seedless
II	10	Brazilian Sapota, Kalipatti, Kirthibarthi, Pala, CO-2, CO-3, DHS-1, DHS-2, PKM-3 and PKM-4
III	7	Calcutta Round, Gavarayya, Gutti, Mirandi, Singapore, Tagarampudi and CO-1,
IV	6	Dwarapudi, Hybrid, Krishna Rao, Virudhnagar, PKM-1 and PKM-2
V	1	Cricket Ball
VI	1	Columbian Sapota

Table 15 Clustering pattern of sapota genotypes by Tocher’s method

Cluster Number	Number of genotypes	Name of genotypes
I	6	Cricket Ball, Hybrid, Badami, Krishna Rao, Seedless and Columbian Sapota
II	6	Pakala Round, CO-1,CO-2,Pakala,CO-3 and Pakala Oval
III	8	Gavarayya, Gutti, Calcutta Round, PKM-3, Singapore, Dwarapudi, Simhoudi and DHS-1
IV	3	Pala, PKM-1 and Kirthibarthi
V	4	Brazilian Sapota,DHS-2, Ulvapadu Selection and Tagarampudi
VI	2	Bombay and Mirandi
VII	3	Virudnagar, PKM-2 and Kalipatti
VIII	1	PKM-4

Table 14 Relative contribution of different characters towards divergence in thirty three genotypes of sapota

S.No.	Character	Time ranked first	Contribution (%)
1	Plant height	715	38.72
2	Leaflength	51	3.05
3	Leaf width	42	2.11
4	Leaf area	40	2.08
5	Ovarysize	15	1.07
6	Fruit length	95	5.46
7	Fruit width	84	4.59
8	Fruit weigth	81	4.28
9	Seed number	58	3.83
10	Seed weight	46	2.14
11	Pulp to Seed ratio	22	1.65
12	Per cent fruit set	140	7.07
13	Yield/tree	235	14.19
14	TSS	48	2.25
15	Totalsugars	26	1.87
16	Reducingsugars	19	1.23
17	Non-reducing sugars	27	1.86
18	Ascorbicacid	6	0.54
19	Titrable acidity	4	0.25
20	TSS to Acid ratio	7	0.62
21	Total phenols	2	0.18
22	Pectins	9	0.96

Table 16 Mean inter and intra cluster distance among eight clusters formed by Tocher's method in sapota genotype

	Cluster I	**Cluster II**	**Cluster III**	**Cluster IV**	**Cluster V**	**Cluster VI**	**Cluster VII**	**Cluster VIII**
Cluster I	**27.89**	70.62	42.68	70.71	126.16	168.27	166.79	167.36
Cluster II		**32.12**	102.17	167.24	94.75	270.44	255.65	132.23
Cluster III			**95.64**	89.22	192.84	247.26	160.76	170.16
Cluster IV				**20.15**	203.47	124.35	217.39	252.94
Cluster V					**81.91**	222.83	240.88	248.17
Cluster VI						**25.82**	398.84	364.33
Cluster VII							**78.68**	459.86
Cluster VIII								**0.00**

Table 22 Mean values of six clusters for twenty two characters in sapota genotypes by using Ward’s minimum varience method

Cluster No.	Plant height	Leaflength	Leaf width	Leaf area	Ovary size	Fruit length	Fruit width	Fruit weight	Seed number	Seed weight	Pulp to seed ratio	Yield/tree	Percent fruit set	TSS	Totalsugars	Reducingsugars	Non-reducing sugars	Ascorbicacid	Titrable acidity	TSS: Acid ratio	Total phenols	Pectin
I	**4.92**	**9.16**	**3.33**	**22.54**	**4.02**	**5.92**	**5.85**	**105.4**	**5.00**	**3.07**	**33.71**	**48.96**	**11.92**	**20.47**	**9.52**	**7.84**	**1.66**	**7.01**	**0.25**	**82.65**	**0.52**	**1.83**
II	**5.87**	**10.38**	**3.95**	**29.56**	**3.93**	**6.59**	**6.38**	**161.3**	**4.2**	**4.24**	**34.34**	**90.09**	**16.19**	**22.65**	**10.64**	**8.4**	**2.34**	**9.89**	**0.2**	**114.6**	**0.44**	**1.96**
III	**5.4**	**9.43**	**2.8**	**20.67**	**3.64**	**7.47**	**6.25**	**167.9**	**6.67**	**2.02**	**61.5**	**59.47**	**12.6**	**19.4**	**8.97**	**8.04**	**0.93**	**7.18**	**0.295**	**65.76**	**0.611**	**2.86**
IV	**5.92**	**9.97**	**3.57**	**26.58**	**4.49**	**5.45**	**5.65**	**92.7**	**6.33**	**5.5**	**17.27**	**22.06**	**9.47**	**22.23**	**8.93**	**6.23**	**2.7**	**5.35**	**0.202**	**110.00**	**0.478**	**1.98**
V	**5.55**	**13.81**	**5.87**	**40.17**	**4.03**	**5.32**	**7.34**	**221.1**	**6.33**	**7.81**	**28.8**	**91.97**	**16.03**	**19.9**	**8.97**	**6.87**	**2.1**	**6.22**	**0.252**	**78.96**	**0.425**	**2.61**
VI	**6.56**	**9.83**	**3.47**	**23.25**	**4.27**	**7.91**	**8.2**	**540.3**	**5.33**	**7.07**	**75.67**	**35.01**	**8.6**	**18.6**	**8.61**	**6.6**	**2.01**	**7.69**	**0.245**	**75.91**	**0.551**	**1.34**

Table 21 Mean intra and inter cluster distance among six clusters formed by Ward’s minimum varience method in sapota genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	216.32	456.51	491.00	707.10	1261.65	1105.51
Cluster II		403.44	676.06	643.73	947.50	1367.33
Cluster III			724.99	892.20	885.22	1523.80
Cluster IV				984.02	1019.25	1208.48
Cluster V					0.00	1312.62
Cluster VI						0.00

Table 23 Yield and absorbance ratio of DNA in different genotypes of sapota

S.No	Genotypes	Quantity (ng/µl)	260/280 ratio
1	Badami	527.00	1.78
2	Bombay	610.00	1.92
3	Brazilian Sapota	695.00	1.80
4	Calcutta Round	734.00	1.79
5	Columbian Sapota	702.00	1.94
6	Cricket Ball	855.00	1.88
7	Dwarapudi	660.00	1.82
8	Gavarayya	352.00	1.75
9	Guthi	841.00	1.69
10	Hybrid	790.00	2.00
11	Kalipatti	728.00	1.73
12	Kirthibarthi	680.00	1.70
13	Krishna Rao	530.00	1.75
14	Mirandi	600.00	1.80
15	Pakala	773.00	1.91
16	Pakala Oval	585.00	1.78
17	Pakala Round	620.00	1.69
18	Pala	1055.00	1.65
19	Seedless	1260.00	1.77
20	Simhoudi	1555.00	1.79

21	Singapore	1840.00	1.75
22	Tagarampudi	2045.00	1.82
23	Ulvapadu Selection	2268.00	1.89
24	Virudnagar	1752.00	1.66
25	CO-1	1330.00	2.02
26	CO-2	1971.00	1.94
27	CO-3	1883.00	2.00
28	DHS-1	1955.00	1.86
29	DHS-2	2020.00	1.70
30	PKM-1	2530.00	1.90
31	PKM-2	2095.00	1.85
32	PKM-3	1450.00	1.76
33	PKM-4	1628.00	2.02

Table 24 List of Random primers used for DNA amplification in sapota genotypes

S.No.	Primer	Number of alleles	Allele size (bp)	Polymorphic information content (PIC)
1	OPB-6	8	540-2800	0.78
2	OPC-2	9	750-2600	0.74
3	OPG-3	15	650-3000	0.90
4	OPG-4	8	400-2450	0.77
5	OPG-5	13	500-2900	0.85
6	OPG-6	12	600-2400	0.83
7	OPG-7	5	460-1520	0.61
8	OPG-13	14	300-2400	0.88
9	OPV-3	4	1500-2150	0.63
10	OPV-4	7	300-1350	0.72
11	OPV-5	5	700-1500	0.65
12	OPV-9	8	500-2000	0.69
13	OPV-10	6	800-2740	0.72
14	OPV-17	11	250-1800	0.80
15	OPV-18	12	400-2000	0.83
16	OPX-2	3	350-600	0.55
17	OPX-3	7	300-2200	0.70
18	OPX-5	3	750-1400	0.61
19	OPX-9	4	700-3000	0.63
20	OPX-11	7	450-2450	0.71
21	OPX-12	9	820-2500	0.77

22	OPX-13	6	400-2300	0.68
23	OPX-14	13	750-1550	0.65
24	OPX-15	11	600-2950	0.82
25	OPX-20	4	1800-2500	0.60

Table 25 Details of unique RAPD fragments associated with specific sapota genotypes

S.No.	Name of the genotype	Primer revealing unique RAPD	Size of the fragment (bp)
1	Gutti	OPC-2	1845
2	Tagarampudi	OPG-3	650
3	PKM-4	OPG-3	744
4	Calcutta Round	OPG-4	2450
5	Columbian Sapota	OPG-4	2385
6	Cricket Ball	OPG-4	2250
7	Pakala Oval	OPG-7	365
8	Cricket Ball	OPX-11	180
9	Gavarraya	OPX-11	400
10	Kalipatti	OPX-12	2450
11	DHS-2	OPV-9	2000
12	DHS-1	OPV-10	2650

13	Columbian Sapota	OPX-3	280
14	Cricket Ball	OPX-11	320
15	Kalipatti	OPX-12	2490
16	Pakala Round	OPX-12	130

Table 27 Details of unique SSR fragments associated with specific sapota genotypes

S.No.	**Name of the genotype**	**Primer revealing unique SSR fragment**	**Size of the fragment (bp)**
1	Columbian Sapota	SSR S-1	235
2	DHS-1 and DHS-2	SSR S-1	160
3	CO-3	SSR S-2	190
4	Pakala Round	SSR S-3	180
5	Seedless	SSR S-4	175
6	PKM-2	SSR S-5	555
7	DHS-1 and DHS-2	SSR S-7	250
8	Cricket Ball	SSR S-8	220
9	Calcutta Round	SSR S-8	180
10	CO-2	SSR S-8	220

11	Gavarayya	SSR S-9	135
12	Singapore	SSR S-10	220
13	DHS-1	SSR S-10	280
14	PKM-2	SSR S-10	300
15	CO-2	SSR Mh-26	195
16	Bombay	SSR Mh-20	130
17	Kalipatti	SSR Mh-12	220
18	Pakala Round	SSR Mh-12	230
19	Pala	SSR Mh-17	180

Table-1 List of RAPD primers nucleotide sequences used for amplification of sapota DNA

S.No.	Primer	Nucleotide sequence (5'-3')	S.No.	Primer	Nucleotide sequence (5'-3')
1	OPB-1	GTTTCGCTCC	26	OPC-6	GAACGGACTC
2	OPB-2	TGATCCCTGG	27	OPC-7	GTCCCGACGA
3	OPB-3	CTTCCCCCTG	28	OPC-8	TGGACCGGTG
4	OPB-4	GGACTGGAGT	29	OPC-9	CTCACCGTCC
5	OPB-5	TGCGCCCTTC	30	OPC-10	TGTCTGGGTG
6	OPB-6	TGCTCTGCCC	31	OPC-11	AAAGCTGCGG
7	OPB-7	GGTGACGCAG	32	OPC-12	TGTCATCCCC

8	OPB-8	GTCCACACGG	33	OPC-13	AAGCCTCGTC
9	OPB-9	TGGGGGACTC	34	OPC-14	TGCGTCGTTG
10	OPB-10	CTGCTGGGAC	35	OPC-15	GACGGATCAG
11	OPB-11	GTAGACCCGT	36	OPC-16	CACACTCCAG
12	OPB-12	CCTTGACGCA	37	OPC-17	TTCCCCCCAG
13	OPB-13	TTCCCCCGCT	38	OPC-18	TGAGTGGGTG
14	OPB-14	TCCGCTCTGG	39	OPC-19	GTTGCCAGCC
15	OPB-15	GGAGGGTGTT	40	OPC-20	ACTTCGCCAC
16	OPB-16	TTTGCCCGGA	41	OPG-1	CTACGGAGGA
17	OPB-17	AGGGAACGAG	42	OPG2	GGCACTGAGG
18	OPB-18	CCACAGCAGT	43	OPG-3	GAGCCCTCCA
19	OPB-19	ACCCCCGAAG	44	OPG-4	AGCGTGTCTG
20	OPB-20	GGACCCTTAC	45	OPG-5	CTGAGACGGA
21	OPC-1	TTCGAGCCAG	46	OPG-6	GTGCCTAACC
22	OPC-2	GTGAGGCGTC	47	OPG-7	GAACCTGCGG
23	OPC-3	GGGGGTCTTT	48	OPG-8	TCACGTCCAC
24	OPC-4	CCGCATCTAC	49	OPG-9	CTGACGTCAC
25	OPC-5	GATGACCGCC	50	OPG-10	AGGGCCGTCT

Table 1 cont…

S.No.	Primer	Nucleotide sequence (5'-3')	S.No.	Primer	Nucleotide sequence (5'-3')
51	OPG-11	TGCCCGTCGT	76	OPV-16	GGGCCAATGT
52	OPG-12	CAGCTCACGA	77	OPV-17	GACGTGGTGA
53	OPG-13	CTCTCCGCCA	78	OPV-18	GTGGAGTCAG

54	OPG-14	GGATGAGACC	79	OPV-19	TGAGGGTCCC
55	OPG-15	ACTGGGACTC	80	OPV-20	AGCCGTGAAA
56	OPG-16	AGCGTCCTCC	81	OPX-1	CTGGGCACGA
57	OPG-17	ACGACCGACA	82	OPX-2	TTCCGCCACC
58	OPG-18	GGCTCATGTG	83	OPX-3	TGGCGCAGTG
59	OPG-19	GTCAGGGCAA	84	OPX-4	CCGCTACCGA
60	OPG-20	TCTCCCTCAG	85	OPX-5	CCTTTCCCTC
61	OPV-1	GTGGCATCTC	86	OPX-6	ACGCCAGAGG
62	OPV-2	CATCGCCGCA	87	OPX-7	GAGCGAGGCT
63	OPV-3	ACAGCCTGCT	88	OPX-8	CAGGGGTGGA
64	OPV-4	GGCTGCAATG	89	OPX-9	GGTCTGGTTG
65	OPV-5	GGCTGCGACA	90	OPX-10	CCCTAGACTG
66	OPV-6	AAGGCTCACC	91	OPX-11	GGAGCCTCAG
67	OPV-7	AGAGCCGTCA	92	OPX-12	TCGCCAGCCA
68	OPV-8	AGGCAGAGCA	93	OPX-13	ACGGGAGCAA
69	OPV-9	AGCAGCGCAC	94	OPX-14	ACAGGTGCTG
70	OPV-10	CAAACGTGGG	95	OPX-15	CAGACAAGCC
71	OPV-11	AGACGATGGG	96	OPX-16	CTCTCGTTCGG
72	OPV-12	AAGCCTGCGA	97	OPX-17	GACACGGACC
73	OPV-13	GGGTCTCGGT	98	OPX-18	GACTAGGTGG
74	OPV-14	GGTCGATCTG	99	OPX-19	TGGCAAGGCA
75	OPV-15	AGTCGCCCTT	100	OPX-20	CCCAGCTAGA

Table 2 List of SSR primers nucleotide sequences used for amplification of sapota DNA

S.No.	Primer		Nucleotide sequence (5'-3')
1	SSR S-1	F	TCTCAGCAACACAACTTCGT
		R	ACAAATTGGTGTTGTGCCCA
2	SSR S-2	F	GTAATAAAGGAGCAATAACGCCC
		R	TGCTCATAACTTCCCTCTAGACC
3	SSR S-3	F	ATCGTTACAAAGGGCGATGC
		R	GGCGGTCCTTGGAAAGTTTT
4	SSR S-4	F	TTCCTTCGACTTTCTGGGCT
		R	TCTGATAAATCCGCCCAGACT
5	SSR S-5	F	ACCGCTACAACCCTTCCTAC
		R	CAGAGCAACGCCATTCCTAC
6	SSR S-6	F	ATCGTTACAAAGGGCGATGC
		R	GGCGGACCTTGGAAAGTTTT
7	SSR S-7	F	CCGCTTCTATGTTGTCACCG
		R	ACCTCCGTTGGCAATATGGA
8	SSR S-8	F	GGGAGCTGGAGTGGGTAAAA
		R	GGCGGTTCATTCATCTGACC
9	SSR S-9	F	CCAGTCTGGGCGGATTTATC
		R	TACGAGCCAAAGTCCTAGCA
10	SSR S-10	F	TCTCAGCAACACAACTTCGT
		R	GCTAGTTGATTCGGCAGGTG
11	SSR Mh-03	F	CACTTCTGTCTCTCTCCTCGT
		R	GGGTGATTGCAGAGACGTA

12	SSR Mh-04	F	GCACTCTCCATGGTTCCAGT
		R	AAAGAGTCAATGGCGTGAGC
13	SSR Mh-06	F	ACACGCACAAAACAAACCAA
		R	TTCTTGAAGGAGGGTTGCTC
14	SSR Mh-07	F	ATTGCAGCATATCCACACCA
		R	GCAAAGGGTGATGGGTTAGA
15	SSR Mh-08	F	GTAATGGGAGCCGTTTGAGA
		R	CTGGGTAGCATTTGTTGCAT

Table 2 Cont…

16	SSR Mh-12	F	TGCGGAACTGTGGAAAGAGT
		R	ATCCACAGCAATGACTGACG
17	SSR Mh-17	F	CACGATGACCTCTCAGTGGA
		R	CCTGTGTATGCGTTCGATTG
18	SSR Mh-19	F	AATTACAACCAAAGCTCCACTT
		R	TGAGAGTCTTTTCGCACTTTCA
19	SSR Mh-20	F	GAAGTTTTGACCATTTGGGAAT
		R	GACATAACACTAACCCTTCACGA
20	SSR Mh-22	F	CCCATTATAGCCCTCCCACCT
		R	AGAGAGCACATGCAAGCTCA
21	SSR Mh-24	F	CCACTTCTGTCTCTCTCCTCGT
		R	GACATTGTGGGTGATTGCAG
22	SSR Mh-26	F	TGCTCAGACTGCTTTCTTTTTG

R	TGCAATAAGTGTGATTTGGAGAA

Table-8 Fruit biochemical characters of sapota genotypes

S.No.	Genotypes	TSS (^{O}B)	Titrable Acidity (%)	TSS:Acid ratio
1	Badami	20.57	0.266	77.33
2	Bombay	18.63	0.283	65.83
3	Brazilian Sapota	23.79	0.234	101.64
4	Calcutta Round	22.47	0.194	115.82
5	Columbian Sapota	18.6	0.245	75.91
6	Cricket Ball	19.9	0.252	78.96
7	Dwarapudi	22.23	0.202	110.04
8	Gavarayya	21.87	0.192	113.9
9	Gutti	23.5	0.205	114.63
10	Hybrid	19.5	0.248	78.62
11	Kalipatti	22.97	0.191	120.26
12	Kirthibarthi	22.83	0.168	135.89
13	Krishna Rao	21.6	0.282	78.59
14	Mirandi	19.4	0.295	65.76
15	Pakala	20.63	0.223	92.51
16	Pakala Oval	20.6	0.231	89.22
17	Pakala Round	20.73	0.24	86.38

18	Pala	24.33	0.181	134.41
19	Seedless	21.63	0.199	108.69
20	Simhoudi	20.37	0.253	80.51
21	Singapore	22.93	0.206	111.3
22	Tagarampudi	20.93	0.217	96.45
23	Ulvapadu Selection	21.6	0.223	96.86
24	Virudnagar	23.07	0.184	125.38
25	CO-1	21.57	0.241	89.5
26	CO-2	23.17	0.256	90.5
27	CO-3	19.77	0.21	94.14
28	DHS-1	22.43	0.191	117.42
29	DHS-2	21.53	0.213	101.07
30	PKM-1	23.9	0.177	135
31	PKM-2	24.03	0.195	123.23
32	PKM-3	24.6	0.219	112.31
33	PKM-4	25.33	0.169	149.88
	Mean	21.74	0.22	101.99
	SEm (±)	0.604	0.01	11.2
	CD (5%)	1.71	0.03	25.6

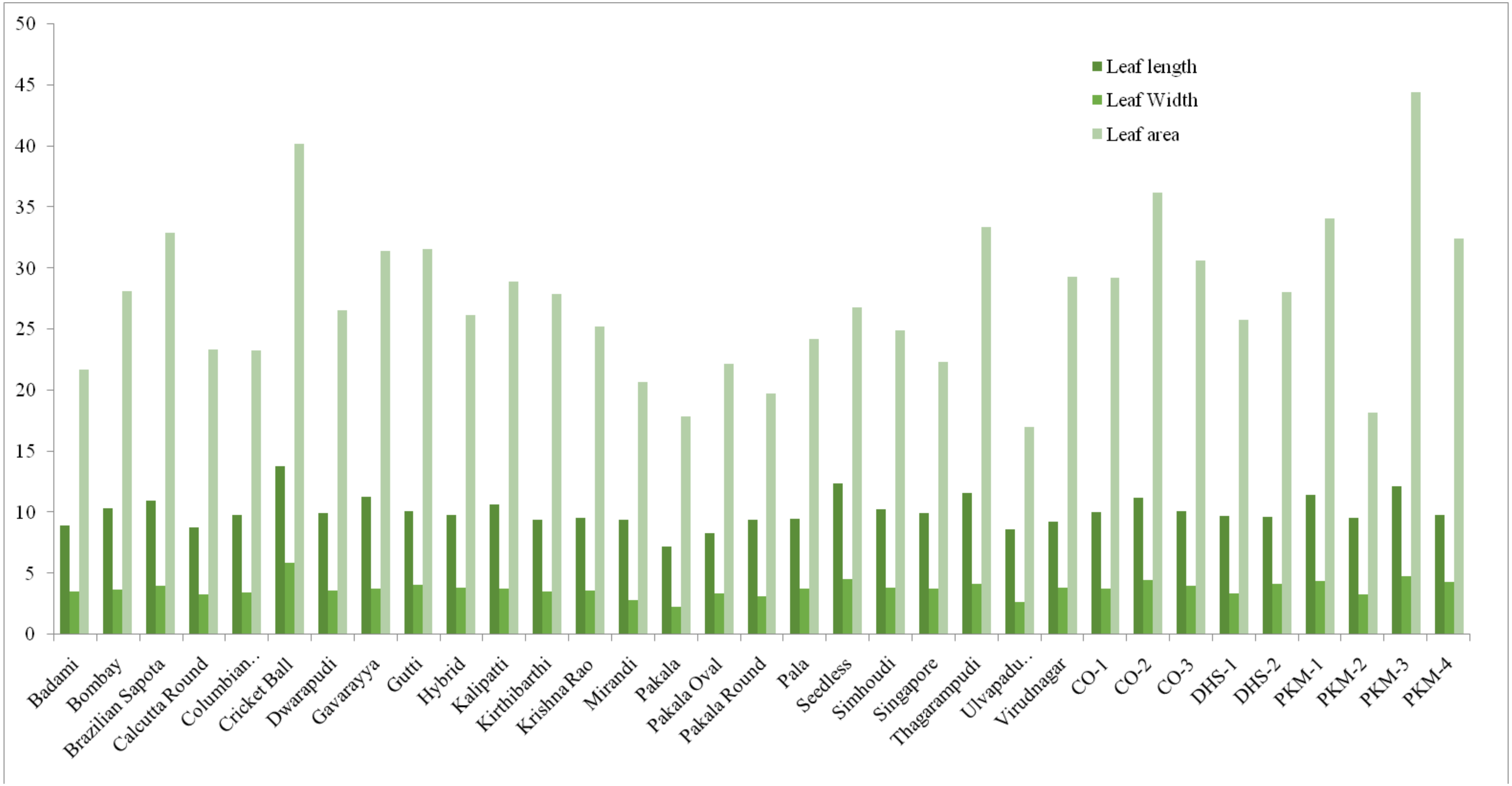


Fig-1 Graph showing leaf parameters of sapota genotypes

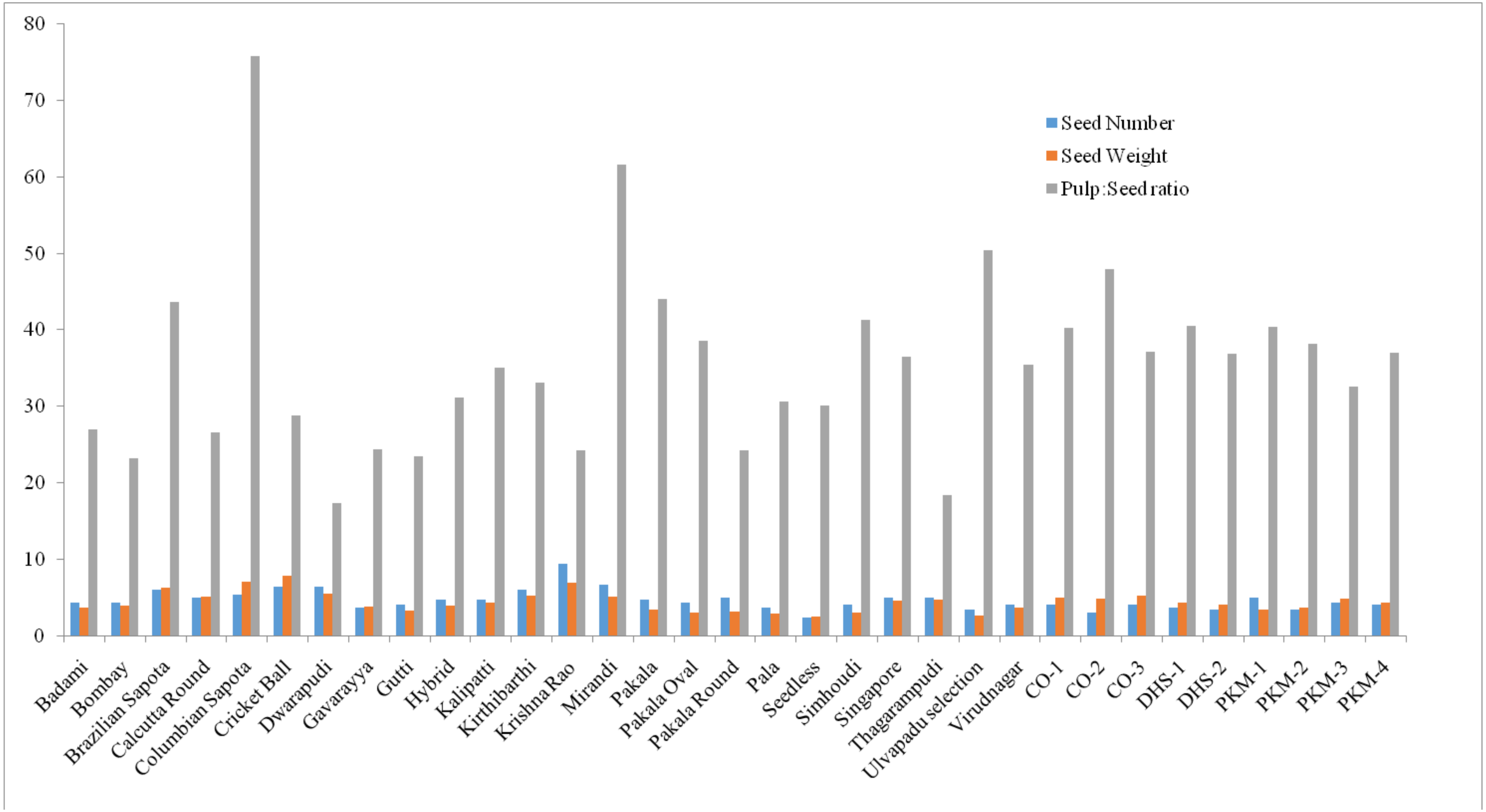


Fig-2 Graph showing seed number, seed weight and pulpto seed ratio of sapota genotypes

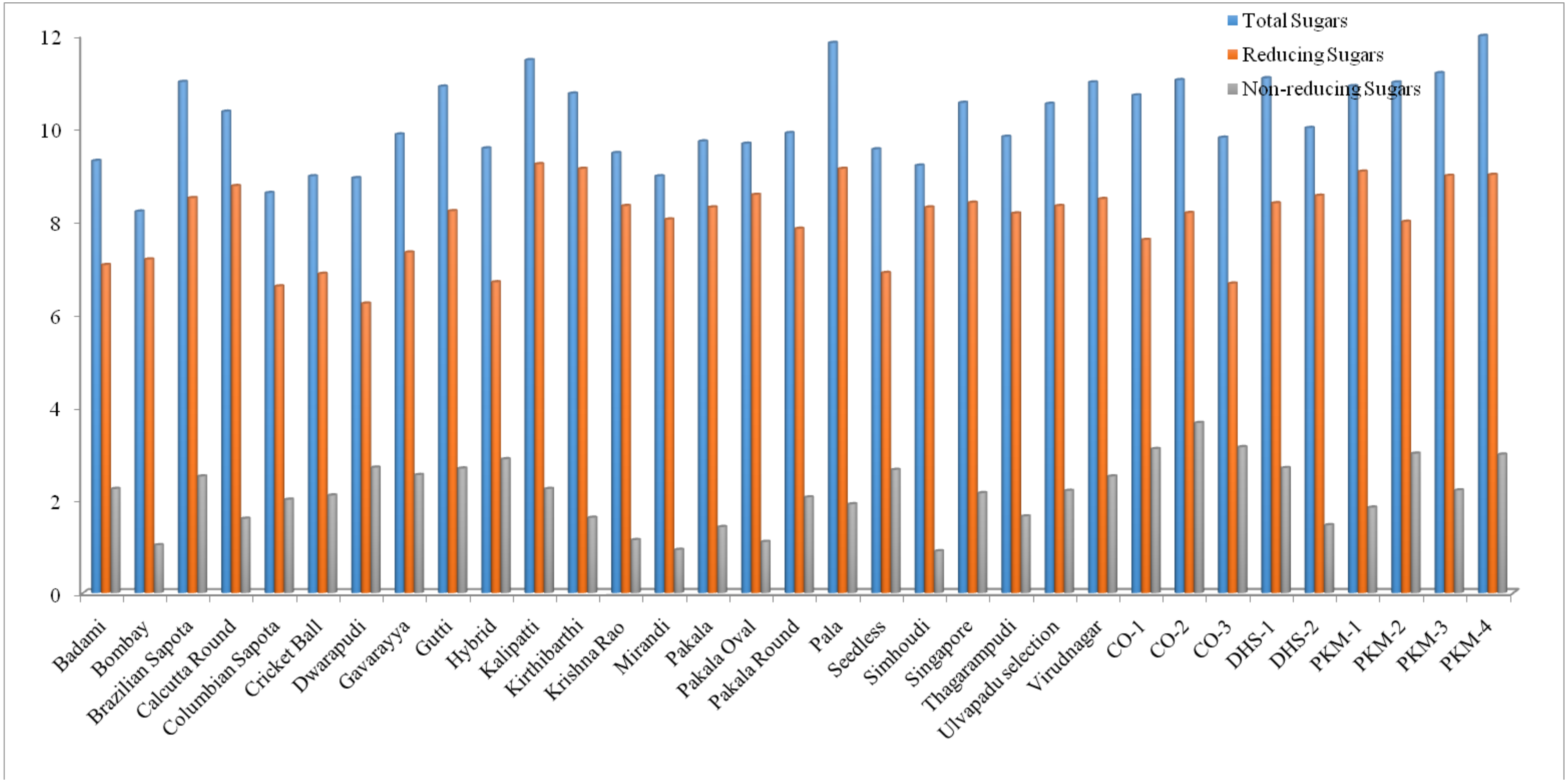


Fig-3 Graph representing Total sugars, Reducing sugars and Non-reducing sugars of sapota genotypes

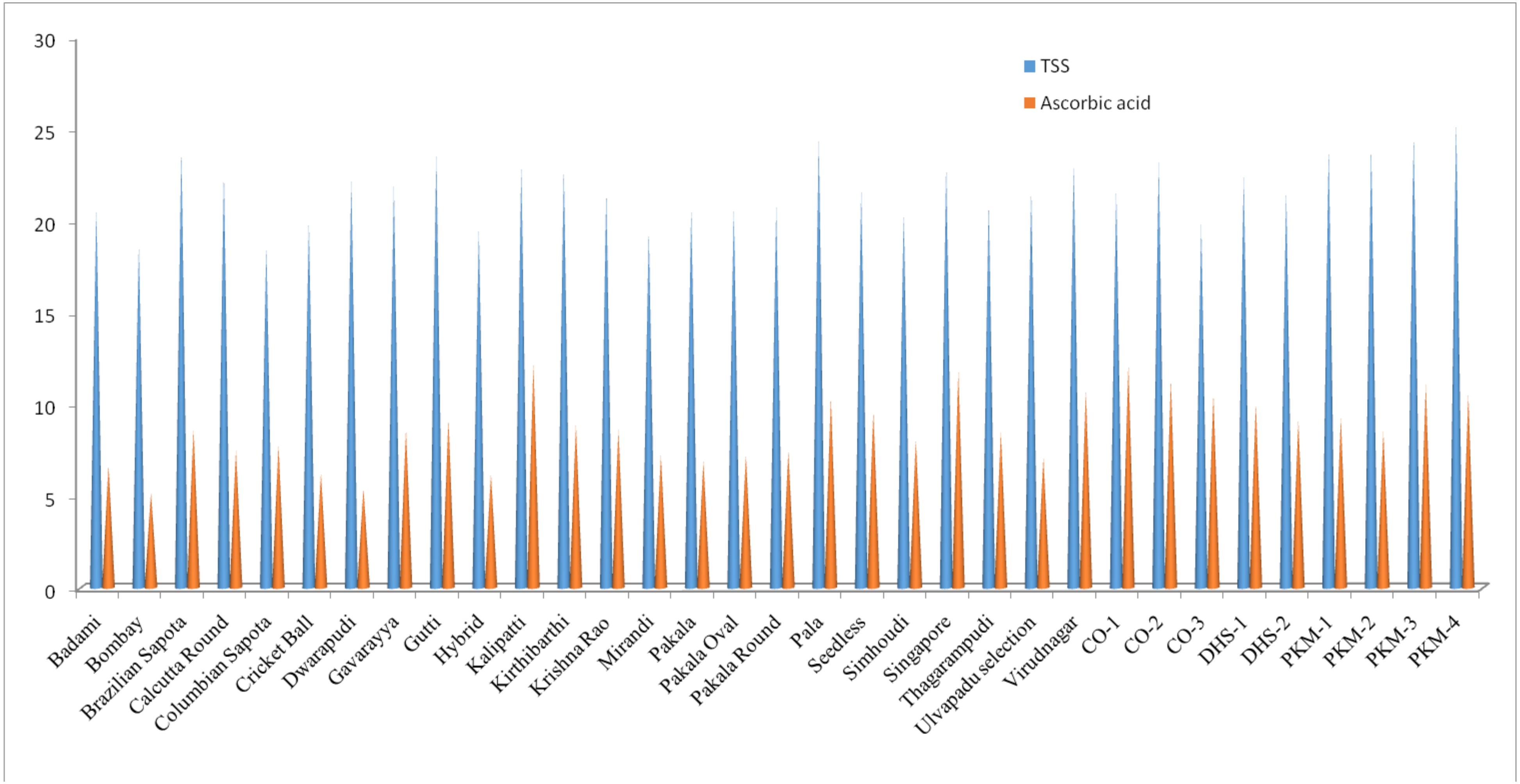


Figure-4 Graph showing TSS and Ascorbic acid content of sapota genotypes

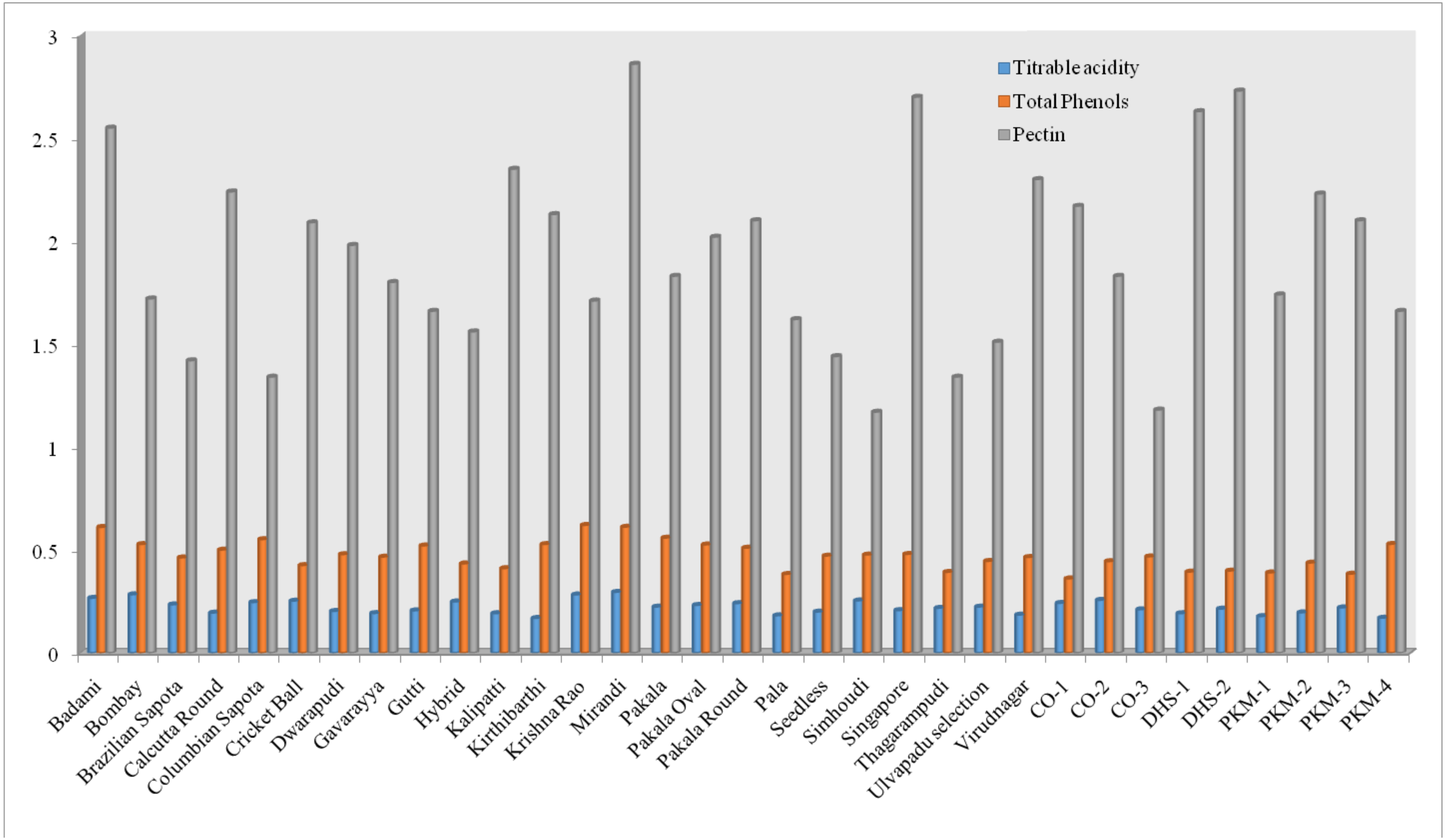


Fig-5 Graph presenting Titrable acidity, total phenols and pectins of sapota genotypes

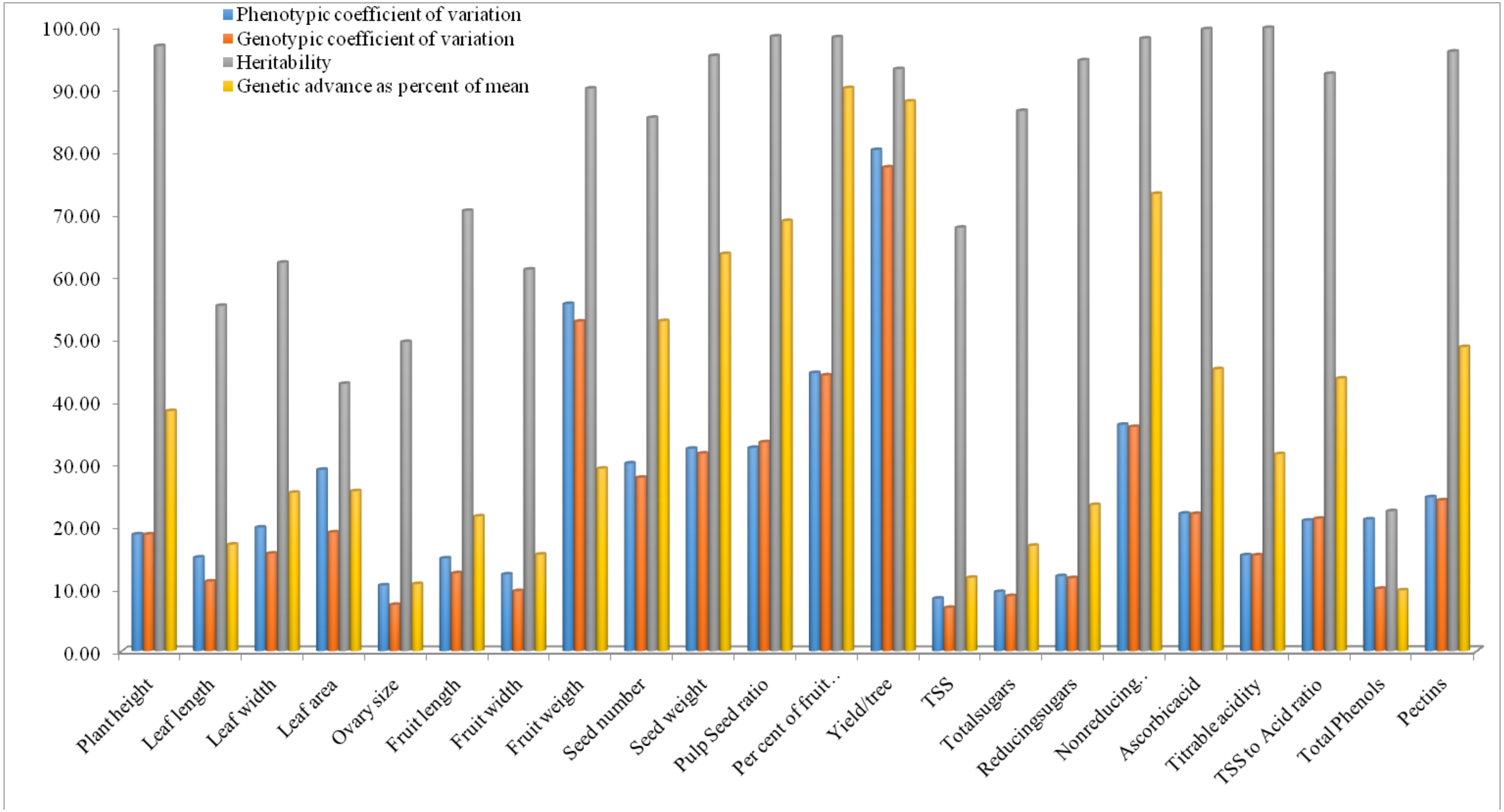
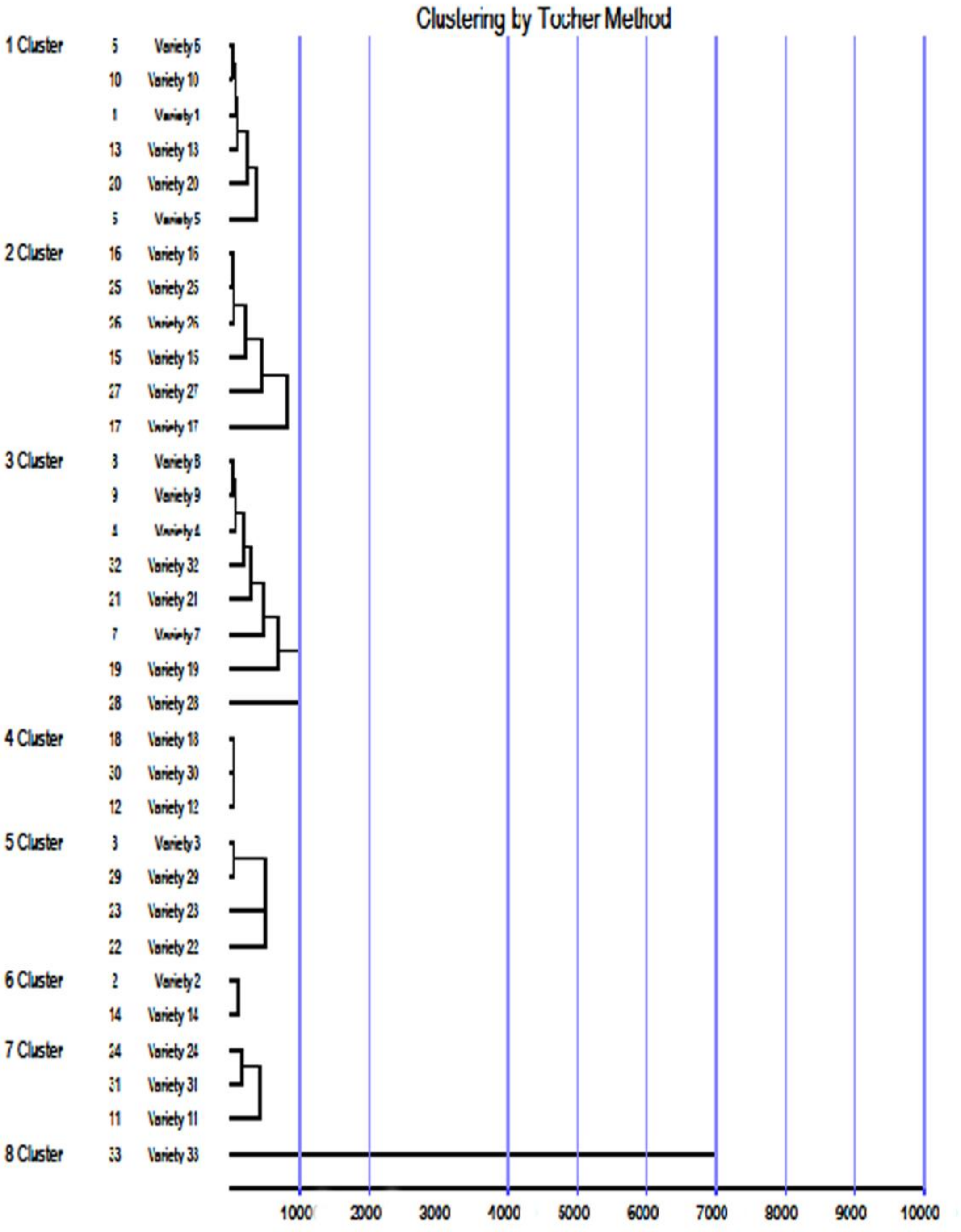


Fig 5 Graph showing distribution of genetic parameters in different sapota genotypes

Fig-9 Dendrogram showing clustering pattern of 33 sapota genotypes by Tocher's method



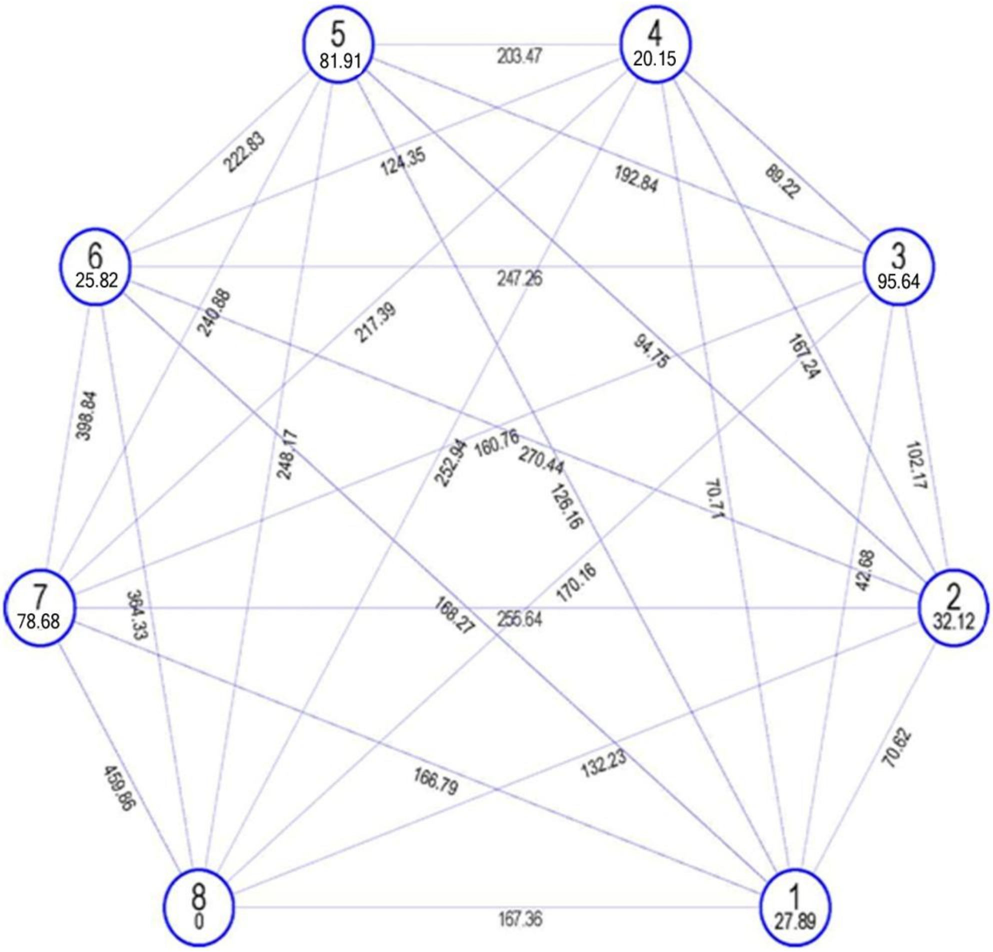


Fig 10 Inter and Intra cluster distances for morphological and fruit bio-chemical characters of sapota genotypes by Tocher's method

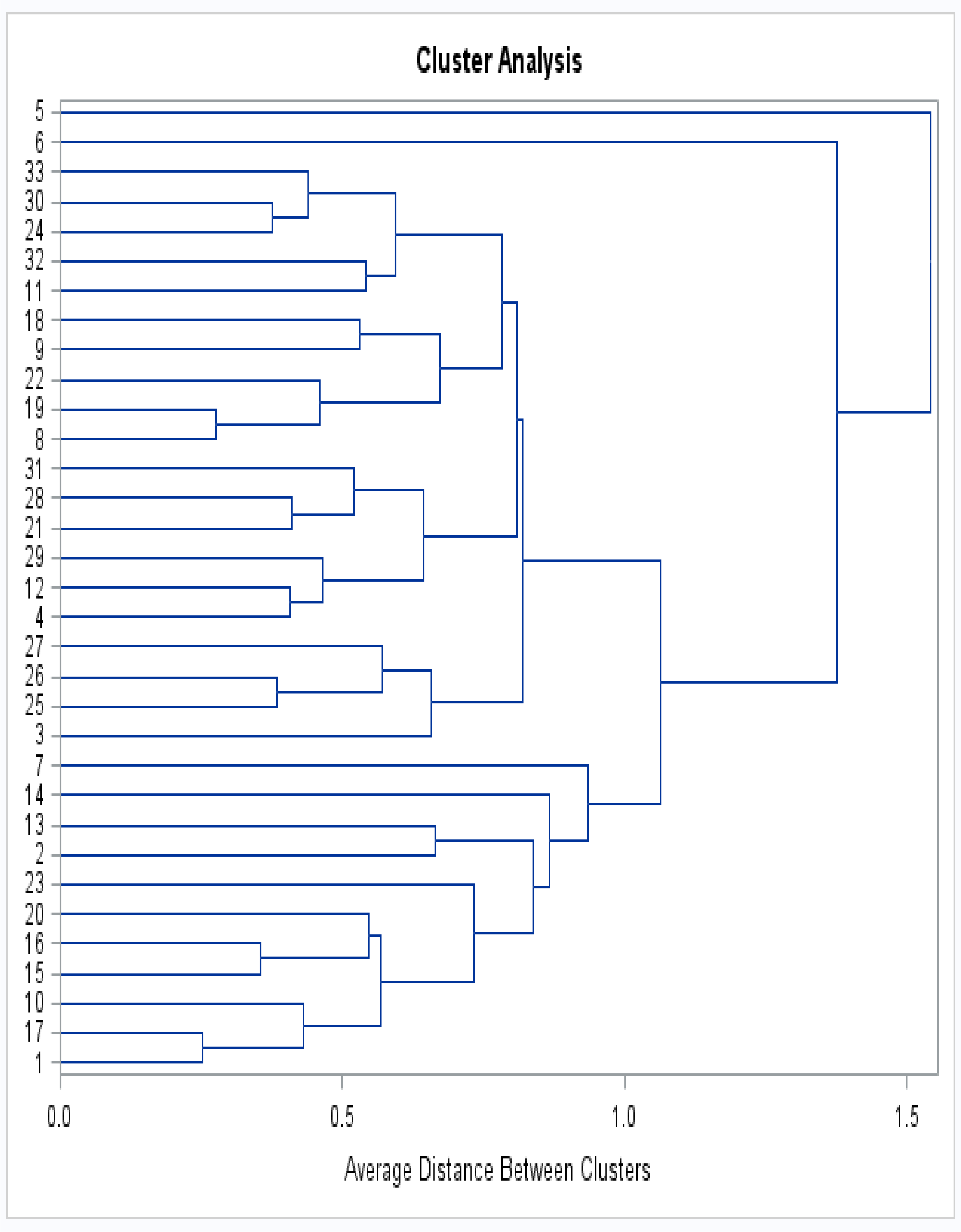


Fig 11 Dendrogram showing clustering pattern of 33 sapota genotypes by Ward's minimum variance method

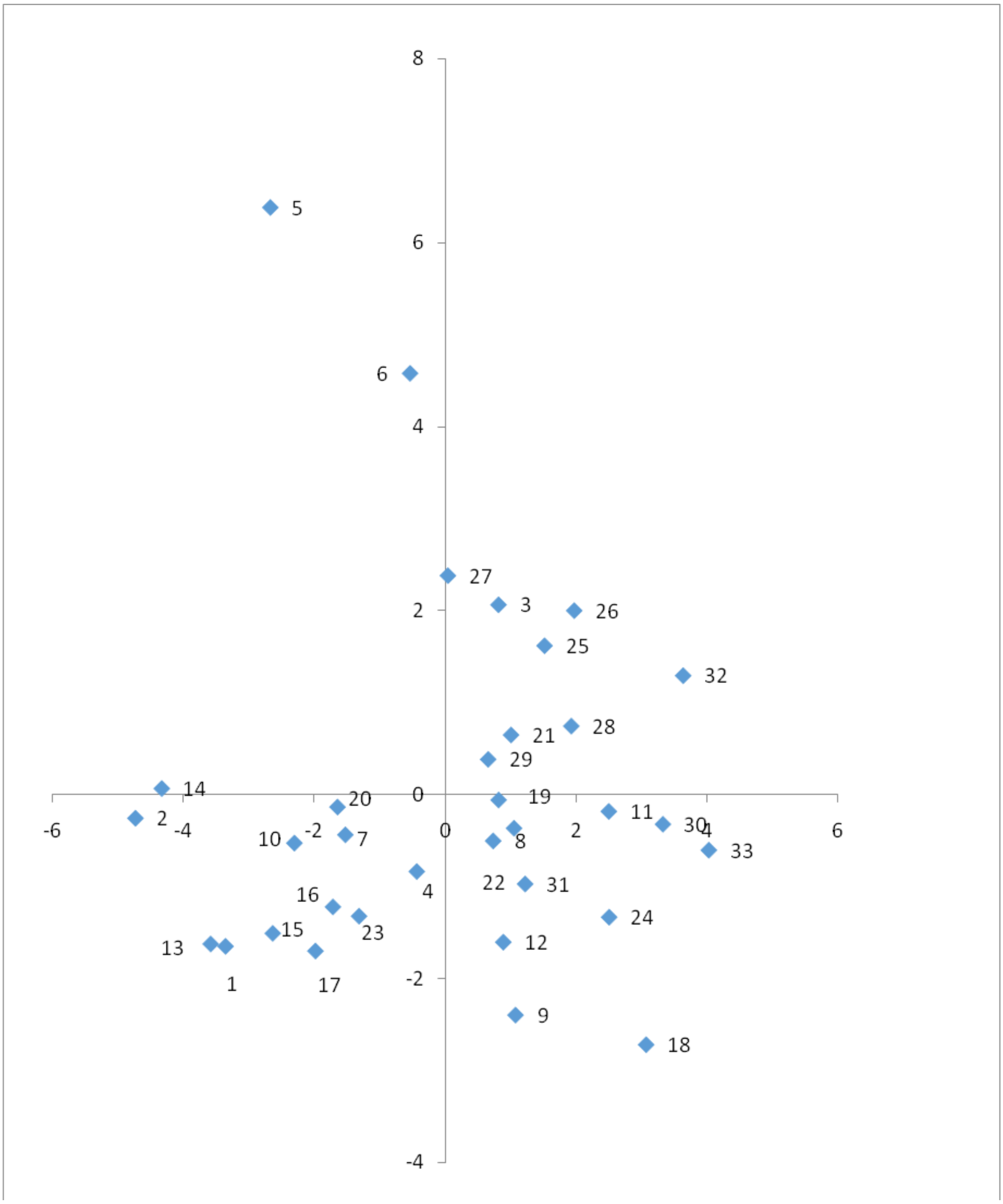


Fig-12 Loading of principal component scores (score plot) in sapota genotypes

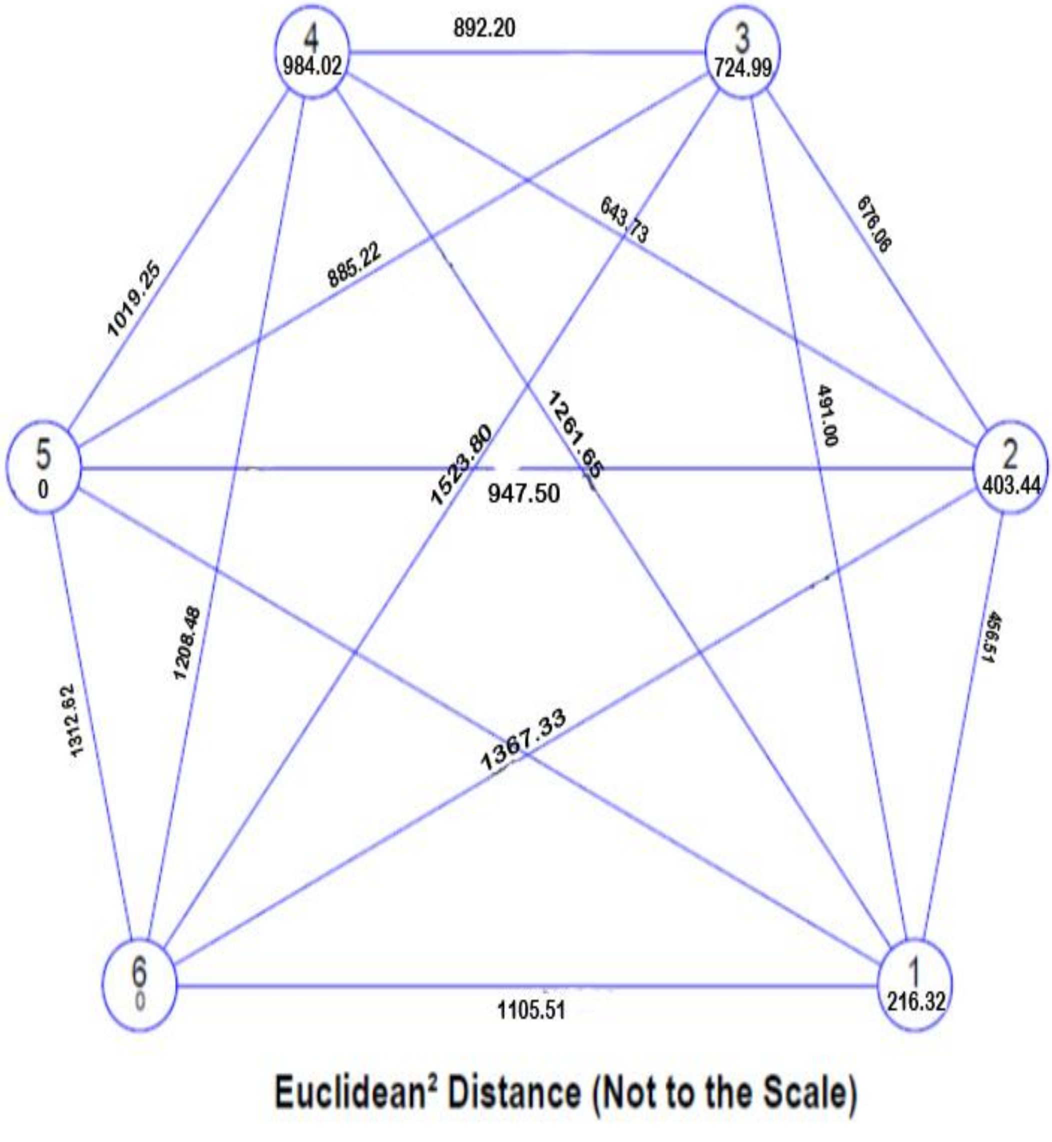


Fig-13 Inter and intra cluster distances for morphological and fruit biochemical characters of sapota genotypes (Ward's minimum variance method)

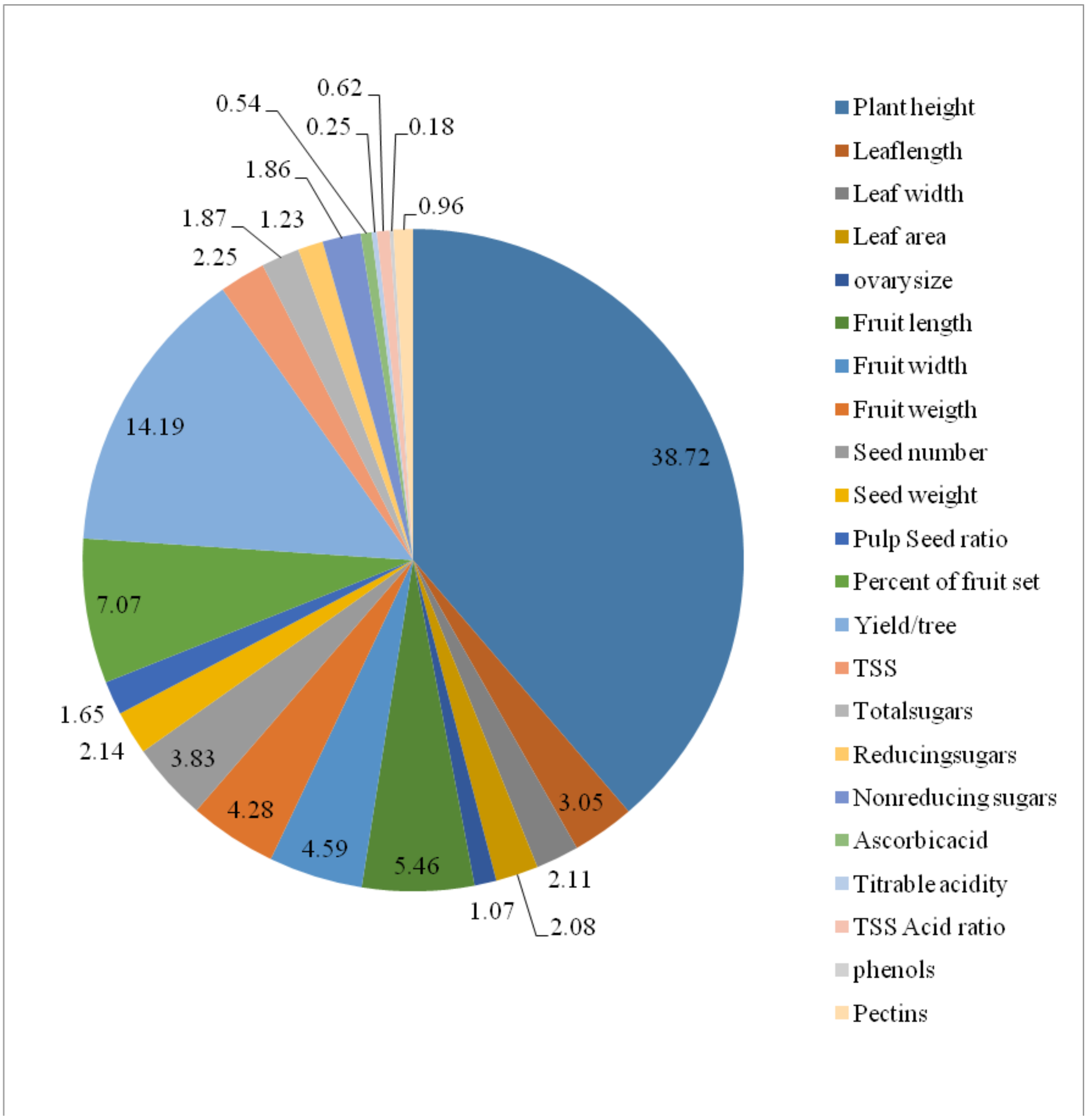


Fig-6 Relative contribution of morphological and fruit bio-chemical characters towards divergence

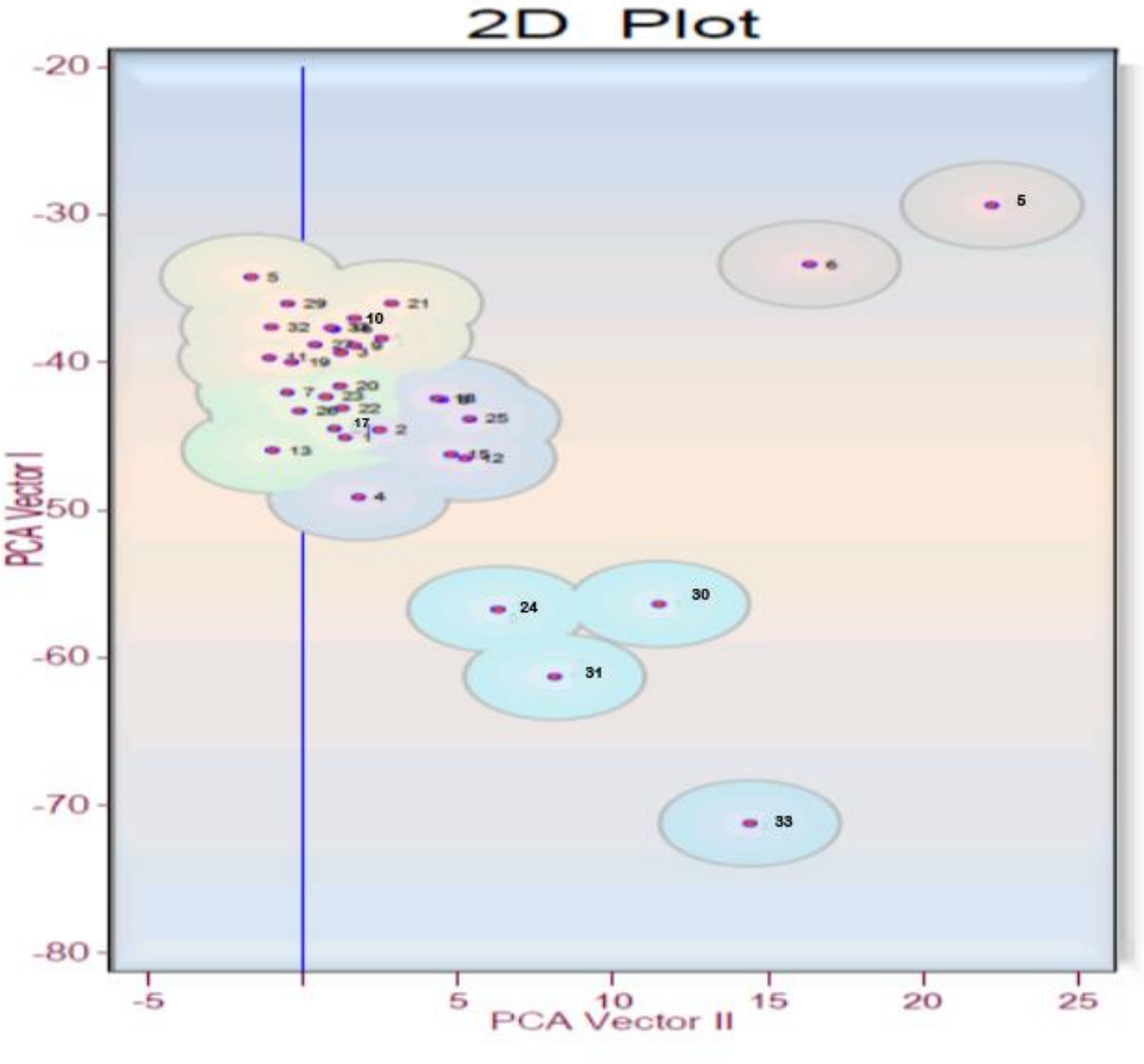
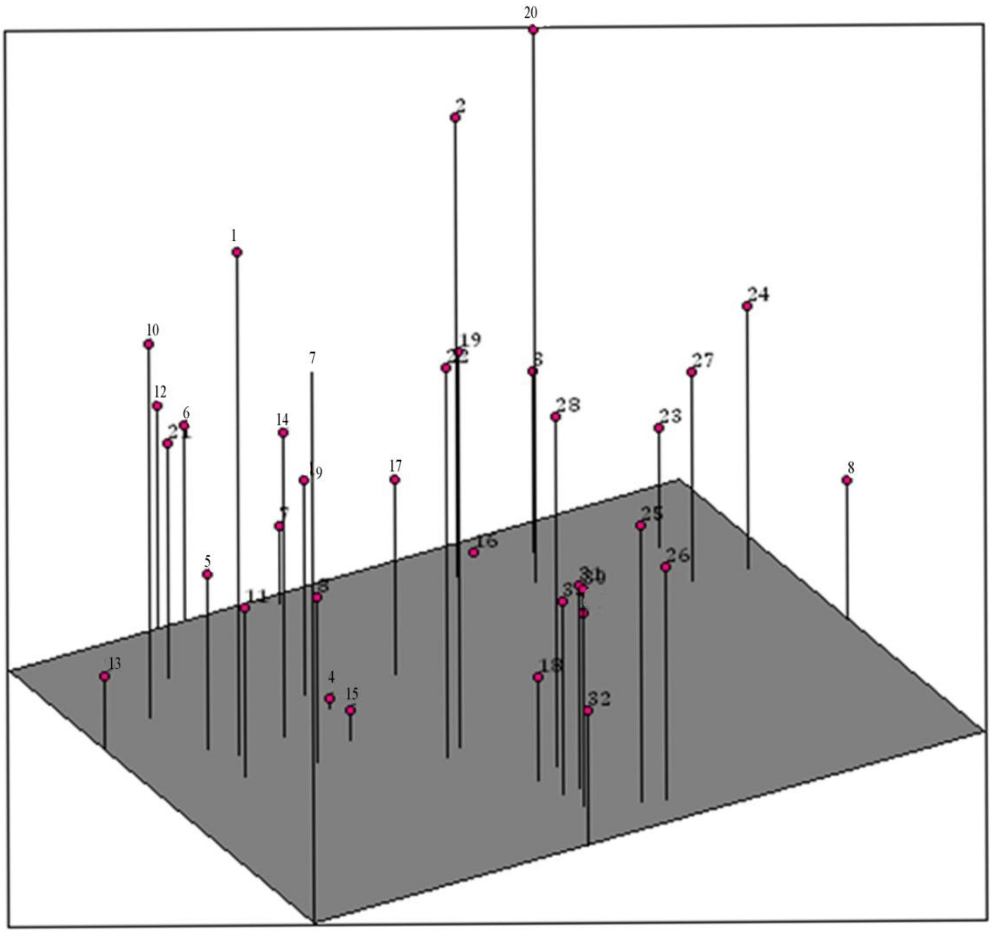


Fig 14 The relative position of sapota genotypes based on PCA scores of morphological and bio-chemical characters

Fig 16 The relative position of sapota genotypes based on RAPD markers



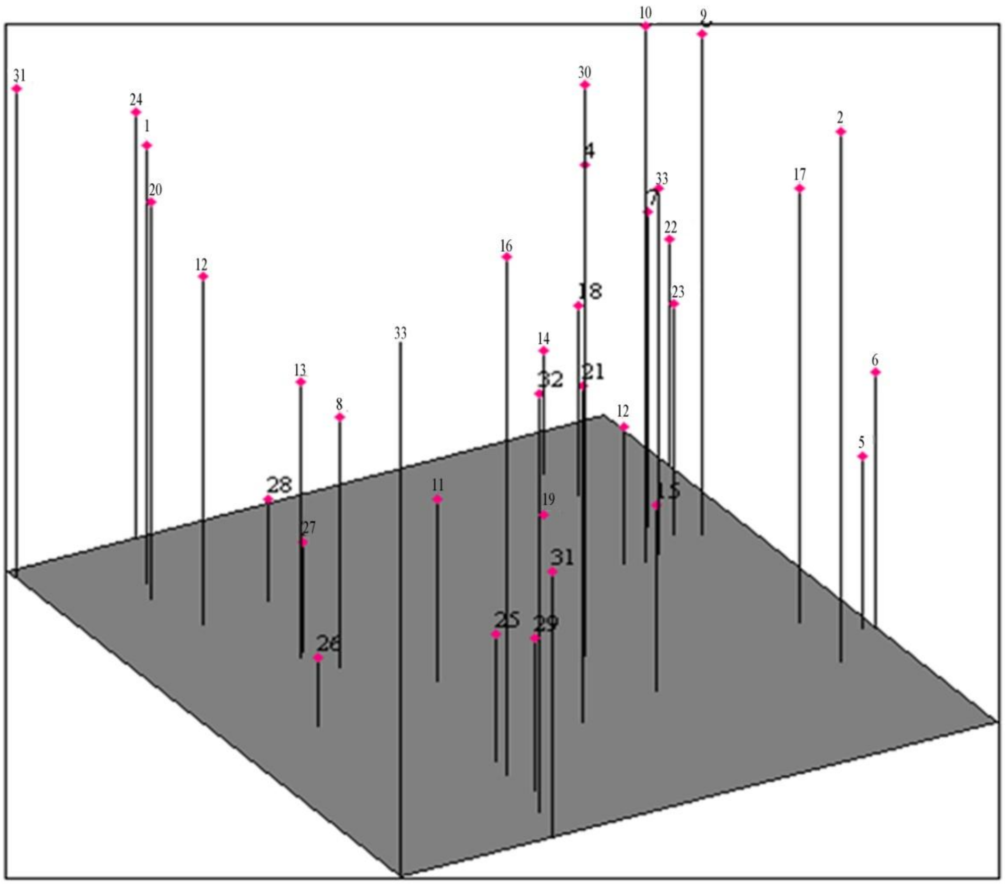
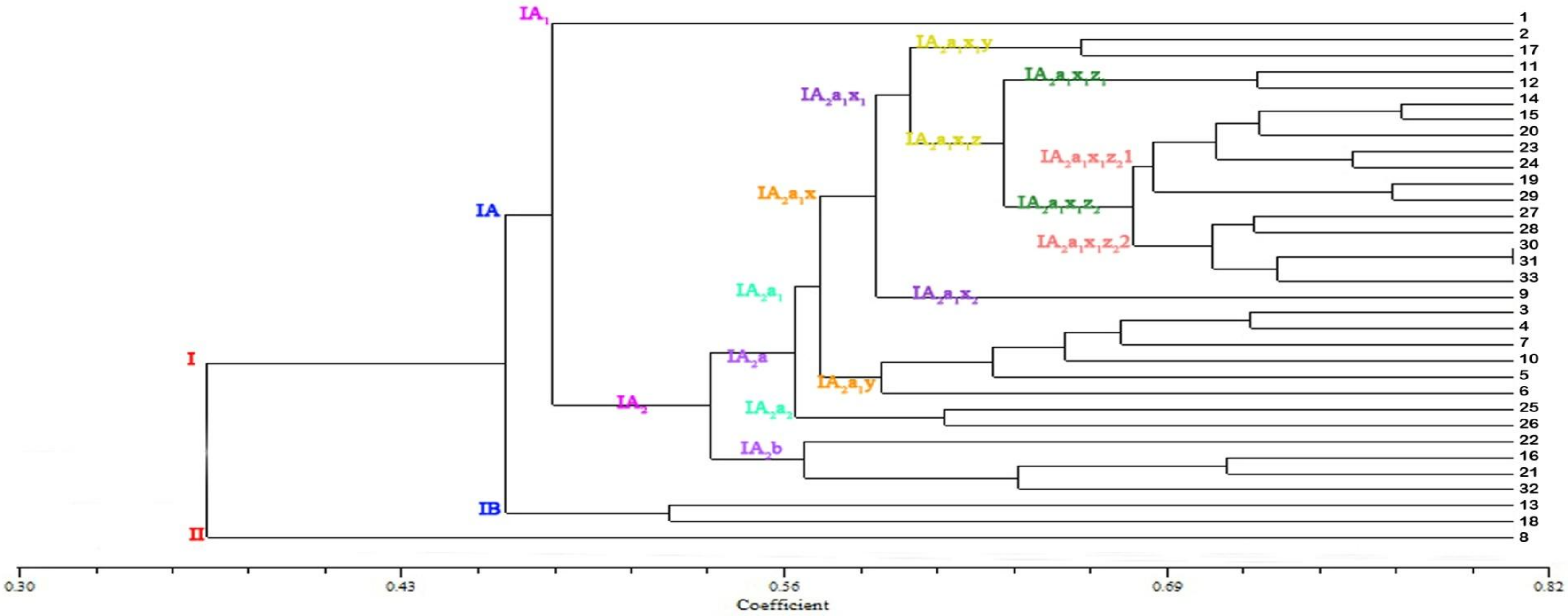


Fig 18 The relative position of sapota genotypes based on SSR markers

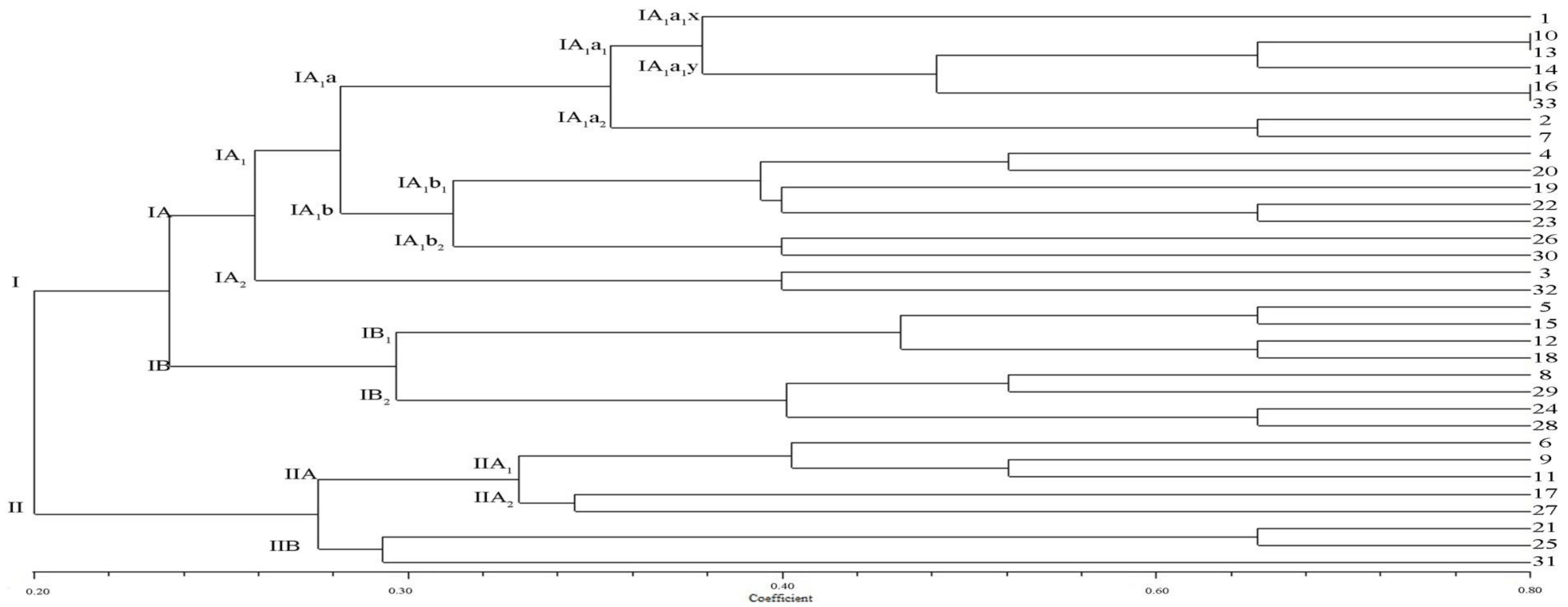


1- Badami
2-Bombay
3- Brazilian Sapota
4 - Calcutta Round
5- Columbian Sapota
6- Cricket Ball
7 - Dwarapudi
8 - Gavarayya
9 - Gutti
10 -Hybrid
11 -Kalipatti

12 -Kirthibarthi
13 -Krishna Rao
14 Mirandi
15 - Pakala
16- Pakala Oval
17- Pakala Round
18 -Pala
19 -Seedless
20 -Simbhoudi
21 -Singapore
22 -Tagarampudi

23- Ulvapadu Selection
24 - Virudnagar
25 - CO-1
26 - CO-2
27 - CO-3
28 - DHS-1
29 - DHS-2
30 - PKM-1
31 - PKM-2
32 - PKM 3
33 - PKM 4

Fig-17 Dendrogram of sapota genotypes based on SSR markers

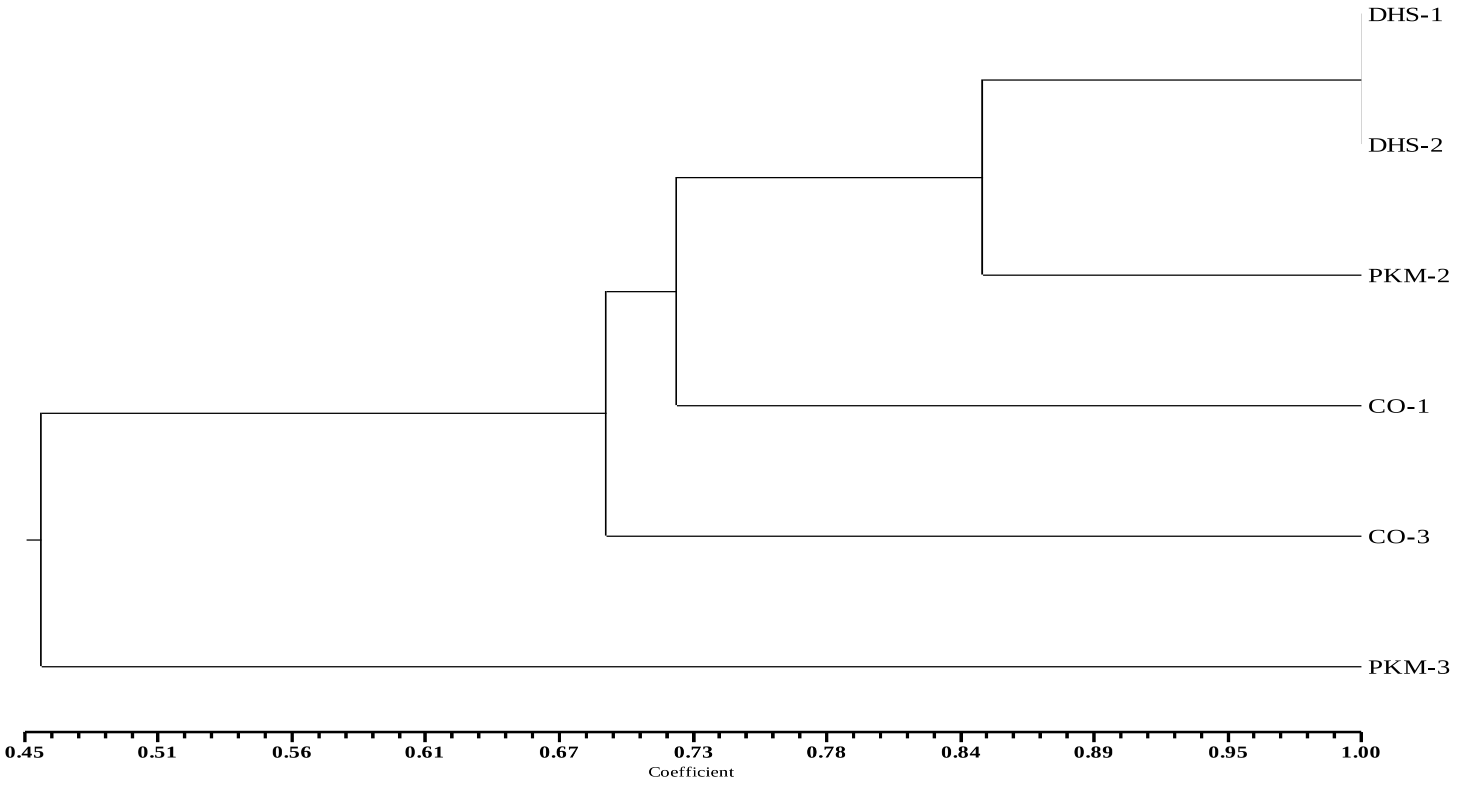


1- Badami
2-Bombay
3- Brazilian Sapota
4 - Calcutta Round
5- Columbian Sapota
6- Cricket Ball
7 - Dwarapudi
8 - Gavarayya
9 - Gutti
10 -Hybrid
11 -Kalipatti

12 -Kirthibarthi
13 -Krishna Rao
14 Mirandi
15 - Pakala
16- Pakala Oval
17- Pakala Round
18 -Pala
19 -Seedless
20 -Simhoudi
21 -Singapore
22 -Tagarampudi

23- Ulvapadu Selection
24 - Virudnagar
25 - CO-1
26 - CO-2
27 - CO-3
28 - DHS-1
29 - DHS-2
Lane 30 - PKM-1
Lane 31 - PKM-2
Lane 32 - PKM 3
Lane 33 - PKM 4

Fig-19 Dendrogram showing variation among different sapota hybrids

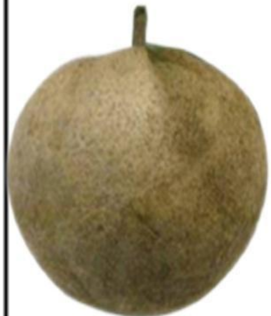



BADAMI


BOMBAY


BRAZILIAN SAPOTA


CALCUTTA ROUND


COLUMBIAN SAPOTA


CRICKET BALL


DWARAPUDI


GAVARAYYA


GUTTI


HYBRID


KALIPATTI


KIRTHIBARTHI


KRISHNA RAO


MIRANDI


PAKALA


PAKALA ROUND


1- Badami


2-Bombay


3- Brazilian sapota

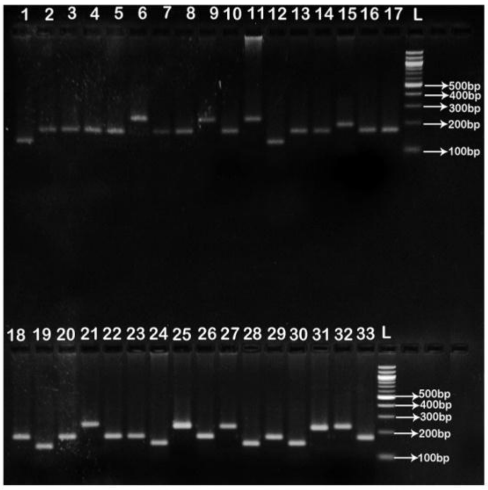

4- Calcutta Round


5- Columbian Sapota

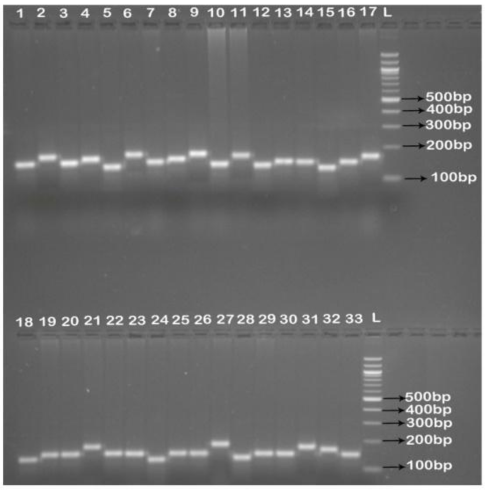

6-Cricket Ball


7-Dwarapudi

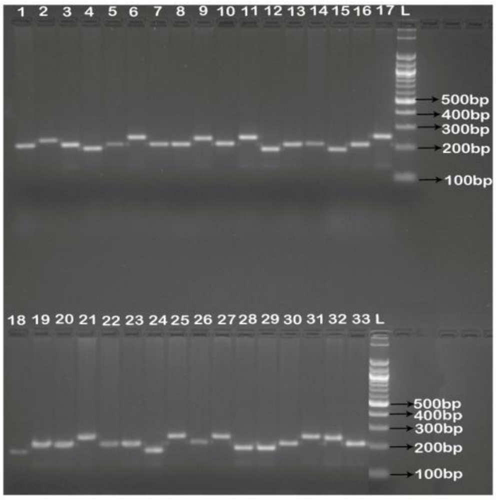

8-Gavarayya



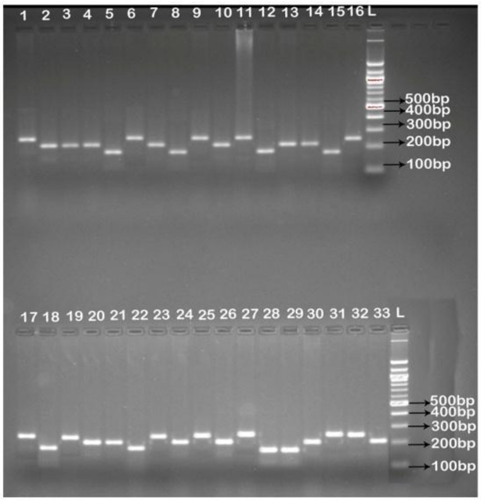
SSR S-1



SSR S-2



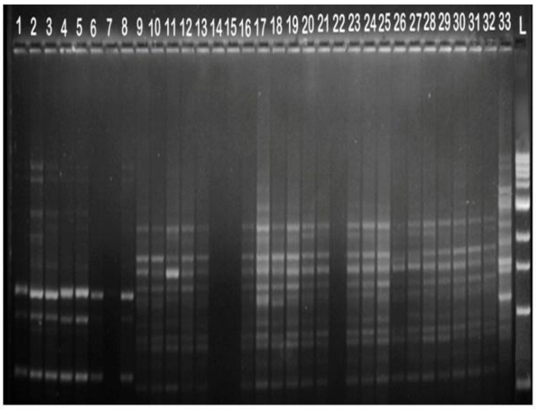
SSR S-3



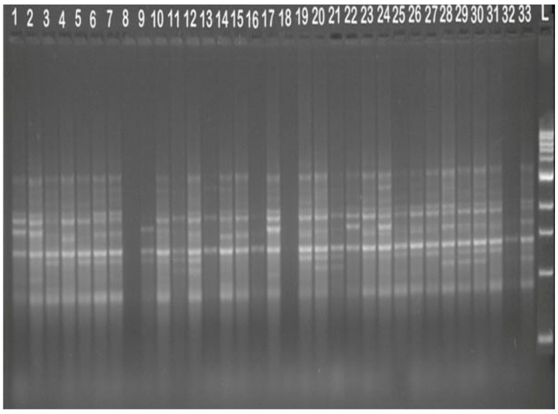
SSR S-4

Lane 1- Badami
Lane 2-Bombay
Lane 3- Brazilian Sapota
Lane 4 - Calcutta Round
Lane 5- Columbian Sapota
Lane 6- Cricket Ball
Lane7 - Dwarapudi
Lane8 - Gavarayya
Lane9 - Gutti
Lane10 -Hybrid
Lane11 -Kalipatti
Lane12 -Kirthibarthi
Lane13 -Krishna Rao
Lane14 Mirandi
Lane 15 - Pakala
Lane 16- Pakala Oval
Lane 17- Pakala Round

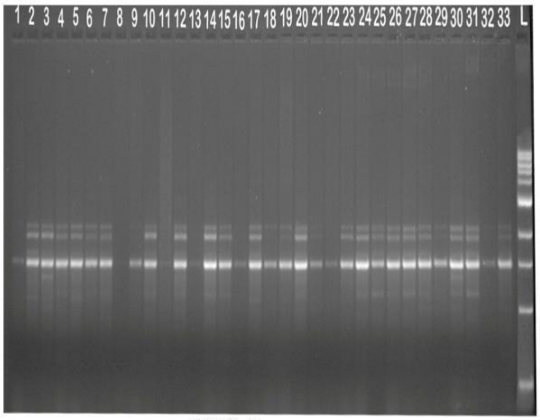
Lane 18 -Pala
Lane 19 -Seedless
Lane 20 -Simhoudi
Lane 21 -Singapore
Lane 22 -Tagarampudi
Lane 23- Ulvapadu Selection
Lane 24 - Virudnagar
Lane 25 - CO-1
Lane 26 - CO-2
Lane 27 - CO-3
Lane 28 - DHS-1
Lane 29 - DHS-2
Lane 30 - PKM-1
Lane 31 - PKM-2
Lane 32 - PKM 3
Lane 33 - PKM 4
Lane 34- 100 Base pair Ladder



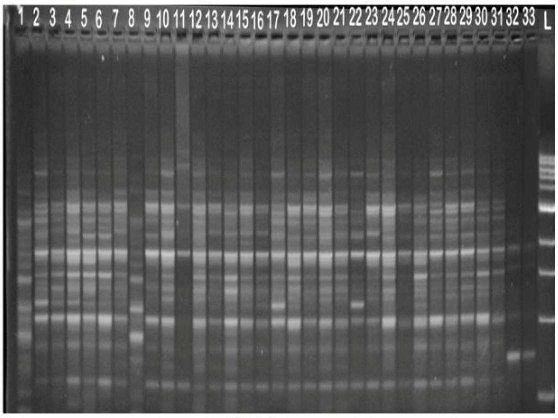
OPB-6



OPC-2

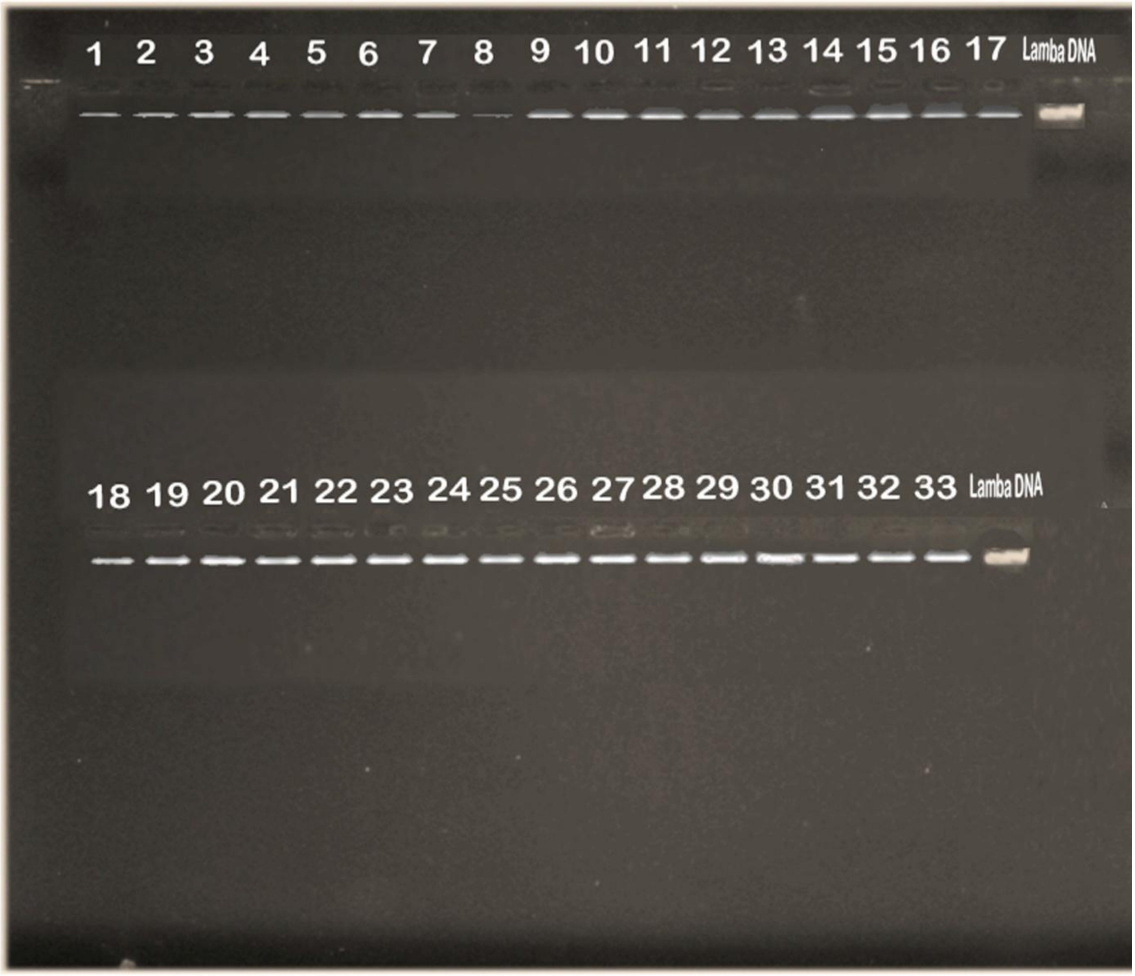


OPC-3



OPG-3

Lane 1- Badami
Lane 2-Bombay
Lane 3- Brazilian Sapota
Lane 4 - Calcutta Round
Lane 5- Columbian Sapota
Lane 6- Cricket Ball
Lane7 - Dwarapudi
Lane8 - Gavarayya
Lane9 - Gutti
Lane10 -Hybrid
Lane11 -Kalipatti
Lane12 -Kirthibarthi
Lane13 -Krishna Rao
Lane14 -Mirandi
Lane 15 - Pakala
Lane 16- Pakala Oval
Lane 17- Pakala Round
Lane 18 -Pala
Lane 19 -Seedless
Lane 20 -Simhoudi
Lane 21 -Singapore
Lane 22 -Tagarampudi
Lane 23- Ulvapadu Selection
Lane 24 - Virudnagar
Lane 25 - CO-1
Lane 26 - CO-2
Lane 27 - CO-3
Lane 28 - DHS-1
Lane 29 - DHS-2
Lane 30 - PKM-1
Lane 31 - PKM-2
Lane 32 - PKM-3
Lane 33 - PKM-4
Lane L- 1000 Base pair Ladder



Lane 1- Badami
Lane 2-Bombay
Lane 3- Brazilian Sapota
Lane 4 - Calcutta Round
Lane 5- Columbian Sapota
Lane 6- Cricket Ball
Lane7 - Dwarapudi
Lane8 - Gavarayya
Lane9 - Gutti
Lane10 -Hybrid
Lane11 -Kalipatti
Lane12 -Kirthibarthi
Lane13 -Krishna Rao
Lane14 Mirandi
Lane 15 - Pakala
Lane 16- Pakala Oval
Lane 17- Pakala Round

Lane 18 -Pala
Lane 19 -Seedless
Lane 20 -Simhoudi
Lane 21 -Singapore
Lane 22 -Tagarampudi
Lane 23- Ulvapadu Selection
Lane 24 - Virudnagar
Lane 25 - CO-1
Lane 26 - CO-2
Lane 27 - CO-3
Lane 28 - DHS-1
Lane 29 - DHS-2
Lane 30 - PKM-1
Lane 31 - PKM-2
Lane 32 - PKM 3
Lane 33 - PKM 4
Lane- Lamda DNA

CHAPTER V

SUMMARY AND CONCLUSIONS

The present investigation entitled "Studies on the diversity in morphological, bio-chemical and molecular characterization in sapota (*Manilkaraachras* (Mill.) Fosberg) genotypes" was conducted at Horticultural Research Station, Venkatramannagudem, Andhra Pradesh during the period from 2013-2014 with thirty three sapota genotypes to document information on morphological and bio-chemical characters and to determine the relationship between different characters through character association studies and path coefficient analysis. The study also aimed to assess the extent of genetic diversity by D^2 analysis and also at molecular level using RAPD and SSR markers to test the reliability of each marker technology i.e., RAPD and SSR markers in finger printing of sapota genotypes.

Studies have been carried out on tree morphological and fruit biochemical characters along with twenty four RAPD primers and sixteen SSR primers were tested to study the genetic diversity in 33sapota genotypes. The results of the investigation are summarized below.

5.1 GENETIC DIVERSITY ANALYSIS OF MORPHOLOGICAL AND BIO-CHEMICAL CHARACTERS

5.1.1 Mean performance of the genotypes

The analysis of variance for the twenty three quantitaive traits revealed significant differences for all the characters studied thus indicating wide variation among the genotypes.

The genotype Kirthibarthirecorded maximum plant height, while the genotype Cricket Ball recorded maximum leaf length, leaf width, leaf area and seed weight. The mean fruit length was highest in PKM-4 and maximum fruit width, fruit weight and pulp to seed ratio was recorded in Columbian Sapota. The genotype Singapore recorded maximum ovary size, while per cent fruit set and yield/tree was highest in Tagarampudi. Further, Krishna Rao recorded maximum seed number per fruit.

The genotype PKM-4 recorded maximum TSS and total sugars, while maximum reducing sugars was reported in Kalipatti and highest non-reducing sugars was recorded in the genotype CO-2. Highest amount of ascorbic acid content was recorded in CO-1, while maximum titrable acidity and pectin was reported in Mirandi. Further, the genotype PKM-4 recorded maximum TSS to acid ratio, and highest total phenols was reported in Krishna Rao.

5.1.2 Genetic variability studies

Genetic variability studies revealed that the range of PCV was from 8.40 to 80.25 per cent, while the range of GCV was varied from 7.40 to 77.46 per cent. The estimates for heritability ranged fom 22.40 to 99.60 per cent and genetic advance as per cent of mean ranged from 9.73 to 90.16 per cent for morphological and fruit bio-chemical characters. The high values of PCV and GCV indicates that variation among the genotypes was also high. Higher estimates of heritability for a trait suggested that simple selection would be more efficient on the basis of phenotypic performance of genotypes. The higher estimates of genetic advance indicated that a character was governed by additive genes and selection would be helpful for their improvement. High heritability in conjugation with high genetic advance as per cent of mean observed for a trait indicates the preponderance of additive gene action.

The characters, namely, fruit weight, seed number, seed weight, pulp to seed ratio, per cent fruit set, yield per tree, non-reducing sugars, ascorbic acid, TSS to acid ratio and pectins had recorded higher estimates for phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance as per cent mean.

5.1.3 Character association studies

Correlation coefficient analysis revealed that the association of plant height, leaf width, leaf area, fruit width, fruit weight, seed weight, pulp to seed ratio and per cent fruit set with yield/tree and and among themselves was positive and highly significant and these traits were identified as fruit yield components. Similarly the association of TSS, total sugars, reducing sugars, non-reducing sugars, ascorbic acid and TSS to acid ratio with yield per tree and among themselves was positive and highly significant. This indicated possibility of simultaneous selection of all these characters for improvement of sapota genotypes.

Path coefficient analysis revealed that the traits plant height, leaf area, fruit weight, seed number, seed weight, pulp to seed ratio, per cent fruit set were exerted high positive direct influence on yield per tree. This indicated that direct selection of yield improvement via these traits would be rewarding.

5.1.4 Genetic divergence

The multivariate analysis indicated the presence of considerable genetic divergence among the sapota genotypes. The 33 genotypes were grouped into VIII clusters in D^2 analysis and VI clusters in principle component analysis combined with Ward’s minimum variance method. Among all the characters maximum genetic divergence 38.72%, was contributed by plant height followed by yield/tree (14.19%), per cent fruit set (7.07%), fruit length (5.46%), fruit width (4.59%), fruit weight (4.28%), seed number (3.83%), leaf length (3.05%), leaf width (2.11%), leaf area (2.08%), seed weight (2.14%) and total soluble solids (2.25%). The characters viz., pulp to seed ratio (1.65%), total sugars (1.87%), reducing sugars (1.23%), non-reducing sugars (1.86%), ovary size (1.07%), ascorbic acid (0.54%), titrable acidity (0.25%), sugars to acid ratio (0.62%), total phenols (0.18%) and pectin (0.96%) contributed very less towards genetic divergence in the sapota genotypes under the study. The characters with maximum contribution towards diversity are important should also be given due consideration during selection indices for sapota crop improvement.

The inter-cluster D^2 values ranged from 42.68 (cluster I and III) to 459.86 (cluster VII and VIII). The maximum inter cluster distance (459.86) was observed between VII and VIII clusters followed by clusters VI and VII (398.84) and cluster VI and VIII (364.33) suggesting that the crosses involving varieties from these clusters would give desirable recombination.Intra-cluster D^2 values ranged from zero (cluster VIII) to 95.64 (cluster III). Maximum intra cluster distance was observed in cluster III (95.64), followed by cluster V (81.91), Cluster VII (78.68), cluster II (32.12), cluster I (27.89), cluster VI (25.82) and cluster IV (20.15) indicating that the genotypes of these clusters may be used as parents in the crossing programme to generate breeding material with high diversity.

The clusters means for all the characters under study showed that cluster VIII had high mean value for leaf width, leaf area, fruit length, per cent fruit set, TSS, total sugars, non-reducing sugars, ascorbic aid and TSS to acid ratio ; cluster I had high mean value for fruit weight, seed number and seed weight ; cluster VI had high mean value pulp to seed ratio and phenols cluster VII had high mean value for yield per tree, reducing sugars and phenols. The result indicates that selection of genotypes having high values for particular trait could be made and used in the hybridization programme for improvement of that character.

Principal component analysis combined with clustering by ward's method in genetic diversity among the 33 genotypes of sapota were grouped into six clusters. Clusters II was largest comprising of 10 genotypes (Brazilian Sapota, Kalipatti, Kirthibarthi, Pala, CO-2, CO-3, DHS-1, DHS-2, PKM-3 and PKM-4) followed by cluster I comprising 8 genotypes (Badami, Bombay, Pakala, Pakala Round, Pakala Oval, Simhoudi, Ulvapadu Selection, Seedless), followed by clusters III consisting 7 genotypes (Calcutta Round, Gavarayya, Gutti, Mirandi, Singapore, Thagarampudi and CO-1) followed by cluster IV having six genotypes (Dwarapudi, Hybrid, Krishna Rao, Virudhnagar, PKM-1 and PKM-2), and cluster V, cluster VI consisting each with one genotype Cricket Ball and Columbian Sapota respectively.

The principal component analysis sorted out the total 22 characters into three main principal components. The contribution of the main characters for variance easily identified by the characters loaded on the PC_1 as it explained maximum variance. By PCA, the in-depth analysis for genetic diversity can be made. In D^2 analysis, the characters *viz.*, leaf width, leaf area, fruit length, per cent fruit set, TSS, total sugars, non-reducing sugars, ascorbic aid and TSS to acid ratio are more for the divergence. In PCA, the characters *viz.*, yield per tree, total sugars, TSS, ascorbic acid, titrable acidity, TSS to acid ratio, phenols and per cent fruit set in PC_1 contributing more towards variability.

5.2GENETIC DIVERSITY ANALYSIS AT MOLECULAR LEVEL

Good quality DNA (with OD value ranging from 1.69-2.02) was obtained by using the modified CTAB (NNN, CetylTrimethyl Ammonium Bromide).

5.2.1 RAPD analysis

The RAPD analysis with twenty four primers produced 204 polymorphic fragments with an average of 8.2 polymorphic bands per primer. The RAPD analysis detected a moderate level of genetic variation among different genotypes with average similarity coefficient of 0.56. Out of total 204 polymorphic fragments, sixteen fragments were unique to particular genotype viz., Gutti (OPC-2), Tagarampudi and PKM-4 (OPG-3), Calcutta Round, Columbian Sapota and Cricket Ball (OPG-4), Pakala Oval (OPG-7), DHS-2 (OPV-9), DHS-1 (OPV-10), Columbian Sapota (OPX-3), Cricket Ball (OPX-11), Kalipatti and Pakala Round (OPX-12) which could be exploited for DNA fingerprinting of these accessions by converting RAPD markers into STS markers and this was highly useful for detecting mixes between genotypes.

The dendrogram derived from the UPGMA cluster analysis revealed one major cluster with 32 genotypes further it grouped into several sub clusters and cluster II with one genotype.

Cluster I was further divided into sub cluster IA with 30 genotypes and IB with 2 genotypes (Krishna Rao and Pala), at similarity coefficient value of 0.45 as shown in the dendrogram. Cluster IA was further divided into sub cluster IA_1 containing only one genotype (Badami) and IA_2 with 29 genotypes at similarity coefficient value of 0.53. The sub cluster IA_2 further divided into two clusters IA_2a possessed 25 genotypes and IA_2b comprised of 4 genotypes (Tagarampudi, Pakala Oval, Singapore and PKM-3), while IA_2a was further divided into 2 clusters IA_2a_1 containing 23 genotypes and IA_2a_2 comprising 2 genotypes (CO-1 and CO-2) at similarity coefficient value of 0.58 in the dendrogram. The sub cluster IA_2a_1 further divided into two clusters IA_2a_1x comprising of 17 genotypes and IA_2a_1y containing 6 genotypes (Brazilian Sapota, Calcutta Round, Dwarapudi, Hybrid, Columbian Sapota and Cricket Ball), while IA_2a_1x is further divided into $IA_2a_1x_1$ consisting 16 genotypes and $IA_2a_1x_2$ comprising one genotype (Gutti) at similarity coefficient value of 0.60 as shown in the dendrogaram. Cluster $IA_2a_1x_1$ was further divided into 2 clusters $IA_2a_1x_1y$ consisting of two genotypes (Bombay and Pakala Round) and $IA_2a_1x_1z$ comprising of 14 genotypes) at similarity coefficient value of 0.67 as shown in the dendrogram. Further, $IA_2a_1x_1z$ was divided into two clusters $IA_2a_1x_1z_1$ comprised of 2 genotypes (Kalipatti and Kirthibarthi) and $IA_2a_1x_1z_2$ cluster possessed 12 genotypes. At similarity coefficient value of 0.68 cluster $IA_2a_1x_1z_2$ further divided into two clusters $IA_2a_1x_1z_21$ consisted 7 genotypes (Mirandi, Pakala, Simhoudi, Ulvapadu selection, Virudnagar, Seedless and DHS-2) and cluster $IA_2a_1x_1z_22$ with 5 genotypes (CO-3, DHS-1, PKM-1, PKM-2 and PKM-4).

5.2.2 SSR analysis

The sixteenselected SSR primers amplified alleles were found among 33 genotypes with varied degree of polymorphism. Eighty eight alleles were produced by examining the 33 sapota genotypes with sixteen microsatellite loci with an average number of 2.66 alleles per locus. High level of polymorphism was observed with primers SSR S-4 and SSR Mh-12. The PCR product size obtaind by amplification of SSR primers was ranged from 135 to 555 bp.

Identification of genotypes for finger prints, the most important function of a primer is to descriminate as many genotypes as possible. Out of the total 88 polymorphic alleles, twenty one fragments were unique to particular genotypes viz., Columbian Sapota, DHS-1 and DHS-2 (SSR S-1), CO-3 (SSR S-2), Pakala Round (SSR S-3), Seedless (SSR S-4), PKM-2 (SSR S-5), DHS-1 and DHS-2 (SSR S-7), Cricket Ball, Calcutta Round and CO-2 (SSR S-8), Gavarayya (SSR S-9), Singapore, DHS-1 and PKM-2 (SSR S-10), CO-2 (SSR Mh-26), Bombay (SSR Mh-20), Kalipatti and Pakala Round (SSR Mh-12), Pala (SSR Mh-17) and DHS-2 and PKM-2 (Mh-22) which could be exploited for DNA fingerprinting of these genotypes.

The UPGMA cluster analysis for SSR markers revealed one major cluster (I) with 25 genotypes further it grouped into several sub clusters and another major cluster (II) with 8 genotypes.Cluster I was further divided into sub cluster IA with 17 genotypes and IB with 8 genotypes, at similarity coefficient value of 0.25. Cluster IA was further divided into sub cluster IA_1 containing 15 genotypes and IA_2 with 2 genotypes (Brazilian Sapota and PKM-3) at similarity coefficient value 0.28. The sub cluster IA_1 further divided into two clusters IA_1a possessing 8 genotypes and IA_1b comprised of 7 genotypes, while IA_1b was further divided into 2 clusters IA_1b_1 containing 5 genotypes (Calcutta Round, Simhoudi, Seedless, Tagarampudi and Ulvapadu Selection) and IA_1b_2 comprising 2 genotypes (CO-2 and PKM-3) at similarity coefficient value of 0.34. The sub cluster IA_1a further divided into two clusters IA_1a_1 comprising of 6 genotypes and IA_1a_2 containing 2 genotypes (Bombay and Dwarapudi), while IA_1a_1 is further divided into IA_1a_1x consisting one genotype (Badami) and IA_1a_1y comprising 5 genotypes (Hybrid, Krishna Rao, Mirandi, Pakala Oval and PKM-4) at similarity coefficient value of 0.37. Cluster IA_1a_1x formed as a solitary cluster at similarity coefficient value of 0.37. Cluster IB was further divided into sub cluster IB_1 containing 4 genotypes (Columbian Sapota, Pakala, Kirthibarthi and Pala) and IB_2 with 4 genotypes (Gavarayya, Virudnagar, DHS-1 and DHS-2) at similarity coefficient value 0.30.

Cluster II was further divided into IIA consisting of 5 genotypes and IIB which comprising of 3 genotypes (Singapore, CO-1 and PKM-2) at similarity coefficient value of 0.28. At similarity coefficient value 0.34 cluster IIA was further divided into 2 sub clusters IIA_1 containing 3 genotypes (Cricket Ball, Gutti and Kalipatti) and IIA_2 comprised 2 genotypes (Pakala Round and CO-3).

In comparision of morphological characters with SSR markers in sapota indicated that, all round, elliptic, oblong and oval shaped fruit bearing genotypes are grouped together (Cluster I) are Badami, Bombay, Calcutta Round, Columbian Sapota, Kirthibarthi, Pala, Pakala Round, Tagarampudi and DHS-2 were round shaped fruits, Gavarayya, Gutti, Hybrid and Simhoudi were oblong fruits, Brazilian Sapota, Ulvapadu Selection, PKM-1, PKM-3 and PKM-4 were oval shaped fruits. Pakala Oval and PKM-4 are closely associated to each other may be due to oval shape fruits and broadly pyramidal shape of tree. Similarly, Hybrid and Krishna Rao are closely associated to each other may be due to semierect growth habit of the tree and having acorn shape of fruits. Sub cluster IA_2 included 2 genotypes, Brazilian Sapota and PKM-3 are grouped near due to their oval shape of fruit. Further, sub-cluster IA_1b_1 containing 5 genotypes, Calcutta Round, Simhoudi, Seedless, Tagarampudi and Ulvapadu Selection are grouped near due to their dense branching density habit and sub-cluster IA_1b_2 comprising 2 genotypes CO-2 and PKM-3 are closely associated may be due to spreading type of growth habit and verticillate branching pattern. The sub-cluster IA_1a_2 containing 2 genotypes Bombay and Dwarapudi which are closely associated to each other may be due to dense branching density. Cluster IB was further divided into sub cluster IB_1 containing 4 genotypes, Columbian Sapota, Pakala, Kirthibarthi and Pala are closely grouped in a dendrogram due to their broadly pyramidal shape of tree and globose shape of fruit, while in sub-cluster IB_2 comprising 4 genotypes, Virudnagar, DHS-1 and DHS-2 are grouped near due to semierect growth habit of tree and verticillate branching pattern except Gavarayya which is having irregular branching pattern but similar in growth habit.In cluster II sub-cluster IIA consisting of 5 genotypes, Cricket Ball, Gutti, Kalipatti, Pakala Round and CO-3 are grouped together may be due to horizontal branching pattern and brown flesh color and Singapore, CO-1 and PKM-2 has been placed in subcluster IIB due to solitary fruit bearing habit.

5.2.3 Variation among hybrids

Hybrids are distributed into two distinctive groups. Group-I contains DHS-1, DHS-2, PKM-2, CO-1 and CO-3 which are grouped together because of their acorn and round shaped fruits with spreading and single bearing habit. Within this group DHS-1 and DHS-2 are 100 per cent similar as they are having same parentage (Kalipatti X Cricket Ball). PKM-2 is closely related with DHS-1 and DHS-2 as it is having acorn shape fruits as that of DHS-1. CO-1 and CO-3 are closely linked with DHS-2 due to round shaped fruits. PKM-3 has been placed in separate cluster (group-II).

Based on outcome of the present investigation, we conclude that molecular markers for fingerprinting as well as estimation of genetic diversity and genetic relatedness in sapota genotypes is effective, precise and more efficient than morphological markers. It offers a general association between molecular profile and common morphological characters. This helps in identifying suitable parents for effecting crosses and selecting desirable types at early stages.

Study on identification of markers associated with specific traits of interest, which could be converted into SCAR markers needs attention to speed up the crop improvement programme. The degree of genetic variability among genotypes of sapota evaluated through molecular characterization can form the basis for future breeding programme and a finer molecular analysis with additional SSR loci and use of other molecular markers like AFLP, SNP’s is required to differentiate identical genotypes. Hybridization among genotypes of different clusters with high heterotic effects and QTL mapping for sapota crop improvement has been suggested.

Future line of work

1.The genotype with high genetic divergence as revealed from this study can be used for hybridization process which would result in heterotic hybrids.

2. Studies on identification of markers linked to traits of interest could be converted into SCAR markers and it can be used for marker assisted selection (MAS).

3.For protection of valuablegermplasm, molecular markers can be employed for characterization and conservation of elite genotypes.

4. Finger printing of genotypes will facilitate conservation and useful in the IPR regime.

5. Need for development DUS characters for sapota for registration of new germplasm.

6. Identification of better parents for hybrid programme using diversity results supported by male and female fertility and hybrid regeneration studies.

APPENDIX

Morphological and fruit biochemical and molecular characterization in sapota genotypes

S.No.	Character	Badami	Bombay	Brazilan Sapota
1	Growth habit	Semi-erect	Semi-erect	Semi-erect
2	Tree shape	Broadly Pyramidal	Semi-circular	Spherical
3	Branching pattern	Verticillate	Verticillate	Verticillate
4	Branching density	Dense	Dense	Medium dense
5	Plant height(m)	5.11	2.84	4.92
6	Leaf length (cm)	8.93	10.33	11.00
7	Leaf width (cm)	3.5	3.67	4.03
8	Leaf area (cm^2)	21.67	28.17	32.92
9	Fruit length (cm)	6.03	5.07	6.36
10	Fruit width (cm)	5.49	5.74	6.44
11	Fruit weigth (g)	83.8	100.74	221.08
12	Seed number	4.33	4.33	6.00
13	Seed weight (g)	3.60	3.85	6.22
14	Pulp to Seed ratio	26.91	23.10	43.54
15	Fruit shape	Globose	Globose	Oval
16	Fruit surface texture	Rough	Rough	Rough
17	Peel color	Brown	Greenish brown	Greenish brown
18	Flesh color	Brown	Brown	Greenish brown
19	Flesh texture	Soft	Firm	Sandy
20	Flesh taste	Sweet	Sweet	Starchy
21	Fruit availability	April- June	March-June	March-June, October-November
22	Fruit clustering habit	Solitary	Solitary	Solitary
23	Ovary size (mm)	3.37	4.00	4.47
24	Per cent fruit set	6.00	6.8	7.13
25	Yield/tree (Kg)	14.67	17.63	20.49
26	TSS(^{O}B)	20.57	18.63	23.79
27	Titrable acidity (%)	0.266	0.283	0.234
28	TSS to Acid ratio	77.33	65.83	101.64
29	Totalsugars (%)	9.3	8.21	11
30	Reducing sugars (%)	7.06	7.18	8.5
31	Non-reducing sugars (%)	2.24	1.03	2.51
32	Ascorbic acid (mg/100g)	6.63	5.15	8.62
33	Total Phenols (%)	0.61	0.527	0.462
34	Pectins (%)	2.55	1.72	1.42
35	Cluster analysis by Tochers method	Cluster I	Cluster VI	Cluster V
36	Cluster analysis by Ward's minimum varience method	Cluster I	Cluster I	Cluster II

37	Molecular markers	It forms close association with Hybrid, Krishna Rao, Pakala Oval in SSR maker analysis	Genotype specific fragment was amplified with SSR primer Mh-20 at 130 bp	It forms close association with PKM-3 in SSR maker analysis

	Character	**Calcutta Round**	**Columbian Sapota**	**Cricket Ball**
1	Growth habit	Semi-erect	Upright	Semi-erect
2	Tree shape	Broadly Pyramidal	Broadly Pyramidal	Broadly Pyramidal
3	Branching pattern	Verticillate	Verticillate	Horizontal
4	Branching density	Medium dense	Sparse	Medium dense
5	Plant height(m)	6.52	6.56	5.55
6	Leaf length (cm)	8.77	9.83	13.81
7	Leaf width (cm)	3.33	3.47	5.87
8	Leaf area (cm^2)	23.33	23.25	44.42
9	Fruit length (cm)	5.37	7.91	5.32
10	Fruit width (cm)	6.76	8.20	7.34
11	Fruit weigth (g)	144.93	540.27	259.71
12	Seed number	5.00	5.33	6.33
13	Seed weight (g)	5.09	7.07	7.81
14	Pulp to Seed ratio	26.53	75.67	28.80
15	Fruit shape	Globose	Globose	Globose
16	Fruit surface texture	Rough	Web pattern on the surface	Rough
17	Peel color	Brown	Greenish brown	Brown
18	Flesh color	Reddish brown	Yellowish brown	Yellowish brown
19	Flesh texture	Firm	Firm	Soft
20	Flesh taste	Starchy	Starchy	Starchy
21	Fruit availability	March-June, October-November	March to June	March to June, October -November
22	Fruit clustering habit	Solitary	Solitary	Solitary
23	Ovary size (mm)	3.87	4.27	4.03
24	Per cent fruit set	5.43	8.61	16.03
25	Yield/tree (Kg)	16.41	35.01	91.97
26	TSS(^{O}B)	22.47	18.61	19.90
27	Titrable acidity (%)	0.194	0.245	0.252
28	TSS to Acid ratio	115.82	75.91	78.96
29	Totalsugars (%)	10.36	8.61	8.97
30	Reducing sugars (%)	8.76	6.6	6.87
31	Non-reducing sugars (%)	1.60	2.01	2.11
32	Ascorbic acid (mg/100g)	7.46	7.69	6.22
33	Total Phenols (%)	0.52	0.551	0.425
34	Pectins (%)	2.24	1.34	2.09
35	Cluster analysis by	Cluster III	Cluster I	Cluster I

36	Tochers method PCA by Ward's minimum varience method	Cluster III	Cluster VI	Cluster V
37	Molecular markers	Genotype specific fragment was amplified with RAPD primer OPG-4 at 2450 bp and with SSR primer S-8 at 180 bp	Genotype specific fragment was amplified with RAPD primer OPG-4 at 2385 bp and with SSR primer S-1 at 235 bp	Genotype specific fragment was amplified with RAPD primers OPG-4 at 2250 bp, OPX-11 at 320 bp and with SSR primer S-8 at 220 bp

S.No.	**Character**	**Dwarapudi**	**Gavarayya**	**Gutti**
1	Growth habit	Semi-erect	Semi-erect	Semi-erect
2	Tree shape	Elliptical	Spherical	Elliptical
3	Branching pattern	Verticillate	Irregular	Verticillate
4	Branching density	Dense	Medium dense	Medium dense
5	Plant height(m)	5.92	5.73	4.69
6	Leaf length (cm)	9.97	11.27	10.13
7	Leaf width (cm)	3.57	3.77	4.07
8	Leaf area (cm^2)	26.58	31.42	31.58
9	Fruit length (cm)	5.45	6.83	5.83
10	Fruit width (cm)	5.65	6.04	5.09
11	Fruit weigth (g)	92.7	105.91	78.95
12	Seed number	6.33	3.67	4.00
13	Seed weight (g)	5.50	3.74	3.21
14	Pulp to Seed ratio	17.27	24.32	23.41
15	Fruit shape	Acorn	Oblong	Oblong
16	Fruit surface texture	Smooth	Rough	Smooth
17	Peel color	Brown	Brown	Brown
18	Flesh color	Brown	Brown	Brown
19	Flesh texture	Firm	Firm	Soft
20	Flesh taste	Sweet	Sweet	Sweet
21	Fruit availability	March-June, October-November	March-June, October-November	March-June, October-November
22	Fruit clustering habit	Solitary	Solitary	Cluster
23	Ovary size (mm)	4.49	3.73	3.61
24	Per cent fruit set	9.47	11.52	14.30
25	Yield/tree (Kg)	22.06	79.54	87.20
26	TSS(^{O}B)	22.23	21.87	23.50
27	Titrable acidity (%)	0.202	0.192	0.205
28	TSS to Acid ratio	110.04	113.91	114.63
29	Totalsugars (%)	8.93	9.87	10.9
30	Reducing sugars (%)	6.23	7.33	8.22
31	Non-reducing sugars (%)	2.7	2.54	2.68

32	Ascorbic acid (mg/100g)	5.35	8.57	9.14
33	Total Phenols (%)	0.478	0.466	0.52
34	Pectins (%)	1.98	1.80	1.66
35	Cluster analysis by Tochers method	Cluster III	Cluster III	Cluster III
36	Cluster analysis by Ward's minimum varience method	Cluster IV	Cluster III	Cluster III
37	Molecular markers	It forms a close association with Bombay in SSR marker analysis	Genotype specific fragment was amplified with RAPD primers OPX-11 at 400 bp and with SSR primer S-9 at 135 bp	Genotype specific fragment was amplified with RAPD primer OPC-2 at 1845 bp

S.No.	**Character**	**Hybrid**	**Kalipatti**	**Kirthibarthi**
1	Growth habit	Semi-erect	Spreading	Spreading
2	Tree shape	Elliptical	Spherical	Broadly Pyramidal
3	Branching pattern	Verticillate	Verticillate	Horizontal
4	Branching density	Medium dense	Dense	Dense
5	Plant height(m)	6.03	5.88	7.74
6	Leaf length (cm)	9.77	10.67	9.4
7	Leaf width (cm)	3.87	3.77	3.53
8	Leaf area (cm^2)	26.15	28.92	27.87
9	Fruit length (cm)	6.05	6.83	5.59
10	Fruit width (cm)	5.18	6.52	6.44
11	Fruit weigth (g)	106.55	169.46	149.52
12	Seed number	4.63	4.67	6.00
13	Seed weight (g)	3.9	4.28	5.26
14	Pulp to Seed ratio	31.03	35.03	33.05
15	Fruit shape	Globose	Acorn	Globose
16	Fruit surface texture	Smooth	Smooth	Smooth
17	Peel color	Yellowish brown	Yellowish brown	Yellowish brown
18	Flesh color	Brown	Brown	Brown
19	Flesh texture	Firm	Firm	Soft
20	Flesh taste	Sweet	Sweet	Sweet
21	Fruit availability	March-June, October-November	March-June, October to November	March-June, October to November
22	Fruit clustering habit	Solitary	Solitary	Solitary
23	Ovary size (mm)	4.16	4.02	3.67
24	Per cent fruit set	8.47	17.00	9.70
25	Yield/tree (Kg)	33.08	76.69	46.60

26	TSS(^{O}B)	19.51	22.97	22.83
27	Titrable acidity (%)	0.248	0.191	0.168
28	TSS to Acid ratio	78.62	120.26	135.89
29	Totalsugars (%)	9.57	11.47	10.75
30	Reducing sugars (%)	6.69	9.23	9.13
31	Nonreducing sugars (%)	2.88	2.24	1.62
32	Ascorbic acid (mg/100g)	6.16	12.10	8.85
33	Total Phenols (%)	0.433	0.41	0.527
34	Pectins (%)	1.56	2.35	2.13
35	Cluster analysis by Tochers method	Cluster I	Cluster VII	Cluster IV
36	Cluster analysis by Ward's minimum varience method	Cluster IV	Cluster II	Cluster II
37	Molecular markers	Hybrid and Krishna Rao shows 100 per cent close association in SSR marker analysis	Genotype specific fragment was amplified with RAPD primer OPX-12 at 2450 bp and with SSR primer Mh-12 at 230 bp	It forms close association with Pala in SSR marker analysis

S.No.	**Character**	**Krishna Rao**	**Mirandi**	**Pakala**
1	Growth habit	Semi-erect	Semi-erect	Semi-erect
2	Tree shape	Broadly Pyramidal	Broadly Pyramidal	Broadly Pyramidal
3	Branching pattern	Horizontal	Verticillate	Verticillate
4	Branching density	Medium dense	Medium dense	Medium dense
5	Plant height(m)	3.51	5.4	5.62
6	Leaf length (cm)	9.57	9.43	7.2
7	Leaf width (cm)	3.60	2.80	2.30
8	Leaf area (cm^2)	25.25	20.67	17.83
9	Fruit length (cm)	5.8	7.47	5.39
10	Fruit width (cm)	5.51	6.25	6.58
11	Fruit weigth (g)	73.6	167.91	142.49
12	Seed number	9.33	6.67	4.67
13	Seed weight (g)	6.93	5.02	3.33
14	Pulp to Seed ratio	24.16	61.5	43.95
15	Fruit shape	Acorn	Oblong	Oblong
16	Fruit surface texture	Smooth	Web pattern on the surface	Smooth
17	Peel color	Brown	Greenish brown	Brown
18	Flesh color	Reddish brown	Greenish brown	Brown

19	Flesh texture	Firm	Firm	Soft
20	Flesh taste	Sweet	Starchy	Sweet
21	Fruit availability	March-June	April-June	March-June October-November
22	Fruit clustering habit	Solitary	Solitary	Solitary
23	Ovary size (mm)	3.93	3.64	4.08
24	Per cent fruit set	10.21	12.60	16.71
25	Yield/tree (Kg)	50.64	59.47	71.95
26	TSS(^{O}B)	21.6	19.4	20.63
27	Titrable acidity (%)	0.282	0.295	0.223
28	TSS to Acid ratio	78.59	65.76	92.51
29	Totalsugars (%)	9.47	8.97	9.72
30	Reducing sugars (%)	8.33	8.04	8.34
31	Non-reducing sugars (%)	1.14	0.93	1.42
32	Ascorbic acid (mg/100g)	8.6	7.18	6.87
33	Total Phenols (%)	0.62	0.6	0.558
34	Pectins (%)	1.71	2.86	1.83
35	Cluster analysis by Tochers method	Cluster I	Cluster VI	Cluster II
36	Cluster analysis by Ward's minimum varience method	Cluster IV	Cluster III	Cluster I
37	Molecular markers	It shows cent per cent association with Hybrid in SSR marker analysis	It shows close association with Krishna Rao and Hybrid in SSR marker analysis	It form close association with Columbian Sapota

S.No.	**Character**	**Pakala Oval**	**Pakala Round**	**Pala**
1	Growth habit	Semi-erect	Semi-erect	Spreading
2	Tree shape	Broadly Pyramidal	Broadly Pyramidal	Broadly Pyramidal
3	Branching pattern	Horizontal	Verticillate	Horizontal
4	Branching density	Medium dense	Medium dense	Medium dense
5	Plant height(m)	6.00	5.98	6.29
6	Leaf length (cm)	8.33	9.43	9.51
7	Leaf width (cm)	3.40	3.13	3.73
8	Leaf area (cm^2)	22.17	19.75	24.25
9	Fruit length (cm)	6.88	4.84	5.64
10	Fruit width (cm)	5.77	6.22	5.37
11	Fruit weigth (g)	120.14	75.61	94.25
12	Seed number	4.33	5.00	3.67

13	Seed weight (g)	2.99	3.10	2.84
14	Pulp to Seed ratio	38.46	24.19	30.62
15	Fruit shape	Oval	Globose	Oval
16	Fruit surface texture	Smooth	Smooth	Rough
17	Peel color	Brown	Brown	Brown
18	Flesh color	Brown	Brown	Brown
19	Flesh texture	Firm	Soft	Soft
20	Flesh taste	Sweet	Sweet	Sweet
21	Fruit availability	March-June, October-November	March-June, October-November	March-June, October-November
22	Fruit clustering habit	Solitary	Solitary	Solitary
23	Ovary size (mm)	4.03	4.01	3.13
24	Per cent fruit set	15.8	16.11	17.85
25	Yield/tree (Kg)	61.43	69.7	71.92
26	TSS(^{O}B)	20.6	20.73	24.33
27	Titrable acidity (%)	0.231	0.24	0.181
28	TSS to Acid ratio	89.22	86.38	134.41
29	Totalsugars (%)	9.67	9.90	11.04
30	Reducing sugars (%)	8.57	7.84	9.13
31	Non-reducing sugars (%)	1.1	2.06	1.91
32	Ascorbic acid (mg/100g)	7.2	7.43	10.32
33	Total Phenols (%)	0.525	0.509	0.382
34	Pectins (%)	2.02	2.11	1.62
35	Cluster analysis by Tochers method	Cluster II	Cluster II	Cluster IV
36	PCA by Ward's minimum varience method	Cluster I	Cluster I	Cluster II
37	Molecular markers	Genotype specific fragment was amplified with RAPD primer OPG-7 at 365 bp	Genotype specific fragment was amplified with SSR primer S-3 at 180 bp and Mh-12 at 230 bp	Genotype specific fragment was amplified with SSR primer Mh-17 at 180 bp

S.No.	**Character**	**Seedless**	**Simhoudi**	**Singapore**
1	Growth habit	Semi-erect	Semi-erect	Spreading
2	Tree shape	Semi-circular	Spherical	Broadly Pyramidal
3	Branching pattern	Horizontal	Verticillate	Horizontal
4	Branching density	Medium dense	Dense	Dense
5	Plant height(m)	5.51	5.95	5.62
6	Leaf length (cm)	12.37	10.27	9.97

7	Leaf width (cm)	4.57	3.83	3.77
8	Leaf area (cm^2)	26.83	24.92	22.33
9	Fruit length (cm)	6.19	6.67	6.6
10	Fruit width (cm)	6.42	6.14	6.67
11	Fruit weigth (g)	102.15	108.76	198.61
12	Seed number	2.33	4.00	5.00
13	Seed weight (g)	2.45	2.93	4.58
14	Pulp to Seed ratio	30.03	41.23	36.46
15	Fruit shape	Oblong	Oblong	Acorn
16	Fruit surface texture	Smooth	Smooth	Smooth
17	Peel color	Yellowish brown	Brown	Brown
18	Flesh color	Greenish brown	Brown	Brown
19	Flesh texture	Firm	Soft	Firm
20	Flesh taste	Sweet	Sweet	Sweet
21	Fruit availability	March-June, October-November	March-June, October-November	March-June, October-November
22	Fruit clustering habit	Solitary	Solitary	Solitary
23	Ovary size (mm)	3.78	4.09	4.79
24	Per cent fruit set	15.20	15.43	11.10
25	Yield/tree (Kg)	60.70	63.42	34.79
26	TSS(^{O}B)	21.63	20.37	22.93
27	Titrable acidity (%)	0.199	0.253	0.206
28	TSS to Acid ratio	108.69	80.51	111.30
29	Totalsugars (%)	9.55	9.20	10.55
30	Reducing sugars (%)	6.89	8.30	8.40
31	Non-reducing sugars (%)	2.65	0.90	2.15
32	Ascorbic acid (mg/100g)	9.58	8.02	11.76
33	Total Phenols (%)	0.471	0.476	0.479
34	Pectins (%)	1.44	1.17	2.70
35	Cluster analysis by Tochers method	Cluster I	Cluster III	Cluster III
36	Cluster analysis by Ward's minimum varience method	Cluster I	Cluster I	Cluster III
37	Molecular markers	Seedless is closely related to Ulvapadu Selection and Tagarampudi in SSR marker analysis	It is closely related to Calcutta Round in SSR marker analysis	Genotype specific fragment was amplified with SSR primer S-10 at 220 bp

S.No.	**Character**	**Tagarampudi**	**Ulvapadu**	**Virudnagar**

			Selection	
1	Growth habit	Semi-erect	Spreading	Spreading
2	Tree shape	Broadly Pyramidal	Elliptical	Broadly Pyramidal
3	Branching pattern	Horizontal	Verticillate	Verticillate
4	Branching density	Dense	Medium dense	Dense
5	Plant height(m)	6.80	3.27	5.55
6	Leaf length (cm)	11.57	8.63	9.27
7	Leaf width (cm)	4.17	2.7	3.83
8	Leaf area (cm^2)	33.35	17	29.33
9	Fruit length (cm)	5.81	6.59	6.99
10	Fruit width (cm)	5.8	5.99	5.87
11	Fruit weigth (g)	89.25	138.56	147.33
12	Seed number	5.00	3.33	4.00
13	Seed weight (g)	4.71	2.58	3.68
14	Pulp to Seed ratio	18.29	50.40	35.32
15	Fruit shape	Globose	Oval	Oblong
16	Fruit surface texture	Rough	Smooth	Rough
17	Peel color	Brown	Brown	Brown
18	Flesh color	Reddish brown	Yellowish brown	Brown
19	Flesh texture	Sandy	Soft	Soft
20	Flesh taste	Starchy	Sweet	Sweet
21	Fruit availability	March-June,	March-June	February-June, October-November
22	Fruit clustering habit	Solitary	Solitary	Solitary
23	Ovary size (mm)	3.67	4.49	3.67
24	Per cent fruit set	32.00	11.80	27.00
25	Yield/tree (Kg)	187.14	58.08	184.57
26	TSS(^{O}B)	20.93	21.6	23.07
27	Titrable acidity (%)	0.217	0.223	0.184
28	TSS to Acid ratio	96.45	96.86	125.38
29	Totalsugars (%)	9.82	10.53	10.99
30	Reducing sugars (%)	8.17	8.33	8.48
31	Non-reducing sugars (%)	1.65	2.22	2.51
32	Ascorbic acid (mg/100g)	8.46	7.05	10.68
33	Total Phenols (%)	0.392	0.455	0.464
34	Pectins (%)	1.34	1.51	2.30
35	Cluster analysis by Tochers method	Cluster V	Cluster V	Cluster VII
36	Cluster analysis by Ward's minimum varience method	Cluster III	Cluster I	Cluster IV
37	Molecular markers	Genotype specific fragment was amplified with RAPD primer OPG-3 at 650 bp	Ulvapadu Selection was closely related to Tagarampudi in SSR marker analysis.	Virudnagar was closely related to DHS-1 in SSR marker analysis. Cluster II

S.No.	Character	CO-1	CO-2	CO-3
1	Growth habit	Upright	Spreading	Semi-erect
2	Tree shape	Elliptical	Irregular	Irregular
3	Branching pattern	Verticillate	Verticillate	Verticillate
4	Branching density	Sparse	Sparse	Sparse
5	Plant height(m)	4.99	6.19	4.65
6	Leaf length (cm)	10.03	11.20	10.13
7	Leaf width (cm)	3.73	4.43	3.97
8	Leaf area (cm^2)	29.25	36.17	30.68
9	Fruit length (cm)	7.81	6.28	6.85
10	Fruit width (cm)	6.88	6.78	7.63
11	Fruit weigth (g)	222.33	239.18	217.86
12	Seed number	4.00	3.00	4.00
13	Seed weight (g)	5.00	4.83	5.23
14	Pulp to Seed ratio	40.21	47.82	37.09
15	Fruit shape	Turbinate	Turbinate	Acorn
16	Fruit surface texture	Smooth	Rough	Web pattern on the surface
17	Peel color	Brown	Brown	Yellowish brown
18	Flesh color	Yellowish brown	Yellowish brown	Brown
19	Flesh texture	Sandy	Sandy	Sandy
20	Flesh taste	Sweet	Sweet	Sweet
21	Fruit availability	April-June, October-November	April-June, October-November	April-June, October-November
22	Fruit clustering habit	Solitary	Solitary	Solitary
23	Ovary size (mm)	3.66	4.32	3.98
24	Per cent fruit set	18.00	17.00	13.97
25	Yield/tree (Kg)	91.8	82.18	55.07
26	TSS(^{O}B)	21.57	23.17	19.77
27	Titrable acidity (%)	0.241	0.256	0.21
28	TSS to Acid ratio	89.5	90.5	94.14
29	Totalsugars (%)	10.71	11.84	9.80
30	Reducing sugars (%)	7.6	8.18	6.66
31	Non-reducing sugars (%)	3.1	3.66	3.14
32	Ascorbic acid (mg/100g)	12.16	11.27	10.50
33	Total Phenols (%)	0.332	0.444	0.467
34	Pectins (%)	2.17	1.83	1.18
35	Cluster analysis by Tochers method	Cluster II	Cluster II	Cluster II
36	PCA by Ward's minimum varience method	Cluster III	Cluster II	Cluster II
37	Molecular markers	CO-1 was closely related to	Genotype specific fragment was amplified	Genotype specific

		Singapore in SSR marker analysis	with SSR primers S-8 at 220 bp and Mh-26 at 195 bp	fragment was amplified with SSR primers S-2 at 190 bp

	Character	**DHS-1**	**DHS-2**	**PKM-1**
1	Growth habit	Semi-erect	Semi-erect	Spreading
2	Tree shape	Semi-circular	Spherical	Broadly Pyramidal
3	Branching pattern	Verticillate	Verticillate	Verticillate
4	Branching density	Medium dense	Dense	Dense
5	Plant height(m)	6.90	7.14	5.47
6	Leaf length (cm)	9.73	9.67	11.43
7	Leaf width (cm)	3.37	4.13	4.37
8	Leaf area (cm^2)	25.75	28.08	34.08
9	Fruit length (cm)	7.19	6.69	6.69
10	Fruit width (cm)	7.02	6.59	6.25
11	Fruit weigth (g)	205.85	165.82	146.6
12	Seed number	3.67	3.33	5.00
13	Seed weight (g)	4.35	4.00	3.37
14	Pulp to Seed ratio	40.46	36.75	40.35
15	Fruit shape	Acorn	Globose	Elliptical
16	Fruit surface texture	Rough	Rough	Smooth
17	Peel color	Brown	Brown	Brown
18	Flesh color	Yellowish brown	Reddish brown	Brown
19	Flesh texture	Soft	Firm	Soft
20	Flesh taste	Sweet	Sweet	Sweet
21	Fruit availability	March-June, October-November	March-June	March-June, October-November
22	Fruit clustering habit	Solitary	Solitary	Cluster
23	Ovary size (mm)	4.34	3.83	4.08
24	Per cent fruit set	9.20	8.50	28.47
25	Yield/tree (Kg)	44.41	32.77	151.83
26	TSS(^{O}B)	22.43	21.53	23.90
27	Titrable acidity (%)	0.191	0.213	0.177
28	TSS to Acid ratio	117.42	101.07	135
29	Totalsugars (%)	11.08	10.01	10.91
30	Reducing sugars (%)	8.39	8.55	9.07
31	Non-reducing sugars (%)	2.69	1.46	1.84
32	Ascorbic acid (mg/100g)	10.00	9.11	9.23
33	Total Phenols (%)	0.393	0.398	0.381

34	Pectins (%)	2.63	2.73	1.74
35	Cluster analysis by Tochers method	Cluster III	Cluster V	Cluster IV
36	PCA by Ward's minimum varience method	Cluster II	Cluster II	Cluster IV
37	Molecular markers	Genotype specific fragment was amplified with RAPD primer OPV-10 at 2650 bp and with SSR primers S-1 at 160 bp, S-7 at 250 and S-10 at 280 bp	Genotype specific fragment was amplified with RAPD primer OPV-9 at 2000 bp and with SSR primers S-1 at 160 bp, S-7 at 250 bp	It shows close association with CO-2 with SSR cluster analysis and it fall under cluster I

S.No.	**Character**	**PKM-2**	**PKM-3**	**PKM-4**
1	Growth habit	Semi-erect	Semi-erect	Semi-erect
2	Tree shape	Broadly Pyramidal	Broadly Pyramidal	Broadly Pyramidal
3	Branching pattern	Verticillate	Verticillate	Verticillate
4	Branching density	Dense	Medium dense	Medium dense
5	Plant height(m)	5.65	6.48	4.81
6	Leaf length (cm)	9.53	12.17	9.83
7	Leaf width (cm)	3.27	4.82	4.30
8	Leaf area (cm^2)	18.16	40.17	32.42
9	Fruit length (cm)	6.67	7.27	8.39
10	Fruit width (cm)	6.46	6.38	6.16
11	Fruit weigth (g)	152.1	164.03	181.64
12	Seed number	3.33	4.33	4.00
13	Seed weight (g)	3.72	4.77	4.23
14	Pulp to Seed ratio	38.07	32.49	36.88
15	Fruit shape	Acorn	Oval	Elliptical
16	Fruit surface texture	Smooth	Smooth	Smooth
17	Peel color	Brown	Brown	Brown
18	Flesh color	Brown	Yellowish brown	Reddish brown
19	Flesh texture	Firm	Firm	Soft
20	Flesh taste	Sweet	Sweet	Sweet
21	Fruit availability	March-June, October-November	March-June, October-November	March-June, October-November
22	Fruit clustering habit	Cluster	Cluster	Cluster
23	Ovary size (mm)	4.02	4.00	3.93
24	Per cent fruit set	20.03	17.91	22.60
25	Yield/tree (Kg)	101.13	94.8	111.83
26	TSS(^{O}B)	24.03	24.6	25.33

27	Titrable acidity (%)	0.195	0.219	0.169
28	TSS to Acid ratio	123.23	112.31	149.88
29	Totalsugars (%)	10.99	11.19	11.99
30	Reducing sugars (%)	7.99	8.98	9.00
31	Non-reducing sugars (%)	3.00	2.21	2.98
32	Ascorbic acid (mg/100g)	8.51	11.05	10.51
33	Total Phenols (%)	0.437	0.383	0.528
34	Pectins (%)	2.23	2.10	1.66
35	Cluster analysis by Tochers method	Cluster VII	Cluster III	Cluster VIII
36	PCA by Ward's minimum varience method	Cluster IV	Cluster II	Cluster II
37	Molecular markers	Genotype specific fragment was amplified with SSR primers S-5 at 555 bp and S-10 at 300 bp	It is closely related to Brazilian Sapota in SSR analysis and in cluster I	Genotype specific fragment was amplified with RAPD primer OPG-3 at 744 bp

Monthly data on meteorological parameters during the period of investigation at Horticultural Research Station, Venkataramannagudem

Month and Year	Temperature (^{o} C)		Relative Humidity (%)	Rainfall (mm)
	Maximum	Minimum		
May,2013	39.45	26.87	57.16	9.00
June,2013	33.83	25.83	74.76	56.70
July,2013	29.77	24.54	70.48	107.20
August,2013	31.74	25.93	66.08	139.90
September,2013	32.40	25.00	67.00	219.30
October, 2013	30.83	24.51	73.37	341.00
November,2013	30.60	20.90	63.70	31.70
December,2013	30.09	18.61	55.74	0.00
January, 2014	29.93	18.70	62.66	0.00
February, 2014	30.66	19.55	61.20	0.00
March, 2014	33.68	22.00	60.39	0.00
April, 2014	38.00	25.37	57.53	0.00
May, 2014	38.13	27.03	58.74	42.40
June, 2014	39.13	28.10	54.90	27.70
July, 2014	33.26	24.90	68.85	261.80
August, 2014	33.55	25.29	66.68	113.80
September, 2014	31.73	24.37	74.00	137.60