

**MORPHOLOGICAL, BIOCHEMICAL AND ISOZYME
CHARACTERIZATION IN SAPOTA (*Manilkara achras* (Mill.)
Fosberg) GERMPLASM ACCESSIONS USING
PRINCIPAL COMPONENT ANALYSIS**

By

MARRI RAJASEKHAR

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**DEPARTMENT OF HORTICULTURE
COLLEGE OF AGRICULTURE
ACHARYA N. G. RANGA AGRICULTURAL UNIVERSITY
RAJENDRANAGAR, HYDERABAD – 500 030**

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CERTIFICATE

Mr. MARRI RAJASEKHAR has satisfactorily prosecuted the course of research and that the thesis entitled, 'MORPHOLOGICAL, BIOCHEMICAL AND ISOZYME CHARACTERIZATION IN SAPOTA (*Manilkara achras* (Mill.) Fosberg) GERMPLASM ACCESSIONS USING PRINCIPAL COMPONENT ANALYSIS', submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any other University.

Date:

(Dr. D. V. RAGHAVA RAO)

Major Advisor

CERTIFICATE

This is to certify that the thesis entitled, 'MORPHOLOGICAL, BIOCHEMICAL AND ISOZYME CHARACTERIZATION IN SAPOTA (*Manilkara achras* (Mill.) Fosberg) GERMPLASM ACCESSIONS USING PRINCIPAL COMPONENT ANALYSIS' submitted in partial fulfillment for the degree of DOCTOR OF PHILOSOPHY IN HORTICULTURE of the Acharya N G Ranga Agricultural University, Hyderabad, is a record of the bonafide research work carried out by Mr. MARRI RAJASEKHAR under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

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

(Dr. D. V. RAGHAVA RAO)
Chairman of the Advisory Committee

Thesis approved by the Student's Advisory Committee

Chairman:	Dr. D. V. RAGHAVA RAO Dean of Horticulture Andhra Pradesh Horticultural University Venkataramannagudem – 534 101 West Godavari District, A. P.	_____
Member:	Dr. D. SRIHARI Professor, Dept. of Horticulture College of Horticulture Rajendranagar, Hyderabad – 500 030	_____
Member:	Dr. B. SRINIVASULU Controller of Examinations Andhra Pradesh Horticultural University Venkataramannagudem – 534 101 West Godavari District, A. P.	_____
Member:	Dr. N. A. ANSARI Associate Dean College of Agriculture Aswaraopet, Khammam Dt.	_____
Member:	Dr. A. SIVASANKAR Professor, Dept. of Plant Physiology College of Agriculture Rajendranagar, Hyderabad – 500 030	_____

DECLARATION

I, MARRI RAJASEKHAR hereby declare that the thesis entitled 'MORPHOLOGICAL, BIOCHEMICAL AND ISOZYME CHARACTERIZATION IN SAPOTA (*Manilkara achras* (Mill.) Fosberg) GERMPLASM ACCESSIONS USING PRINCIPAL COMPONENT ANALYSIS' submitted to the Acharya N G Ranga Agricultural University for the degree of Doctor of Philosophy is a result original research work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

Date:

(MARRI RAJASEKHAR)

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(M. Rajasekhar)

LIST OF ABBREVIATIONS

%	- Per cent
Σ	- Sum of
<i>et al</i>	- And others
CD	- Critical Difference
cm	- Centimetre
°C	- Degree centigrade
Fig.	- Figure
g	- Gram
ha	- Hectare
IIHR	- Indian Institute of Horticultural Research
kg	- Kilogram
l	- Litre
µl	- Microlitre
µm	- Micrometre
m	- Metre
mg	- Milligram
ml	- Millilitre
mm	- Millimeter
mM	- Millimolar
NS	- Not Significant
SEm	- Standard Error mean
Sq. cm	- Square centimetre

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ABSTRACT

Sapota is one of the important and remunerative fruits grown in the country and Andhra Pradesh is one of the important growers. However, systematic work regarding characterization of sapota germplasm and study of genetic diversity is limited in India. Hence, the present investigation was conducted at Horticultural Research Station, Venkataramannagudem, during the years 2006-2008 on 25 genotypes of sapota, planted during 1996.

The genotypes studied recorded erect, semi-erect and spreading type of tree growth habit, while the tree shapes were found to be broadly pyramidal, spherical, semi-circular, elliptical and irregular. Verticillate, horizontal and irregular branching pattern and sparse to dense branching were also observed to a limited extent.

The fruit weight of sapota genotypes ranged from 44.83 g (Ulavapadu Selection) to 198.74 g (Cricket Ball), while the mean fruit weight was 96.63 g. The range of fruit yield/tree recorded was 26.20 kg/tree (CO – 2) to 93.35 kg/tree (PKM – 3), while the mean fruit yield was found to be 57.66 kg/tree. The various fruit shapes among the genotypes were found to be turbinate, acron, globose, oblong, elliptical and oval, while the flesh colour recorded was greenish brown, brown, yellowish brown and reddish brown.

The range of TSS content in fruits was observed to be 20.20^o brix (Cricket Ball) to 24.30^o brix (Pala), while the total sugars content of sapota genotypes ranged from 8.21 per cent (Badami) to 11.93 per cent (Pala). The ascorbic acid content of the sapota genotypes studied in the present investigation varied from 5.12 mg/100g (Dwarapudi) to 11.37 mg/100g (Kalipatti), while the total phenols content ranged from 0.2 mg/100g (Pala) to 0.78 mg/100g (Dwarapudi).

Peroxidase isozyme was found to be the most efficient in identifying 15 out of 25 genotypes studied, while a combination of peroxidase with either polyphenol oxidase or catalase isozymes was found to efficiently distinguish all the 25 sapota genotypes studied in the present investigation.

Genetic studies indicated high variability, heritability and genetic advance for the characters, namely, leaf area, fruit weight, fruit shape, seed number/fruit, seed weight, pulp:seed ratio, total soluble solids, sugar:acid ratio, ascorbic acid and total phenols indicating the effectiveness of direct phenotypic selection for improvement of these traits in sapota.

Principal component (PC) analysis revealed contribution of maximum variance by PC I and major characters contributing to formation of the PC's were also identified, while the dendrogram constructed resulted in formation of six clusters in all the three sets of PC analyses. Clustering of the fruit and biochemical characters revealed higher number of genotypes (nine) in Cluster I (Badami, Gede Sapota, Kalipatti, PKM – 2, Tagarampudi, Singapore, Krishna Rao, Calcutta Round and Dwarapudi), followed by six in Cluster V (Gutti, Pakala Round, Pala, Kirthibarthi, Ulavapadu Selection and DHS – 2) while lower number of genotypes (one) were recorded for Cluster VI (CO - 2).

From the results obtained from the present investigation on morphological and biochemical characterization of sapota genotypes in this study, a vision is obtained about the extent of variability in sapota genotypes. It was found that qualitative and quantitative traits of fruit could be employed in determining the level of variation and the 25 genotypes studied could be grouped into six clusters.

CHAPTER I

INTRODUCTION

Sapota (*Manilkara achras* (Mill.) Fosberg), originated in the tropical America, is an important commercially cultivated fruit in India, The Philippines, Sri Lanka, Malaysia, British Honduras, Indonesia, Guatemala, Mexico, Venezuela, Florida, West Indies and other countries of Central America. In South America, it is cultivated mostly for chicle, extracted from bark of the tree and used as base material for preparation of chewing gum. However, in India it is cultivated for its fruit, which is mainly used as dessert.

The fruit of sapota is oval or conical in shape resembling a smooth potato in appearance. Its flesh is honey brown, soft mellow and very sweet. It is a good source of digestible sugar which ranges from 12 to 18 per cent and has appreciable amount of protein, fat, fibre and mineral content (Chadha, 1992). It has fairly good amount of Vitamin A and C. From processing point of view it has been rated very low but there is a good scope for dehydration of pulp to be used as sapota fig. It is becoming an important adjunct in ice-creams and milk shakes in its fresh form. The fruit can also be used for preparing liquor and alcohol because of its richness in sugars (Chundawat, 1998).

The crop comes next in importance to mango, banana, citrus, apple and guava in India. It is now being grown commercially in Gujarat, Maharashtra, Karnataka, Andhra Pradesh, West Bengal, Tamil Nadu and Kerala in an area of about 1,40,000 ha, with a production of 11,17,100 t (www.indiastat.com 2005-

06). In Andhra Pradesh it is cultivated in about 15,400 ha yielding about 1,53,600 t of fruits (www.indiastat.com 2005-06).

Large area is being brought under this crop every year in India and Andhra Pradesh. Popularity of this crop is increasing among the farmers due to its high production per unit area, continuous fruiting and thereby income throughout the year, low cost of production, liking to Indian palate, and very little incidence of diseases and pests. Besides, the tree is quite hardy and can tolerate wide range of soils, salinity and water stress to a great extent. Since the crop is becoming popular both among the farmers and consumers, there is need for taking up crop improvement programmes for developing improved varieties suitable for various purposes.

Germplasm is a vital resource in any crop and if properly described, characterized and evaluated once, it can be utilized for long term and systematic crop improvement. Many of the named sapota varieties are the result of local selections and many synonyms are known to exist. Since varieties are identified based on fruit size, shape and quality, it does not satisfy the descriptive need for a cultivar. Existing confusion in classification and nomenclature of varieties can be eliminated only by proper characterization. Limited efforts seem to have been made in sapota to characterize the genetic resources with precision (Brito and Narain, 2002) using modern and systematic methods. Further, information on genetic parameters of variability, namely, genotypic coefficient of variation, phenotypic coefficient of variation, heritability and genetic advance for different characters of economic importance, a major pre-requisite for crop improvement programmes is also lacking in sapota. Hence, there is a need to analyze and

identify the varieties for desirable traits and extent of variability for use in future breeding programmes. Off late, to characterize germplasm in any species, it is necessary to consider many morphological characters along with molecular markers.

Isozyme analysis is being used to distinguish cultivars, breeding lines and germplasm in various horticultural crops. Identification of cultivars and germplasm accessions through isozyme analysis offers far precise results than using morphological descriptors alone. Asiedu (1992) reported that isozyme markers are free from environmental influence, pleiotrophy and epistatic effects and hence, are more reliable than morphological markers. Isozyme markers also facilitate establishment of genetic purity, location of duplicate accessions and classification of the genotypes into distinct clusters.

Principal component analysis is a method that can be used to identify patterns in data set and to eliminate redundancy in univariate analysis, when multicollinear data are involved. Here the data sets containing many correlated variables are restructured into smaller sets of components of the original variables, where each set is uncorrelated with any other, but components within sets are related. The resulting combination may suggest a biological meaning for the grouping of variables or their components. The method was first introduced by Pearson (1901) and developed by Hotelling (1933), but its widespread use started only after the advent of computers. This method is currently being used extensively for classification of genotypes according to their degree of similarity (Smith *et al.*, 1995), helpful in the development of core subsets of germplasm collections.

Systematic work regarding characterization of sapota germplasm and study of genetic diversity for different traits of economic importance has not been carried out in India. Hence, the present investigation was planned with the following objectives :

- i. To identify variations in tree morphology and biochemical characters of elite and local cultivars.
- ii. To study variations and similarities among the accessions in the germplasm by isozyme banding patterns.
- iii. To test the reliability of isozyme markers for the identification of sapota varieties.
- iv. To characterize and classify these accessions into homogenous clusters based on PC analysis using variables from (i) and (ii) above.
- v. To identify genotypes and also clusters or groups of accessions with desirable traits.

CHAPTER II

REVIEW OF LITERATURE

Sapota is an evergreen tree, native of tropical America especially the Southern Mexico or Central America. This fruit tree is mainly cultivated for its edible fruits of exclusive quality but latex is also tapped from the bark for chicle gum, the base for chewing gum. It belongs to the family Sapotaceae and the botanical name is *Manilkara achras* (Mill.) Fosberg (Syn. *Achras sapota* L., *Manilkara zapotilla* Jacq. Gilly). Among the 31 Genera and 400 species of the family only a few species have edible fruits and sapota is one among them. The fruit is known by a number of vernacular names like sapota, sapota plum, sapodilla, zapote, chiko zapote, chicku, dilly, naseberry and nispero. The term sapota provides considerable confusion in literature as a number of other fruits are also called sapota. Sapodilla or chicku are more appropriate and most satisfactory for *Manilkara achras*.

In this chapter, the literature of sapota and other related fruits in respect of genetic resources, botanical description, biochemical characters and isozyme studies for characterization and principal component analysis are reviewed and presented below.

2.1 STATUS OF SAPOTA GERMPLASM

Systematic study of any fruit crop is one of the fundamental aspects of pomology, that the proper selection of varieties provide the key for success in commercial farming. The taxonomic studies are mainly concerned with the description, classification and cataloguing of different genotypes belonging to a

species grown under uniform field or under ex-situ conditions. In crops like mango the first record of the good and bad varieties of mango is available in Ain-i-Akbari by Abul Fazal written in 1590 A.D. Since the commercial cultivation of sapota in India was started in 1890's not much information was gathered on the characters of the available genotypes with the help of a descriptor. Description of sapota genotypes which are popular in respective regions was attempted on a small scale and Naik during 1949 has listed out nine genotypes in sapota which are of moderate to major importance in commercial marketing. Presently major sapota collections are at Venkataramannagudem of Andhra Pradesh (33 collections), Periyakulam of Tamil Nadu (26), Bangalore of Karnataka (19), etc. under the AICRP centres of tropical fruits. At international level major centres of germplasm collections are at Costa Rica (118), Philippines (73), India (41), Puerto Rico (32) and Mexico (31) (Chundawat, 1998).

Most of the commercial varieties in India are the consequence of extensive study and selection of clonal material. Names of different clones are assigned based on the shape of the tree (Chaatri), leaf colour (Pilipatti, Kalipatti), fruiting habit (Jhumakia, Baramasi), shape of the fruit (Cricket Ball, Oval, Round), and location of the selection (Bombay, Calcutta) (Chundawat, 1998).

2.2 GENERAL DESCRIPTION OF SAPOTA TREE

Sapota is a medium sized, spreading evergreen tree with milky sap, growing to a height of 20 m. The tender parts are rusty hairy, bark brown to blackish brown, rough, longitudinally fissured, stem short, branching from lower part forming umbrella shaped crown. Leaves are elliptic, oblong or oblong

lanceolate, leathery, and pale green beneath, shining dark green and glabrous above. Flowering occurs almost throughout the year in tropics, flowers are dull white, axillary, solitary, small. Sepals six in two whorls, tomentose, free, corolla six, imbricate, stamens also six alternating with petaloid staminodes. Ovary superior 10-12 celled, style bifid, protogynous. Fruit globose to oval, fleshy berry, rough, rusty greyish to brown with yellow brown flesh. Seeds 0-12 shining black, hard, 1.5 -2.5 cm long, elliptic lanceolate, thick easily separated from pulp.

2.3 VARIETAL DESCRIPTION

Though this crop is not native to India several varieties have been developed by extensive study and selection of clonal material. Some of the sapota varieties of India, namely, Kalipatti, Pala, Cricket Ball, Kirthibarthi, Dwarapudi, Gutti, Calcutta Round, CO -1, PKM – 1 and DHS - 1 are briefly described in the following table.

Description of certain sapota varieties of India:

S. No. 1	Name of the variety 2	State in which grown 3	Salient characters 4
1	Kalipatti	Gujarat, Maharashtra, North Karnataka	Leaves broad, thick and green in colour, fruits borne in single, fruit quality high, main picking season winter.
2	Pala	Andhra Pradesh, Tamil Nadu	It is a popular cultivar in Andhra Pradesh and Tamil Nadu. The fruits are small to medium in size 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 flavor.

1	2	3	4
3	Cricket Ball	Andhra Pradesh	Fruits are of very large size, round, very sweet, but not of any distinctive flavor
4	Kirthibarthi	Andhra Pradesh, Tamil Nadu	Bears large fruits, round, sweet and much in demand
5	Dwarapudi	Andhra Pradesh	Large sized fruits, round, sweet and much in demand
6	Calcutta Round	West Bengal, Karnataka, 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.
7	Gutti	Andhra Pradesh, Tamil Nadu	Tree medium in height, conical in shape, leaves elliptic, lanceolate, slightly folded, entire, acute, fruit elliptic, small, snuff brown, flesh soft, not juicy, gritty, brown in colour, aroma slight, flavor slight. tastes mild sweet, seeds very few
8	PKM-1	Tamil Nadu	A selection from Gutti, short stature and dense crown, fruits medium sized, elliptic to oblong with thin skin, rich in sugars, high TSS of 24 ^o brix, profuse bearing.
9	CO - 1	Tamil Nadu	This is a hybrid between Cricket Ball x Oval. The combination was designed to get together the large fruit size of the female parent and superior taste of the male parent. Fruit weight is 125g with reddish brown flesh and TSS 18 ^o brix.
10	DHS - 1	Karnataka	Fruit length 6.72 cm, fruit diameter 6.26 cm, fruit weight 154 g, little or no scurf on the surface, colour of the pulp light orange, texture soft and mellow, TSS 26 ^o brix.

(Chundawat, 1998)

2.4 TREE MORPHOLOGICAL CHARACTERS OF SAPOTA

The literature available in respect of different tree morphological characters like growth habit of the tree, shape of the tree, branching pattern, branch density, leaf length, leaf width, leaf area, pollen size, pollen production / anther, pollen fertility and style length were reviewed hereunder.

2.4.1 Growth habit of the tree

Sulladmath and Narayana Reddy (1990) classified sapota cultivars based on the growth habit of the tree into four types, namely, trees with erect growth habit, drooping habit, spreading habit with light green foliage and spreading habit with dark green foliage. They have reported that in the trees with erect habit and drooping habit the branches appear in whorls and in trees with spreading habit the branching is irregular.

In another report, Mathew *et al.* (2001) reported that the cultivar Cricket Ball had spreading type of branching, PKM-1 and PKM-3 had upright branching and in Jhargram Local and Guthi it was medium spreading.

2.5 FRUIT AND BIOCHEMICAL CHARACTERS

Chadha (1992) reported that the fruit of sapota is oval or conical in shape resembling a smooth potato in appearance. Further, it's flesh is honey brown, soft mellow and very sweet. It is a good source of digestable sugar which ranges from 12-18 per cent and has appreciable amount of protein, fat, fibre and mineral content. Though the amount of work on the description of the tree was very little, the fruit chemical composition was described by many workers. The literature

available on fruit biochemical characters, namely, total soluble solids, reducing sugars, non-reducing sugars, total sugars, titrable acidity, sugar:acid ratio, ascorbic acid, total phenols is presented hereunder.

2.5.1 Total soluble solids (TSS)

Large variation exists in the total soluble solids (TSS) content of fruit of sapota varieties. Several workers have reported regarding the TSS of the sapota genotypes which were reviewed here under.

Madhava Rao *et al.* (1971) had studied the total soluble solids of sapota genotype Oval and reported that the TSS was 19.0 °brix at ripe stage, while Suryanarayana and Goud (1984) stated that the TSS of genotype Oval to be 13.92 ° brix at ripe stage. Further, Shanmugavelu and Srinivasan (1973) reported that the TSS of sapota genotypes varied from 24° in Guthi to 13° brix in Dwarapudi, while Lakshminarayana (1980) had reported that the TSS of certain Mexican varieties ranged from 19.7° in SCH-08 to 25.0° in SCH-02.

Ponnuswamy and Irulappan (1987) stated that the TSS of sapota genotypes ranged from 15.02° in CO-1 to 26.28° in PKM-2 but also observed that the TSS ranged from 15.02° in CO – 1 to 23.35° brix in Kirthibarthi (Ponnuswamy and Irulappan, 1989). Paralkar *et al.* (1987) reported that the TSS of sapota genotype Kalipatti was 21.6° brix at maturity, while Shanmugavelu *et al.* (1987) reported that the TSS varied from 18° in Cricket Ball to 25° brix in Long Oval genotype. Similar observations were reported by Waskar and Nikam (1996), who reported that the TSS ranged between 18° to 25° brix in sapota fruits. Further, Mone *et al.*,

(1991) reported that the TSS varied from 17^o in Tagarampudi to 24^o brix in cultivar Kalipatti.

Mathew *et al.* (2001) reported that the TSS varied from 21^o in Guthi to 18^o brix in Local (Narkeli), while Brito and Narain (2002) have reported that the TSS of Brazilian sapota variety Itapirema-31 was 23.20 ^obrix.

2.5.2 Sugars content

The fruits of sapota are rich in sugars, hence recommended for patients convalescing from prolonged illness. The content of reducing, non-reducing and total sugars in sapota fruits is presented below

2.5.2.1 Reducing sugars

Literature available on the reducing sugars content of sapota genotypes has been compiled in the following paragraphs.

The reducing sugars content of sapota genotype Oval was reported to be 3.61 per cent by Madhava Rao *et al.* (1971) and 5.83 per cent by Suryanarayana and Goud (1984). According to Shanmugavelu and Srinivasan (1973) the reducing sugars in sapota cultivars ranged from a minimum of 4.7 per cent in CO - 1 to 10.3 per cent in Kirthibarthi. Further, the reducing sugars content of Mexican varieties ranged from 12.97 per cent (SCH-08) to 6.66 per cent (SCH - 02) (Lakshminarayana, 1980). In another report Selvaraj and Pal (1984) have stated that the reducing sugars content of genotype Cricket ball was 11.95 per cent and that of Oblong was 11.96 per cent.

Paralkar *et al.* (1987) reported that the reducing sugars of cultivar Kalipatti was 8.92 per cent at maturity, while Ponuswamy and Irulappan (1987) stated that

the reducing sugars differed from 6.5 per cent in Tagarampudi to 12.30 per cent in cross No. 2/2 between Guthi and Kirthibarthi. However, Mathew *et al.* (2001) reported that the reducing sugars varied from 3.6 per cent in PKM-3 to 5.04 per cent in PKM-1, while Brito and Narain (2002) have observed that the reducing sugars content was 9.3 per cent in sapota variety Itpirema-31.

2.5.2.2 Non-reducing sugars

The non-reducing sugars content of sapota genotypes ranged from 1.0 per cent in Baramasi to 4.9 per cent in Cricket Ball (Shanmugavelu and Srinivasan, 1973), while Suryanarayana and Goud (1984) have reported that the non-reducing sugars content of sapota variety Oval was 6.77 per cent. However, the non-reducing sugars content of certain Mexican sapota varieties was reported to vary from 4.81 per cent (SCH-08) to 7.35 per cent (SCH-28) by Lakshminarayana (1980). Further, Selvaraj and Pal (1984) have reported that the non-reducing sugars content of sapota genotype Cricket ball to be 2.12 per cent, while that of Oblong to be 3.10 per cent.

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

2.5.2.3 Total Sugars

The total sugars content of sapota genotype Oval was reported to be 14.0 per cent at ripe stage by Madhava Rao *et al.* (1971) and 12.60 per cent by

Suryanarayana and Goud (1984). Further, the total sugars content in sapota genotypes was reported to range from 7.0 per cent in cultivar Oval to 12.3 per cent in Long Oval by Shanmugavelu and Srinivasan (1973).

Lakshminarayana (1980) reported that the total sugars content of certain Mexican varieties ranged from 16.16 per cent (SCH-07) to 19.67 per cent (SCH-02), while the total sugars content in cultivar Kalipatti was observed to be 15.4 per cent at maturity by Paralkar *et al.* (1987). According to Ponnuswamy and Irulappan (1987) the total sugars content ranged from 6.94 per cent in Cricket ball to 14.04 per cent in cross 2/2 between Guthi and Kirthibarthi.

The total sugars content was observed to range from 11.10 per cent in Cricket Ball to 7.70 per cent in Local (Narkeli) by Mathew *et al.* (2001), while Brito and Narain (2002) have reported that the total sugars content of variety Itapirema-31 was 14.8 per cent.

2.5.3 Titrable acidity

The acidity in sapota fruits is predominantly malic acid (Selvaraj and Pal, 1984), while citric and tartaric acids are present to a lesser extent.

It was reported that the titrable acidity content of sapota genotype Oval was 0.13 per cent at ripe stage (Madhava Rao *et al.*, 1971), while the tirtable acidity content of the same genotype was reported to be 0.18 per cent by Suryanarayana and Goud (1984). Further, Shanmugavelu and Srinivasan (1973) reported that the titrable acidity of sapota genotypes ranged from 0.02 per cent in Cricket Ball to 0.09 per cent in Dwarapudi. However, Lakshminarayana (1980) reported that the titrable acidity of Mexican sapota varieties ranged from 0.09 per cent (SCH-07

and SCH-08) to 0.15 per cent (SCH-28), while Selvaraj and Pal (1984) reported that the titrable acidity of sapota genotype Cricket ball to be 0.11 per cent and 0.41 per cent in the genotype Oblong.

Paralkar *et al.* (1987) stated that the titrable acidity in sapota genotype Kalipatti was 0.24 per cent at maturity, while Ponnuswamy and Irulappan (1987) reported that the acidity ranged from 0.02 per cent in CO-1 to 0.108 per cent in Pala. However, Mathew *et al.* (2001) reported that the titrable acidity ranged from 0.064 per cent in Cricket Ball to 0.128 per cent in Local (Narkeli), while the titrable acidity of Brazilain genotype Itapirema-31 was reported to be 3.3 m Eq NaOH/100g (Brito and Narain, 2002).

2.5.4 Sugar acid ratio

Ponnuswamy and Irulappan (1987) have reported that the sugar acid ratio of sapota genotypes ranged from 103.58 in Cricket Ball to 659.17 in the cross No. 2/2 between Guthi x Kirthibarthi.

2.5.5 Ascorbic acid

Madhava Rao *et al.* (1971) have reported that the ascorbic acid content of the sapota genotype Oval was 35 mg/100g, while the ascorbic acid content of the same genotype was observed to be 19.0 mg/100g by Suryanarayana and Goud (1984). Further, Shanmugavelu and Srinivasan (1973) have reported that the ascorbic acid content of sapota genotypes varied from 0.90 per cent in Oval to 11.90 per cent in CO - 1.

Lakshminarayana (1980) observed that the ascorbic acid content of Mexican sapota varieties ranged from 10mg/100g (SCH-07) to 41.4 mg/100g (SCH-28). In another report Selvaraj and Pal (1984) observed that the ascorbic acid content in the genotype Cricket ball was 8 mg/100g, while it was 4 mg/100g in the genotype Oblong.

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 in sapota cultivars ranged from 1.65 mg/100g in Oval to 3.99 mg/100g in cross 2/2 between Guthi and Kirthibarthi. Further, the ascorbic acid content of variety Itapirema-31 was observed to be 18.0 mg/100g (Brito and Narain, 2002).

2.5.6 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 *et al.* (1969) reported that the total phenolic content of two months old fruit was 0.018 g and in mature sapota at eight months of age it was 4.82 g, while the phenolics content of sapota variety Oval was reported to be 6900 µg of catechol/100g (Madhava Rao *et al.* 1971). Further, Selvaraj and Pal (1984) reported that the tannins content was 0.10 per cent and 0.20 per cent respectively in sapota genotypes Cricket Ball and Oblong, while Paralkar *et al.* (1987) recorded 0.67 per cent of tannins at maturity in the genotype Kalipatti.

However, Brito and Narain (2002) observed 0.3 per cent tannin (as tannic acid) in the Brazilian variety Itapirema-31.

2.6 ISOZYME ANALYSIS

Isozyme analysis is commonly used to distinguish cultivars, breeding lines and germplasm in many crop species. Markert and Moller (1959) coined the term isozyme (Isoenzyme) to describe multiple molecular forms of an enzyme, having same catalytic activity. More precise results are obtained through isozyme analysis for the identification of cultivars and germplasm accessions than the study of morphological descriptors alone. Isozyme analysis facilitates establishment of genetic purity, location of duplicate accessions and identification of somaclonal variants. Confirmation of the authenticity of hybrids can be difficult in the absence of reliable genetic markers. Isozyme loci have been used effectively for this purpose (Asiedu, 1992, Moore and Durham, 1992).

In most cases isozyme patterns have a simple genetic basis with the isozyme bands often denoting an allele at a single locus. Allozymes display codominant expression. When a plant is heterozygous at a locus for a monomeric (one unit) enzyme (eg. peroxidase) the hybrid will have two allozyme bands where as three bands will be formed for a dimeric (2 sub unit) enzyme (eg., aspartate amino transferase). Tetrameric (4-sub unit) enzymes (eg., catalase) heterozygote form five types; two homotetramers and three heterotetramers. These allozymes usually migrate to different positions on the gel, resulting in 5 banded phenotypes (Kephart, 1990; Moore and Durham, 1992). The most useful isozyme loci are those where a number of alleles exist within a given taxon. If the

enzymatic products are not well resolved on gels, heterozygotes may display a single broad zone of activity instead of the predicted two or more distinct bands, in such cases 2-dimensional electrophoresis would be helpful to detect the band positions (Kephart, 1990).

Many factors determine the number of bands observed on a gel: (1) the number of coding genes, (2) their allelic states (homozygous or heterozygous), (3) quaternary structure of the protein products and (4) their subcellular compartmentalization (Wendel and Weeden, 1989).

2.6.1 Isozyme analysis for identification of sapota cultivars

There are no reports of isozyme analysis in sapota but several reports have been published in case of other fruit crops.

2.6.1.1 Avocado (*Persia americana*)

Torres and Bergh (1980) have analysed hundred avocado cultivars and 8 other *Persea* spp. for isozyme variation of peroxidase (PX), malate dehydrogenase (MDH), leucine aminopeptidase (LAP) and phospho glucose mutase (PGM). MDH, LAP and PGM isozymes of leaf and mesocarp were identical. Isozymes of alcohol dehydrogenase (ADH) and glutamate oxalo acetate transaminase (GOT) were detected in mesocarps but were not available from leaves. Leaf PX was specified by 3 genes having 11 alleles and MDH isozymes by 1 gene with 3 alleles. Six enzyme systems specified by 12 genes with 37 codominant alleles are available as genetic markers of possible use in problems of avocado systematics, measurement of out crossing rate, documentation of parentages and screening of seedlings in breeding programmes.

2.6.1.2 *Banana (Musa sps.)*

Jarret and Litz (1986b) analysed five isozymes in twenty four clones of banana and plantain representing various levels of ploidy and diploid *M. balbisiana*. Polymorphism was detected in all the five enzyme systems. In addition, the four principal Cavendish clones Robusta, Giant Cavendish, Dwarf Cavendish and Pisang Masak Hijau were found to be monomorphic for isozymes of 10 additional enzymes. Isozymes of glutamate oxaloacetate transaminase were the most useful for discriminating among the clones of a particular genomic group.

In another study, Bhat *et al.* (1992b) have studied four isozyme systems in forty four cultivars of Indian bananas and plantains. They found that the degree of polymorphism was high with peroxidase and superoxide dismutase, while the polymorphism was less with shikimate dehydrogenase and malate dehydrogenase.

2.6.1.3 *Mango (Mangifera indica)*

Degani *et al.* (1990) characterized 41 cultivars of mango electrophoretically, using six isozyme systems viz., aconitase (ACO), isocitrate dehydrogenase (IDH), leucine amino peptidase (LAP), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM) and triose phosphate isomerase (TPI). Differences in banding patterns among the cultivars were observed for all the enzyme systems studied. They identified a zone each for TPI, IDH, PGM and ACO and two for PGI and LAP. The relative mobility of all these enzyme systems were worked out and the bands were designated in each zone based on R_m values. Further, Degani *et al.* (1992) analysed leaf phosphoglucose isomerase (PGI) isozymes from 139 cultivars and seedlings of mango by starch gel electrophoresis

and reported that six distinct banding patterns of PGI-2 consisting of single and triple banded phenotypes were detected. The genetic control of PGI-2 isozymes was identified from segregating progenies of self-pollinated parent cultivars having triple banded phenotypes. Comparison of the banding patterns of PGI-2 isozymes extracted from the pollen and leaf of the same heterozygous cultivar demonstrates the allelism of the PGI-2 locus.

In addition genetic diversity among 20 monoembryonic and 14 polyembryonic genotypes were observed through four isozyme systems (ACP, EST, PRX and AAT), in a study carried out at Bangalore (Mustaffa, 1993). Based on these studies, 20 monoembryonic mango genotypes could be classified into 7 distinct groups. The North Indian varieties were genetically divergent from the South Indian ones, due to different banding patterns. Fourteen polyembryonic mango varieties exhibited polymorphism for 3 isozymes showing 3 groups. A unique triple banded GPI-2 was observed only in polyembryonic cv. Prior. The monomorphism observed in this cultivar may be due to obligate apomixis. Identical banding patterns were observed in Kitchner and Peach cultivars indicating their identical origin. Out of the 10 enzyme systems, namely, ACP, ALP, EST, PRX, AAT, GPI, PGM, G6PDH, HK and GDH included in the study, only 4 enzymes EST, PRX, AAT and ACP showed polymorphism in 19 loci.

Further, Subedi *et al.*, (2005) have also characterized the mango genetic diversity in Nepal by isozyme analysis. Esterase enzyme provided evidence of genetic variation among the germplasm studied.

2.6.1.4 Papaya (*Carica papaya*)

Coppens d'Eeckenbrugge *et al.* (2007) have characterized papaya accessions based on isozymes and found that four isozymes out of eight allowing good resolution, revealed polymorphism. However they could identify eight zymotypes.

2.6.1.5 Jackfruit (*Artocarpus heterophyllus*)

Azad *et al.* (2007) have assessed morphological and isozyme variation in jackfruit accessions from Bangladesh. Isozyme analysis confirmed field studies of morphological characters and ten potentially superior types were selected.

2.6.1.6 Cashew (*Anacardium occidentale*)

Maranan and Mendioro (2008) have analysed isozymes of cashew germplasm from Philippine and found that acid phosphatase and esterase were polymorphic.

2.6.1.7 Cacao (*Theobroma cacao*)

Atkinson *et al.* (1986) screened twenty four isozymes in cacao with a view to use the data for characterizing cacao germplasm. Seventeen of these showed activity and in seven enzyme systems reproducible variation in banding pattern was obtained. Peroxidase activity was observed only in woody tissues.

2.6.1.8 Citrus *sps.*

Torres *et al.* (1982) extracted leaf isozymes in different citrus species and analysed them by starch gel electrophoresis for isozymes hexokinase (HK), isocitrate dehydrogenase (IDH), leucine amino peptidase (LAP), malate dehydrogenase (MDH) and malic enzyme (ME). The first three showed a single region of activity suggesting each is specified by a single gene. Banding patterns

indicate HK and LAP are monomeric, and IDH is dimeric. Evidence of four alleles of *Hk* and *Idh* and three of *Lap* was found in the nine species of cultivated Citrus and Poncirus trifoliata. Isozymes of MDH occurred in three gel regions, are apparently specified by four genes two of which *Mdh-1* and *Mdh-2* are polymorphic. Zymograms indicated that MDH is dimeric, but the sub units of *Mdh-1* do not dimerize with those of *Mdh-2*.

2.6.1.10 Apple (*Malus pumila*)

Weeden and Lamb (1985) have characterized electrophoretically fifty four apple cultivars using six isozyme systems and reported that the intracultivar variation in isozyme phenotypes was not observed, where as inter cultivar polymorphism was sufficient to permit reliable and unambiguous identification of nearly every cultivar. The most useful isozyme systems for distinguishing among the cultivars were 6-phosphogluconate dehydrogenase and aspartate aminoatransferase.

Iamtham *et al.* (1998) have also characterized temperate fruits from high lands of Thailand and indicated that peroxidase enzyme was suitable for separating these fruit tree species, apple being one of them.

Further, Wagner *et al.* (2004) have studied the isozyme polymorphism in *Malus sylvestris* (L.) belonging to Central Europe and they have investigated twelve enzyme systems GPI, TPI, AAT, PGM, DIA, 6PGD and MDH from fresh leaf samples and found that the various gene pools differ in their mean number of alleles per locus and genotypes per locus, mean heterozygosity total differentiation and diversity. Most loci showed major polymorphism. It was also

observed that there is relatively high genetic distance between *M. sylvestris* and *M. x domestica*.

Simo-Santalla *et al.* (2000) have investigated the leaf isozymes of crab apple for acid phosphatase, catechol oxidase, diaphorase, endopeptidase, esterase, leucine aminopeptidase, phosphoglucosomerase and superoxide dismutase. With the help of these enzyme systems 22 clones could be distinguished by specific patterns or combinations of patterns.

2.6.1.11 Sweet Cherry (*Prunus avium*)

Morales *et al.* (2008) have characterized sour and sweet cherry varieties with five isozymes and found that the varieties analysed showed low isozyme polymorphism.

2.6.1.12 Camellia sps.

Sen *et al.* (2000) have characterized *Camellia sps.* based on isozymes and found that there are both qualitative and quantitative variations in the isozyme banding pattern among the different clones and species studied.

Jeyaramaraja and Manian (2005) have characterized *Camellia sps.* and found that in drought susceptible clones there is high activity of acid phosphatase indicating disturbances in Pi nutrition during drought conditions. The esterase isozyme was present in all clones except in drought tolerant clone.

2.6.1.9 Rubber (*Hevea brasiliensis*)

Sreelatha *et al.* (1993) analysed leaf extracts of diploid, triploid and tetraploid clones of *Hevea brasiliensis* for isozyme variation of five enzyme systems using polyacrylamide gel electrophoresis. Esterase, peroxidase, acid phosphatase, diaphorase and isocitrate dehydrogenase produced sharp and well

resolved bands. The qualitative zymograms studies showed marked variation in banding patterns among the cytotypes. Esterase showed a decrease in the mean number of bands with increase in ploidy level. An enhancement in the number and intensity of bands in tetraploid was observed in the case of isocitrate dehydrogenase and diaphorase. Banding site polymorphism between cytotypes indicated the usefulness of isozymes for clone characterization.

2.7 GENETIC VARIABILITY STUDIES

Genetic variability studies included the study of genotypic coefficient of variation, phenotypic coefficient of variation, heritability, genetic advance and genetic advance as per cent mean. Variability refers to the presence of differences among the individuals of plant population, which is essential for crop improvement. Hence, insight into the magnitude of genetic variability present in a population is of paramount importance to a plant breeder for starting a judicious breeding programme. Heritability and genetic advance refer to the relative degree at which a character is transmitted from parent to offspring and the genetic gain that could be obtained in the later generations.

The reports about genotypic coefficient of variation, phenotypic coefficient of variation, heritability, genetic advance and genetic advance as per cent mean in various crops are provided in this section.

2.7.1 Genotypic coefficient of variation

The literature available on genotypic coefficient of variation in various fruit crops is presented here under.

Genotypic coefficient of variation of various fruit crops

S. No	Character	Crop	GCV	Range	Reference
1	2	3	4	5	6
1.	Fruit length	Litchi	9.12	Low	Ghanta <i>et al.</i> (1994)
		Mango	0.638		Attri <i>et al.</i> (1999)
		Banana	19.20	Moderate	Rosamma and Namboodiri (1990)
			9.00	Low	Sirisena and Senanayake (2000)
2.	Fruit width	Mango	19.72	Moderate	Karibasappa (1995)
		Litchi	8.20	Low	Ghanta <i>et al.</i> (1994)
		Mango	0.983		Attri <i>et al.</i> (1999)
		Banana	13.01	Moderate	Rosamma and Namboodiri (1990)
3.	Fruit weight		5.00	Low	Sirisena and Senanayake (2000)
		Mango	17.17	Moderate	Karibasappa (1995)
		Litchi	21.65	High	Ghanta <i>et al.</i> (1994)
		Mango	1.123		Attri <i>et al.</i> (1999)
4.	Fruit yield/tree	Acid lime	11.76	Moderate	Ranpise and Desai (1994)
		Banana	48.34	High	Rosamma and Namboodiri (1990)
			7.00	Low	Sirisena and Senanayake (2000)
		Mango	48.41	High	Karibasappa, (1995)
5.	Seed weight	Banana	45.17	High	Rosamma and Namboodiri (1990)
			20.90	High	Rekha and Prasad (1993)
			58.58	High	Uma <i>et al.</i> (2000)
			12	Moderate	Sirisena and Senanayake (2000)
6.	Titrable acidity	Acid lime	67.63	High	Ranpise and Desai (1994)
		Litchi	31.03	High	Ghanta <i>et al.</i> (1994)
		Mango	30.24	High	Karibasappa (1995)
		Mango	1.57		Attir <i>et al.</i> (1999)
6.	Titrable acidity	Banana	16.26	Moderate	Rekha and Prasad (1993)

1	2	3	4	5	6
		Acid	6.46	Low	Ranpise and Desai (1994)
		Lime			
		Mango	89.09	High	Karibasappa, (1995)
7.	TSS	Mango	0.468		Attri <i>et al.</i> (1999)
		Banana	11.97	Moderate	Rekha and Prasad (1993)
		Acid	6.40	Low	Ranpise and Desai (1994)
		Lime			
		Mango	19.69	Moderate	Karibasappa (1995)
8.	Reducing sugars	Mango	0.643		Attri <i>et al.</i> (1999)
		Mango	64.23	High	Karibasappa (1995)
9.	Total sugars	Mango	0.564		Attri <i>et al.</i> (1999)
		Mango	52.38	High	Karibasappa (1995)
10.	Ascorbic acid	Mango	1.74		Attri <i>et al.</i> (1999)
		Acid	9.50	Low	Ranpise and Desai (1994)
		Lime			
		Mango	75.70	High	Karibasappa (1995)
11.	Sugar:acid ratio	Mango	42.02	High	Karibasappa (1995)
12.	Skin colour	Mango	29.00	High	Karibasappa (1995)
13.	Pulp colour	Mango	19.08	Moderate	Karibasappa (1995)

2.7.2 Phenotypic coefficient of variation

The literature available on phenotypic coefficient of variation in various fruit crops is furnished here under.

Phenotypic coefficient of variation in various fruit crops

S. No	Character	Crop	PCV	Range	Reference
1	2	3	4	5	6
1.	Fruit length	Litchi	10.99	Moderate	Ghanta <i>et al.</i> (1994)
		Mango	0.648		Attri <i>et al.</i> (1999)
		Banana	66.98	High	Rosamma and Namboodiri (1990)
			14.00	Moderate	Sirisena and Senanayake (2000)
		Mango	19.89	Moderate	Karibasappa (1995)
2.	Fruit width	Litchi	9.81	Low	Ghanta <i>et al.</i> (1994)

1	2	3	4	5	6
		Mango	0.449		Attri <i>et al.</i> (1999_
		Banana	14.95	Moderate	Rosamma and Namboodiri (1990)
			7.00	Low	Sirisena and Senanayake (2000)
3.	Fruit weight	Mango	17.36	Moderate	Karibasappa (1995)
		Litchi	21.92	High	Ghanta <i>et al.</i> (1994)
		Mango	1.125		Attri <i>et al.</i> (1999)
		Acid lime	12.48	Moderate	Ranpise and Desai (1994)
		Banana	51.95	High	Rosamma and Namboodiri (1990)
			20.00	Moderate	Sirisena and Senanayake (2000)
		Mango	48.85	High	Karibasappa, (1995)
4.	Fruit yield/tree	Banana	48.38	High	Rosamma and Namboodiri (1990)
			41.16	High	Rekha and Prasad (1993)
			65.49	High	Uma <i>et al.</i> (2000)
			24.00	High	Sirisena and Senanayake (2000)
		Acid lime	72.16	High	Ranpise and Desai (1994)
5.	Seed weight	Litchi	32.46	High	Ghanta <i>et al.</i> (1994)
		Mango	32.58	High	Karibasappa (1995)
6.	Titration acidity	Mango	1.57		Attri <i>et al.</i> (1999)
		Banana	14.93	Moderate	Rekha and Prasad (1993)
		Acid Lime	9.04	Low	Ranpise and Desai (1994)
		Mango	92.97	High	Karibasappa, (1995)
7.	TSS	Mango	0.480		Attri <i>et al.</i> (1999)
		Banana	7.22	Low	Rekha and Prasad (1993)
		Acid Lime	7.80	Low	Ranpise and Desai (1994)
		Mango	21.58	High	Karibasappa (1995)
8.	Reducing sugars	Mango	0.644		Attri <i>et al.</i> (1999)

1	2	3	4	5	6
		Mango	67.98	High	Karibasappa (1995)
9.	Total sugars	Mango	0.566		Attri <i>et al.</i> (1999)
		Mango	54.38	High	Karibasappa (1995)
10.	Ascorbic acid	Mango	1.74		Attri <i>et al.</i> (1999)
		Acid	11.69	Moderate	Ranpise and Desai (1994)
		Lime			
		Mango	79.02	High	Karibasappa (1995)
11.	Sugar:acid ratio	Mango	48.91	High	Karibasappa (1995)
12.	Skin colour	Mango	29.01	High	Karibasappa (1995)
13.	Pulp colour	Mango	19.73	Moderate	Karibasappa (1995)

2.7.3 Heritability

Heritability for different traits reported in various fruit crops is presented here under.

Heritability (Broad sense) in various fruit crops

S. No	Character	Crop	Heritability	Range	Reference
1	2	3	4	5	6
1.	Fruit length	Litchi	68.82	High	Ghanta <i>et al.</i> (1994)
		Mango	96.80	High	Attri <i>et al.</i> (1999)
		Banana	8.22	Low	Rosamma and Namboodiri (1990)
			43.00	Moderate	Sirisena and Senanayake (2000)
		Mango	98.40	High	Karibasappa (1995)
2.	Fruit width	Litchi	69.89	High	Ghanta <i>et al.</i> (1994)
		Mango	98.30	High	Attri <i>et al.</i> (1999)
		Banana	75.72	High	Rosamma and Namboodiri (1990)
			46.00	Moderate	Sirisena and Senanayake (2000)
		Mango	98.70	High	Karibasappa (1995)
3.	Fruit weight	Litchi	97.54	High	Ghanta <i>et al.</i> (1994)
		Mango	99.50	High	Attri <i>et al.</i> (1999)
1	2	3	4	5	6
		Acid lime	88.90	High	Ranpise and Desai (1994)

		Banana	86.58	High	Rosamma and Namboodiri (1990)
			13.00	Low	Sirisena and Senanayake (2000)
4.	Fruit yield/tree	Banana	87.14	High	Rosamma and Namboodiri (1990)
			79.50	High	Rekha and Prasad (1993)
			80.00	High	Uma <i>et al.</i> (2000)
			24.00	Low	Sirisena and Senanayake (2000)
		Acid lime	87.80	High	Ranpise and Desai (1994)
5.	Seed weight	Mango	98.20	High	Karibasappa, (1995)
		Litchi	91.40	High	Ghanta <i>et al.</i> (1994)
		Mango	86.10	High	Karibasappa (1995)
6.	Titration acidity	Mango	95.20	High	Attri <i>et al.</i> (1999)
		Banana	54.20	Moderate	Rekha and Prasad (1993)
		Acid Lime	61.00	High	Ranpise and Desai (1994)
7.	TSS	Mango	91.80	High	Karibasappa, (1995)
		Mango	95.20	High	Attri <i>et al.</i> (1999)
		Banana	73.30	High	Rekha and Prasad (1993)
		Acid Lime	78.00	High	Ranpise and Desai (1994)
8.	Reducing sugars	Mango	83.20	High	Karibasappa (1995)
		Mango	93.90	High	Attri <i>et al.</i> (1999)
9.	Total sugars	Mango	89.30	High	Karibasappa (1995)
		Mango	99.40	High	Attri <i>et al.</i> (1999)
		Mango	92.80	High	Karibasappa (1995)
10.	Ascorbic acid	Mango	99.90	High	Attri <i>et al.</i> (1999)
		Acid Lime	66.00	High	Ranpise and Desai (1994)
		Mango	91.80	High	Karibasappa (1995)
11.	Sugar:acid ratio	Mango	73.80	High	Karibasappa (1995)
12.	Skin colour	Mango	99.30	High	Karibasappa (1995)
13.	Pulp colour	Mango	93.40	High	Karibasappa (1995)

2.7.4 Genetic advance

The literature available on genetic advance in various fruit crops is furnished below.

Genetic advance in various fruit crops

S. No	Character	Crop	Genetic Advance	Range	Reference
1	2	3	4	5	6
1.	Fruit length	Mango	5.63	Low	Attri <i>et al.</i> (1999)
		Banana	1.73		Rosamma and Namboodiri (1990)
			8.00	Low	Sirisena and Senanayake (2000)
2.	Fruit width	Mango	4.21	Low	Karibasappa (1995)
		Mango	2.79	Low	Attri <i>et al.</i> (1999)
		Banana	2.52	Low	Rosamma and Namboodiri (1990)
			5.80	Low	Sirisena and Senanayake (2000)
		Mango	2.71	Low	Karibasappa (1995)
3.	Fruit weight	Mango	266.23	High	Attri <i>et al.</i> (1999)
		Acid lime	8.82	Low	Ranpise and Desai (1994)
		Banana	72.50	High	Rosamma and Namboodiri (1990)
			7.20	Low	Sirisena and Senanayake (2000)
4.	Fruit yield/tree	Banana	8.76	Low	Rosamma and Namboodiri (1990)
			107.94	High	Uma <i>et al.</i> (2000)
			14.50	Moderate	Sirisena and Senanayake (2000)
		Acid lime	2.13	Low	Ranpise and Desai (1994)
		Mango	304.04	High	Karibasappa, (1995)
5.	Stone weight	Mango	20.84	High	Karibasappa (1995)
6.	Titration acidity	Mango	0.454	Low	Attri <i>et al.</i> (1999)

1	2	3	4	5	6
		Acid	0.66	Low	Ranpise and Desai (1994)
		Lime			
		Mango	0.69	Low	Karibasappa, (1995)
7.	TSS	Mango	4.40	Low	Attri <i>et al.</i> (1999)
		Acid	0.78	Low	Ranpise and Desai (1994)
		Lime			
		Mango	6.35	Low	Karibasappa (1995)
8.	Reducing sugars	Mango	1.988	Low	Attri <i>et al.</i> (1999)
		Mango	9.78	Low	Karibasappa (1995)
9.	Total sugars	Mango	4.66	Low	Attri <i>et al.</i> (1999)
		Mango	25.78	High	Karibasappa (1995)
10.	Ascorbic acid	Mango	77.36	High	Attri <i>et al.</i> (1999)
		Acid	3.77	Low	Ranpise and Desai (1994)
		Lime			
		Mango	14.75	Moderate	Karibasappa (1995)
11.	Sugar:acid ratio	Mango	57.74	High	Karibasappa (1995)
12.	Skin colour	Mango	2.53	Low	Karibasappa (1995)
13.	Pulp colour	Mango	0.88	Low	Karibasappa (1995)

2.7.5 Genetic advance as per cent mean

The literature available on genetic advance as per cent mean, in various fruit crops is furnished below.

Genetic advance as per cent mean in various fruit crops

S. No	Character	Crop	Genetic advance as per cent Mean	Range	Reference
1	2	3	4	5	6
1.	Fruit length	Litchi	15.58	Moderate	Ghanta <i>et al.</i> (1994)
2.	Fruit width	Litchi	14.12	Moderate	Ghanta <i>et al.</i> (1994)
3.	Fruit weight	Litchi	44.04	High	Ghanta <i>et al.</i> (1994)
4.	Fruit yield/tree	Banana	75.52	High	Rekha and Prasad (1993)
		Acid lime	63.38	High	Ranpise and Desai
5.	Seed weight	Litchi	61.12	High	Ghanta <i>et al.</i> (1994)
6.	Titration acidity	Banana	23.84	High	Rekha and Prasad

7.	TSS	Banana	21.11	High	(1993) Rekha and Prasad (1993)
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Further, the GCV, PCV, heritability and genetic advance in some other fruit crops is presented hereunder.

2.7.6 Cashew (*Anacardium occidentale*)

Reddy *et al.* (2002) have studied genotypic and phenotypic variation, heritability and genetic advance for yield components in 25 cashew genotypes of India. PCV was higher than the GCV. High PCV was recorded for nut yield, kernel yield, kernel weight, 100-nut weight, fruit weight, number of nuts per panicle, per cent of flowering shoots/m² and stem girth. High GCV was recorded for nut yield, kernel yield, kernel weight, 100-nut weight, fruit weight, number of nuts per panicle, per cent flowering shoots, number of flowering shoots/m² and per cent hermaphrodite flowers. Very high heritability estimates were recorded for the number of flowering shoots/m², 100-nut weight, kernel weight, panicle length, fruit weight, per cent flowering shoots/m² and stem girth. Number of flowering shoots/m² showed high heritability with high genetic advance.

2.7.7 Guava (*Psidium guajava*)

Yadava and Saxena (2005) have studied the genetic variability in 68 accessions of guava and reported that the GCV was maximum for pulp:seed ratio, number of seeds per fruit, 100 seed weight and number of seeds per 100 g fruit. PCV ranged from 33.85 per cent for average fruit weight to 609.75 per cent for

pulp:seed ratio. Heritability in the broad sense ranged from 0.558 for seed content to 0.843 for pulp::seed ratio and genetic advance ranged from 43.76 for seed percentage to 1058.89 for pulp:seed ratio. Genetic advance as per cent of mean for pulp:seed ratio, number of seeds per fruit and 100 seed weight was higher indicating additive gene control.

2.8 PRINCIPAL COMPONENT ANALYSIS

The degree of relatedness among individuals within or between species or among genotypes within a breeding population can be approximated with Principal Component (PC) analysis. The analysis can reveal clusters of morphologically similar phenotypes when plotted against the major principal components, genotypes within a cluster are likely related (Iezzoni and Pritts, 1991). PC analysis along with calculation of Euclidian distances between cultivars is highly correlated with genetic relationships based on pedigrees. Thus it would be helpful in germplasm characterization and breeding decisions to exploit hybrid vigor or minimize inbreeding depression. The PC analysis is extended to also explain evolutionary questions, to study species overlap that is to identify specimens of hybrid origin and their putative parental species, by taxonomists.

Variables in PC analysis, which are strongly associated in the same group, may share some underlying biological relationships. These associations are often useful for generating hypotheses or for understanding the behaviour of complex traits. The response of groups of variables or complex traits to imposed treatments or evolutionary pressures can be quantified and tested using a combination of PC analysis and simple univariate statistics (Broschat, 1979).

The interpretation assigned to PC groups will depend on the particular set of traits used, the diversity of entries and the strength of association among the variables and the judgement of the researcher. The utility of PC analysis in fruit crops is illustrated in the following paragraphs.

2.8.1 Mango (*Mangifera indica*)

Eighty four cultivars of mango were studied for association among fruit characters by Suman *et al.* (1985), where in factor –I was designated as fruit size and shape factor, was the major and accounted for 58.3 per cent of total variation among the cultivars. Factor –II, represented TSS and stone thickness and accounted for 12 per cent of the total variation, though TSS and stone thickness were not related to each other directly. Thus fruit weight and shape index were important, where as pulp weight and stone weight were less important.

Further, Mitra *et al.* (2008) have studied the physico-chemical characters of some mango varieties in West Bengal. PCA revealed that 67.15 per cent of variance among the cultivars, showed that Daudia, Himsagar, Zardalu and Chandankosa were superior cultivars with respect to biochemical composition. Dilshad, Daudia and Chini Champa recorded higher fruit weights.

2.8.2 Guava (*Psidium guajava*)

Hernandez-Delagado *et al.* (2007) have characterized guava germplasm by PCA and found that it explained 30 per cent of total variation and fourteen characteristics from trees (1), leaves (2) and fruits (11) were most informative. PC

analysis has separated the germplasm into three major groups of accessions based on fruit size and weight, stem diameter and leaf size.

2.8.3 Date palm (*Phoenix dactylifera*)

Khan *et al.* (2008) have characterized date varieties using multivariate analysis and found that multivariate analysis is an important statistical tool for separation of samples on the basis of geographical origin and maturation.

2.8.4 Apple (*Malus pumila*)

One of the first reports on the use of principal component analysis (PCA) in apple crop was that of Moore (1965) in qualifying yield component interactions in apple cultivars Cox Orange Pippin and Worcester Pearmain. Three major vectors were identified which represented vegetative vigor growth, fruitfulness vector and the third of a balance between fruit buds and crop weight. With this interpretation of plant vigour, it has been now possible to apply different treatments to increase yield and to determine whether the yield increase was the result of an increase in vegetative vigour, a change in the balance of vegetative growth and fruit production, a reduction in fruit set or a combination of these factors.

Currie *et al.* (2000) have also performed quantitative evaluation of apple fruit shape by PCA and found out the variation accounted by fruit aspect (76.8%), asymmetric crown (7.8%), fruit conicity (6.0%), asymmetric sides (4.3%) and fruit squareness (2.0%).

2.8.5 Cherry (*Prunus avium*)

The process of fruit ripening in cherry was analysed using principal components by Fils-Laycaon *et al.* (1988). The data represented with 21 traits of physiological and chemical characteristics of fruit over a range of harvest dates. Principal component analysis was able to separate unripe, ripe and over ripe fruits.

2.8.6 Peach (*Prunus persica*)

In a study on the analysis of peach germplasm in Central Mexico, the grouping of peach phenotypes was made using PC analysis. Based on the first three PCs that accounted for 63 per cent of the total variability among 52 accessions with 28 variables which included morphological, phenological and adaptability traits, the analysis provided a comprehensive back ground for peach improvement with defined goals and breeding strategies (Perez *et al.*, 1993).

2.8.7 Sour Cherry (*Prunus cerasus*)

Hillig and Iezzoni (1988) have evaluated morphological traits of 16 sour cherry cultivars and of hybrid and open pollinated seedlings of germplasm collected in Eastern Europe with principal component analysis. Due to the character loadings of the first three PC's in each analysis, some of the PC's can be interpreted as representing gradations between morphologies characteristic of the two presumed progenitor species, sweet cherry and ground cherry. Genetically related cultivars and families tend to cluster, indicating that there is a significant genetic component to the underlying patterns of morphological variation. Families of cold hardy Russian cultivars generally show a greater morphological

resemblance to ground cherry than do families of less cold hardy cultivars, suggesting that selective forces may also have contributed to the patterns of morphological variation detected. PC1 characterized the length and width of leaves and petioles and less number of serrations per cm. PC2 characterized leaf thickness, petiole width:length ratio and number of swollen glands on basal leaf edge generally increase, while the petiole:leaf ratio tends to decrease. PC3 is characterized by an increase in the number of petiole glands and a decrease in both internodal length and pubescence.

2.8.8 Quince (*Cydonia oblonga*)

Silva *et al.* (2005) have characterized the quince fruit based on principal component analysis and found that difference between the pulp and peel phenolic profiles was more apparent during PCA. Two PC's accounted for 81.29% of the total variability, PC1 (74.14%) and PC2 (7.15%).

2.8.9 Walnut (*Juglans regia*)

Pandey *et al.* (2007) have characterized 103 walnut germplasm through regression and PCA. PCA has clearly characterized the germplasm into nine components based on their total variance and latent roots. The first PC has accounted for more than 55% of total variation and the kernel percentage, kernel weight, kernel oil and nut length are important with largest positive loading while the shell thickness and shell nut weight had the largest negative loading.

From the review of literature on sapota and other crops it is evident that:

- a. The varieties cannot be identified precisely only based on botanical or morphological description which leads to confusion.
- b. Through biochemical markers or isozyme analyses genetic resources can be precisely distinguished by removing confusion in nomenclature and germplasm/varieties/hybrids can be precisely identified irrespective of location and environment. However, usage of isozyme analysis seems to be almost scarce in an important fruit crop like sapota.
- c. In several fruit crops Principal component analysis proved very useful in determining genetic diversity and the varieties could be grouped into clusters based on genetic distance. The application of PC analysis in an important fruit crop like sapota seems to be scarce and there are no earlier reports.

Conclusions:

From the fore going review it is also indicative that only limited reports are available in sapota in respect of studies on utility of isozyme analysis and PC analysis and confusion still exists in classification and identification of genetic resources, and varieties and hybrids of sapota. A few attempts were made for characterization of sapota varieties but were limited to study of morphological characters. Therefore, there is a scope and need for application of biochemical markers and PC analysis in precise classification and characterization of sapota varieties. Hence, the present studies on sapota in classification and characterization of varieties or genetic resources are planned in the present context after a thorough review of literature.

CHAPTER III

MATERIALS AND METHODS

The study entitled “Morphological, biochemical and isozyme characterization in sapota (*Manilkara achras* (Mill.) Fosberg) germplasm accessions using principal component analysis” was conducted at Horticultural Research Station, Andhra Pradesh Horticultural University, Venkataramannagudem, West Godavari district of Andhra Pradesh.

The materials used and methods adopted in conducting the present investigations are detailed hereunder:

3.1 SITE AND PERIOD OF THE EXPERIMENT

The investigations were conducted during 2006-2008 in the experimental plots maintained in about eight hectares at Horticultural Research Station, Venkataramannagudem.

3.2 LOCATION

Horticultural Research Station, Venkataramannagudem is situated at 16°53'120" North latitude and 81°27'568" East longitude at an elevation of 34 m above mean sea level.

3.3 AGRO-CLIMATIC CONDITIONS

Average rainfall of the region is 970.0 mm per annum and temperatures range from 16 °C during winter to about 48 °C during summer.

3.4 SOIL TYPE

The soils are sandy to red sandy loam with 1.80 m depth. Further, the soil properties are as follows:

Organic matter content	-	0.19 % - 0.29 %
Water holding capacity	-	29 % – 48 %
pH	-	7.40 – 7.90
EC	-	0.14 – 0.33 m mhos cm ⁻¹
N ₂	-	132 – 188 kg ha ⁻¹
P ₂ O ₅	-	5 – 21 kg ha ⁻¹
K ₂ O	-	77 – 220 kg ha ⁻¹

3.5 EXPERIMENTAL CONDITIONS

The trees were planted during 1996 with a spacing of 10 m x 10 m under rainfed conditions, while drip irrigation was provided from eleventh year onwards. Fertilizers were applied at the rate of 400 g Nitrogen, 200 g Phosphorus and 450 g Potash per tree per annum. Timely intercultural and weeding was adopted along with proper plant protection measures in the experimental plots to raise healthy trees.

3.6 GERMPLASM ACCESSIONS

Grafted trees of the following genotypes of sapota were used in the present study:

1. Badami	14. Singapore
2. Brazilian Sapota	15. Tagarampudi
3. Calcutta Round	16. Ulavapadu Selection
4. Cricket Ball	17. CO – 1
5. Dwarapudi	18. CO – 2
6. Gavarayya	19. CO – 3
7. Gede Sapota	20. DHS – 1
8. Gutti	21. DHS – 2
9. Kalipatti	22. PKM -1
10. Kirthibarthi	23. PKM – 2
11. Krishna Rao	24. PKM – 3
12. Pakala Round	25. PKM – 4
13. Pala	

3.7 OBSERVATIONS RECORDED

Timely and periodical observations were recorded in the field starting from May 2006 to February 2008 for various characters. All the morphological observations were taken as per the descriptor for sapota (given in Appendix I) developed at IIHR, Bangalore with slight modifications (Singh, 1991). The observations recorded and the methodology adopted for biochemical and isozyme analysis and the units of measurement are presented hereunder:

3.7.1 Tree Morphological characters

3.7.1.1 *Growth habit of the tree*

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

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

3.7.1.2 *Shape of the tree*

The shape of the tree was recorded as per the seven point score given below:

Tree shape	Score
Irregular	- 1
Elliptical	– 2
Oblong	– 3
Semi-circular	– 4
Spherical	– 5
Broadly pyramidal	– 6
Pyramidal	– 7

3.7.1.3 *Branching pattern*

Branching pattern of the tree was recorded based on five point score

Branching pattern	Score
Irregualr	– 1

Horizontal	– 2
Verticillate	– 3
Erect	– 4
Opposite	– 5

3.7.1.4 Branch Density

It was recorded based on a three point score.

Branch Density	Score
Sparse	– 1
Medium dense	– 2
Dense	-3

3.7.1.5 Leaf length

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

3.7.1.6 Leaf width

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

3.7.1.7 Leaf area

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

3.7.1.8 Pollen size

For measuring the pollen size, ocular micrometer calibrated with the help of a stage micrometer was used under a compound microscope and the pollen size was

recorded and expressed in μm . Ten pollen grains from five random flowers were measured and the average was recorded as pollen size.

3.7.1.9 Pollen production anther⁻¹

The number of pollen grains per anther was recorded as per the procedure outlined by Oberle and Goertzen (1952) with slight modifications. Anthers from five flowers were used for estimating the number of pollen grains per anther. The anther was crushed on a slide under a microscope to release the pollen, anther tissue was removed under the microscope and a cover slip with gridlines was used to cover the pollen. The number of pollen grains was then counted and the average was recorded as pollen production per anther.

3.7.1.10 Pollen fertility

The pollen fertility was estimated by staining the pollen with 1 per cent acetocarmine and calculating the number of pollen stained pink. The ratio of number of stained pollen grains to the total number of pollen was computed and expressed as per cent. Pollen obtained from five flowers was used for the study.

3.7.1.11 Style length

Average length of five randomly selected styles from five flowers was measured and expressed in centimetres.

3.7.2 Fruit morphological characters

3.7.2.1 Fruit length

Five randomly selected fruits were measured from proximal end to distal end excluding the stalk and the average length was expressed in centimetres.

3.7.2.2 Fruit width

The fruit diameter as measured at the widest portion of the fruit for five randomly selected fruits was recorded and the average was computed and expressed in centimetres.

3.7.2.3 Fruit weight

Five randomly selected fruits were weighed and the average weight was recorded in grams.

3.7.2.4 Fruit yield tree⁻¹

All the fruits from a tree were weighed and the yield was recorded in kg tree⁻¹.

3.7.2.5 Fruit shape

Fruit shape was measured on seven point score.

Fruit shape	Score
Turbinate	– 1
Acron	– 2
Globose	– 3
Sub-globose	– 4
Elliptic	– 5
Oblong	– 6
Oval	– 7

3.7.2.6 Fruit surface texture

It was recorded on a three point score.

Fruit surface texture	Score
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Web pattern on the surface	– 1
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Scurfy/ Rough	– 2
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Smooth	– 3
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3.7.2.7 Peel colour

It was recorded on a five point score.

Peel colour	Score
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Scurfy brown	– 1
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Brown with streaks	– 2
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Greenish brown	– 3
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Brown	– 4
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Yellowish brown	- 5
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3.7.2.8 Flesh colour

It was recorded on a six point score.

Flesh colour	Score
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Reddish green	- 1
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Pale green	2
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Greenish brown	– 3
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Brown	– 4
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Yellowish brown	– 5
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Reddish brown	- 6
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3.7.298 Flesh texture

Flesh texture was recorded based on a four point score.

Flesh texture	Score
Sandy	– 1
Firm	– 2
Soft	– 3
Melting	– 4

3.7.2.10 Flesh taste

Flesh taste was recorded on a four point score.

Flesh taste	Score
Astringent	– 1
Bland	- 2
Starchy	– 3
Sweet	- 4

3.7.2.11 Fruit Dessert Quality

Fruit dessert quality was recorded based on three point score.

Fruit dessert quality	Score
Fair	-1
Good	-2
Excellent	-3

3.7.2.12 Seed number fruit⁻¹

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

3.7.2.13 Seed weight

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

3.7.2.14 Pulp:seed ratio

The fruit pulp and seed were weighed separately for five randomly selected fruits and their average weights were recorded in grams. Ratio of their average weights was recorded as pulp:seed ratio.

3.7.3 Fruit Biochemical Characters

Mature ripe fruits were selected for studying the biochemical characters of the fruits, namely, total soluble solids (TSS), titrable acidity, total sugars, reducing sugars, non-reducing sugars, ascorbic acid, total phenols and sugar:acid ratio. The observations were recorded on sample size of two fruits per replication for two years (2006-07 and 2007-08) and the mean values of the two seasons were utilized for statistical analysis and formulation of tables.

3.7.3.1 Total soluble solids (TSS)

Total soluble solids of the fruit were measured with the help of a hand refractometer after extraction of the juice from the pulp and the data was expressed in °Brix.

3.7.3.2 Sugars

The total sugars and reducing sugars were estimated adopting the procedure described by Ranganna (1986) and were expressed in percentage. Non-reducing sugars were calculated by deducting the reducing sugars from total sugars.

3.7.3.3 Titrable acidity

Titration acidity was estimated by titrating the sample against 0.1 N NaOH using phenolphthalein indicator and expressed in percentage (Ranganna, 1986).

3.7.3.4 Ascorbic acid

Ascorbic acid was estimated by titrimetric method of reduction of standard 2,6, Dichlorophenol indophenol dye solution as described by Ranganna (1986). The data was expressed in mg 100 g⁻¹.

3.7.3.5 Total phenols

The total phenols were estimated using Folin-Ciocalteu reagent as per the procedure outlined by Sadasivam and Manickam (1996). Catechol standards were prepared and the absorbance was read at 650 nm with a spectrophotometer against reagent blank and the values were expressed in mg 100 g⁻¹ fresh weight.

3.7.4 Other morphological characters

Data related to the following morphological characters was also taken for characterization of the sapota genotypes based on the descriptor for sapota. Five samples each were examined and the data was recorded.

3.7.4.1 Leaf shape

The leaf shapes are recorded based on the following categories, namely, elliptic, broadly elliptic, narrowly elliptic and sub-ovate.

3.7.4.2 Leaf margin

Leaf margins are recorded based on the categories, namely, entire, wavy and crinkled.

3.7.4.3 Shape of the leaf tip

The different shapes of the leaf tip are recorded based on the following categories given, namely, retuse, acute and sub-acuminate.

3.7.4.4 Fruit apex

Fruit apex is recorded based on the following categories, namely, obtuse and acute.

3.7.4.5 Fruit base

Fruit base is recorded based on the following categories, namely, inflated, flattened, depressed and pointed.

3.7.4.6 Seed shape

Seed shape was recorded based on the categories given in the descriptor, namely, broadly clavate with spine, broadly clavate with pointed spine, semi-ovate, semi-deltoid with spine and fusiform.

3.7.4.7 Other characters

Other characters like leaf colour, colour of shoot tip, surface of the shoot tip, inflorescence type, flower position, flower bud colour, arrangement of flowers,

number of sepals/flower, sepal colour, number of petals, petal colour and anther number/flower were recorded directly by taking five samples each.

3.7.5 Isozyme analysis

Isozyme analysis technique in the present study was adopted from Markert and Moller (1959) employing native polyacrylamide gel electrophoresis. The analysis was conducted at Horticultural Research Station, Ambajipeta, East Godavari District, Andhra Pradesh. The following steps were involved in the analysis of isozymes :

1. Tissue sampling
2. Enzyme extraction
3. Preparation of PAGE gel slab
4. Electrophoresis
5. Enzyme assay/staining for enzyme activity
6. Analysis and interpretation of enzyme activity
7. Analysis and interpretation of banding pattern (zymogram analysis)
8. Genetic analysis, gene identity, genetic distance and genetic diversity indices

The above methods were standardized to suit the samples of sapota tissues.

3.7.5.1 Tissue sampling

Preliminary investigations were done to find out suitable tissue for enzyme extraction. Fresh tender leaves, immature green leaves, mature dark green leaves and mature leaves harvested the previous day were analyzed. Enzyme concentration was much higher in mature dark green leaves and bands were more profound when these

leaves were used for extraction. Hence, mature dark green leaves from 6th to 10th position from the branch tip were utilized for the study. The leaf samples were put in a thermocol container with ice immediately after separation from the tree for extraction and analysis of leaf isozymes.

3.7.5.2 Enzyme extraction

Preliminary investigations with extraction buffers with pH 7.2 and pH 8.0 were tried and the extraction buffer with pH 7.2 was found to be suitable for enzyme extraction in sapota. Composition of the extraction buffer is as follows:

Tris HCl with pH 7.2	- 70 mM	- 2 ml
Cysteine HCl	- 10 mM	- 5 µl
EDTA	- 1mM	- 5 µl
Polyvinyl pyrrolidone (PVP)		- 50 mg

Studies on the quantity of leaf sample revealed 600 mg leaf sample to be suitable for the extraction of leaf enzymes in sapota. Hence, 600 mg leaf tissue was taken from the middle portion of the leaf and ground in chilled pestle and mortar on ice along with 2 ml of extraction buffer, 5 µl of EDTA and 50 mg of PVP. For extraction of catalase, the leaf sample, EDTA and PVP quantities were doubled (1200 mg, 10 µl and 100 mg, respectively). The grindate thus obtained, was centrifuged at 10,000 rpm for 20 minutes at 2⁰ C and the extracts were stored immediately in a deep freezer at 0⁰ C.

3.7.5.3 Preparation of gel

Isozyme analysis was carried out on native polyacrylamide gel electrophoresis in anionic system as per the procedure detailed by Laemmli (1970). The following stock solutions were prepared as detailed hereunder for preparation of the gel :

Stock solution A:	Acrylamide	– 14.5 g and
	Bis acrylamide	– 500 mg in 50 ml water

Stock Solution B:	Tri HCl with pH adjusted to 8.8
	Tris – 1.5 M - 18.17 g
	Distilled water – 100 ml

Stock solution C:	Tris HCl with pH adjusted to 6.8	
	Tris – 0.5 M	- 6.075 g
	Distilled water	- 100 ml

Separating gel (8%) was prepared by mixing the following:

Stock A	- 5.3 ml
Distilled water	– 9.4 ml
Tris HCl pH 8.8	- 5.0 ml
Ammonium persulphate	- 200 µl
(0.1 g in 1 ml distilled water made freshly)	
Temed	- 20 µl

For stacking gel (3%), the following ingredients were mixed:

Stock solution A - 1.3 ml

Distilled water - 5.5 ml

Tris HCl pH 6.8 - 1.0 ml

Ammonium per sulphate – 150 µl

(0.1 g in 1 ml distilled water made freshly)

Temed - 20 µl

The solutions were prepared and the resolving and stacking gels were poured between two glass plates and a comb was inserted for formation of wells. After the gel had set the comb was removed carefully.

3.7.5.4 Sample loading

To 90 µl of sample, 30µl of sucrose solution (600 mg 1 ml⁻¹ of extraction buffer) was mixed and about 100 µl of sample was loaded into the wells. Further, Bromophenol blue dye (100 mg in 1 ml of extraction buffer) was loaded in the first well as indicator for completion of the run.

3.7.5.5 Running of the gel

The glass plates were placed in the electrode buffer tank and the power pack was switched on. The electrode buffer consisted of the following:

Tris 0.5 M - 700 mg

Glycine - 2.3 g

Distilled water - 800 ml

pH adjusted to - 8.2

The gels were run at 20 mA and 100 V until the bromophenol dye front reached the cathodal end.

3.7.5.6 Gel staining

After the run was complete, the gel was removed and washed with distilled water and stained. The staining procedures followed for different enzymes are as follows :

3.7.5.6.1 Esterase:

For preparing 0.1 M phosphate buffer at pH 7.0, 1.731 g of di-sodium hydrogen phosphate and 1.224 g of sodium di-hydrogen phosphate were dissolved in 200 ml of distilled water and the pH was adjusted to 7.0. Forty milligrams of Fast blue RR salt was mixed to 50 ml of the above phosphate buffer solution. Similarly, 20 mg of Naphthyl acetate was dissolved in 1 ml acetone and mixed to another 50 ml of the above prepared phosphate buffer solution. Later, both the solutions were mixed and the gel was incubated for 1 hour in dark for staining (Shaw and Prasad, 1970).

3.7.5.6.2 Peroxidase:

- a) 200 mg of benzidine was dissolved in 9 ml of acetic acid
- b) 5 ml of hydrogen peroxide was added to 95 ml of water.

The solutions (a) and (b) were mixed and the gel was incubated in the solution for development of blue coloured bands in 5 minutes (Sadasivam and Manickam, 1996).

3.7.5.6.3 Polyphenol oxidase:

0.1 M phosphate buffer of pH 7.0 was prepared by dissolving 1.731 g of disodium hydrogen phosphate and 1.224 g of sodium di-hydrogen phosphate in 200 ml distilled water and the pH was adjusted to 7.0.

- a) 100 mg of dinitro phenyl amine was dissolved in 100 ml of the above phosphate buffer solution and was filtered. The gel was soaked in the resultant solution for 30 minutes.
- b) 100 mg catechol was dissolved in another 100 ml of the above prepared phosphate buffer.

The gel was taken out from solution (a) after 30 minutes and soaked in solution (b) for development of yellow coloured bands within three hours (Sadasivam and Manickam, 1996).

3.7.5.6.4 Catalase:

- a) The gel was incubated in 0.03% H₂O₂ for 10 min. and washed briefly with distilled water.
- b) The gel was then incubated in a solution containing 1% Ferric chloride and 1% Potassium ferricyanide for 10 minutes. The gel staining was negative staining type (Sadasivam and Manickam, 1996).

3.7.5.7 Analysis and interpretation of isozyme banding patterns

The banding patterns or the zymograms, so obtained were analysed based on the procedures given by Wendel and Weeden (1989), Kephart (1990), Degani *et al.*, (1990). The measurement of band positions was made as below:

$$\text{Rf value} = \frac{\text{Distance traveled by the isozyme band from the origin}}{\text{Distance traveled by the buffer front from the origin}}$$

The relative mobility (Rm) was worked out according to Degani *et al.*, (1990) as:

$$\text{Rm} = \text{Rf} \times 100$$

The band position for each isozyme and for each genotype was presented in the form of figures and supporting photographs.

3.8 STATISTICAL ANALYSIS

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. Principal component analysis was performed on the data obtained as detailed hereunder:

3.8.1 Analysis of variance

Analysis of variance for each character was carried out by the method suggested by Panse and Sukhatme (1985).

3.8.2 Genotypic and phenotypic variances

The genotypic and phenotypic variances were computed based on the expected mean sum of squares as follows:

$$\sigma^2_g = \frac{M_1 - M_2}{r}$$

$$\sigma^2 p = \sigma^2 g - \sigma^2 e \quad \text{or} \quad \frac{M_2 - M_3}{r} + M_3$$

Where,

$\sigma^2 g$ = Genotypic variance (GV)

$\sigma^2 p$ = Phenotypic variance (PV)

$\sigma^2 e$ = Environmental variance (EV)

M_2 = Treatment mean sum of squares

M_3 = Error mean sum of squares

3.8.3 Coefficient of variation

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

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

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

Where,

GCV = Genotypic coefficient of variation

PCV = Phenotypic coefficient of variation

GV = Genotypic variance

PV = Phenotypic variance

—

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

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

0 – 10%	:	Low
10 – 20%	:	Moderate
20% and above	:	High

3.8.4 Heritability

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

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

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

Less than 30%	:	Low
30 – 60%	:	Moderate
More than 60%	:	High

3.8.5 Genetic advance as per cent mean (GAM):

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

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

Where,

$$GA = \text{Genetic advance} = k \times \sigma^2_p \times h^2$$

K : Selection differential which is equal to 2.06 at 5% intensity of selection (Lush, 1940)

σ^2_p : Phenotypic standard deviation

h^2 : Estimated heritability, and

$\bar{X}$: General mean

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

Low	:	Less than 10%
Moderate	:	10 – 20%
High	:	More than 20%

3.8.6 Principal Component (PC) Analysis

Principal component analyses were carried out separately for tree morphological, fruit and biochemical, and leaf isozyme banding patterns separately, according to the procedure described by Banfield (1978) using WINDOWSTAT software package Version 8.1. For PC analysis of leaf isozymes, the average of band centre computed for the different bands was utilized. Further, the PCA was performed on correlation matrix of traits, thereby removing the effects of scale (Jackson, 1991).

The main reasons for the use of the correlation matrix are :

1. The presence of original variables in different units.
2. Existence of differences in the variances, in case of original variables having the same units.

3.8.6.1 Eigen values (Latrent root) and eigen vector (Loading)

The eigen vectors are normally used to aid in the interpretation of a multivariate analysis. The proportion of variation accounted for each principal component (PC) is expressed as the eigen value divided by the sum of the eigen values.

$$\text{Per cent variance explained for PC I} = \frac{\text{Eigen value (PC I)}}{\text{Sum of eigen values}}$$

The eigen vector (loading) defines the correlation of each variable with the principal components. The principal components were identified by the following procedure.

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

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

Where,

A_{ij} are found such that Y_j is uncorrelated

$Y_1, Y_2, \dots, Y_{j-1}$ the j^{th} largest variance

The A_{ij} are the elements of ghe 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) = $\lambda_1 + \lambda_2 + \dots + \lambda_p$

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

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

Where S_i is the standard deviation of X_i

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

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

Where, a, b, ... are coefficients and $x_1, x_2, \dots$ are the variables in such a way that the principal component has a unit variance as reported by Ehrenberg (1985). The PCA scores for each genotype under concerned PC's were computed and utilized to drive a 2D or 3D (dimensional) scatter plot of individuals.

3.8.6.2 Grouping of genotypes into various clusters

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

Obtaining data matrix :

PCA scores for 25 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 characters on the basis of respective variation.

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 25 genotypes were taken in data matrix therefore resemblance coefficient was computed.

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

$$\text{Squared Euclidian distance } [d_{ij}] = \sum_{k=1}^P (X_{ik} - X_{jk})^2$$

Where,

P = number of genotypes i.e., 25

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

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

Execution of the clustering method:

Distance matrix was converted into dendrogram by using Ward's minimum variance 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 sum of squares within the cluster was minimized over all partitions obtained by combining two clusters from previous stage.

CHAPTER IV

RESULTS

Data collected in respect of 25 genotypes of sapota (*Manilkara achras* (Mill.) Fosberg) being maintained at Horticultural Research Station, Venkataramannagudem, (West Godavari District, Andhra Pradesh) for various tree morphological characters, fruit and biochemical characters and leaf isozyme banding pattern were subjected to statistical analysis and the results obtained are presented under the following heads:

- 4.1 Tree morphological characters
- 4.2 Fruit morphological and biochemical characters
- 4.3 Other morphological characters
- 4.4 Isozyme analysis
- 4.5 Genetic variability studies
- 4.6 Principal component analysis

4.1 TREE MORPHOLOGICAL CHARACTERS

The results pertaining to the study of tree morphological characters, namely, growth habit of the tree, shape of the tree, branching pattern, branch density, leaf length, leaf width, leaf area, pollen size, pollen production per anther, pollen fertility and style length are presented in Table 1, 2 and 3 and in Plate 1. Analysis of variance for the different tree morphological characters studied revealed the existence of significant differences among the genotypes for all tree morphological characters, except pollen size.

4.1.1 Growth habit of the tree

The differences in growth habit score points were significant among the genotypes and the growth habit (Table 1) of the sapota genotypes studied varied from spreading (2.00) to erect (4.00). Erect growth habit was recorded for the genotype CO – 1, while Badami, Brazilian Sapota, Calcutta Round, Cricket Ball, Dwarapudi, Gavarayya, Gutti, Krishna Rao, Pakala Round, Tagarampudi, CO – 3, DHS – 1, DHS – 2, PKM – 2, PKM – 3 and PKM - 4 recorded semi-erect (3.00) type of growth. However, spreading growth habit (2.00) was noticed for Gede Sapota, Kalipatti, Kirthibarthi, Pala, Singapore, Ulavapadu Selection, CO – 2 and PKM – 1.

4.1.2 Shape of the tree

The genotypes differed significantly in the score points given for tree shape and the genotypes under study recorded irregular (1.00), elliptical (2.00), semi-circular (4.00), spherical (5.00) and broadly pyramidal (6.00) tree shapes (Table 1). Majority of the genotypes recorded broadly pyramidal tree shape (Badami, Calcutta Round, Cricket Ball, Kalipatti, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Singapore, Tagarampudi, PKM – 1, PKM – 2, PKM – 3 and PKM – 4), while the genotypes, Brazilian Sapota, Gavarayya, Gutti and DHS – 2 recorded spherical shape of the tree. Further, the genotype DHS – 1 recorded semi-circular shape and the genotypes, namely, Dwarapudi, Gede Sapota, Ulavapadu Selection and CO – 1 recorded elliptical shape, while CO – 2 and CO – 3 recorded irregular shape of the tree.

4.1.3 Branching pattern

There were significant differences in the branching pattern in genotypes and the branching pattern among the genotypes (Table 1) ranged from irregular (1.00) to verticillate (3.00). The branching pattern in Gavarayya was found to be irregular (1.00), while Cricket Ball, Kalipatti, Kirthibarthi, Pala, Singapore and Tagarampudi genotypes recorded horizontal branching pattern. The genotypes, namely, Badami, Brazilian Sapota, Calcutta Round, Dwarapudi, Gede Sapota, Gutti, Krishna Rao, Pakala Round, Ulavapadu Selection, CO – 1, CO - 2, CO – 3, DHS – 1, DHS – 2, PKM – 1, PKM – 2, PKM – 3 and PKM – 4, however recorded verticillate branching pattern.

4.1.4 Branch density

The differences in branch density were significant among the genotypes studied and the branch density (Table 1) for the sapota genotypes studied ranged from sparse (1.00), medium dense (2.00) to dense (3.00). The genotypes, namely Badami, Dwarapudi, Gutti, Kalipatti, Singapore, Tagarampudi, DHS – 2, PKM – 1 and PKM – 2 had recorded dense branching, while Brazilian Sapota, Calcutta Round, Cricket Ball, Gavarayya, Gede Sapota, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Ulavapadu Selection, DHS – 1, PKM – 3 and PKM - 4 genotypes recorded medium dense branching. However, in CO – 1, CO – 2 and CO – 3 genotypes the branching was sparse.

4.1.5 Leaf length

The genotypes differed significantly in leaf length and the leaf length (Table 2 and Plate 1) ranged from a minimum of 7.67 cm to a maximum of

12.99 cm for the various sapota genotypes studied. The mean leaf length for the genotypes studied was 9.52 cm. Significantly highest leaf length of 12.99 cm was recorded in Gede Sapota followed by Cricket Ball (10.98 cm) and CO – 2 (10.63 cm), while lowest leaf length of 7.67 cm was recorded in Pala followed by Kalipatti (8.46 cm), PKM – 1 (8.54 cm), PKM – 2 (8.61 cm) and Kirthibarthi (8.68 cm). The other genotypes fell in intermediary range.

4.1.6 Leaf width

Significant differences were revealed in genotypes and the average leaf width was observed to be 3.43 cm for the sapota genotypes studied, while the leaf width ranged from a minimum of 2.53 cm to a maximum of 5.00 cm. Significantly highest leaf width of 5.00 cm was recorded in Gede Sapota (Table 2 and Plate 1) followed by PKM – 3 (4.23 cm) and CO – 3 (4.09 cm), while lowest leaf width of 2.53 cm was recorded in DHS – 1 genotype followed by Ulavapadu Selection (2.66 cm), Kalipatti (2.82 cm) and Badami (2.89 cm) and the other genotypes fell in intermediary range.

4.1.7 Leaf area

The differences in leaf area were significant among the genotypes studied and the leaf area (Table 2 and Plate 1) ranged from a minimum of 14.54 sq. cm to a maximum of 35.98 sq. cm among the sapota genotypes under study, while the mean leaf area recorded was 21.06 sq. cm. Leaf area was found to be significantly highest in Gede Sapota (35.98 sq.cm) followed by PKM – 3 (32.12 sq.cm), CO – 3 (29.37 sq. cm), Cricket Ball (29.10 sq. cm) and PKM – 4 (26.43 sq. cm)

and lowest values were found in DHS - 1 (14.54 sq.cm) followed by Kalipatti (14.72 sq. cm), PKM – 1 (15.10 sq. cm), Ulavapadu Selection (15.35 sq. cm), Kirthibarthi (15.93 sq. cm) and Pala (16.43 sq. cm), while the other genotypes fell in intermediary range.

4.1.8 Pollen size

The differences in pollen size in various genotypes were found to be non-significant. The average pollen size recorded among the sapota genotypes studied was 16.03 μm , while the pollen size ranged from a minimum of 15.33 μm to maximum of 16.86 μm . However, pollen size (Table 3) was maximum in genotypes, namely, Krishna Rao (16.86 μm) followed by Gede Sapota (16.76 μm) and Tagarampudi (16.68 μm). Further, the smallest pollen was observed in PKM – 2 (15.33 μm).

4.1.9 Pollen Production

The differences in pollen production per anther were significant among the genotypes and the pollen production ranged from 494.11 to 902.84, while the average pollen produced (Table 3) was 681.59. Maximum pollen production was noticed in Gutti (902.84) followed by DHS – 1 (795.14), CO – 2 (781.91) and PKM – 1 (768.22), while minimum pollen production was observed in Brazilian Sapota (494.11) followed by Krishna Rao (544.72), Dwarapudi (614.92), PKM – 2 (617.08), Gede Sapota (617.10) and Kalipatti (643.62), while the other genotypes fell in intermediary range.

4.1.10 Pollen fertility

Significant differences were observed among the various genotypes in respect of pollen fertility and the pollen fertility among the sapota genotypes ranged from 96.21 per cent to 98.86 per cent, while average pollen fertility (Table 3) was observed to be 97.38 %. Further, pollen fertility was maximum in Kalipatti (98.86 %) followed by Pala (98.44 %), Cricket Ball (98.41 %) and PKM - 3 (98.17 %) while it was minimum in Dwarapudi (96.21 %) followed by CO – 1 (96.58 %), Gede Sapota (96.61 %), CO – 2 (96.65 %), Tagarampudi (96.72 %), Krishna Rao (96.79 %) and Badami (96.80 %).

4.1.11 Style length

The differences in style length among the various genotypes were found to be significant. The mean style length (Table 1) was 0.74 cm, while it ranged from a minimum of 0.51 cm to a maximum of 0.90 cm. Further, maximum style length was recorded for Dwarapudi, Tagarampudi and DHS – 1 (0.90 cm) followed by Badami (0.89 cm), Gede Sapote (0.83 cm) and PKM – 3 (0.82 cm). The shortest style was however, recorded for Singapore (0.51 cm) genotype followed by Krishna Rao (0.57 cm), Brazilian sapota (0.60 cm), CO – 1 (0.68 cm) and Pala, Ulavapadu Selection and DHS – 2 (0.69 cm).

4.2 FRUIT MORPHOLOGICAL AND BIOCHEMICAL CHARACTERS

The results of the study on fruit morphological and biochemical characters, namely, fruit length, fruit width, fruit weight, fruit yield per tree, fruit shape, fruit surface texture, peel colour, flesh colour, flesh texture, flesh taste, fruit dessert

quality, seed number per fruit, seed weight, pulp:seed ratio, TSS, reducing sugars, non-reducing sugars, total sugars, titrable acidity, sugar:acid ratio and total phenols are presented in Table 4, 5 and 6 and Plate 2. Analysis of variance for the different fruit morphological and biochemical characters studied revealed the existence of significant differences among the genotypes for all the characters studied.

4.2.1 Fruit length

Significant differences were observed among the genotypes in respect of fruit length and the genotypes studied recorded mean fruit length of 5.24 cm (Table 4 and Plate 2), while the fruit length ranged from a minimum of 3.71 cm to maximum of 7.75 cm. Fruit length was maximum in the genotype PKM – 4 (7.75 cm) followed by CO – 1 (7.28 cm), CO – 2 (6.87 cm), CO – 3 (6.85 cm) and Cricket Ball (6.48 cm), while it was minimum in genotype Ulavapadu Selection (3.71 cm) followed by Tagarampudi (3.99 cm), Pakala Round (4.21 cm), Pala (4.48 cm) and DHS – 2 (4.51 cm). Further, fruit length for Gede Sapota (3.82 cm) was noticed to be on par with Ulavapadu Selection.

4.2.2 Fruit width

The fruit width differed significantly among the genotypes studied, while the fruit width in the present study ranged from 2.37 cm to 6.79 cm (Table 4 and Plate 2), the mean fruit width recorded was 4.69 cm. In the present study, significantly maximum fruit width was recorded in Cricket Ball (6.79 cm) followed by CO – 2 (5.89 cm), CO – 3 (5.82 cm), Krishna Rao (5.80 cm), CO – 1

(5.77 cm) and DHS – 2 (5.14 cm), while minimum fruit width was recorded in Ulavapadu Selection (2.37 cm) followed by Gede Sapota (3.35 cm), Badami (3.71 cm), Kalipatti and Kirthibarthi (4.10 cm) and Pala and PKM – 4 (4.17 cm).

4.2.3 Fruit weight

The differences in genotypes in respect of fruit weight were found to be significant. The mean fruit weight recorded in the present study was 96.63 g (Table 4 and Plate 2), while the fruit weight ranged from a minimum of 44.83 g to a maximum of 198.74 g. The genotype Cricket Ball recorded the highest fruit weight (198.74 g) followed by CO – 1 (170.67 g), CO – 2 (150.65 g), CO – 3 (127.229 g) and PKM – 4 (127.04 g), while the lowest figures of 44.83 g was recorded in the genotype Ulavapadu Selection followed by Gede Sapota (63.13 g), Tagarampudi (63.27 g), Pala (63.38 g), Badami (64.35 g), Kalipatti (64.42 g) and Krishna Rao (67.74 g). The rest of the genotypes, namely, Brazilian Sapota, Calcutta Round, Dwarapudi, Gavarayya, Gutti, Krishna Rao, Pakala Round, Singapore, DHS – 1, DHS – 2, PKM – 1, PKM – 2 and PKM - 3 fell in intermediary range

4.2.4 Fruit yield per tree

The differences in fruit yield/tree in respect of the genotypes studied were significant. The fruit yield per tree ranged from 26.20 kg to 93.35 kg (Table 4) among the genotypes studied, while the mean fruit yield per tree recorded was 57.66 kg. It was found that highest fruit yield was recorded in the genotype PKM – 3 (93.35 kg) which was on par with PKM – 1 (89.95 kg) followed by

Kirthibarthi (86.45 kg), Singapore (85.35 kg), Kalipatti (83.40 kg) and DHS – 1 (81.55 kg), while lowest fruit yield was recorded in the genotype CO – 2 (26.20 kg) followed by Krishna Rao (31.45 kg), Gede Sapota (31.70 kg), Badami (32.35 kg), CO – 3 (32.95 kg) and Gutti (36.15 kg). The genotype CO – 1 (26.40 kg) was on par with CO – 2, while the other genotypes fell in intermediary range.

4.2.5 Seed number/fruit

The results presented in Table 4 reveal the average seed number per fruit to be 4.24, while it ranged from a minimum of 2 to a maximum of 8. Maximum seed number per fruit was recorded in Brazilian Sapota and Cricket Ball (8) followed by Dwarapudi (7), Singapore (6); and Calcutta Round, Krishna Rao, Tagarampudi and PKM – 2 (5), while lowest seed number was recorded in the genotype Ulavapadu Selection (2) followed by Badami, Gavarayya, Gede Sapota, Gutti, Kalipatti, Pala, CO – 2, DHS – 1 and DHS – 2 (3).

4.2.6 Seed weight

The seed weight revealed significant differences among the genotypes. The seed weight of the sapota genotypes studied in the present investigation ranged from minimum of 1.48 g to a maximum of 7.78 g (Table 4), while the mean seed weight was 3.13 g. Further, maximum seed weight was observed for Brazilian Sapota (7.78 g) followed by Cricket Ball (6.27 g), Dwarapudi (4.55 g) and Tagarampudi (4.23g) while lowest seed weight was recorded in CO – 2 (1.48 g). The genotypes Kirthibarthi (1.57 g) and Ulavapadu Selection (1.59 g) also

recorded seed weight on par with the genotype CO – 2 followed by Pala (1.64 g), Pakala Round (1.68 g) and Gutti (2.19 g).

4.2.7 Pulp:seed ratio

Significant differences were revealed in pulp:seed ratio and the results presented in Table 4 reveal that the mean pulp:seed ratio to be 34.28, while the range of pulp:seed ratio was from minimum of 11.34 to maximum of 100.80 among the sapota genotypes studied. Significantly maximum pulp:seed ratio was recorded in CO – 2 (100.80) followed by CO – 1 (56.11), Kirthibarthi (53.49), Pakala Round (45.29) and Gutti (45.25), while lowest pulp:seed ratio was recorded for Brazilian Sapota (11.34) followed by Tagarampudi (14.66), Dwarapudi (18.02) and Calcutta Round (20.50).

4.2.8 Fruit shape

There were significant differences among the sapota genotypes in fruit shape and fruit shapes (Table 5 and Plate 2) ranged from turbinate to oval. In the genotypes, CO – 1 and CO – 2, the fruit shape was turbinate (1.00), while in Dwarapudi, Kalipatti, Krishna Rao, Singapore, CO – 3, DHS – 1 and PKM – 2 it was acron (2.00). Further, Badami, Calcutta Round, Cricket Ball, Gede Sapota, Kirthibarthi, 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 and Gutti it was oblong (6.00). However, the genotypes Brazilian Sapota, Pala, Ulavapadu Selection and PKM – 3 recorded oval (7.00) fruit shape.

4.2.9 Fruit surface texture

Fruit surface texture showed significant differences and the fruit surface texture of sapota genotypes studied in the present investigation (Table 5) was observed to be rough (2.00) in Badami, Brazilian Sapota, Calcutta Round, Gavarayya, Krishna Rao, Pakala Round, Pala, Tagarampudi, CO – 2, DHS – 1 and DHS - 2, while it was smooth (3.00) in Cricket Ball, Dwarapudi, Gede Sapota, Gutti, Kalipatti, Kirthibarthi, Singapore, Ulavapadu Selection, CO – 1, CO – 3, PKM – 1, PKM - 2, PKM – 3 and PKM – 4.

4.2.10 Peel Colour

There were significant differences in the peel colour and the peel colour (Table 5) of sapota genotypes studied was observed to be greenish brown (3.00) in Brazilian Sapota, while the peel colour was brown (4.00) in Badami, Calcutta Round, Dwarapudi, Gavarayya, Gede Sapota, Gutti, Krishna Rao, Pakala Round, Pala, Singapore, Tagarampudi, Ulavapadu Selection, CO – 2, CO – 3, DHS - 1, DHS – 2, PKM – 1, PKM – 2, PKM – 3 and PKM – 4 genotypes. Further, the genotypes Cricket Ball, Kalipatti, Kirthibarthi and CO – 1 recorded yellowish brown (5.00) peel colour.

4.2.11 Flesh colour

Flesh colour differed significantly among the sapota genotypes studied. It varied from yellowish brown to reddish brown (Table 5), while it was greenish brown (3.00) in Brazilian Sapota; brown (4.00) in Badami, Dwarapudi, Gavarayya, Gede Sapota, Gutti, Kalipatti, Kirthibarthi, Pakala Round, Pala,

Singapore, CO – 3, DHS – 1, PKM – 1, PKM – 2 and PKM – 4; yellowish brown (5.00) in Cricket Ball, Ulavapadu Selection, CO – 1, CO - 2 and PKM -3; and it was reddish brown (6.00) in Calcutta Round, Krishna Rao, Tagarampudi and DHS – 2.

4.2.12 Flesh texture

Significant differences were noticed in flesh texture and the flesh texture (Table 5) ranged from sandy to soft for the genotypes under study. Brazilian Sapota, Gede Sapota, Singapore, CO – 1, CO – 2, CO – 3 and PKM – 4 possessed sandy (1.00) flesh texture, while flesh texture of the genotypes Calcutta Round, Dwarapudi, Gavarayya, Kalipatti, Krishna Rao, Tagarampudi, DHS - 2, PKM – 2 and PKM – 3 was firm (2.00). Further, the genotypes, Badami, Cricket Ball, Gutti, Kirthibarthi, Pakala Round, Pala, Ulavapadu Selection, DHS – 1 and PKM – 1 were observed to possess soft flesh texture (3.00).

4.2.13 Flesh taste

Flesh taste differed significantly among the sapota genotypes and it ranged from starchy to sweet (Table 5). The flesh of the genotypes, Brazilian Sapota, Calcutta Round, Gavarayya, Gutti and Tagarampudi was starchy (3.00), while the genotypes Badami, Cricket Ball, Dwarapudi, Gede Sapota, Kalipatti, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Singapore, Ulavapadu Selection, CO – 1, CO – 2, CO – 3, DHS – 1, DHS – 2, PKM – 1, PKM – 2, PKM – 3 and PKM – 4 recorded sweet (4.00) flesh.

4.2.14 Fruit dessert quality

There were significant differences in the fruit dessert quality of sapota genotypes which ranged from fair (1.00) to excellent (3.00) (Table 5). The genotype Pala (2.80) possessed excellent fruit dessert quality. Further, the genotypes, Calcutta Round, Cricket Ball, Gavarayya, Kalipatti, Kirthibarthi, Pakala Round, Singapore, Tagarampudi, Ulavapadu Selection, CO – 1, CO – 2, CO – 3, DHS – 1, DHS – 2, PKM – 3 and PKM – 4 possessed good fruit dessert quality (1.80 to 2.40), while the genotypes, Badami, Brazilian Sapota, Dwarapudi, Gede Sapota, Gutti, Krishna Rao, PKM - 1 and PKM – 2 recorded fair fruit dessert quality (1.00 to 1.60).

4.2.15 Total Soluble Solids

The TSS content in fruits of sapota genotypes differed significantly. The results presented in Table 6 reveal that the total soluble solids (TSS) ranged from 20.20 ° brix to 24.30 ° brix among the sapota genotypes studied, while the average TSS recorded was 22.37 ° brix in the genotypes. TSS was observed to be the highest with the genotype, Pala (24.30 ° brix), while the genotypes, namely, PKM – 1 (24.10 ° brix), Kalipatti (23.90 ° brix), PKM – 3 (23.70 ° brix), PKM – 4 (23.50 ° brix), Ulavapadu Selection (23.40 ° brix), Brazilian Sapota (23.10 ° brix), Gutti (23.00 ° brix), Singapore (23.00 ° brix), Calcutta Round (22.90 ° brix) and DHS – 1 (22.90 ° brix) were found to be on par with Pala genotype. Lowest TSS was recorded in the genotype Cricket Ball (20.20 ° brix). Further, the genotypes, CO – 3 (20.70 ° brix), Gede Sapota (21.10 ° brix), Badami

(21.20 ° brix), Dwarapudi and CO – 1 (21.30 ° brix), recorded total soluble solids on par with Cricket Ball.

4.2.16 Reducing sugars

Significant differences were noticed in reducing sugars content and the mean per cent of reducing sugars content for sapota genotypes studied was observed to be 7.94 per cent (Table 6), while it ranged from 5.88 per cent to 10.65 per cent. Highest amount of reducing sugars was recorded in the genotype, Kalipatti (10.65 %) which was on par with Pala (10.05 %) followed by PKM - 3 (9.21 %) and CO – 2 (8.89 %). Further, lowest amount of reducing sugars was recorded in the genotype, Dwarapudi (5.88 %). The genotypes Gede Sapota (6.29 %), CO – 3 (6.39 %), Cricket Ball (6.53 %), CO – 1 (6.88 %) and Gavarayya (6.95 %) were found to be on par with the genotype Dwarapudi.

4.2.17 Non-reducing sugars

The sapota genotypes differed significantly in non-reducing sugars content and the non-reducing sugars (Table 6) content ranged from a minimum of 1.01 per cent to maximum of 4.21 per cent, while the mean non-reducing sugar content was 1.79 per cent. The non-reducing sugars content was found to be significantly higher in the genotype Dwarapudi (4.21 %), Cricket Ball (3.27 %) and Gavarayya (2.76 %), while the genotypes Krishna Rao and PKM - 1 (1.01 %) had recorded lower amount of non-reducing sugars. The genotypes PKM – 2 (1.09 %), Kalipatti (1.13 %), Ulavapadu Selection (1.16 %) and DHS – 1 (1.17 %) were on par with Krishna Rao and PKM – 1.

4.2.18 Total sugars

The differences in the total sugars content among the sapota genotypes investigated was found to be significant and the results presented in Table 6 reveal that the mean total sugars content to be 9.72 per cent, while the range was 8.21 per cent to 11.93 per cent. Maximum total sugars content was recorded in the genotypes, Pala (11.93 %), Kalipatti (11.78 %), Singapore (10.82 %) and Kirthibarthi (10.71 %), while lowest total sugars content was recorded for the genotypes, Badami (8.21 %) followed by CO – 3 (8.38 %), Gede Sapota (8.63 %), PKM – 2 (8.78 %), Krishna Rao (8.89 %), Tagarampudi (9.00 %), and Brazilian Sapota (9.15 %). The other genotypes, namely, Calcutta Round, Cricket Ball, Dwarapudi, Gavarayya, Gutti, Pakala Round, Ulavapadu Selection, CO – 1, CO – 2, DHS – 1, DHS – 2, PKM – 1, PKM – 3, PKM - 4 were found to be intermediary.

4.2.19 Titrable acidity

The range of titrable acidity among the sapota genotypes studied was found to be 0.01 per cent to 0.07 per cent (Table 6). The results revealed highest value for titrable acidity in Gavarayya (0.07 %). The genotypes Cricket Ball, CO – 1, PKM - 1 and PKM – 2 (0.05%) were on par with Gavarayya. Lowest value for titrable acidity was recorded by the genotype DHS – 1 (0.01 %), which was observed to be on par with Badami, Brazilian Sapota, Kirthibarthi, Krishna Rao, Pala, Ulavapadu Selection and PKM - 3 (0.03%); Pakala Round (0.02%) genotypes, while Calcutta Round, Dwarapudi, Gede Sapota, Gutti, Kalipatti, Singapore, Tagarampudi, CO – 2, CO – 3, DHS – 2 and PKM – 4 recorded 0.04

per cent titrable acidity. In addition, the average titrable acidity for the genotypes studied in the present investigation was noticed to be 0.04 per cent.

4.2.20 Sugar:acid ratio

The sapota genotypes under study differed significantly in the sugar:acid ratio. The average sugar:acid ratio (Table 6) for the sapota genotypes studied in the present investigation was 304.31, while the range of sugar:acid ratio recorded was 142.01 to 582.08. Significantly highest sugar:acid ratio was noticed in DHS – 1 (582.08), Pakala Round (492.73), Kirthibarthi (469.25) and PKM – 3 (461.73), while significantly lowest sugar:acid ratio was recorded in the genotypes, namely, Gavarayya (142.01), PKM – 2 (175.1), Cricket Ball (201.59), CO – 1 (214.74), CO – 3 (216.05), Gede Sapota (221.87), PKM – 1 (227.20), PKM – 4 (245.51), Tagarampudi (246.06), Dwarapudi (256.32), DHS – 2 (257.68), Badami (275.23) and CO – 2 (276.04) genotypes which were all on par with each other. The other genotypes were intermediary.

4.2.21 Ascorbic acid

The ascorbic acid content differed significantly among the genotypes and the mean ascorbic acid (Table 6) was 8.48 mg/100g for the sapota genotypes studied in the present investigation, while the range was noticed to be 5.12 mg/100g to 11.37 mg/100g. Kalipatti (11.37 mg/100g), Singapore (11.00 mg/100g), CO – 1 (10.75 mg/100g) and PKM – 1 (10.37 mg/100 g) genotypes recorded significantly higher amount of ascorbic acid and were on par with each other, while significantly lower ascorbic acid content was recorded for Dwarapudi

(5.12 mg/100g), Cricket Ball (5.87 mg/100g), Badami and Pakala Round (6.50 mg/100g), Calcutta Round and Gede Sapota (6.75 mg/100g) which were all on par to each other.

4.2.22 Total phenols

The total phenols content differed significantly among the various sapota genotypes studied. The results presented in Table 6 revealed that the average total phenol content of the sapota genotypes to be 0.47 mg/100g, while the range among the sapota genotypes was noticed to be 0.23 to 0.78 mg/100g. Further, significantly higher phenol content was recorded for Dwarapudi (0.78 mg/100g), Badami (0.69 mg/100g), Gede Sapota (0.67 mg/100g), DHS – 2 (0.66 mg/100g) and Krishna Rao (0.64 mg/100g) genotypes which were all on par to each other, while significantly low phenol content was recorded for Pala (0.23 mg/100g), Ulavapadu Selection (0.25 mg/100g), CO – 1 (0.26 mg/100g), Cricket Ball and CO – 2 (0.33 mg/100g), Singapore (0.34 mg/100g), Gavarayya and Kalipatti (0.36 mg/100g) genotypes and were all on par to each other. The other genotypes were intermediary.

4.3 OTHER MORPHOLOGICAL CHARACTERS

In addition to the above tree and fruit characters studied, certain morphological characters were also described as per the descriptor qualitatively and considered for characterization of the 25 sapota genotypes. The results obtained on the study pertaining to other morphological characters, namely, leaf colour, leaf shape, leaf margin, shape of the leaf tip, colour of the shoot tip,

surface of shoot tip, inflorescence type, inflorescence position, flower bud colour, flowers per cluster, number of sepals, sepal colour, number of petals, petal colour, number of anthers, fruit apex, fruit base and seed shape for the 25 sapota genotypes studied in the present investigation are presented in Table 7. The results are presented hereunder.

4.3.1 Leaf shape

The genotypes fell into three types of leaf shapes (Table 7), namely, elliptic (Badami, Cricket Ball, Gede Sapota, Kalipatti, Pakala Round, Pala, Tagarampudi, Ulavapadu Selection, DHS – 1 and PKM – 4), broadly elliptic (Brazilian Sapota, Dwarapudi, Gavarayya, Gutti, Kirthibarthi, Krishna Rao, Singapore, CO – 1, CO – 2, CO – 3, DHS – 2, PKM – 1, PKM – 2 and PKM – 3) and sub-ovate (Calcutta Round).

4.3.2 Leaf margin

The sapota genotypes recorded two types of leaf margins (Table 7), namely, entire (Badami, Brazilian Sapota, Calcutta Round, Dwarapudi, Gavarayya, Kalipatti, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Singapore, Tagarampudi, DHS – 1 and PKM – 1) and wavy (Cricket Ball, Gede Sapota, Gutti, Ulavapadu Selection, CO – 1, CO – 2, CO – 3, DHS – 2, PKM – 2, PKM – 3 and PKM – 4).

4.3.3 Shape of the leaf tip

The shape of the leaf tip (Table 7) of the sapota genotypes studied was either retuse (Badami, Brazilian Sapota, Calcutta Round, Cricket Ball, Gavarayya, Gutti, Krishna Rao, CO – 3 and PKM – 3) or acute (Dwarapudi, Gede Sapota, Kalipatti, Kirthibarthi, Pala, Tagarampudi, Ulavapadu Selection, CO – 1, DHS – 1, PKM – 1 and PKM - 2) or sub-acuminate (Pakala Round, Singapore, CO – 2, DHS – 2 and PKM - 4).

4.3.4 Fruit apex

The sapota genotypes under investigation possessed obtuse (Badami, Cricket Ball, Gavarayya, Kirthibarthi, Pakala Round, DHS – 1, DHS – 2, PKM – 2, PKM – 3 and PKM - 4) and acute (Brazilian Sapota, Calcutta Round, Dwarapudi, Gede Sapota, Gutti, Kalipatti, Krishna Rao, Pala, Singapore, Tagarampudi, Ulavapadu Selection, CO – 1, CO – 2, CO – 3, PKM – 1) shape of the fruit apex (Table 7).

4.3.5 Fruit base

The fruit base of the sapota genotypes studied ranged from inflated (Dwarapudi, Singapore and PKM – 1) to flattened (Badami, Gede Sapota, Kalipatti, Ulavapadu Selection, DHS – 1, PKM – 2 and PKM - 4) and to depressed (Brazilian Sapota, Calcutta Round, Cricket Ball, Gavarayya, Gutti, Kirthibarthi, Krishna Rao, Pakala Round, Tagarampudi, CO - 1, CO – 2, CO – 3, DHS - 2 and PKM – 3) (Table 7).

4.3.6 Seed shape

The seed shape of the sapota genotypes studied showed variation and found to be broadly clavate with spine in Badami, Brazilian Sapota, Calcutta Round, Dwarapudi, Gede Sapota, Gutti, Krishna Rao, Pala, Singapore, Tagarampudi, Ulavapadu Selection, CO - 1, CO - 3, PKM - 3 and PKM – 4, semi-ovate in Pakala Round, DHS – 1, DHS - 2 and PKM – 2, semi-deltoid with spine in Cricket Ball and CO – 2 and broadly clavate with pointed spine in Gavarayya, Kalipatti, Kirthibarthi and PKM -1 (Table 7).

Certain other characters studied, namely, colour of the shoot tip (brown), surface of the shoot tip (pubescent), leaf colour (green), inflorescence type (cymose), flower position (axillary), colour of flower bud (brown), arrangement of flowers (solitary), number of sepals per flower (6), sepal colour (greenish white), number of petals per flower (6), petal colour (white) and anther number per flower (6) were found to be uniform for all the genotypes studied and considerable variation could not be noticed (Table 7).

4.4 ISOZYME ANALYSIS

Analysis of four isozymes in leaf tissues, namely esterase, peroxidase, polyphenol oxidase and catalase was carried out for characterization of sapota genotypes and the results are presented in Tables 8-11, Figs. 1-4 and Plates 3-6.

4.4.1 Esterase

A perusal of the results on the banding pattern of esterase isozyme (Table 8, Fig. 1 and Plate 3) revealed the existence of bands at Rm 37, 38 and 39

positions. However, the position and band intensity varied considerably with the genotypes studied indicating the prevalence of polymorphism of the isozyme.

At Rm value 37, high intensity bands were noticed for Gavarayya, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Ulavapadu Selection, DHS- 2, PKM – 2 and PKM – 3, while medium intensity bands were recorded for CO – 1 and CO – 2 genotypes. However, Badami, Brazilian Sapota, Calcutta Round, Cricket Ball, Dwarapudi, Gede Sapota, Gutti, Kalipatti, Singapore, Tagarampudi, CO – 3, DHS – 1, PKM – 1 and PKM – 4 did not record any band at Rm 37.

At Rm value 38, high intensity bands were recorded for Badami, Calcutta Round, Gede Sapota, Kalipatti, Singapore, CO – 3, PKM – 1 and PKM – 4, while the other genotypes studied have not shown any band at all.

At Rm 39, the genotypes, Brazilian Sapota, Cricket Ball, Dwarapudi, Gutti, Tagarampudi and DHS – 1 recorded high intensity bands, while other genotypes studied did not record any band at the Rm value. The most frequent esterase band was found to be Rm 37 (eleven genotypes).

4.4.2 Peroxidase

The results pertaining to banding pattern of the isozyme for the 25 sapota genotypes studied were presented in Table 9, Fig. 2 and Plate 4. A perusal of these results revealed presence of bands at Rm 27, 28, 29, 30, 31, 33, 37, 39, 40, 41, 42, 43, 44, 45 and 46 positions. The intensity and band position, however varied with the genotype studied. These results indicate the presence of extensive polymorphism for the isozyme thereby lending scope for identification of various genotypes of sapota.

High intensity band at Rm 27 was observed for Pala genotype alone, while Dwarapudi genotype recorded high intensity band at Rm 28. Further, the genotypes, Gede Sapota, Kalipatti, Tagarampudi and PKM – 3 recorded medium intensity bands, while CO – 1 and PKM – 4 recorded low intensity bands at Rm 28.

The genotype, Brazilian Sapota recorded high intensity band at Rm 29, while Krishna Rao and CO – 3 genotypes recorded medium density bands at Rm 29. However, low intensity bands were observed at Rm 29 for Ulavapadu Selection, CO – 2 and DHS – 2.

At Rm 30, Badami and Gutti genotypes recorded high intensity bands, while Calcutta Round and Pakala Round recorded medium density bands. Low intensity band at Rm 30 was noticed for genotype PKM – 2.

Medium intensity band for Gavarayya and Kirthibarthi; and low intensity band for Cricket Ball and PKM – 1 were recorded at Rm 31. Similarly, the genotype Singapore had recorded medium intensity band, while DHS – 1 genotype had recorded low intensity band at Rm 33 for the isozyme.

At Rm 37, Pakala Round genotype alone had recorded low intensity band, while the other genotypes studied did not record any band. Similarly, at Rm 39, CO – 1 genotype alone recorded low intensity band, while the other genotypes studied did not record any band.

At Rm 40, Cricket Ball, Krishna Rao and CO – 2 genotypes had recorded low intensity bands, while other genotypes studied did not record any band. However, medium intensity band for Dwarapudi; and low intensity bands at Rm

41 were recorded for Badami, Gede Sapota, Kirthibarthi, DHS – 1, CO – 3, DHS – 2 and PKM – 1 genotypes.

At Rm 42, low intensity bands were noticed for Gavarayya, Singapore and PKM – 2 genotypes, while Brazilian Sapota and Gutti recorded medium intensity bands at Rm 43.

The genotype, Pala recorded medium intensity band at Rm 44, while Calcutta Round, Kalipatti and PKM – 3 recorded low intensity bands at Rm 45. Similar low intensity bands were observed at Rm 46 for Pakala Round, Tagarampudi, Ulavapadu Selection and PKM – 4 genotypes.

The most frequent band was observed to be Rm 28 and Rm 29. Further, the bands obtained are unique to 15 genotypes out of the 25 genotypes tested and these results indicate that the isozyme can be extensively utilized for identification of 15 genotypes as mentioned above in the text.

4.4.3 Polyphenol oxidase

The results pertaining to band position and intensity for the isozyme are presented in Table 10, Fig. 3 and Plate5. A perusal of these results revealed bands at Rm 19, 21, 23, 24, 25, 26, 27, 28, 37, 38, 41 and 44 positions and showed extensive polymorphism. However, the band position and intensity varied with the genotype studied. The most frequent band noticed was at Rm 23.

At Rm 19, Kalipatti alone had recorded medium intensity band, while Cricket Ball and CO – 1 had recorded medium intensity bands at Rm 21. The genotypes, CO -2 and DHS – 1, however, recorded low intensity bands at Rm 21. Further, low intensity bands were recorded at Rm 23 for the genotypes, namely,

Dwarapudi, Gavarayya, Gede Sapota, Kirthibarthi, Pala, Singapore, Ulavapadu Selection, PKM – 2 and PKM – 3; at Rm 24 for Badami, Brazilian Sapota, Calcutta Round, PKM – 1 and PKM – 4; at Rm 25 for Gutti and Pakala Round; at Rm 26 for Tagarampudi; and at Rm 27 for CO – 3 genotype. Medium intensity band was also noticed at Rm 27 for genotype DHS – 2. Low intensity bands were also recorded at Rm 28 for Krishna Rao; at Rm 37 for CO – 2; at Rm 38 for PKM – 1; at Rm 41 for Kalipatti; and at Rm 44 for Kirthibarthi and PKM – 2 genotypes.

4.4.4 Catalase

The results pertaining to band position and intensity for the isozyme are presented in Table 11, Fig. 4 and Plate 6. A perusal of these results revealed bands for different sapota genotypes studied at Rm 53, 54, 55, 56, 57, 58, 60, 61, 62 and 63. The band position and intensity, however, varied with the genotype under study. The most frequent bands were recorded at Rm 54 and Rm 58 in case of catalase.

High intensity bands were recorded at Rm 53 for Pakala Round genotype and at Rm 54 for Brazilian Sapota and Ulavapadu Selection, while low intensity bands were recorded at Rm 54 for Kalipatti and Kirthibarthi genotypes.

The genotype CO – 2 had recorded low intensity band at Rm 55, while high intensity bands were recorded at the Rm value for genotypes Badami and CO – 1. Similar high intensity bands were noticed for Dwarapudi, Pala and PKM – 4 genotypes at Rm 56; Gavarayya and Tagarampudi genotypes at Rm 57; Calcutta Round, Gede Sapota, Gutti and DHS – 2 genotypes at Rm 58; CO – 3 at Rm 60;

Cricket Ball, DHS – 1 and PKM – 2 at Rm 61; and PKM – 1 at Rm 62. The genotype, Singapore, however had recorded medium intensity band at Rm 63, while bands were not observed in case of the genotypes, Krishna Rao and PKM – 3.

In this study of four isozymes, namely, esterase, peroxidase, polyphenol oxidase and catalase, peroxidase exhibited a higher polymorphism at 15 Rm values and can be considered extremely useful in identification of as many as 15 genotypes of sapota, while only few genotypes could be identified with other isozymes (seven in case of polyphenol oxidase and four in case of catalase).

4.5 GENETIC VARIABILITY STUDIES

The genetic parameters, namely, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability in broad sense and genetic advance as per cent mean were estimated for all the characters under study and the results are presented in Tables 12 and 13 and Figs. 5-12. Further, the variability studied in different characters is presented character wise under the following heads:

4.5.1 Tree morphological characters

The results obtained for 11 tree morphological characters studied for the 25 sapota genotypes are presented in Table 12 and Figs. 5 and 6.

4.5.1.1 *Growth habit of the tree*

The estimates for phenotypic coefficient of variation (20.28 %) were high, while genotypic coefficient of variation (17.89 %) was found to be moderate for

this character. However, higher values for heritability (88.21 %) and genetic advance as per cent mean (35.01 %) were recorded for the trait (Table 12).

4.5.1.2 Shape of the tree

This character registered high phenotypic coefficient of variation (37.96 %) and genotypic coefficient of variation (37.59 %). The estimates for heritability (99.03 %) and genetic advance as per cent mean (77.14 %) were also found to be high for this character (Table 12).

4.5.1.3 Branching pattern

The trait recorded (Table 12) high phenotypic coefficient of variation (20.77 %), moderate genotypic coefficient of variation (15.14 %), high heritability (72.89 %) and high genetic advance as per cent mean (27.75 %).

4.5.1.4 Branch density

All the variability parameters studied, namely, phenotypic coefficient of variation (28.18 %), genotypic coefficient of variation (25.10 %), heritability (89.07 %) and genetic advance as per cent mean (48.79 %) were found to be high for the character (Table 12).

4.5.1.5 Leaf length

The estimate for phenotypic coefficient of variation was moderate (11.52 %), while it was found to be low for genotypic coefficient of variation (7.39 %) in respect of the trait. Similarly, the estimate for heritability (64.15 %) was found to be high, while the estimates for genetic advance as per cent mean (9.98 %) was low (Table 12).

4.5.1.6 Leaf width

The estimated values (Table 12) of phenotypic coefficient of variation (15.54 %), genotypic coefficient of variation (10.41 %) and genetic advance as per cent mean (14.47 %) were moderate for this character, while the estimate for heritability was high (67.00 %).

4.5.1.7 Leaf area

The estimates for phenotypic coefficient of variation (30.99 %), genotypic coefficient of variation (20.15 %), heritability (65.02 %) and genetic advance as per cent mean (27.00 %) were all high for this character (Table 12).

4.5.1.8 Pollen size

The estimates for phenotypic coefficient of variation (3.63 %), genotypic coefficient of variation (1.52 %) and genetic advance as per cent mean (3.04 %) were low for this character, while the estimate for heritability (41.87 %) was observed to be moderate (Table 12).

4.5.1.9 Pollen production

The estimate for phenotypic coefficient of variation (11.96 %) was observed to be moderate for this trait, while genotypic coefficient of variation (7.91 %) was low. Similarly, the estimate for heritability (66.14 %) was observed to be high, while genetic advance as per cent mean (10.83 %) was moderate (Table 12).

4.5.1.10 Pollen fertility

Low estimates for phenotypic coefficient of variation (0.67 %), genotypic coefficient of variation (0.44 %) and for genetic advance as per cent mean (0.58

%) were recorded for the trait, while the estimate for heritability (65.67 %) was high (Table 12).

4.5.1.11 Style length

The character (Table 12) recorded moderate estimates for phenotypic coefficient of variation (14.91 %) and genotypic coefficient of variation (14.67 %) and high estimates for heritability (97.05 %) and genetic advance as per cent mean (29.27 %).

Genetic studies revealed that the range of phenotypic coefficient of variation was 0.67 to 37.96 among the tree morphological characters, while the range of genotypic coefficient of variation was 0.44 to 37.59. Further, the estimates for heritability ranged from 41.87 to 99.03 and genetic advance as per cent mean ranged from 0.58 to 77.14.

4.5.2 Fruit morphological and biochemical characters

The results of the variability studies conducted in respect of fruit morphological and biochemical characters are presented in Table 13 and Figs. 7 – 12.

4.5.2.1 Fruit length

The estimates of phenotypic coefficient of variation (4.52 %), genotypic coefficient of variation (4.12 %) and genetic advance as per cent mean (8.05 %) were observed to be low for this character, while heritability (91.15 %) recorded high estimate (Table 13).

4.5.2.2 Fruit width

The estimates for phenotypic coefficient of variation (4.73 %), genotypic coefficient of variation (4.61 %) and genetic advance as per cent mean (9.39 %) are low, while heritability (97.46 %) recorded high estimate for this trait (Table 13).

4.5.2.3 Fruit weight

All the variability parameters studied for the trait (Table 13), namely phenotypic coefficient of variation (37.60 %), genotypic coefficient of variation (35.70 %), heritability (94.95 %) and genetic advance as per cent mean (69.88 %) were found to be high.

4.5.2.4 Fruit yield per tree

All the variability parameters studied, namely, phenotypic coefficient of variation (38.03 %) and genotypic coefficient of variation (35.79 %) along with heritability (94.11 %) and genetic advance as per cent mean (69.77 %) were high for the character studied (Table 13).

4.5.2.5 Fruit shape

The estimates for phenotypic coefficient of variation (40.34 %), genotypic coefficient of variation (39.52 %) heritability (97.97 %) and genetic advance as per cent mean (81.51 %) were all high for the trait (Table 13).

4.5.2.6 Fruit surface texture

The estimates for fruit surface texture (Table 13) were moderate for phenotypic coefficient of variation (19.79 %) and genotypic coefficient of variation (16.64 %), while high estimates were recorded for heritability (84.08 %) and genetic advance as per cent mean (31.92 %).

4.5.2.7 Peel colour

Moderate phenotypic coefficient of variation (12.05 %), low genotypic coefficient of variation (8.05 %), high heritability (66.80 %) and moderate genetic advance as per cent mean (14.60 %) were recorded for the trait (Table 13).

4.5.2.8 Flesh colour

The estimates for flesh colour are moderate for both phenotypic coefficient of variation (17.79 %) and genotypic coefficient of variation (15.46 %), while the estimates for heritability (86.90 %) and genetic advance as per cent mean (29.93 %) are high for the trait (Table 13).

4.5.2.9 Flesh texture

The character recorded high estimates (Table 13) for all variability parameters studied, namely, phenotypic coefficient of variation (37.80 %), genotypic coefficient of variation (35.17 %), heritability (93.04 %) and genetic advance as per cent mean (69.80 %).

4.5.2.10 Flesh taste

Moderate phenotypic coefficient of variation (11.17 %), low genotypic coefficient of variation (7.28 %), high heritability (65.17 %) and moderate genetic advance as per cent mean (13.11 %) were noticed for the character (Table 13).

4.5.2.11 Fruit dessert quality

Higher values for phenotypic coefficient of variation (24.28 %), genotypic coefficient of variation (20.14 %), heritability (82.95 %) and genetic advance as per cent mean (38.33 %) were recorded for the trait (Table 13).

4.5.2.12 Seed number/fruit

This character (Table 13) recorded high estimates for phenotypic coefficient of variation (37.41 %), genotypic coefficient of variation (36.27 %) heritability (96.95 %) and genetic advance as per cent mean (73.54 %).

4.5.2.13 Seed weight

The estimates for phenotypic coefficient of variation (46.75 %) and genotypic coefficient of variation (40.69 %) were high for this character (Table 13). Similarly, the estimates for heritability (87.04 %) and genetic advance as per cent mean (73.02 %) were also high for the trait.

4.5.2.14 Pulp:Seed ratio

Higher values for phenotypic coefficient of variation (38.18 %), genotypic coefficient of variation (33.21 %), heritability (86.98 %) and genetic advance as per cent mean (59.55 %) were recorded for this character (Table 13).

4.5.2.15 Total Soluble Solids

The estimates for phenotypic coefficient of variation (26.53 %), genotypic coefficient of variation (23.60 %), heritability (88.96 %) and genetic advance as per cent mean (44.57 %) were high for the trait (Table 13).

4.5.2.16 Reducing sugars

Moderate estimates were noticed for phenotypic coefficient of variation (15.44 %) and genotypic coefficient of variation (14.21 %), while high estimates were registered for heritability (92.03 %) and genetic advance as per cent mean (30.84 %) for the character (Table 13).

4.5.2.17 Non-reducing sugars

The estimates for phenotypic coefficient of variation (5.57 %) and genotypic coefficient of variation (4.62 %) are low for this character, while the estimates for heritability (82.94 %) and genetic advance as per cent mean (81.44 %) were high for the trait (Table 13).

4.5.2.18 Total Sugars

Moderate estimates for phenotypic coefficient of variation (10.21 %), low estimates for genotypic coefficient of variation (9.92 %) and high estimates for heritability (97.16) and genetic advance as per cent mean (22.13 %) were recorded for the character (Table 13).

4.5.2.19 Titrable acidity

The estimates for phenotypic coefficient of variation (35.95 %), genotypic coefficient of variation (20.11 %) and genetic advance as per cent mean (35.74 %) are high, while heritability (55.94 %) registered moderate value for the trait (Table 13).

4.5.2.20 Sugar:acid ratio

Higher values were recorded for phenotypic coefficient of variation (38.82 %), genotypic coefficient of variation (26.04 %), heritability (67.08 %) and genetic advance as per cent mean (47.30 %) for the trait (Table 13).

4.5.2.21 Ascorbic acid

The estimates for phenotypic coefficient of variation (22.35 %), genotypic coefficient of variation (21.23 %), heritability (95.00 %) and genetic advance as per cent mean (45.49 %) were all high for the character (Table 13).

4.5.2.22 Total phenols

Higher values were recorded for phenotypic coefficient of variation (33.89 %), genotypic coefficient of variation (26.45 %), heritability (78.05 %) and genetic advance as per cent mean (48.89 %) for the character (Table 13).

The phenotypic coefficient of variation ranged from 4.52 to 46.75 among the fruit and biochemical characters, while the genotypic coefficient of variation ranged from 4.12 to 40.69. Further, the heritability values ranged from 55.94 to 97.97, while the genetic advance as per cent mean ranged from 8.05 to 81.51.

From the results presented from the aforesaid paragraphs it is evident that the characters, namely, shape of the tree, branch density, leaf area, fruit weight, fruit yield/tree, fruit shape, flesh texture, fruit dessert quality, seed number/fruit, seed weight, pulp:seed ratio, TSS, sugar:acid ratio, ascorbic acid and total phenols had recorded higher estimates for phenotypic coefficient of variation, genotypic coefficient of variation, heritability and genetic advance as per cent mean.

4.6 PRINCIPAL COMPONENT ANALYSIS

Principal component analyses (PCA) for tree morphological characters, fruit and biochemical characters and leaf isozyme banding pattern were performed adopting correlation matrix method and the results are presented in Tables 14 – 31 and Figs. 13 - 24. The character loading values for principal components represented the weights defining the contribution of different characters for the respective principal components. Further, the loadings signs (+ / -) are indicative of the direction of contribution, similar to that of regression coefficients.

4.6.1 Tree morphological characters

The principal components, eigen values, per cent variability, cumulative per cent of variability and component loading of different tree morphological characters studied are furnished in Table 14 and 15. The principal components with eigen values less than one were considered non-significant as per the procedure. In the present investigation, the first three principal components with eigen values more than one contributed to 74.23 per cent of cumulative variability among the 25 sapota genotypes evaluated for 11 tree morphological characters studied. The first principal component (PC I) contributed highest towards variability (40.81%). Characters like pollen size (-0.44), leaf area (0.41), leaf length (0.38) and pollen production per anther (0.34) explained maximum variability in the first principal component (Table 14 and 15) and among these characters leaf area, leaf length and pollen production per anther were positively correlated, while pollen size was negatively correlated.

The second principal component was noticed to explain 21.72% of the variability and showed high loadings for style length (0.49), branch density (0.48), pollen fertility (-0.47), shape of the tree (-0.38) and branching pattern (0.30), while the third principal component explained 11.71 % of variability and showed high positive correlation for growth habit of tree (0.72) and negative correlation for branch density (-0.40) and pollen fertility (-0.31).

The PCA scores or genotype mean scores for the 25 genotypes in the first three principal components with eigen value more than one were computed and are presented in Table 16. These three PCA scores for the 25 sapota genotypes were plotted on graph to get the 2D (PCA I as X axis and PCA II as Y axis) and

3D (PCA I as X axis, PCA II as Y axis and PCA III as Z axis) scatter diagrams (Figs. 13 and 14, respectively).

To group the genotypes into various clusters, agglomerative hierarchical cluster analysis using Ward's minimum variance method was followed based on Euclidean² distance (dissimilarity coefficient). 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. Dendrogram, was also constructed based on Euclidian² distance computed from the PCA scores of genotypes by utilizing Ward's minimum variance method (Fig. 15).

The 25 sapota genotypes studied were thus grouped into six clusters and their distribution is presented in Table 17. Cluster I comprised of six genotypes (Badami, PKM – 1, Ulavapadu Selection, Gutti, Tagarampudi and Dwarapudi), while Cluster II comprised of nine genotypes (Brazilian Sapota, Kirthibarthi, Cricket Ball, Pakala Round, Krishna Rao, DHS – 2, PKM – 2, Kalipatti and Pala). Further, Cluster III comprised of three genotypes (Gavarayya, PKM – 4 and Singapore), while Cluster IV comprised of two genotypes (CO – 1 and CO – 2). Similarly, Cluster V comprised of three genotypes (Cricket Ball, PKM – 3 and DHS – 1), while Cluster VI consisted of two genotypes (Gede Sapota and CO – 3).

A perusal of the intra and inter-cluster distances (Table 18 and Fig. 16), estimated based on Ward's minimum variance dendrogram revealed maximum inter-cluster Euclidian² distances between Cluster I and V (5818.70), followed by Cluster II and V (4732.20), Cluster II and VI (4681.55) and Cluster I and VI

(4249.59), while minimum inter-cluster distance (727.55) was observed between Clusters I and II.

Intra-cluster Euclidian² distances were maximum in Cluster VI (680.52) and Cluster V (435.24), while minimum intra-cluster distance was recorded in Cluster II (244.15) followed by Clusters I, III and IV.

Cluster means were also estimated for various tree morphological characters studied and are presented in Table 19. A perusal of these results revealed the existence of considerable differences between clusters. Higher differences were observed in respect of pollen production/anther, tree shape, leaf length and leaf area, while moderate differences were noticed for branch density, leaf width and pollen fertility. Further, lower differences were recorded in case of style length, pollen size, growth habit of the tree and branching pattern.

Maximum cluster mean of 3.25 (Cluster IV) and minimum of 2.67 (Cluster I, II and III) was recorded for growth habit of the tree. The cluster mean for shape of the tree was highest for Cluster II (5.78) followed by Cluster III (5.67) and Cluster V (5.33) and lowest for Cluster VI (1.50). Maximum cluster mean for branching pattern was recorded in Cluster IV and cluster VI (3.00 each) and minimum in Cluster III (2.17). Higher cluster mean was recorded for Cluster I (2.83) in case of branch density while lower mean was recorded for Cluster IV (1.25). Maximum cluster mean for leaf length and leaf width were recorded for Cluster VI (11.79 and 4.55 respectively) and in Cluster V (34.20) for leaf area, while minimum mean for leaf length was recorded in Cluster II (8.69), for leaf width in Cluster I (3.15) and Cluster II for leaf area (17.74).

The maximum mean for pollen size was recorded for Cluster V (16.15), for pollen production per anther in Cluster V (744.18), for pollen fertility in Cluster V (98.06) and for style length in Cluster V and Cluster VI (0.81 each). Lower means was recorded in Cluster III for pollen size (15.93) Cluster II (623.90) for pollen production, in Cluster IV (96.62) for pollen fertility and in Cluster III (0.68) for style length.

By principal component analysis, the tree morphological characters could be classified into three principal components. The first principal component (PC I) was found to contain maximum variability over the rest of the PCs. Agglomerative hierarchical clustering based on Ward's minimum variance method was able to cluster the genotypes into six clusters and scatter diagrams were constructed to depict the position of the genotypes in relation to the other genotypes. Cluster diagram could depict the cluster to cluster relationships, revealing the inter-cluster distances (maximum between Cluster I and Cluster V) and intra-cluster distances (maximum for Cluster VI) along with cluster means.

4.6.2 Fruit morphological and biochemical characters

The eigen values (variances), per cent variability, cumulative per cent of variability and component loading of the different fruit and biochemical characters are given in Tables 20 and 21, respectively.

The first three principal components with eigen values more than one were noticed to contribute to 89.79 per cent of cumulative variability among the 25 sapota genotypes evaluated for the 22 fruit morphological and biochemical characters (Table 20). Further, the first principal component (PC I) contributed to

high variability (59.07 %) and the characters, namely, fruit surface texture, (0.27), titrable acidity (0.27), TSS (-0.27), reducing sugars (-0.27), fruit weight (0.26), ascorbic acid (-0.26), peel colour (0.25), flesh colour (-0.25) and flesh taste (-0.25) were observed to explain maximum variability in the first principal component.

The second principal component explained 18.93 % of the variability and showed high loadings of total sugars (0.47), pulp:seed ratio (0.46), total phenols (-0.42), sugar:acid ratio (0.38) and non-reducing sugars (0.26), while the third principal component explained 11.78 % of variability and showed conspicuously high loading for fruit shape (0.52), flesh texture (0.51), fruit yield/ tree (0.32), fruit dessert quality (0.31) and seed number per fruit (-0.28). The fourth principal component, though considered not significant, however explained 4.36 % of variability and showed high positive loadings of fruit yield/tree (0.72), and negative loadings of fruit width (-0.43) and fruit shape (-0.43).

The PCA scores for the 25 genotypes in the first three principal components with eigen value more than one were computed and are presented in Table 22. These three PCA scores were plotted on graph to get the 2D (PCA I as X axis and PCA II as Y axis) and 3D (PCA I as X axis, PCA II as Y axis and PCA III as Z axis) scatter diagrams (Figs. 17 and 18, respectively).

Dendrogram for clustering of genotypes into different clusters was constructed based on Euclidian² distance computed from the PCA scores of genotypes by utilizing Ward's minimum variance method and is presented in Fig. 19. The 25 genotypes were grouped into six clusters and their distribution is presented in Table 23. Cluster I comprised of seven genotypes (Badami, Gede

Sapota, Kalipatti, Calcutta Round, PKM – 2, Krishna Rao, Tagarampudi, Singapore and Dwarapudi), while Cluster II had four genotypes (Gavarayya, DHS – 1, PKM – 1 and PKM – 3); Cluster III comprised of three genotypes (CO – 3, PKM – 4 and CO – 1); Cluster IV comprised of two genotypes (Brazilian Sapota and Cricket Ball); Cluster V comprised of six genotypes (Gutti, DHS – 2, Pakala Round, Pala, Kirthibarthi and Ulavapadu Selection) and Cluster VI had one genotype (CO – 2).

The average intra and inter-cluster distances estimated from Ward's minimum variance dendrogram are presented in Table 24 and Fig. 20. The maximum inter-cluster Euclidian² distances were recorded between Cluster IV and VI (193942.40) followed by Cluster IV and V (132432.70), Cluster I and VI (84216.74) and Cluster III and VI (82496.05). Further, maximum intra-cluster Euclidian² distance was observed in Cluster IV (21468.48), followed by Cluster V (6804.23) and Cluster III (5490.01), while minimum intra-cluster distance was observed in Cluster VI (0.00).

Cluster means were also estimated for the fruit and biochemical characters studied and are presented in Table 25. The results revealed the existence of considerable differences between clusters. Further, greater differences were observed in respect of sugar:acid ratio, fruit weight, pulp:seed ratio and fruit yield per tree, while smaller differences were recorded for titrable acidity, total phenols, flesh taste, fruit dessert quality, peel colour, flesh colour and total sugars.

The maximum cluster mean for fruit length was recorded in Cluster III (7.30) followed by Cluster VI (6.88) and lowest being in Cluster V (4.18) (Table 25). In case of fruit width, higher cluster mean was recorded in Cluster IV (5.97)

followed by cluster VI (5.89), while lowest mean was recorded in Cluster V (4.23). Cluster mean for fruit weight was highest in Cluster VI (150.65) followed by Cluster IV (148.15) and lowest in Cluster I (73.18). Maximum cluster mean for yield per tree was recorded in Cluster II (80.00) and minimum in Cluster VI (26.20).

The maximum cluster means for seed number was recorded in Cluster VI (8), while minimum was recorded in Cluster V (3.17). The cluster mean for seed weight was higher in Cluster IV (7.03), while it was lowest in Cluster VI (1.49). Maximum cluster mean for pulp:seed ratio was recorded in Cluster VI (100.80), for fruit shape in Cluster II (5.00), for fruit surface texture in Cluster III (3.00), for peel colour in Cluster III (4.50), for flesh colour in Cluster V (4.58), for flesh texture in Cluster V (2.75) for flesh taste in Cluster III and VI (both 4.00), for fruit dessert quality in Cluster II and VI (both 2.00), for TSS in Cluster II (23.08), for reducing sugars in Cluster VI (8.90), for non-reducing sugars in Cluster IV (2.36), for total sugars in Cluster V (10.20), for titrable acidity in Clusters I, II, III, IV and VI (all 0.04), for sugar:acid ratio in Cluster V (379.50), for ascorbic acid in Cluster VI (10.25), for total phenols in Cluster I (0.55), while minimum cluster mean for pulp:seed ratio was recorded in Cluster IV (21.10), for fruit shape in Cluster VI (1.00), for fruit surface texture in Cluster VI (2.00), for peel colour in Cluster (3.50), for flesh colour in Cluster IV and VI (both 4.00), for flesh texture in Cluster III and VI (both 1.00), for flesh taste in Cluster IV (3.50), for fruit dessert quality in Cluster I (1.59), for TSS in Cluster IV (21.65), for reducing sugars in Cluster IV (7.12), for non-reducing sugars in Cluster VI (1.25), for total sugars in Cluster III (9.12), for titrable acidity in Cluster V (0.03), for sugar:acid

ratio in Cluster III (225.44), for ascorbic acid in Cluster IV (6.63) and for total phenols in Cluster VI (0.33).

In the principal component analysis of the fruit and biochemical characters three principal components could be obtained. The first principal component (PC I) was found to contain maximum variability over the rest of the PC's. Agglomerative hierarchical clustering based on Ward's minimum variance method was able to cluster the genotypes into six clusters and scatter diagrams were constructed to depict the position of the genotypes in relation to the other genotypes. Cluster diagram could depict the cluster to cluster relationships, revealing the inter-cluster distances (maximum between Cluster IV and Cluster VI) and intra-cluster distances (maximum for Cluster IV) along with cluster means.

4.6.3 Isozyme bands

The principal components, eigen values, per cent variability, cumulative per cent of variability and component loading of different isozymes are presented in Tables 26 and 27, respectively. The first two principal components with eigen values more than one contributed to 73.04 per cent of cumulative variability (Table 26) among the 25 sapota genotypes evaluated for four isozymes.

The first principal component contributed maximum towards variability (45.40 %). Further, the isozymes namely, esterase (0.60), peroxidase (0.58) and Polyphenol oxidase (0.54) were found to explain maximum variability in the first principal component (Table 27). The second principal component explained 27.64 % of the variability and showed high loadings of catalase (-0.90) isozyme,

while the third principal component explained 16.45 % of variability and showed conspicuously high loading of polyphenol oxidase (-0.80) and peroxidase (0.47).

The PCA scores for the 25 genotypes in the first three principal components with eigen value more than one were computed and are presented in Table 28. These three PCA scores for the 25 sapota genotypes were also plotted on graph to get the 2D (PCA I as X axis and PCA II as Y axis) and 3D (PCA I as X axis, PCA II as Y axis and PCA III as Z axis) scatter diagrams (Figs. 21 and 22, respectively).

The dendrogram of 25 sapota genotypes for clustering into different clusters was also constructed based on Euclidian² distance computed from the PCA scores of genotypes by utilizing Ward's minimum variance method and is presented in Fig. 23. The 25 sapota genotypes were grouped into six clusters and their distribution among the clusters is presented in Table 29. Cluster I comprised of twelve genotypes namely, Brazilian Sapota, Tagarampudi, Ulavapadu Selection, Badami, Gavarayya, Gutti, Dwarapudi, PKM – 4, Pakala Round, Gede Sapota, Pala and CO - 3, while Cluster II had two genotypes, namely, Calcutta Round and DHS – 2. The Cluster III comprised of four genotypes, namely, Singapore, DHS – 1, Cricket Ball and CO - 1, while Cluster IV comprised of two genotypes namely, Kalipatti and CO – 2. Further, Cluster V comprised of three genotypes, namely, Kirthibarthi, PKM – 2 and PKM – 1, while Cluster VI had two genotypes, namely, Krishna Rao and PKM - 3.

The average intra and inter-cluster distances were also estimated based on Ward's minimum variance dendrogram and are presented in Table 30 and Fig. 24.

Maximum inter-cluster Euclidian² distances were recorded between Cluster II and VI (34.22), followed by Cluster V and VI (33.53), Cluster I and VI (33.48) and Cluster III and VI (30.52), while maximum intra-cluster distance was observed in Cluster VI (0.21), followed by Cluster III (0.14) and Cluster I (0.10).

The cluster means estimated for different isozymes studied are presented in Table 31. A perusal of these results revealed the existence of considerable differences between clusters. Higher differences were observed in respect of catalase, while lower differences were recorded in case of esterase and peroxidase. However, in respect of polyphenol oxidase, moderate differences were noticed. Maximum cluster means was recorded in case of Cluster II and Cluster V (both 4.50) for esterase isozyme and minimum for Cluster I (4.00). Cluster means for peroxidase isozyme was maximum in Cluster III (4.63) and minimum in Cluster VI (4.21). Higher cluster mean was recorded for polyphenol oxidase isozyme in Cluster V (3.52) and lower means was recorded in Cluster III (2.06). The isozyme catalase recorded high cluster mean in Cluster II (6.20) and low cluster mean of 0.00 in Cluster VI.

In a nutshell, principal component analysis of leaf isozyme banding pattern resulted in two principal components. The first principal component (PC I) was found to contain maximum variability over the rest of the PC's. Agglomerative hierarchical clustering based on Ward's minimum variance method was able to cluster the genotypes into six clusters and scatter diagrams were constructed to depict the position of the genotypes in relation to the other genotypes. Cluster diagram could depict the cluster to cluster relationships, revealing the inter-cluster

distances (maximum between Cluster II and Cluster VI) and intra-cluster distances (maximum for Cluster VI) along with cluster means.

Table 7: Morphological characterization of sapota genotypes

S. No.	Character	Description	Genotype
1	Leaf colour	Green	All genotypes under study
2	Leaf shape	Elliptic	Badami, Cricket Ball, Gede Sapota, Kaliaptti, Pakala Round, Pala, Tagarampudi, Ulavapadu Selection, DHS – 1, PKM – 4
		Broadly elliptic	Brazilian Sapota, Dwarapudi, Gavarayya, Gutti, Kirthibarthi, Krishna Rao, Singapore, CO – 1, CO – 2, CO – 3, DHS -2, PKM – 1, PKM – 2, PKM - 3
		Sub-ovate	Calcutta Round
3	Leaf margin	Entire	Badami, Brazilian Sapota, Calcutta Round, Dwarapudi, Gavarayya, Kaliaptti, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Singapore, Tagarampudi, DHS -1, PKM - 1
		Wavy	Cricket Ball, Gede Sapota, Gutti, Ulavapadu Selection, CO – 1, CO – 2, CO – 3, DHS – 2, PKM – 2, PKM – 3, PKM – 4
4	Shape of the leaf tip	Retuse	Badami, Brazilian Sapota, Calcutta Round, Cricket Ball, Gavarayya, Gutti, Krishna Rao, CO – 3, PKM - 3
		Acute	Dwarapudi, Gede Sapota, Kalipatti, Kirthibarthi, Pala, Tagarampudi, Ulavapadu Selection, CO – 1, DHS – 1, PKM – 1, PKM – 2
		Sub-acuminate	Pakala Round, Singapore, CO – 2, DHS – 2, PKM – 4
5	Colour of shoot tip	Brown	All genotypes under study
6	Surface of shoot tip	Pubescent	All genotypes under study
7	Inflorescence type	Cymose	All genotypes under study
8	Flower position	Axillary	All genotypes under study
9	Flower bud Colour	Brown	All genotypes under study
10	Arrangement of Flowers	Soitary	All genotypes under study
11	Number of sepals	6	All genotypes under study
12	Sepal colour	Greenish brown	All genotypes under study
13	Number of petals	6	All genotypes under study
14	Petal colour	White	All genotypes under study
15	Anther number flower ¹	6	All genotypes under study
16	Fruit apex	Obtuse	Badami, Cricket Ball, Gavarayya, Kirthibarthi, Pakala Round, DHS – 1, DHS – 2, PKM – 2, PKM – 3, PKM - 4
		Acute	Brazilian Sapota, Calcutta Round, Dwarapudi, Gede Sapota, Gutti, Kaliaptti, Krishna Rao, Pala, Singapore, Tagarampudi, Ulavapadu Selection, CO – 1, CO – 2, CO – 3, PKM -1
17	Fruit base	Inflated	Dwarapudi, Singapore, PKM – 1
		Flattened	Badami, Gede Sapota, Kalipatti, Pala, Ulavapadu Selection, DHS – 1, PKM – 2, PKM – 4
		Depressed	Brazilian Sapota, Calcutta Round, Cricket Ball, Gavarayya, Gutti, Kirthibarthi, Krishna Rao, Pakala Round, Tagarampudi, CO – 1, CO – 2, CO – 3, DHS – 2, PKM – 3
18	Seed shape	Broadly clavate with spine	Badami, Brazilian Sapota, Calcutta Round, Dwarapudi, Gede Sapota, Gutti, Krishna Rao, Pala, Singapore, Tagarampudi, Ulavapadu Selection, CO – 1, CO -3, PKM -3, PKM - 4
		Semi-ovate	Pakala Round, DHS – 1, DHS – 2, PKM – 2
		Semi-deltoid with spine	Cricket Ball, CO – 2
		Broadly clavate with pointed spine	Gavarayya, Kalipatti, Kirthibarthi, PKM – 1

Table 8: Banding pattern of sapota genotypes for the isozyme esterase

Rm value	37	38	39
Genotype			
Badami	-	+++	-
Brazilian Sapota	-	-	+++
Calcutta Round	-	+++	-
Cricket Ball	-	-	+++
Dwarapudi	-	-	+++
Gavarayya	+++	-	-
Gede Sapota	-	+++	-
Gutti	-	-	+++
Kalipatti	-	+++	-
Kirthibarthi	+++	-	-
Krishna Rao	+++	-	-
Pakala Round	+++	-	-
Pala	+++	-	-
Singapore	-	+++	-
Tagarampudi	-	-	+++
Ulavapadu Selection	+++	-	-
CO – 1	++	-	-
CO – 2	++	-	-
CO – 3	-	+++	-
DHS – 1	-	-	+++
DHS – 2	+++	-	-
PKM – 1	-	+++	-
PKM – 2	+++	-	-
PKM – 3	+++	-	-
PKM - 4	-	+++	-

+++ High intensity band; ++ Medium intensity band; + Low intensity band; ' - ' Band absent

Table 9: Banding pattern of sapota genotypes for the isozyme peroxidase

Rm value	27	28	29	30	31	33	37	39	40	41	42	43	44	45	46
Genotype															
Badami	-	-	-	+++	-	-	-	-	-	+	-	-	-	-	-
Brazilian Sapota	-	-	+++	-	-	-	-	-	-	-	-	++	-	-	-
Calcutta Round	-	-	-	++	-	-	-	-	-	-	-	-	-	+	-
Cricket Ball	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
Dwarapudi	-	+++	-	-	-	-	-	-	-	++	-	-	-	-	-
Gavarayya	-	-	-	-	++	-	-	-	-	-	+	-	-	-	-
Gede Sapota	-	++	-	-	-	-	-	-	-	+	-	-	-	-	-
Gutti	-	-	-	+++	-	-	-	-	-	-	-	++	-	-	-
Kalipatti	-	++	-	-	-	-	-	-	-	-	-	-	-	+	-
Kirthibarthi	-	-	-	-	++	-	-	-	-	+	-	-	-	-	-
Krishna Rao	-	-	++	-	-	-	-	-	+	-	-	-	-	-	-
Pakala Round	-	-	-	++	-	-	+	-	-	-	-	-	-	-	+
Pala	+++	-	-	-	-	-	-	-	-	-	-	-	++	-	-
Singapore	-	-	-	-	-	++	-	-	-	-	+	-	-	-	-
Tagarampudi	-	++	-	-	-	-	-	-	-	-	-	-	-	-	+
Ulavapadu	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
Selection															
CO – 1	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
CO – 2	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
CO – 3	-	-	++	-	-	-	-	-	-	+	-	-	-	-	-
DHS – 1	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-
DHS – 2	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-
PKM – 1	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-
PKM – 2	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-
PKM – 3	-	++	-	-	-	-	-	-	-	-	-	-	-	+	-
PKM - 4	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+

+++ High intensity band;

++ Medium intensity band;

+ Low intensity band;

' - ' Band absent

Table 10: Banding pattern of sapota genotypes for the isozyme polyphenol oxidase

Rm value	19	21	23	24	25	26	27	28	37	38	41	44
Genotype												
Badami	-	-	-	+	-	-	-	-	-	-	-	-
Brazilian Sapota	-	-	-	+	-	-	-	-	-	-	-	-
Calcutta Round	-	-	-	+	-	-	-	-	-	-	-	-
Cricket Ball	-	++	-	-	-	-	-	-	-	-	-	-
Dwarapudi	-	-	+	-	-	-	-	-	-	-	-	-
Gavarayya	-	-	+	-	-	-	-	-	-	-	-	-
Gede Sapota	-	-	+	-	-	-	-	-	-	-	-	-
Gutti	-	-	-	-	+	-	-	-	-	-	-	-
Kalipatti	++	-	-	-	-	-	-	-	-	-	+	-
Kirthibarthi	-	-	+	-	-	-	-	-	-	-	-	+
Krishna Rao	-	-	-	-	-	-	-	+	-	-	-	-
Pakala Round	-	-	-	-	+	-	-	-	-	-	-	-
Pala	-	-	+	-	-	-	-	-	-	-	-	-
Singapore	-	-	+	-	-	-	-	-	-	-	-	-
Tagarampudi	-	-	-	-	-	+	-	-	-	-	-	-
Ulavapadu Selection	-	-	+	-	-	-	-	-	-	-	-	-
CO – 1	-	++	-	-	-	-	-	-	-	-	-	-
CO – 2	-	+	-	-	-	-	-	-	+	-	-	-
CO – 3	-	-	-	-	-	-	+	-	-	-	-	-
DHS – 1	-	+	-	-	-	-	-	-	-	-	-	-
DHS – 2	-	-	-	-	-	-	++	-	-	-	-	-
PKM – 1	-	-	-	+	-	-	-	-	-	+	-	-
PKM – 2	-	-	+	-	-	-	-	-	-	-	-	+
PKM – 3	-	-	+	-	-	-	-	-	-	-	-	-
PKM - 4	-	-	-	+	-	-	-	-	-	-	-	-
+++ High intensity band;	++ Medium intensity band;	+	Low intensity band;			' - ' Band absent						

Table 11: Banding pattern of sapota genotypes for catalase isozyme

Rm value	53	54	55	56	57	58	60	61	62	63
Genotype										
Badami	-	-	+++	-	-	-	-	-	-	-
Brazilian Sapota	-	+++	-	-	-	-	-	-	-	-
Calcutta Round	-	-	-	-	-	+++	-	-	-	-
Cricket Ball	-	-	-	-	-	-	-	+++	-	-
Dwarapudi	-	-	-	+++	-	-	-	-	-	-
Gavarayya	-	-	-	-	+++	-	-	-	-	-
Gede Sapota	-	-	-	-	-	+++	-	-	-	-
Gutti	-	-	-	-	-	+++	-	-	-	-
Kalipatti	-	+	-	-	-	-	-	-	-	-
Kirthibarthi	-	+	-	-	-	-	-	-	-	-
Krishna Rao	-	-	-	-	-	-	-	-	-	-
Pakala Round	+++	-	-	-	-	-	-	-	-	-
Pala	-	-	-	+++	-	-	-	-	-	-
Singapore	-	-	-	-	-	-	-	-	-	++
Tagarampudi	-	-	-	-	+++	-	-	-	-	-
Ulavapadu Selection	-	+++	-	-	-	-	-	-	-	-
CO – 1	-	-	+++	-	-	-	-	-	-	-
CO – 2	-	-	+	-	-	-	-	-	-	-
CO – 3	-	-	-	-	-	-	+++	-	-	-
DHS – 1	-	-	-	-	-	-	-	+++	-	-
DHS – 2	-	-	-	-	-	+++	-	-	-	-
PKM – 1	-	-	-	-	-	-	-	-	+++	-
PKM – 2	-	-	-	-	-	-	-	+++	-	-
PKM – 3	-	-	-	-	-	-	-	-	-	-
PKM - 4	-	-	-	+++	-	-	-	-	-	-

+++ High intensity band; ++ Medium intensity band; + Low intensity band; ' - ' Band absent

Table 12: Genetic variability in sapota genotypes for tree morphological characters

S. No.	Character	Phenotypic coefficient of variation (%)	Genotypic coefficient of variation (%)	Heritability (%)	Genetic advance as per cent mean (%)
1	Growth habit of the tree	20.28	17.89	88.21	35.01
2	Shape of the tree	37.96	37.59	99.03	77.14
3	Branching pattern	20.77	15.14	72.89	27.75
4	Branch density	28.18	25.10	89.07	48.79
5	Leaf length	11.52	7.39	64.15	9.98
6	Leaf width	15.54	10.41	67.00	14.47
7	Leaf area	30.99	20.15	65.02	27.00
8	Pollen size	3.63	1.52	41.87	3.04
9	Pollen production anther ⁻¹	11.96	7.91	66.14	10.83
10	Pollen fertility	0.67	0.44	65.67	0.58
11	Style length	14.91	14.67	97.05	29.27

Index:

PCV, GCV and genetic advance as per cent mean: Low = 0 – 10 %; Medium = 10 – 20 %;
High = 20 % and above

Heritability: Low = Less than 30 %; Medium = 30 – 60 %; High = More than 60 %

Table 13: Genetic variability in sapota genotypes for fruit morphological and biochemical characters

S. No.	Character	Phenotypic coefficient of variation (%)	Genotypic coefficient of variation (%)	Heritability (%)	Genetic advance as per cent mean (%)
1	Fruit length	4.52	4.12	91.15	8.05
2	Fruit width	4.73	4.61	97.46	9.39
3	Fruit weight	37.60	35.70	94.95	69.88
4	Fruit yield tree ⁻¹	38.03	35.79	94.11	69.77
5	Fruit shape	40.34	39.52	97.97	81.51
6	Fruit surface texture	19.79	16.64	84.08	31.92
7	Peel colour	12.05	8.05	66.80	14.60
8	Flesh colour	17.79	15.46	86.90	29.93
9	Flesh texture	37.80	35.17	93.04	69.80
10	Flesh taste	11.17	7.28	65.17	13.11
11	Fruit dessert quality	24.28	20.14	82.95	38.33
12	Seed number fruit ⁻¹	37.41	36.27	96.95	73.54
13	Seed weight	46.75	40.69	87.04	73.02
14	Pulp:seed ratio	38.18	33.21	86.98	59.55
15	TSS	26.53	23.60	88.96	44.57
16	Reducing sugars	15.44	14.21	92.03	30.84
17	Non- reducing sugars	5.57	4.62	82.94	81.44
18	Total sugars	10.21	9.92	97.16	22.13
19	Titration acidity	35.95	20.11	55.94	35.74
20	Sugar : acid ratio	38.82	26.04	67.08	47.30
21	Ascorbic acid	22.35	21.23	95.00	45.49
22	Total phenols	33.89	26.45	78.05	48.89

Index:

PCV, GCV and Genetic advance as per cent mean: Low = 0 – 10 %; Medium = 10 – 20 %;
High = 20 % and above

Heritability: Low = Less than 30 %; Medium = 30 – 60 %; High = More than 60 %

Table 1: Tree morphological characters (based on scores) of sapota genotypes

S. No.	Genotype	Tree characters			
		Growth habit	Shape of the tree	Branching pattern	Branch density
1	Badami	3.00	6.00	3.00	3.00
2	Brazilian Sapota	3.00	5.00	3.00	2.00
3	Calcutta Round	3.00	6.00	3.00	2.00
4	Cricket Ball	3.00	6.00	2.00	2.00
5	Dwarapudi	3.00	2.00	3.00	3.00
6	Gavarayya	3.00	5.00	1.00	2.00
7	Gede Sapota	2.00	2.00	3.00	2.00
8	Gutti	3.00	5.00	3.00	3.00
9	Kalipatti	2.00	6.00	2.00	3.00
10	Kirthibarthi	2.00	6.00	2.00	2.00
11	Krishna Rao	3.00	6.00	3.00	2.00
12	Pakala Round	3.00	6.00	3.00	2.00
13	Pala	2.00	6.00	2.00	2.00
14	Singapore	2.00	6.00	2.00	3.00
15	Tagarampudi	3.00	6.00	2.00	3.00
16	Ulavapadu Selection	2.00	2.00	3.00	2.00
17	CO – 1	4.00	2.00	3.00	1.00
18	CO – 2	2.00	1.00	3.00	1.00
19	CO – 3	3.00	1.00	3.00	1.00
20	DHS – 1	3.00	4.00	3.00	2.00
21	DHS – 2	3.00	5.00	3.00	3.00
22	PKM – 1	2.00	6.00	3.00	3.00
23	PKM – 2	3.00	6.00	3.00	3.00
24	PKM – 3	3.00	6.00	3.00	2.00
25	PKM - 4	3.00	6.00	3.00	2.00
Mean		2.72	4.72	2.68	2.24
<i>Range</i>					
<i>Minimum</i>		<i>2.00</i>	<i>1.00</i>	<i>1.00</i>	<i>1.00</i>
<i>Maximum</i>		<i>4.00</i>	<i>6.00</i>	<i>3.00</i>	<i>3.00</i>
SE		0.14	0.14	0.20	0.14
CD (0.05)		0.40	0.42	0.59	0.42

Table 2: Leaf morphological characters of sapota genotypes

S. No.	Genotype	Leaf characters		
		Leaf length (cm)	Leaf width (cm)	Leaf area (sq. cm)
1	Badami	9.03	2.89	17.44
2	Brazilian Sapota	8.85	3.18	17.65
3	Calcutta Round	8.93	3.20	17.84
4	Cricket Ball	10.98	3.98	29.10
5	Dwarapudi	9.09	3.69	20.37
6	Gavarayya	9.35	3.70	22.34
7	Gede Sapota	12.99	5.00	35.98
8	Gutti	8.95	3.15	18.00
9	Kalipatti	8.46	2.82	14.72
10	Kirthibarthi	8.68	3.14	15.93
11	Krishna Rao	8.71	3.28	19.17
12	Pakala Round	9.24	3.24	17.50
13	Pala	7.67	3.21	16.43
14	Singapore	9.93	3.41	21.37
15	Tagarampudi	9.48	3.43	20.84
16	Ulavapadu Selection	10.42	2.66	15.35
17	CO – 1	10.30	3.30	24.06
18	CO – 2	10.63	3.63	24.32
19	CO – 3	10.59	4.09	29.37
20	DHS – 1	9.13	2.53	14.54
21	DHS – 2	8.99	3.62	19.93
22	PKM – 1	8.54	3.07	15.10
23	PKM – 2	8.67	3.42	20.49
24	PKM – 3	10.15	4.23	32.12
25	PKM - 4	10.11	3.78	26.43
Mean		9.52	3.43	21.06
<i>Range</i>				
<i>Minimum</i>		7.67	2.53	14.54
<i>Maximum</i>		12.99	5.00	35.98
SE		0.11	0.03	0.13
CD (0.05)		0.33	0.09	0.39

Table 3: Floral characters of sapota genotypes

S. No.	Genotype	Floral characters			
		Pollen Size (μm)	Pollen production anther ⁻¹	Pollen fertility (%)	Style length (cm)
1	Badami	16.44	692.89	96.80	0.89
2	Brazilian Sapota	15.67	494.11	97.17	0.60
3	Calcutta Round	15.76	694.93	97.53	0.81
4	Cricket Ball	16.65	758.11	98.41	0.70
5	Dwarapudi	16.04	614.92	96.21	0.90
6	Gavarayya	15.71	700.80	97.50	0.70
7	Gede Sapota	16.76	617.10	96.61	0.83
8	Gutti	15.82	902.84	97.18	0.80
9	Kalipatti	16.32	643.62	98.86	0.81
10	Kirthibarthi	16.24	648.06	97.46	0.59
11	Krishna Rao	16.86	544.72	96.79	0.57
12	Pakala Round	15.93	661.66	97.79	0.72
13	Pala	15.54	658.26	98.44	0.69
14	Singapore	16.43	714.69	97.97	0.51
15	Tagarampudi	16.68	698.04	96.72	0.90
16	Ulavapadu Selection	16.20	673.85	97.72	0.69
17	CO – 1	16.44	662.30	96.58	0.68
18	CO – 2	15.63	781.91	96.65	0.78
19	CO – 3	15.46	702.78	97.29	0.78
20	DHS – 1	16.12	795.14	97.59	0.90
21	DHS – 2	15.80	652.66	97.34	0.69
22	PKM – 1	15.66	768.22	96.91	0.60
23	PKM – 2	15.33	617.08	97.53	0.70
24	PKM – 3	15.66	679.28	98.17	0.82
25	PKM - 4	15.64	671.87	97.29	0.81
	Mean	16.03	681.59	97.38	0.74
	<i>Range Minimum</i>	<i>15.33</i>	<i>494.11</i>	<i>96.21</i>	<i>0.51</i>
	<i>Maximum</i>	<i>16.86</i>	<i>902.84</i>	<i>98.86</i>	<i>0.90</i>
	SE	0.37	4.51	0.03	0.012
	CD (0.05)	NS	13.18	0.09	0.04

Table 4: Fruit morphological characters of sapota genotypes

S. No.	Genotype	Fruit morphological characters						
		Fruit length (cm)	Fruit width (cm)	Fruit weight (g)	Fruit yield tree ⁻¹ (kg)	Seed number fruit ⁻¹	Seed weight (g)	Pulp: seed ratio
1	Badami	4.70	3.71	64.35	32.35	3	2.49	25.07
2	Brazilian Sapota	6.01	5.15	97.57	52.80	8	7.78	11.34
3	Calcutta Round	4.72	4.72	77.75	45.15	5	3.63	20.50
4	Cricket Ball	6.48	6.79	198.74	55.40	8	6.27	30.86
5	Dwarapudi	4.56	4.70	83.63	37.65	7	4.55	18.02
6	Gavarayya	6.16	4.90	104.03	55.15	3	2.30	43.89
7	Gede Sapota	3.82	3.35	63.13	31.70	3	2.72	21.99
8	Gutti	4.03	5.10	102.01	36.15	3	2.19	45.25
9	Kalipatti	5.19	4.10	64.42	83.40	3	2.42	25.10
10	Kirthibarthi	4.72	4.10	86.72	86.45	4	1.57	53.49
11	Krishna Rao	4.53	5.80	67.74	31.45	5	3.06	21.67
12	Pakala Round	4.21	4.57	76.37	55.25	4	1.68	45.29
13	Pala	4.48	4.17	63.38	65.75	3	1.64	39.60
14	Singapore	5.10	4.35	92.44	85.35	6	3.53	25.42
15	Tagarampudi	3.99	4.21	63.27	60.45	5	4.23	14.66
16	Ulavapadu Selection	3.71	2.37	44.83	63.50	2	1.59	27.09
17	CO – 1	7.28	5.77	170.67	26.40	4	2.97	56.11
18	CO – 2	6.87	5.89	150.65	26.20	3	1.48	100.80
19	CO – 3	6.85	5.82	127.29	32.95	4	3.12	39.79
20	DHS – 1	5.29	4.78	120.21	81.55	3	2.89	40.57
21	DHS – 2	4.51	5.14	89.06	75.80	3	2.20	40.63
22	PKM – 1	5.38	4.71	93.30	89.95	4	3.02	29.39
23	PKM – 2	4.68	4.30	81.89	66.75	5	3.55	21.10
24	PKM – 3	5.92	4.65	105.08	93.35	4	3.52	27.88
25	PKM - 4	7.75	4.17	127.04	70.50	4	3.84	31.49
	Mean	5.24	4.69	96.63	57.66	4.24	3.13	34.28
	<i>Range Minimum</i>	<i>3.71</i>	<i>2.37</i>	<i>44.83</i>	<i>26.20</i>	<i>2.00</i>	<i>1.48</i>	<i>11.34</i>
	<i>Maximum</i>	<i>7.75</i>	<i>6.79</i>	<i>198.74</i>	<i>93.35</i>	<i>8.00</i>	<i>7.78</i>	<i>100.80</i>
	SE	0.05	0.03	0.57	1.70	0.19	0.05	0.28
	CD (0.05)	0.13	0.09	1.66	4.96	0.55	0.13	0.82

Table 5: Fruit characters (based on scores) of sapota genotypes

S. No.	Genotype	Fruit characters						
		Fruit shape	Fruit surface texture	Peel colour	Flesh colour	Flesh texture	Flesh taste	Fruit dessert quality
1	Badami	3.00	2.00	4.00	4.00	3.00	4.00	1.20
2	Brazilian Sapota	7.00	2.00	3.00	3.00	1.00	3.00	1.40
3	Calcutta Round	3.00	2.00	4.00	6.00	2.00	3.00	1.80
4	Cricket Ball	3.00	3.00	5.00	5.00	3.00	4.00	1.80
5	Dwarapudi	2.00	3.00	4.00	4.00	2.00	4.00	1.40
6	Gavarayya	6.00	2.00	4.00	4.00	2.00	3.00	2.00
7	Gede Sapota	3.00	3.00	4.00	4.00	1.00	4.00	1.00
8	Gutti	6.00	3.00	4.00	4.00	3.00	3.00	1.60
9	Kalipatti	2.00	3.00	5.00	4.00	2.00	4.00	2.40
10	Kirthibarthi	3.00	3.00	5.00	4.00	3.00	4.00	1.80
11	Krishna Rao	2.00	2.00	4.00	6.00	2.00	4.00	1.40
12	Pakala Round	3.00	2.00	4.00	4.00	3.00	4.00	1.80
13	Pala	7.00	2.00	4.00	4.00	3.00	4.00	2.80
14	Singapore	2.00	3.00	4.00	4.00	1.00	4.00	2.20
15	Tagarampudi	3.00	2.00	4.00	6.00	2.00	3.00	1.80
16	Ulavapadu Selection	7.00	3.00	4.00	5.00	3.00	4.00	2.00
17	CO – 1	1.00	3.00	5.00	5.00	1.00	4.00	2.00
18	CO – 2	1.00	2.00	4.00	5.00	1.00	4.00	2.00
19	CO – 3	2.00	3.00	4.00	4.00	1.00	4.00	1.80
20	DHS – 1	2.00	2.00	4.00	4.00	3.00	4.00	2.00
21	DHS – 2	3.00	2.00	4.00	6.00	2.00	4.00	1.80
22	PKM – 1	5.00	3.00	4.00	4.00	3.00	4.00	1.60
23	PKM – 2	2.00	3.00	4.00	4.00	2.00	4.00	1.20
24	PKM – 3	7.00	3.00	4.00	5.00	2.00	4.00	2.40
25	PKM - 4	5.00	3.00	4.00	4.00	1.00	4.00	2.00
	Mean	3.60	2.56	4.12	4.48	2.08	3.80	1.81
	<i>Range Minimum</i>	<i>1.00</i>	<i>2.00</i>	<i>3.00</i>	<i>3.00</i>	<i>1.00</i>	<i>3.00</i>	<i>1.20</i>
	<i>Maximum</i>	<i>7.00</i>	<i>3.00</i>	<i>5.00</i>	<i>6.00</i>	<i>3.00</i>	<i>4.00</i>	<i>2.80</i>
	SE	0.20	0.14	0.20	0.20	0.14	0.18	0.13
	CD (0.05)	0.59	0.42	0.58	0.59	0.42	0.51	0.36

Table 6: Fruit biochemical characters of sapota genotypes

S. No.	Genotype	Fruit biochemical characters			
		TSS (°brix)	Reducing sugars (%)	Non-reducing sugars (%)	Total sugars (%)
1	Badami	21.20	6.75	1.46	8.21
2	Brazilian Sapota	23.10	7.71	1.44	9.15
3	Calcutta Round	22.90	8.48	1.24	9.72
4	Cricket Ball	20.20	6.53	3.27	9.80
5	Dwarapudi	21.30	5.88	4.21	10.09
6	Gavarayya	21.60	6.95	2.76	9.71
7	Gede Sapota	21.10	6.29	2.34	8.63
8	Gutti	23.00	7.94	2.02	9.96
9	Kalipatti	23.90	10.65	1.13	11.78
10	Kirthibarthi	22.60	8.47	2.23	10.71
11	Krishna Rao	22.10	7.87	1.01	8.89
12	Pakala Round	21.70	7.91	1.99	9.90
13	Pala	24.30	10.05	1.88	11.93
14	Singapore	23.00	8.50	2.32	10.82
15	Tagarampudi	22.10	7.77	1.23	9.00
16	Ulavapadu Selection	23.40	8.15	1.16	9.31
17	CO – 1	21.30	6.88	2.39	9.27
18	CO – 2	22.30	8.89	1.24	10.14
19	CO – 3	20.70	6.39	1.99	8.38
20	DHS – 1	22.90	8.11	1.17	9.28
21	DHS – 2	21.70	8.20	1.20	9.40
22	PKM – 1	24.10	8.81	1.01	9.83
23	PKM – 2	21.50	7.68	1.09	8.78
24	PKM – 3	23.70	9.21	1.45	10.66
25	PKM - 4	23.50	8.31	1.37	9.68
	Mean	22.37	7.94	1.79	9.72
	<i>Range</i> <i>Minimum</i>	<i>20.20</i>	<i>5.88</i>	<i>1.01</i>	<i>8.21</i>
	<i>Maximum</i>	<i>24.30</i>	<i>10.65</i>	<i>4.21</i>	<i>11.93</i>
	SE	0.93	0.45	0.05	0.34
	CD (0.05)	2.72	1.29	0.15	0.99

(Contd....)

Table 6: Contd.....

S. No.	Genotype	Fruit biochemical characters			
		Titration acidity (%)	Sugar: acid ratio	Ascorbic acid (mg 100g ⁻¹)	Total phenols (mg 100g ⁻¹)
1	Badami	0.03	275.23	6.50	0.69
2	Brazilian Sapota	0.03	399.14	7.37	0.50
3	Calcutta Round	0.04	303.20	6.75	0.40
4	Cricket Ball	0.05	201.59	5.87	0.33
5	Dwarapudi	0.04	256.32	5.12	0.78
6	Gavarayya	0.07	142.01	7.87	0.36
7	Gede Sapota	0.04	221.87	6.75	0.67
8	Gutti	0.04	311.03	9.00	0.61
9	Kalipatti	0.04	293.17	11.37	0.36
10	Kirthibarthi	0.03	469.25	7.50	0.53
11	Krishna Rao	0.03	298.40	7.50	0.64
12	Pakala Round	0.02	492.73	6.50	0.49
13	Pala	0.03	399.00	9.50	0.23
14	Singapore	0.04	295.30	11.00	0.34
15	Tagarampudi	0.04	246.06	8.00	0.41
16	Ulavapadu Selection	0.03	347.28	9.75	0.25
17	CO – 1	0.05	214.74	10.75	0.26
18	CO – 2	0.04	276.04	10.25	0.33
19	CO – 3	0.04	216.05	9.75	0.51
20	DHS – 1	0.01	582.08	9.00	0.43
21	DHS – 2	0.04	257.68	8.12	0.66
22	PKM – 1	0.05	227.20	10.37	0.40
23	PKM – 2	0.05	175.10	7.50	0.60
24	PKM – 3	0.03	461.73	10.25	0.48
25	PKM - 4	0.04	245.51	9.62	0.42
	Mean	0.04	304.31	8.48	0.47
	<i>Range Minimum</i>	<i>0.01</i>	<i>142.01</i>	<i>5.12</i>	<i>0.23</i>
	<i>Maximum</i>	<i>0.07</i>	<i>582.08</i>	<i>11.37</i>	<i>0.78</i>
	SE	0.01	47.94	0.57	0.05
	CD (0.05)	0.02	139.92	1.66	0.16

Table 20: Eigen values, per cent variability and cumulative variability for principal components of fruit morphological and biochemical characters in sapota genotypes

	PC1	PC2	PC3	PC4
Eigen value	12.99	4.17	2.59	0.96
Per cent variance explained	59.07	18.93	11.78	4.36
Cumulative variance explained	59.07	78.01	89.79	94.15

Table 21: Character loading of principal components for fruit morphological and bio- chemical characters in sapota genotypes

Character	PC1	PC2	PC3	PC4
Fruit length (cm)	0.24	0.06	0.01	0.08
Fruit width (cm)	0.22	0.06	-0.06	-0.43
Fruit weight (g)	0.26	0.11	-0.06	-0.04
Fruit yield/tree (kg)	0.11	0.03	0.32	0.72
Fruit shape	0.05	-0.12	0.52	-0.43
Fruit surface texture	0.27	0.08	-0.01	-0.08
Peel colour	0.25	0.15	-0.14	0.09
Flesh colour	-0.25	-0.12	0.05	-0.05
Flesh texture	-0.12	-0.12	0.51	-0.04
Flesh taste	-0.25	-0.13	0.18	-0.12
Fruit dessert quality	0.23	0.06	0.31	-0.08
Seed number/ fruit	-0.23	-0.14	-0.28	-0.06
Seed weight (g)	0.24	-0.19	-0.06	-0.09
Pulp: seed ratio	-0.09	0.46	0.06	-0.08
TSS (^o brix)	-0.27	0.05	-0.09	-0.08
Reducing sugars (%)	-0.27	-0.05	-0.14	-0.02
Non- reducing sugars (%)	-0.22	0.26	0.18	-0.07
Total sugars (%)	0.03	0.47	0.08	-0.003
Titration acidity (%)	0.27	0.04	0.09	-0.07
Sugar:acid ratio	-0.11	0.38	-0.15	-0.09
Ascorbic acid (mg/100g)	-0.26	0.07	0.17	0.16
Total phenols (mg/100g)	0.14	-0.42	-0.09	0.05

Table 22: PCA mean scores for fruit morphological and biochemical characters in sapota genotypes

Genotype	PC1	PC2	PC3
Badami	239.78	-0.99	92.97
Brazilian Sapota	412.79	-180.39	81.59
Calcutta Round	293.47	-42.66	84.77
Cricket Ball	550.00	-134.85	63.35
Dwarapudi	314.75	-78.99	66.43
Gavarayya	331.86	43.87	131.86
Gede Sapota	222.34	-6.85	77.58
Gutti	285.22	68.56	124.55
Kalipatti	269.22	14.71	115.63
Kirthibarthi	248.00	138.01	132.18
Krishna Rao	283.94	-29.62	88.59
Pakala Round	232.39	100.35	116.99
Pala	224.37	92.09	151.99
Singapore	319.76	-26.49	87.09
Tagarampudi	263.04	-47.91	82.89
Ulavapadu Selection	152.26	76.84	132.40
CO – 1	440.52	24.35	73.16
CO – 2	335.02	247.53	115.19
CO – 3	404.00	-21.90	87.16
DHS – 1	340.23	33.87	101.58
DHS – 2	281.84	66.08	115.75
PKM – 1	327.31	-1.40	129.95
PKM – 2	297.53	-40.33	80.33
PKM – 3	361.54	-25.49	135.28
PKM - 4	414.58	-61.72	102.60

Table 23: Clustering of sapota genotypes for fruit morphological and biochemical characters

Cluster	Genotypes
Cluster I	Badami, Gede Sapota, Kalipatti, Calcutta Round, PKM – 2, Krishna Rao, Tagarampudi, Singapore, Dwarapudi
Cluster II	Gavarayya, DHS – 1, PKM – 1, PKM - 3
Cluster III	CO – 3, PKM – 4, CO - 1
Cluster IV	Brazilian Sapota, Cricket Ball
Cluster V	Gutti, DHS - 2, Pakala Round, Pala, Kirthibarthi, Ulavapadu Selection
Cluster VI	CO – 2

Table 24: Intra and inter-cluster distances for fruit morphological and biochemical characters in sapota genotypes

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	4281.48	10113.25	23894.97	65431.30	22496.14	84216.74
Cluster II		3104.60	11782.73	58271.89	20692.39	58265.89
Cluster III			5490.01	30243.73	51744.36	82496.05
Cluster IV				21468.48	132432.70	193942.40
Cluster V					6804.23	38492.73
Cluster VI						0.00

Note: Figures in bold indicate intra-cluster distances

Table 25: Cluster means for fruit morphological and biochemical characters in sapota genotypes

Cluster	Fruit Length (cm)	Fruit width (cm)	Fruit weight (g)	Fruit yield/tree (kg)	Fruit shape	Fruit surface texture	Peel colour	Flesh colour	Flesh texture	Flesh taste	Fruit dessert quality	Seed number/fruit
Cluster I	4.59	4.36	73.18	52.69	2.50	2.50	4.22	4.56	2.00	3.83	1.59	4.67
Cluster II	5.69	4.76	105.66	80.00	5.00	2.50	4.00	4.38	2.50	3.88	2.00	3.38
Cluster III	7.30	5.26	141.67	43.28	2.67	3.00	4.50	4.33	1.00	4.00	1.93	4.00
Cluster IV	6.25	5.97	148.15	54.10	4.75	2.50	3.50	4.00	2.25	3.50	1.65	8.00
Cluster V	4.18	4.23	77.07	63.82	4.83	2.58	4.08	4.58	2.75	3.67	1.97	3.17
Cluster VI	6.88	5.89	150.65	26.20	1.00	2.00	4.00	4.00	1.00	4.00	2.00	3.50

Table 25 contd....

Cluster	Seed weight (g)	Pulp:seed ratio	TSS (^o brix)	Reducing sugars (%)	Non-reducing sugars (%)	Total sugars (%)	Titration acidity (%)	Sugar:acid ratio	Ascorbic acid (mg/100g)	Total phenols (mg/100g)
Cluster I	3.36	21.51	22.12	7.77	1.78	9.55	0.04	262.74	7.83	0.55
Cluster II	2.93	35.43	23.08	8.27	1.60	9.87	0.04	353.26	9.38	0.42
Cluster III	3.31	42.46	21.83	7.20	1.92	9.12	0.04	225.44	10.14	0.40
Cluster IV	7.03	21.10	21.65	7.12	2.36	9.48	0.04	300.38	6.63	0.42
Cluster V	1.81	41.89	22.78	8.46	1.75	10.20	0.03	379.50	8.40	0.46
Cluster VI	1.49	100.80	22.30	8.90	1.25	10.14	0.04	276.05	10.25	0.33

Table 26: Eigen values, per cent variability and cumulative variability for principal components for isozyme bands in sapota genotypes

	PC1	PC2	PC3
Eigen value	1.82	1.11	0.66
Per cent variance explained	45.40	27.64	16.45
Cumulative variance explained	45.40	73.04	89.49

Table 27: Character loading of principal components for isozyme bands in sapota genotypes

Isozyme	PC1	PC2	PC3
Esterase	0.60	0.27	0.30
Peroxidase	0.58	-0.29	0.47
Polyphenol oxidase	0.54	0.16	-0.80
Catalase	0.09	-0.90	-0.21

Table 28: PCA mean scores for isozyme bands in sapota genotypes

Genotype	PC1	PC2	PC3
Badami	6.54	-5.35	0.27
Brazilian Sapota	6.58	-5.35	0.13
Calcutta Round	7.06	-5.51	0.35
Cricket Ball	7.06	-5.18	0.61
Dwarapudi	6.61	-5.30	0.35
Gavarayya	6.49	-5.52	0.26
Gede Sapota	6.58	-5.20	0.37
Gutti	6.74	-5.48	0.28
Kalipatti	7.51	-4.86	-0.31
Kirthibarthi	7.54	-4.82	-0.58
Krishna Rao	6.10	0.31	1.24
Pakala Round	6.70	-5.24	0.31
Pala	6.24	-5.42	0.11
Singapore	7.13	-5.24	0.72
Tagarampudi	6.54	-5.34	0.10
Ulavapadu Selection	6.56	-5.37	0.18
CO – 1	6.85	-4.81	0.54
CO – 2	7.48	-4.62	-0.34
CO – 3	6.72	-5.49	-0.12
DHS – 1	7.03	-5.30	0.73
DHS – 2	6.96	-5.29	0.16
PKM – 1	7.83	-5.15	-0.44
PKM – 2	7.81	-4.86	-0.64
PKM – 3	6.43	0.25	1.55
PKM - 4	6.69	-5.33	0.36

Table 29: Clustering of sapota genotypes for isozyme bands

Cluster	Genotype
Cluster I	Brazilian Sapota, Tagarampudi, Ulavapadu Selection, Badami, Gavarayya, Gutti, Dwarapudi, PKM – 4, Pakala Round, Gede Sapota, Pala and CO – 3
Cluster II	Calcutta Round and DHS – 2
Cluster III	Singapore, DHS – 1, Cricket Ball and CO - 1
Cluster IV	Kalipatti and CO – 2
Cluster V	Kirthibarthi, PKM – 2 and PKM - 1
Cluster VI	Krishna Rao and PKM – 3

Table 30: Intra and inter-cluster distances for isozyme bands in sapota genotypes

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	0.10	0.25	0.53	1.58	2.32	33.48
Cluster II		0.09	0.30	1.05	1.54	34.22
Cluster III			0.14	1.40	2.17	30.52
Cluster IV				0.06	0.23	29.71
Cluster V					0.09	33.53
Cluster VI						0.21

Note: Figures in bold indicate intra-cluster distances

Table 31: Cluster means for isozyme bands in sapota genotypes

Isozyme	Esterase	Peroxidase	Polyphenol oxidase	Catalase
Cluster				
Cluster I	4.00	4.27	2.14	6.12
Cluster II	4.29	4.52	2.33	6.20
Cluster III	4.50	4.63	2.06	5.88
Cluster IV	4.43	4.48	3.21	5.68
Cluster V	4.50	4.60	3.52	5.94
Cluster VI	4.27	4.21	2.32	0.00

Table 14: Eigen values, per cent variability and cumulative variability for principal components of tree morphological characters in sapota genotypes

	PC1	PC2	PC3	PC4
Eigen value	4.90	2.61	1.41	0.85
Per cent variance explained	40.81	21.72	11.71	7.04
Cumulative variance explained	40.81	62.53	74.23	81.27

Table 15: Character loading of principal components for tree morphological characters in sapota genotypes

Character	PC1	PC2	PC3	PC4
Growth habit of the tree	0.16	0.02	0.72	0.07
Shape of the tree	-0.26	-0.38	-0.14	-0.26
Branching pattern	0.04	0.30	0.27	-0.86
Branch density	-0.04	0.48	-0.40	-0.19
Leaf length (cm)	0.38	0.17	-0.06	0.14
Leaf width (cm)	0.28	0.11	-0.27	-0.06
Leaf area (cm ²)	0.41	-0.16	-0.04	-0.16
Pollen size (µm)	-0.44	0.10	-0.001	0.03
Pollen production / anther	0.34	0.003	-0.04	0.092
Pollen fertility	0.02	-0.47	-0.31	-0.26
Length of style (cm)	-0.15	0.49	-0.16	0.17

Table 16: PCA mean scores for tree morphological characters in sapota genotypes

Genotype	PC1	PC2	PC3
Badami	129.60	-935.17	-640.06
Brazilian Sapota	127.93	-949.52	-639.64
Calcutta Round	135.95	-947.36	-642.35
Cricket Ball	183.03	-973.47	-655.06
Dwarapudi	142.27	-916.25	-644.99
Gavarayya	155.36	-953.82	-646.13
Gede Sapota	206.78	-943.44	-660.97
Gutti	144.65	-933.54	-642.53
Kalipatti	116.96	-956.43	-654.11
Kirthibarthi	126.03	-951.18	-643.49
Krishna Rao	133.23	-951.55	-637.01
Pakala Round	135.76	-951.65	-643.46
Pala	124.17	-960.10	-651.16
Singapore	148.59	-960.87	-652.68
Tagarampudi	145.03	-936.03	-644.30
Ulavapadu Selection	129.96	-933.75	-645.38
CO – 1	164.32	-939.51	-632.11
CO – 2	168.77	-929.95	-643.64
CO – 3	186.25	-944.24	-646.64
DHS – 1	206.89	979.83	-648.77
DHS – 2	142.26	-944.39	-647.59
PKM – 1	126.16	-938.23	-642.18
PKM – 2	138.53	-953.43	-647.65
PKM – 3	185.34	-974.16	-657.74
PKM - 4	166.45	-957.53	-647.42

Table 17: Clustering of sapota genotypes for tree morphological characters

Cluster	Genotypes
Cluster I	Badami, PKM – 1, Ulavapadu Selection, Gutti, Tagrampudi, Dwarapudi
Cluster II	Brazilian Sapota, Kirthibarthi, Calcutta Round, Pakala Round, Krishna Rao, DHS – 2, PKM – 2, Kalipatti, Pala
Cluster III	Gavarayya, PKM – 4, Singapore
Cluster IV	CO – 1, CO – 2
Cluster V	Cricket Ball, PKM – 3, DHS – 1
Cluster VI	Gede Sapota, CO - 3

Table 18: Intra and inter-cluster distances for tree morphological characters in sapota genotypes

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	294.09	727.55	1417.80	1137.40	5818.70	4249.59
Cluster II		244.15	902.49	1868.40	4723.20	4681.55
Cluster III			273.95	1021.70	1935.50	2051.82
Cluster IV				246.64	3291.30	1568.55
Cluster V					435.24	1514.09
Cluster VI						680.52

Note: Figures in bold indicate intra-cluster distances

Table 19: Cluster means for tree morphological characters in sapota genotypes

Cluster	Tree characters				Leaf characters			Floral characters			
	Growth habit	Shape of the tree	Branching pattern	Branch density	Leaf length (cm)	Leaf width (cm)	Leaf area (cm ²)	Pollen size (µm)	Pollen production / anther	Pollen fertility (%)	Length of style (cm)
Cluster I	2.67	4.42	2.75	2.83	9.26	3.15	17.85	16.14	725.13	96.93	0.80
Cluster II	2.67	5.78	2.72	2.28	8.69	3.24	17.74	15.94	623.90	97.66	0.69
Cluster III	2.67	5.67	2.17	2.33	9.80	3.63	23.39	15.93	695.79	97.59	0.68
Cluster IV	3.25	1.75	3.00	1.25	10.47	3.47	24.19	16.04	722.11	96.62	0.73
Cluster V	3.00	5.33	2.50	2.00	10.09	3.58	34.20	16.15	744.18	98.06	0.81
Cluster VI	2.75	1.50	3.00	1.50	11.79	4.55	32.54	16.11	659.94	96.95	0.81

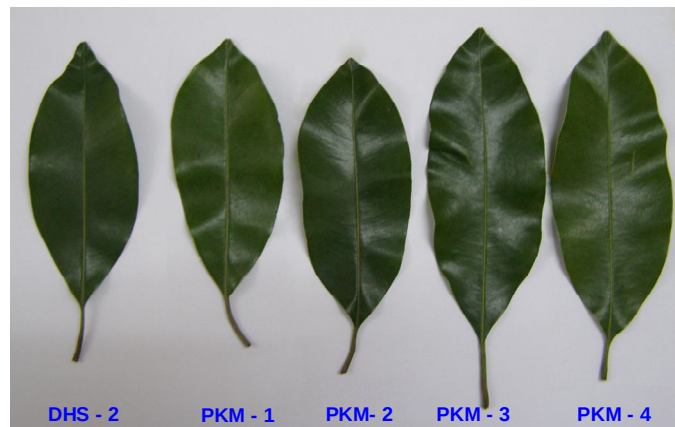
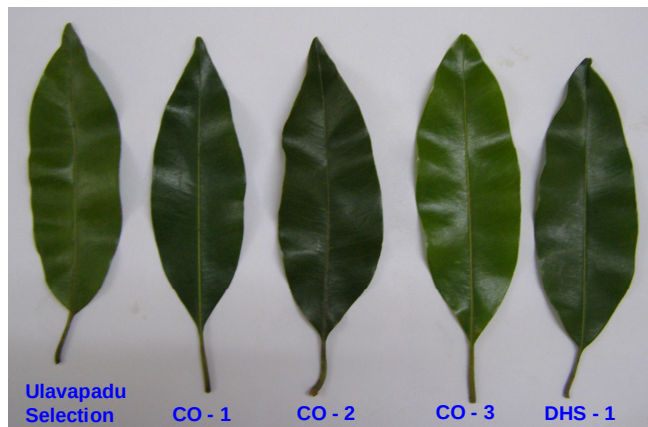
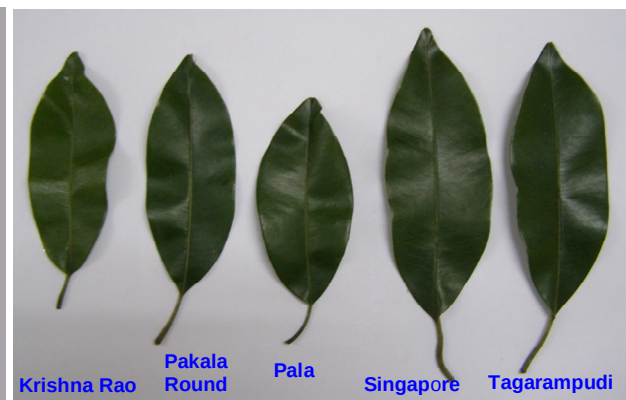
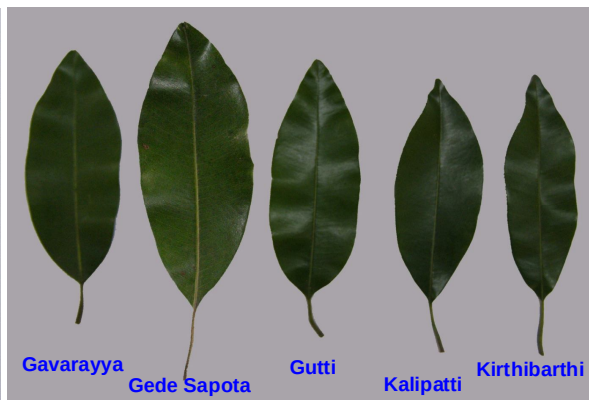


Plate 1: Variation in leaf size and shape among sapota genotypes

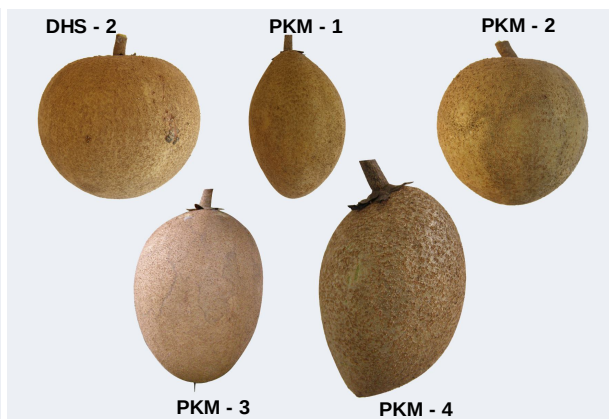
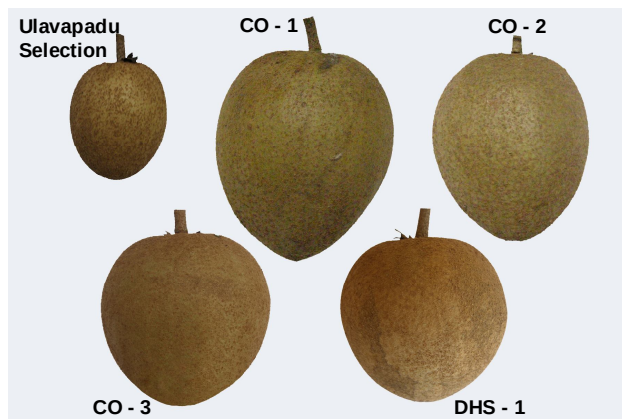
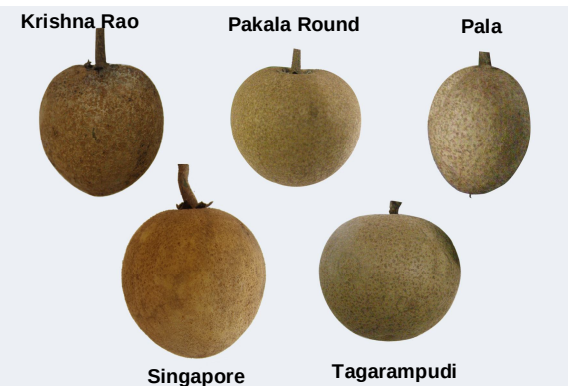
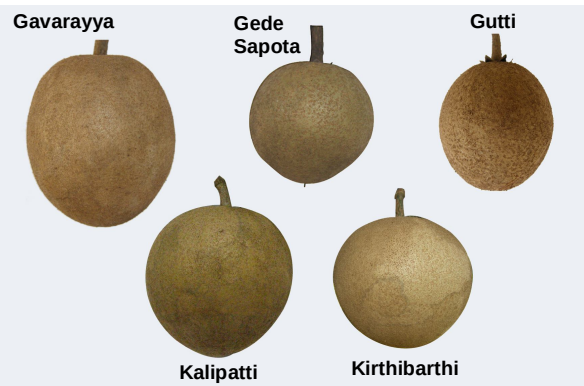
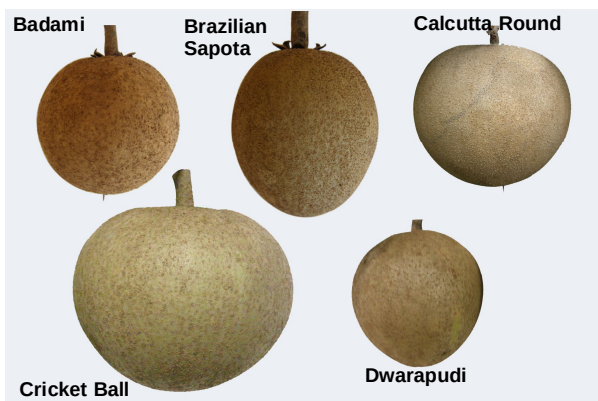


Plate 2: Variation in fruit size and shape among sapota genotypes

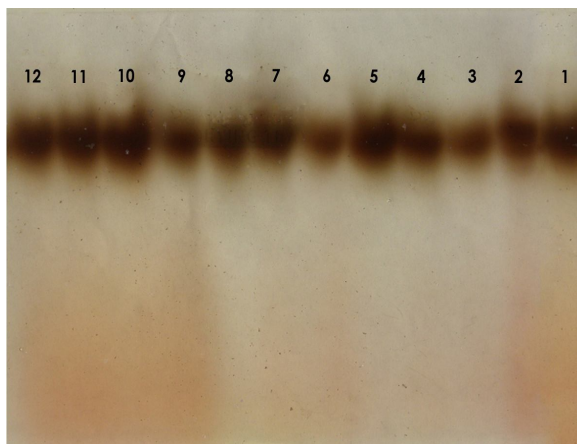
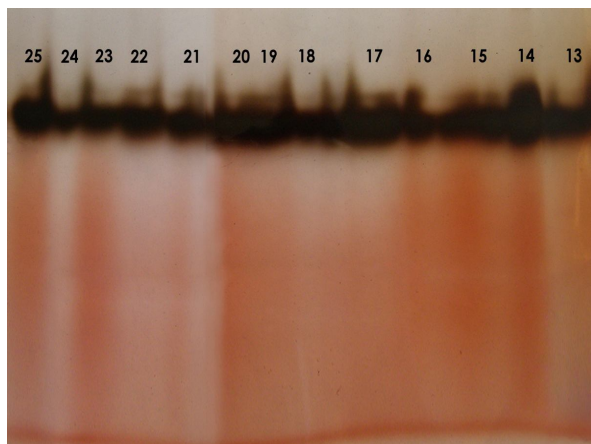


Plate 3: Esterase isozyme bands in sapota genotypes

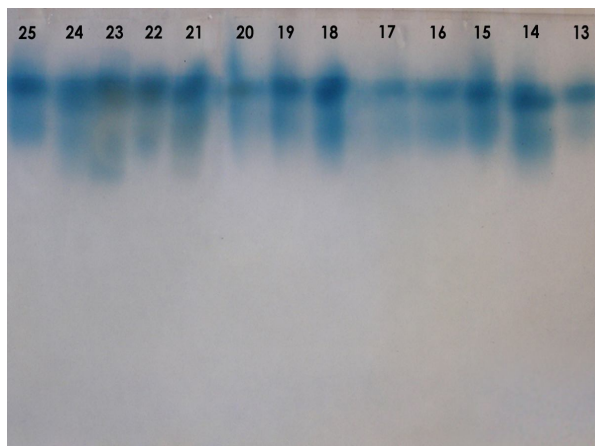


Plate 4: Peroxidase isozyme bands in sapota genotypes

1. Kalipatti
2. Calcutta Round
3. CO – 2
4. Kirthibarthi
5. PKM – 3
6. CO – 1
7. DHS – 2
8. Singapore
9. PKM – 2
10. Cricket Ball
11. DHS – 1
12. PKM – 1
13. Ulavapadu Selection
14. Gutti
15. Krishna Rao
16. PKM – 4
17. Gede Sapota
18. Brazilian Sapota
19. Dwarapudi
20. Tagarampudi
21. Badami
22. CO – 3
23. Pakala Round
24. Gavarayya
25. Pala

1. Cricket Ball
2. PKM – 3
3. Kalipatti
4. CO – 1
5. DHS – 2
6. Kirthibarthi
7. DHS – 1
8. CO – 2
9. Singapore
10. CO – 3
11. PKM – 2
12. PKM – 1
13. Ulavapadu Selection
14. Calcutta Round
15. Brazilian Sapota
16. Pala
17. Krishna Rao
18. Gavarayya
19. Dwarapudi
20. Gede Sapota
21. Badami
22. PKM – 4
23. Tagarampudi
24. Gutti
25. Pakala Round

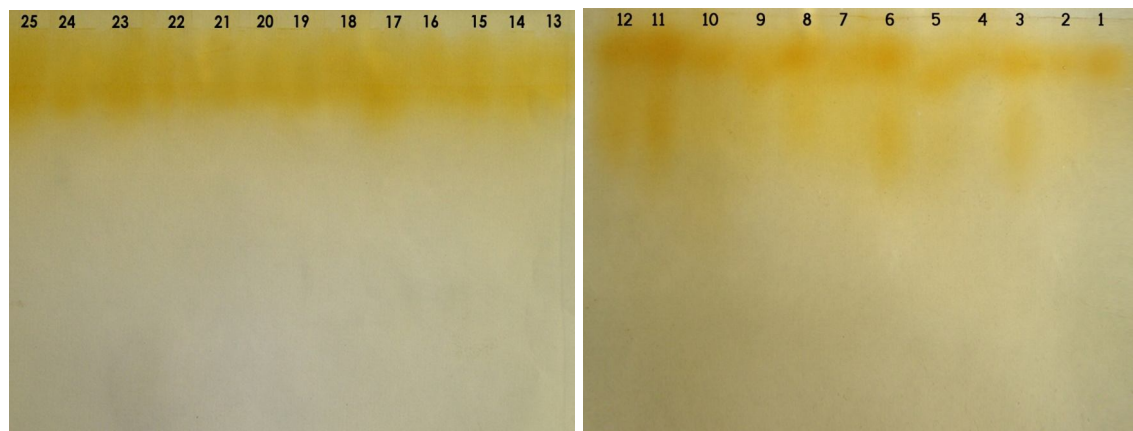


Plate 5: Polyphenol oxidase isozyme bands in sapota genotypes

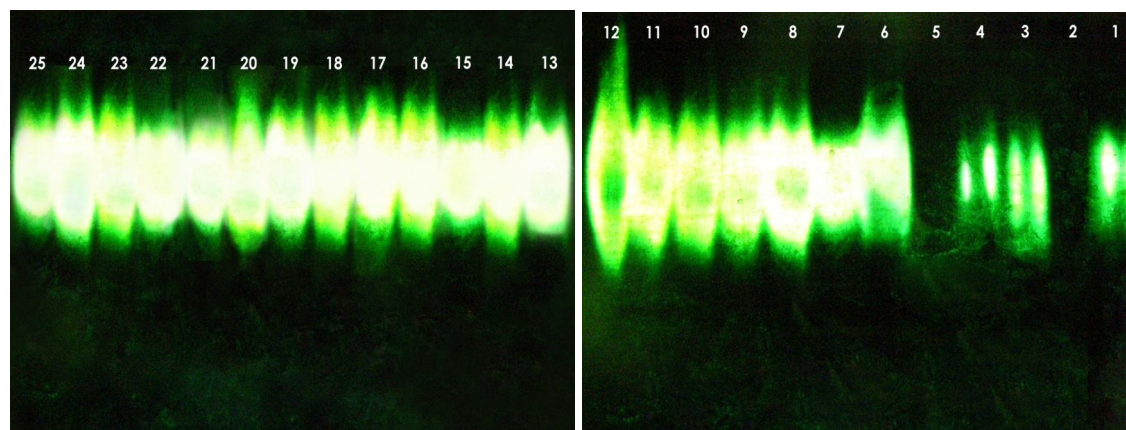


Plate 6: Catalase isozyme bands in sapota genotypes

1. Kalipatti
2. Krishna Rao
3. CO – 2
4. Kirthibarthi
5. PKM – 3
6. CO – 1
7. DHS – 2
8. PKM – 2
9. Cricket Ball
10. DHS – 1
11. PKM – 1
12. Singapore
13. Ulavapadu Selection
14. Calcutta Round
15. Brazilian Sapota
16. Pala
17. CO – 3
18. Gavarayya
19. Dwarapudi
20. Gede Sapota
21. Badami
22. PKM – 4
23. Tagarampudi
24. Gutti
25. Pakala Round

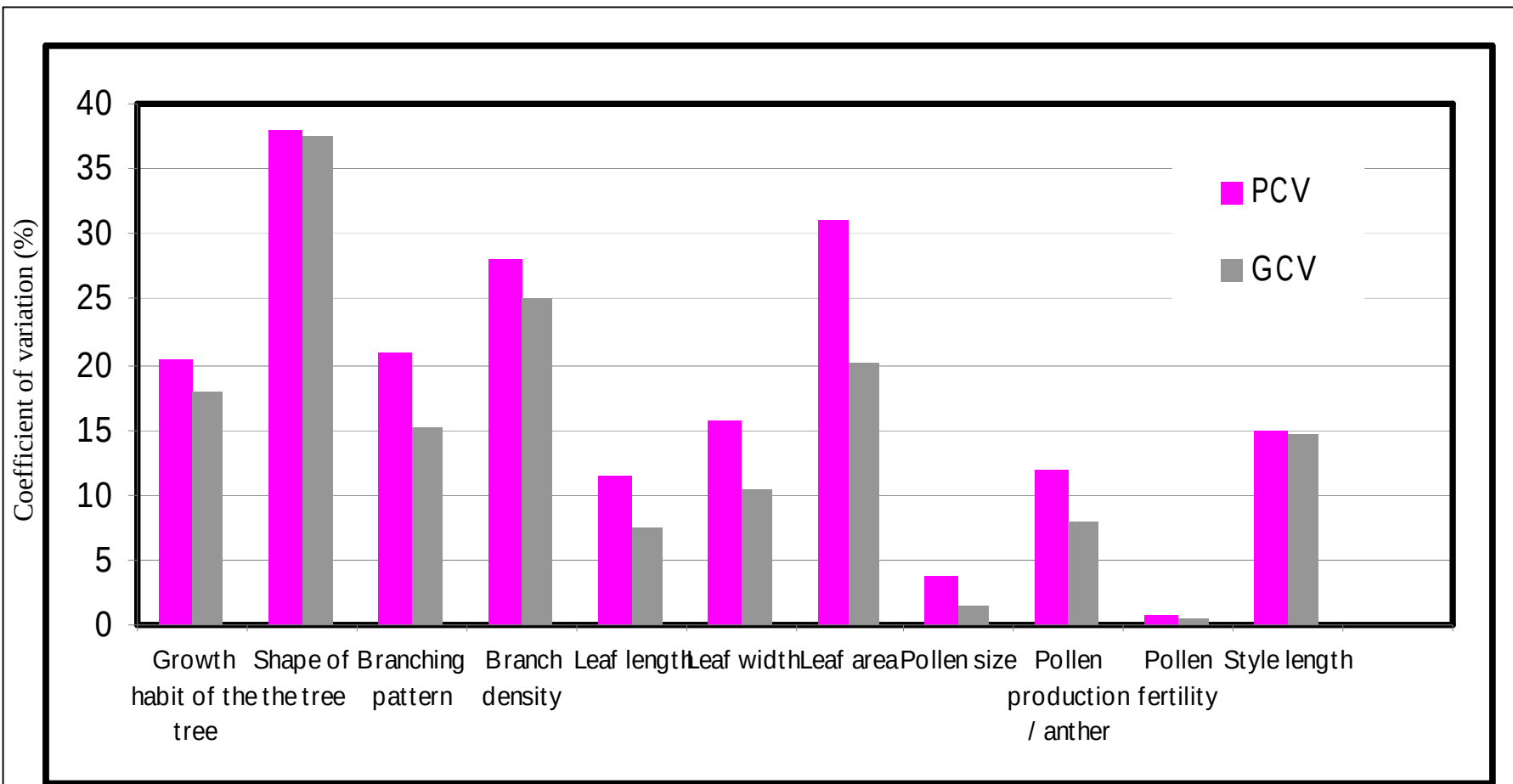


Fig. 5: Phenotypic and genotypic coefficients of variation for tree morphological characters in sapota genotypes

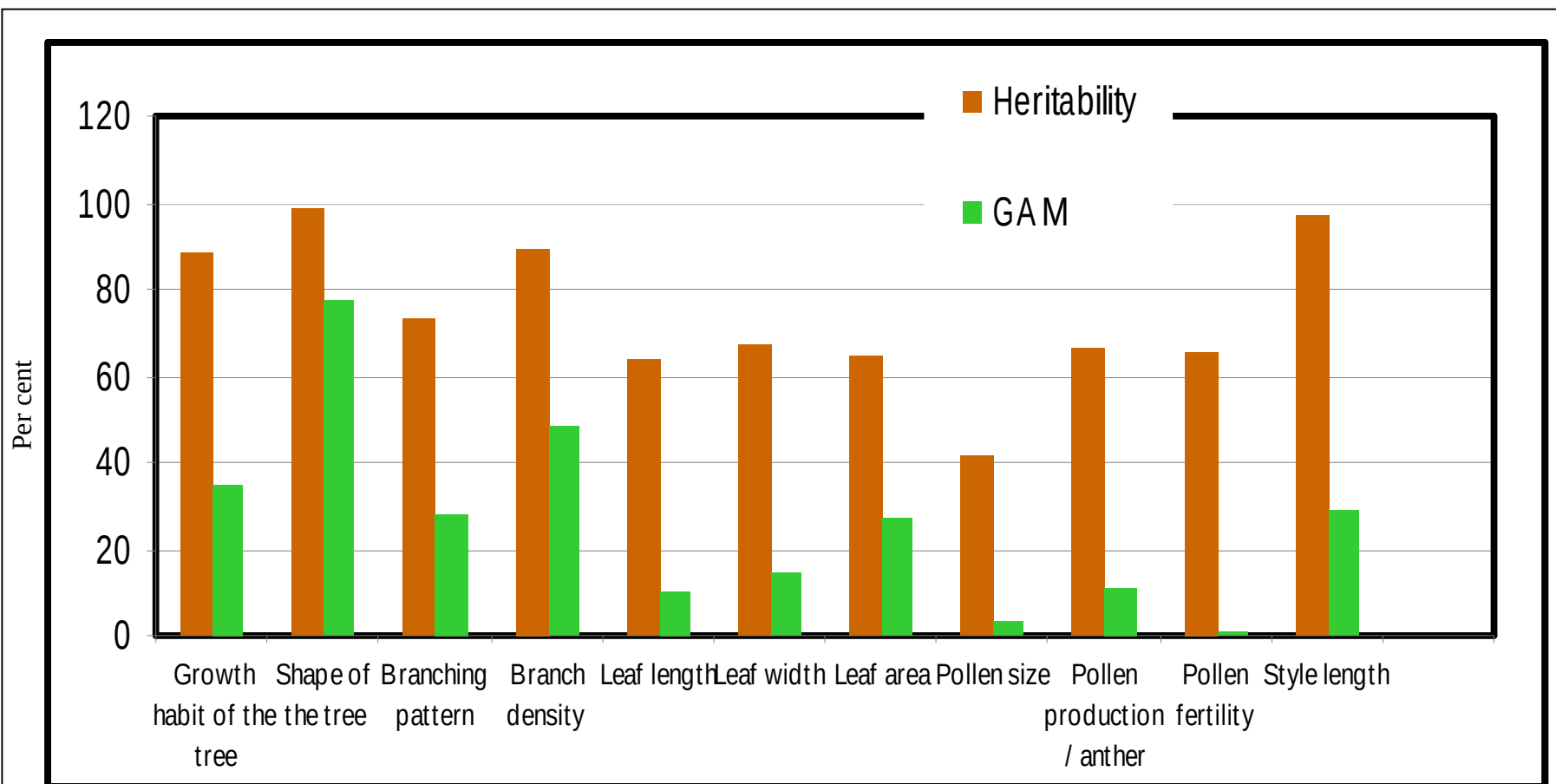


Fig. 6 Heritability and genetic advance as per cent mean for tree morphological characters in sapota genotypes

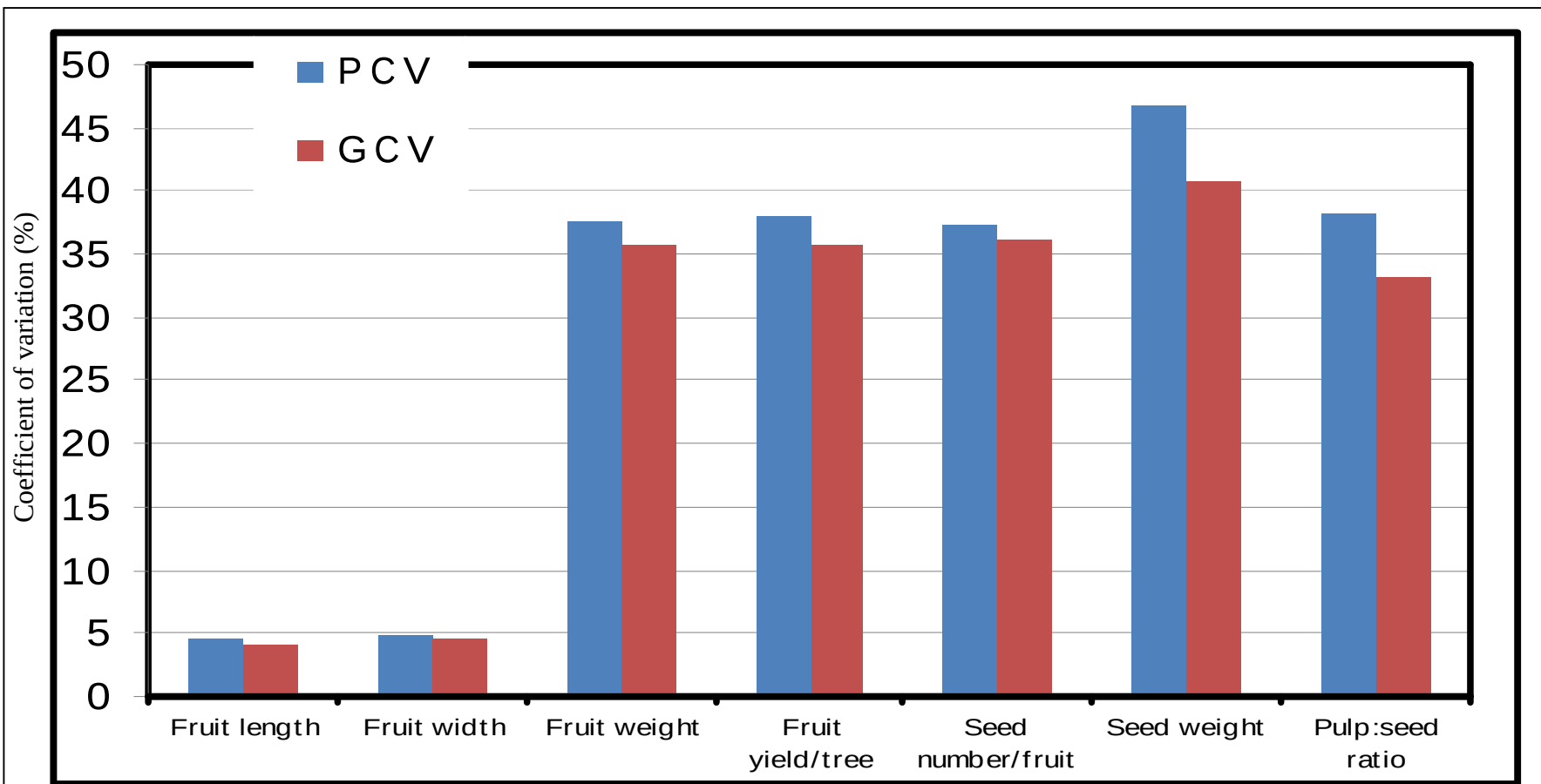


Fig. 7 Phenotypic and genotypic coefficients of variation for fruit morphological characters in saopta genotypes

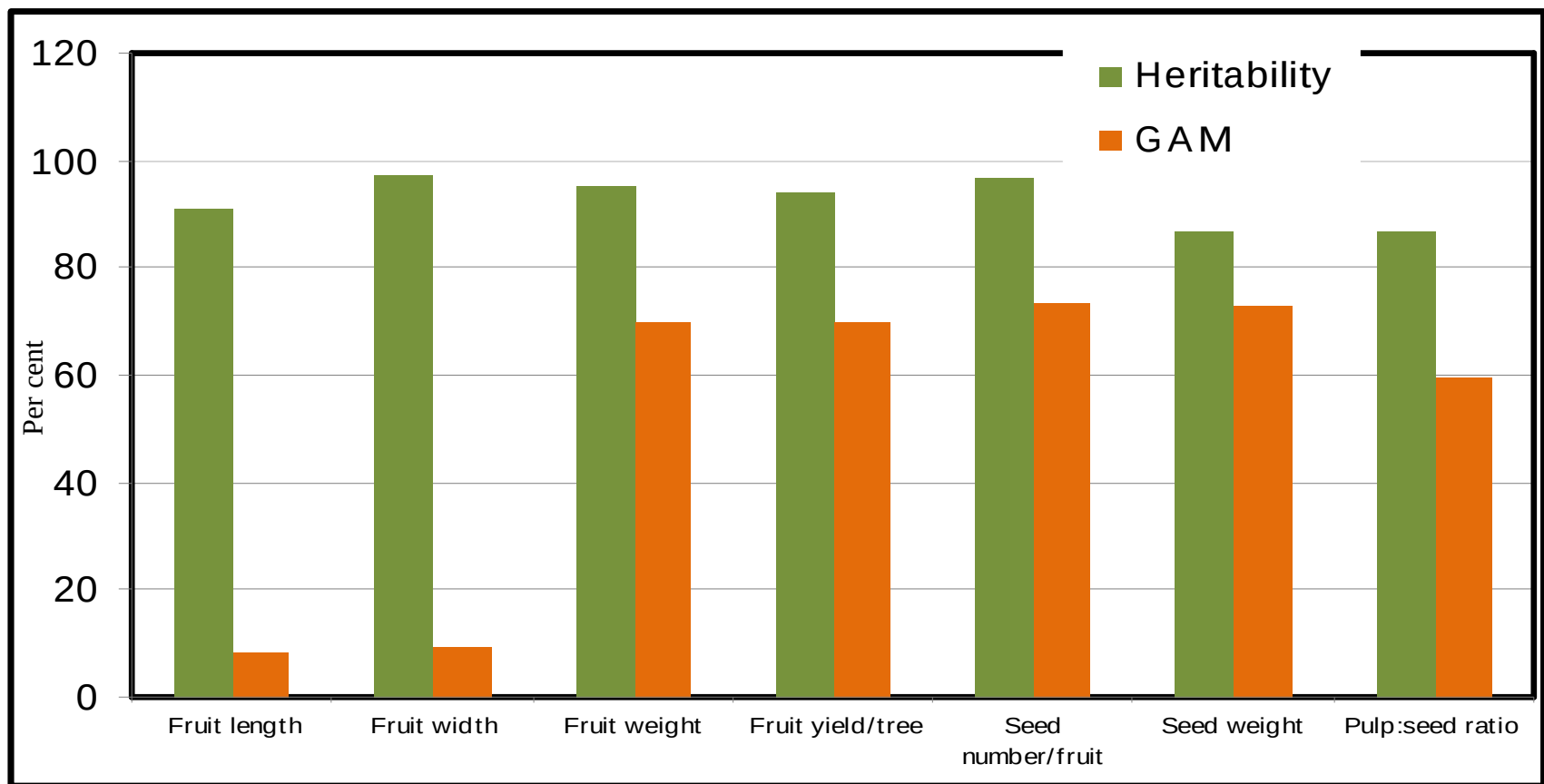


Fig. 8 Heritability and genetic advance as per cent mean for fruit morphological characters in sapota genotypes

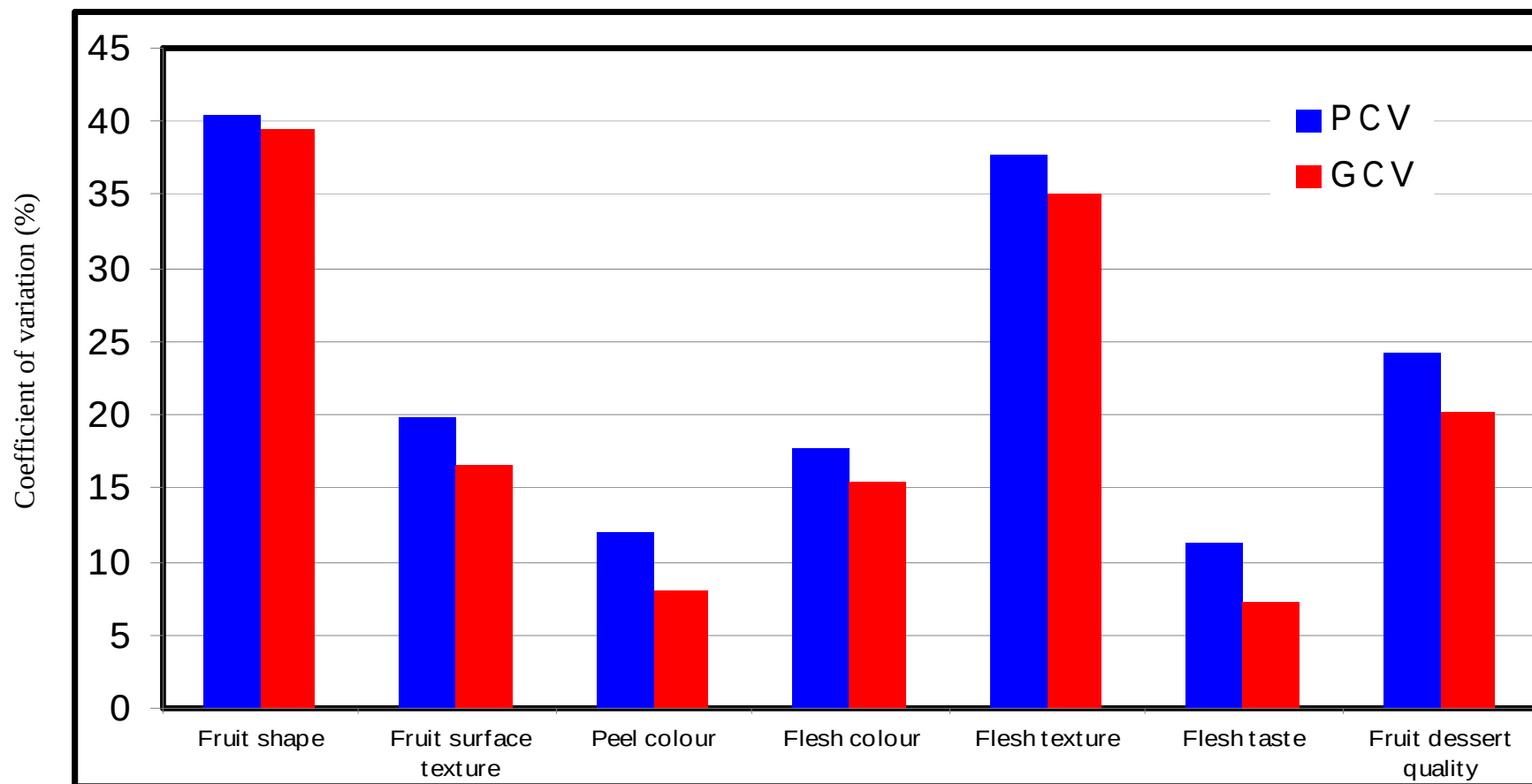
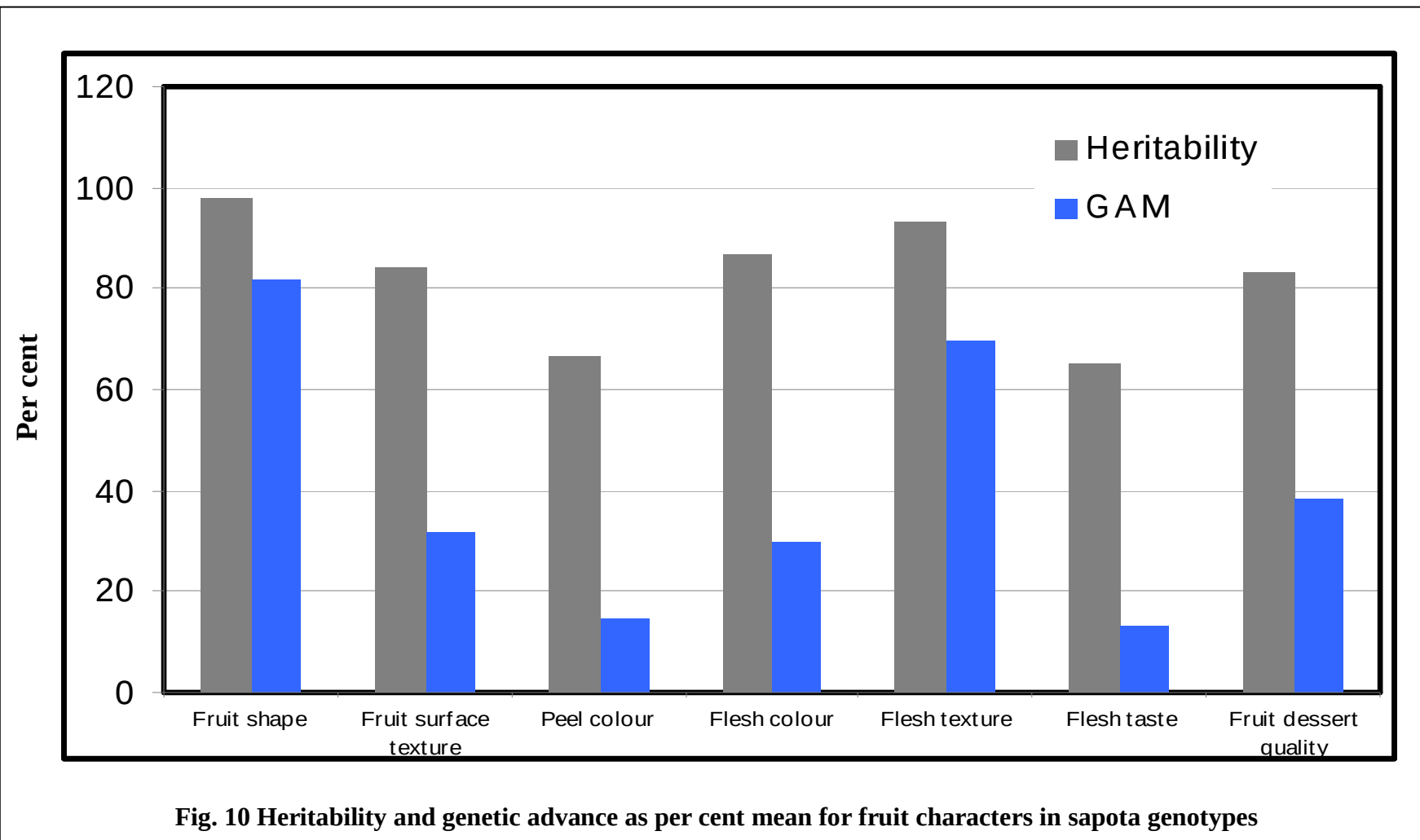
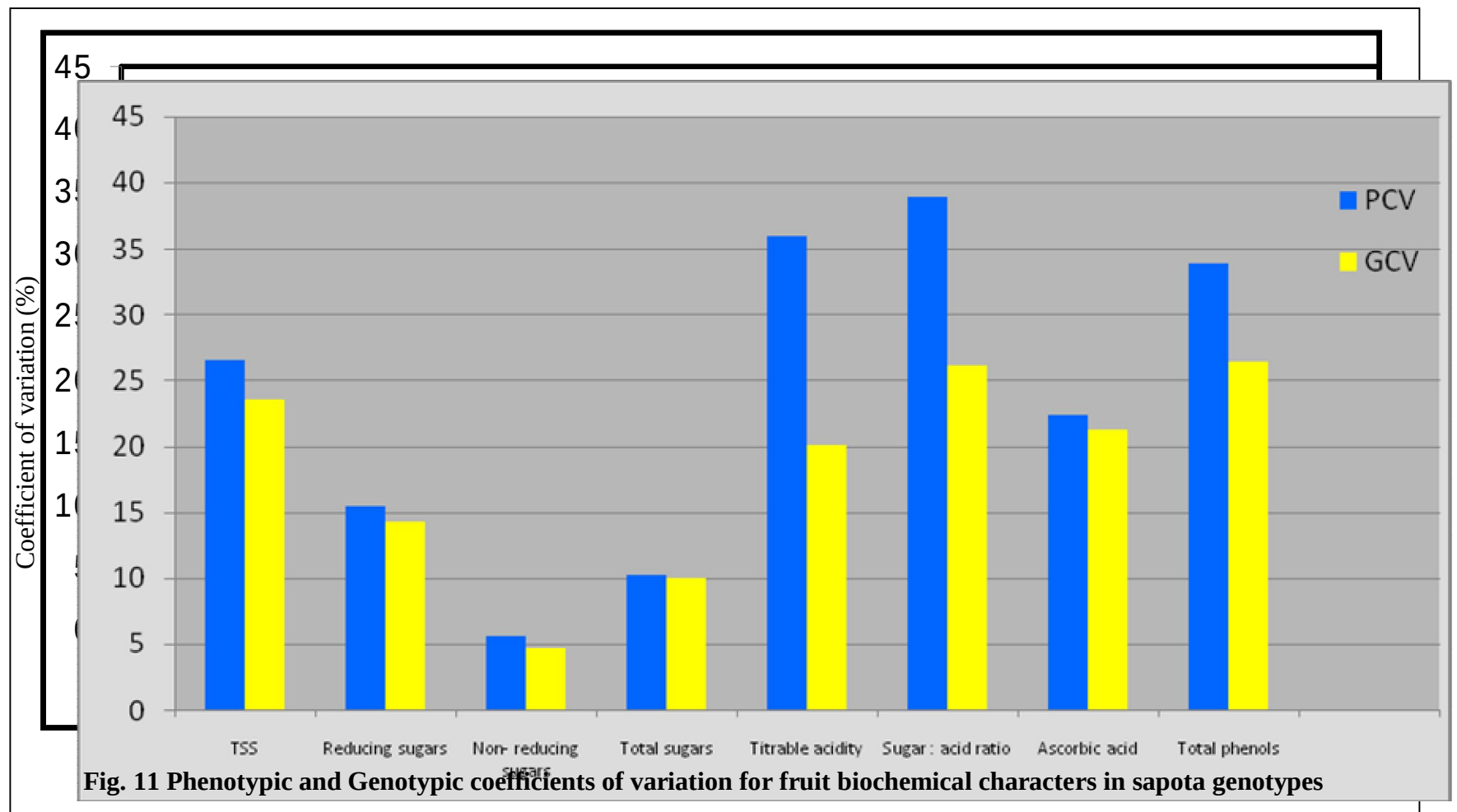


Fig. 9 Phenotypic and genotypic coefficients of variation for fruit characters in sapota genotypes





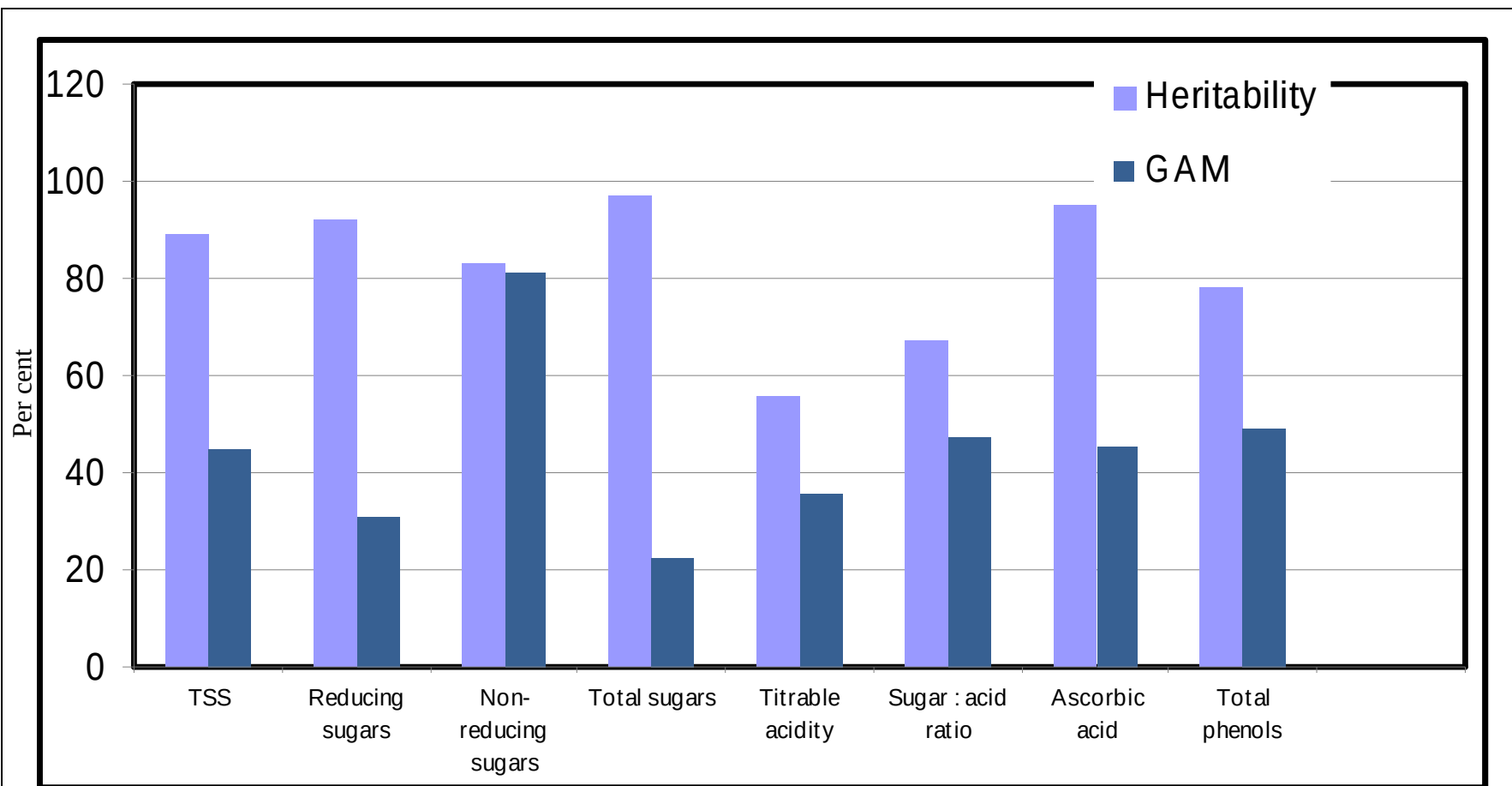
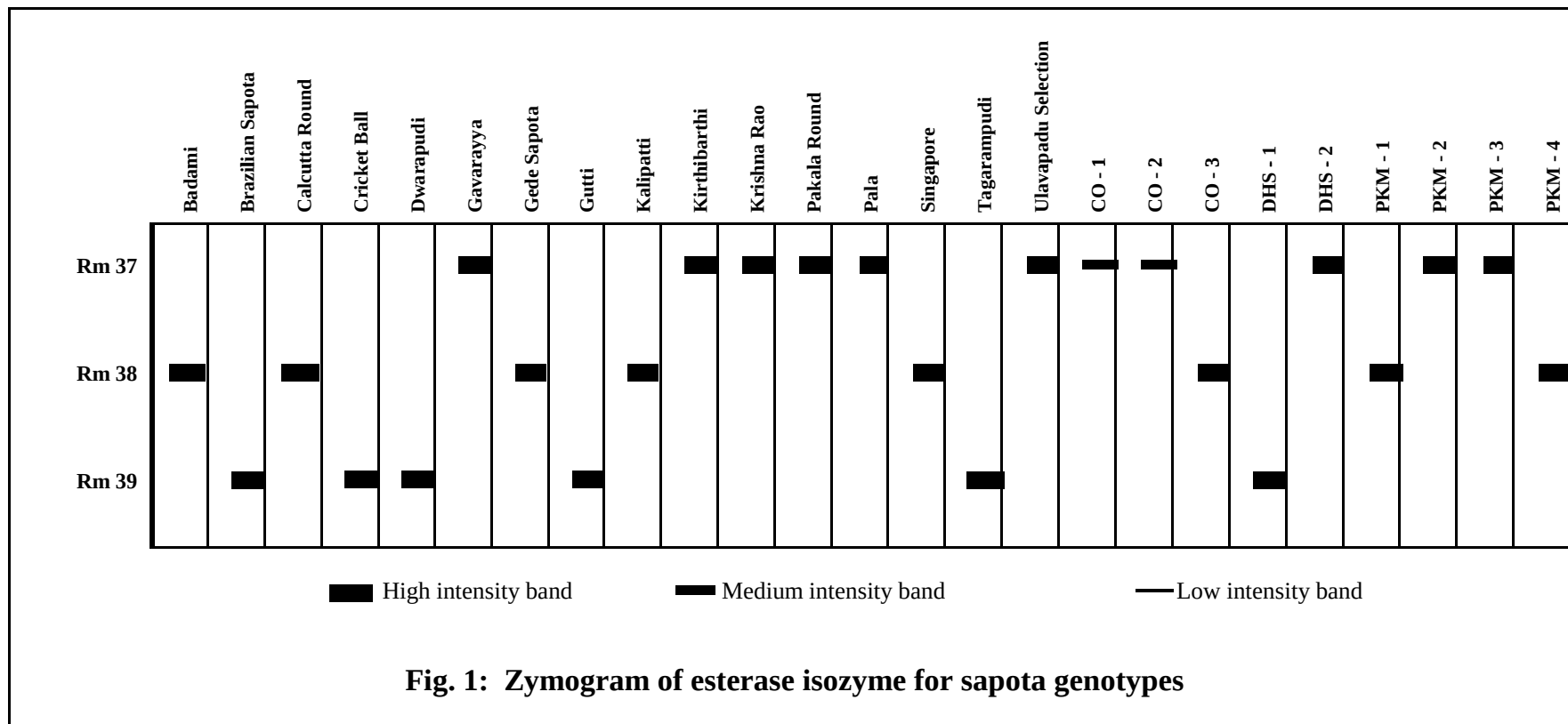


Fig. 12 Heritability and genetic advance as per cent mean for fruit biochemical characters in sapota genotypes



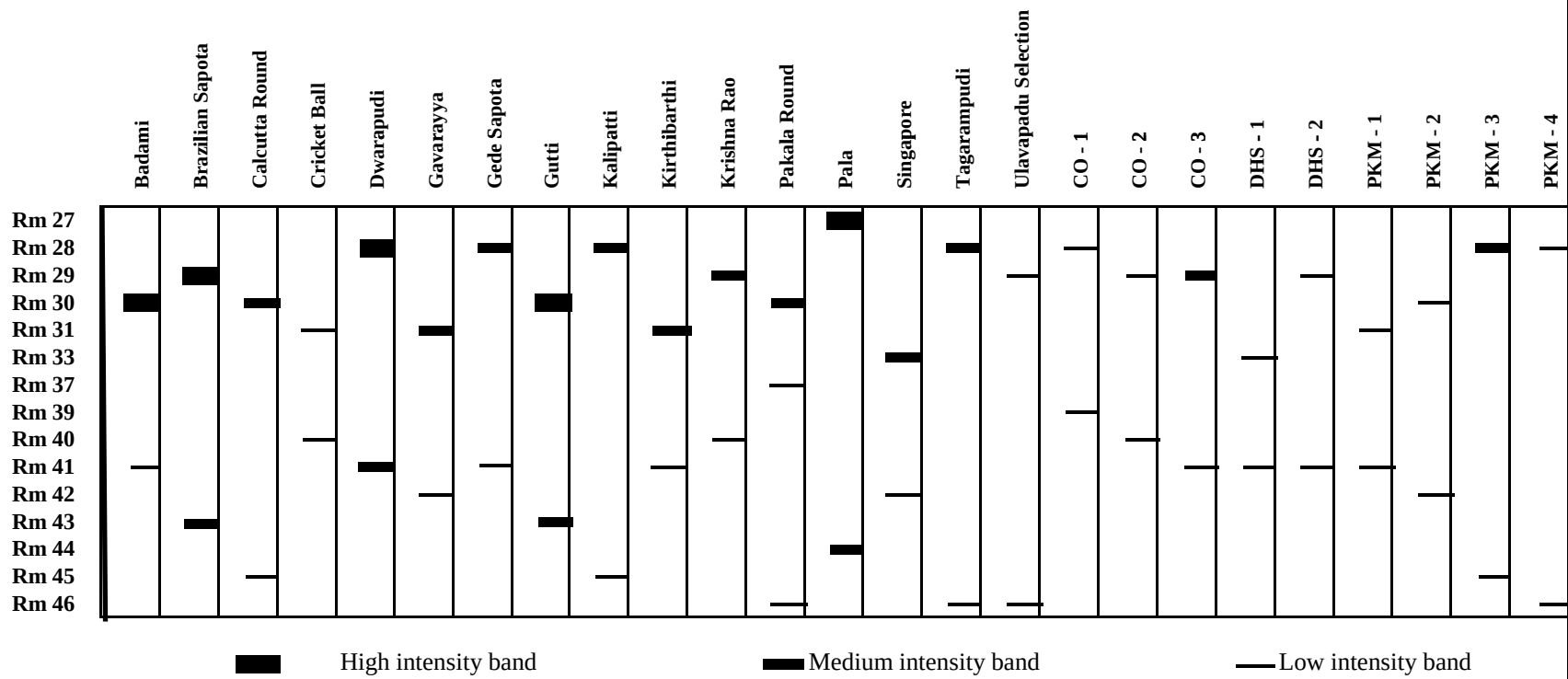


Fig. 2: Zymogram of peroxidase isozyme for sapota genotypes

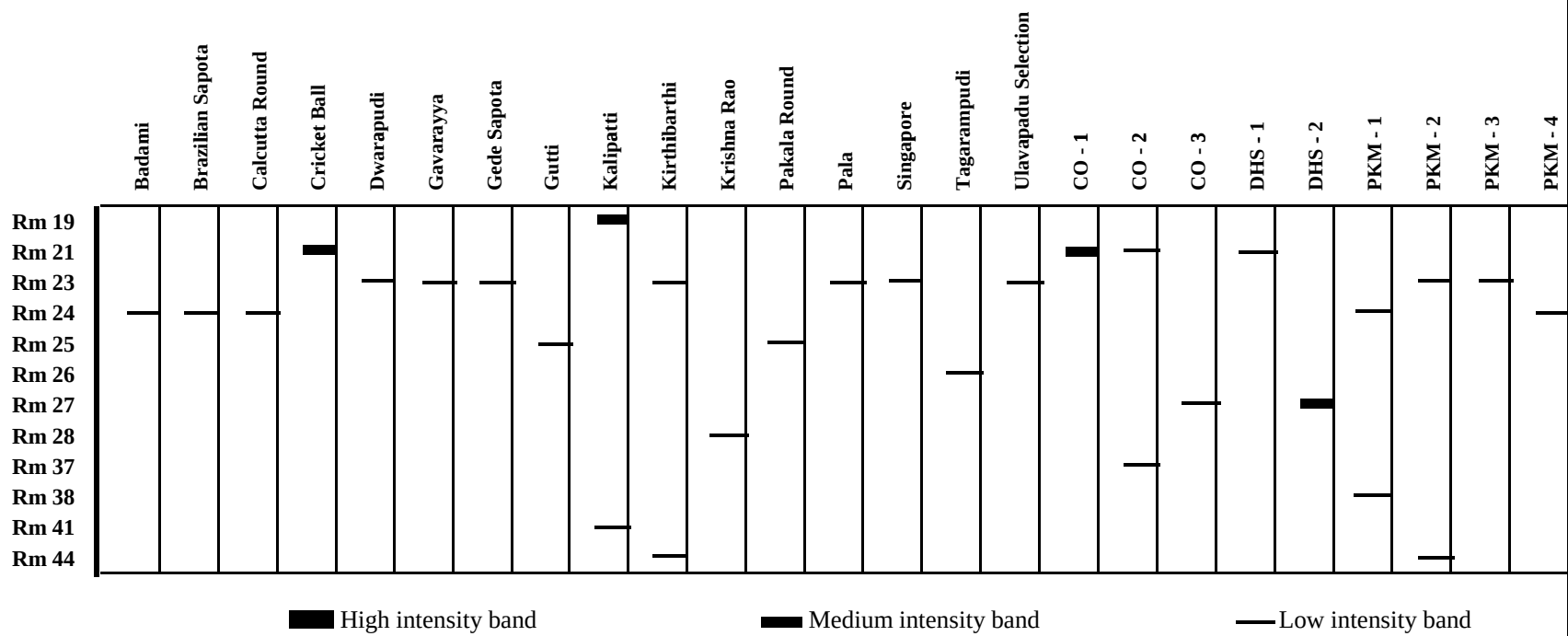


Fig. 3: Zymogram of polyphenol oxidase isozyme for sapota genotypes

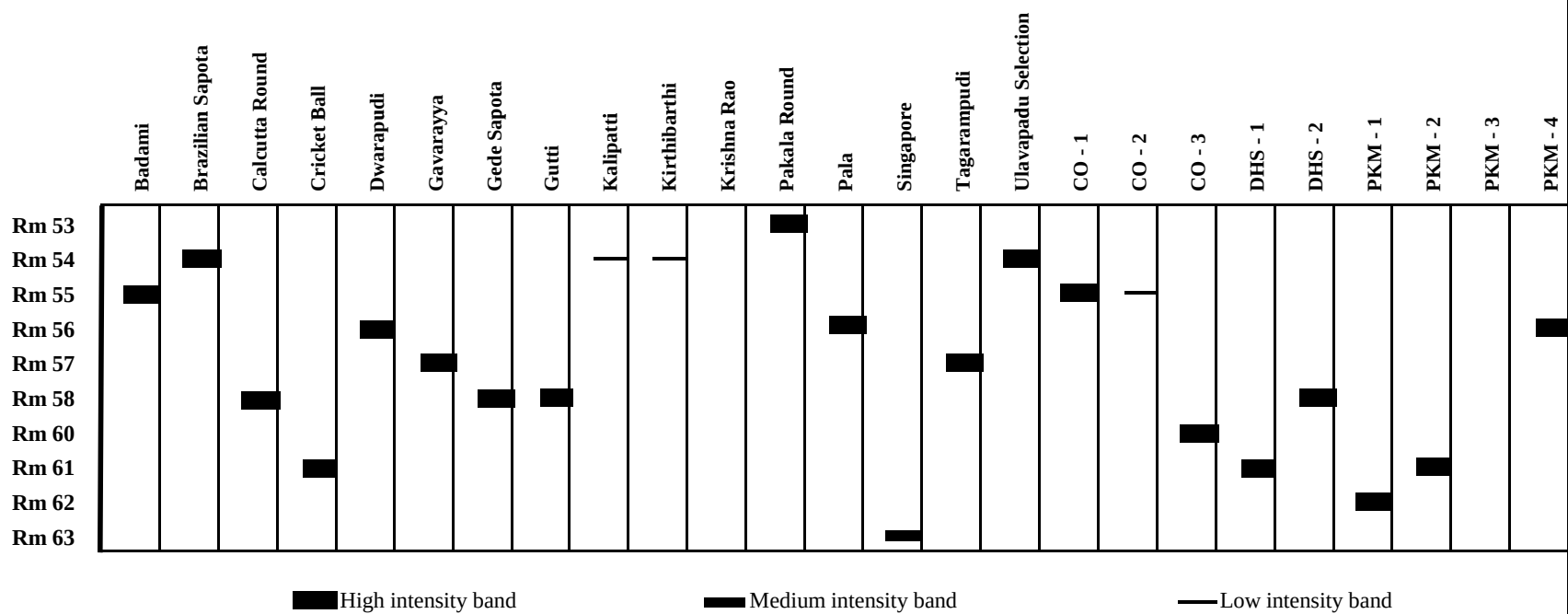
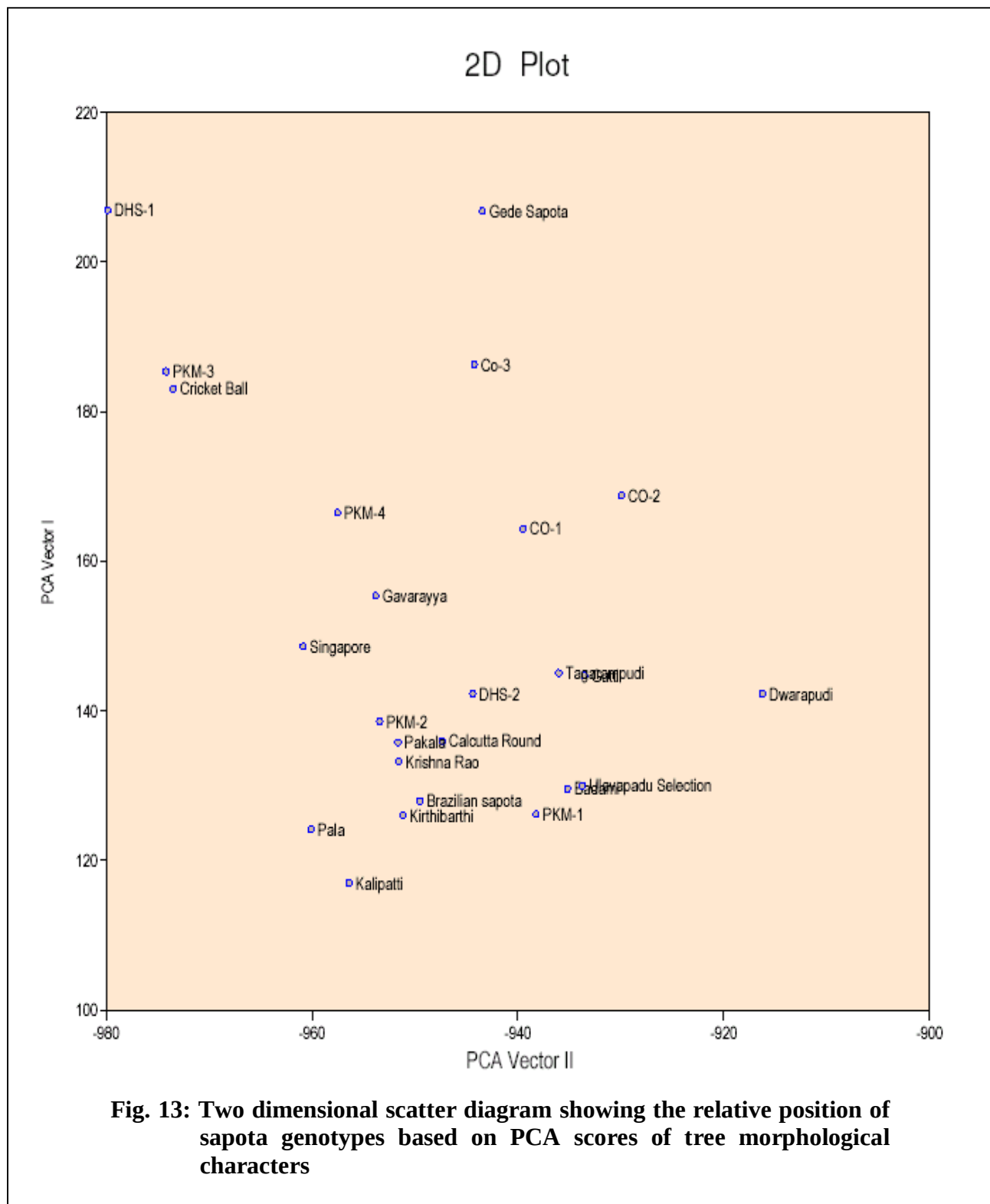


Fig. 4: Zymogram of catalase isozyme for sapota genotypes



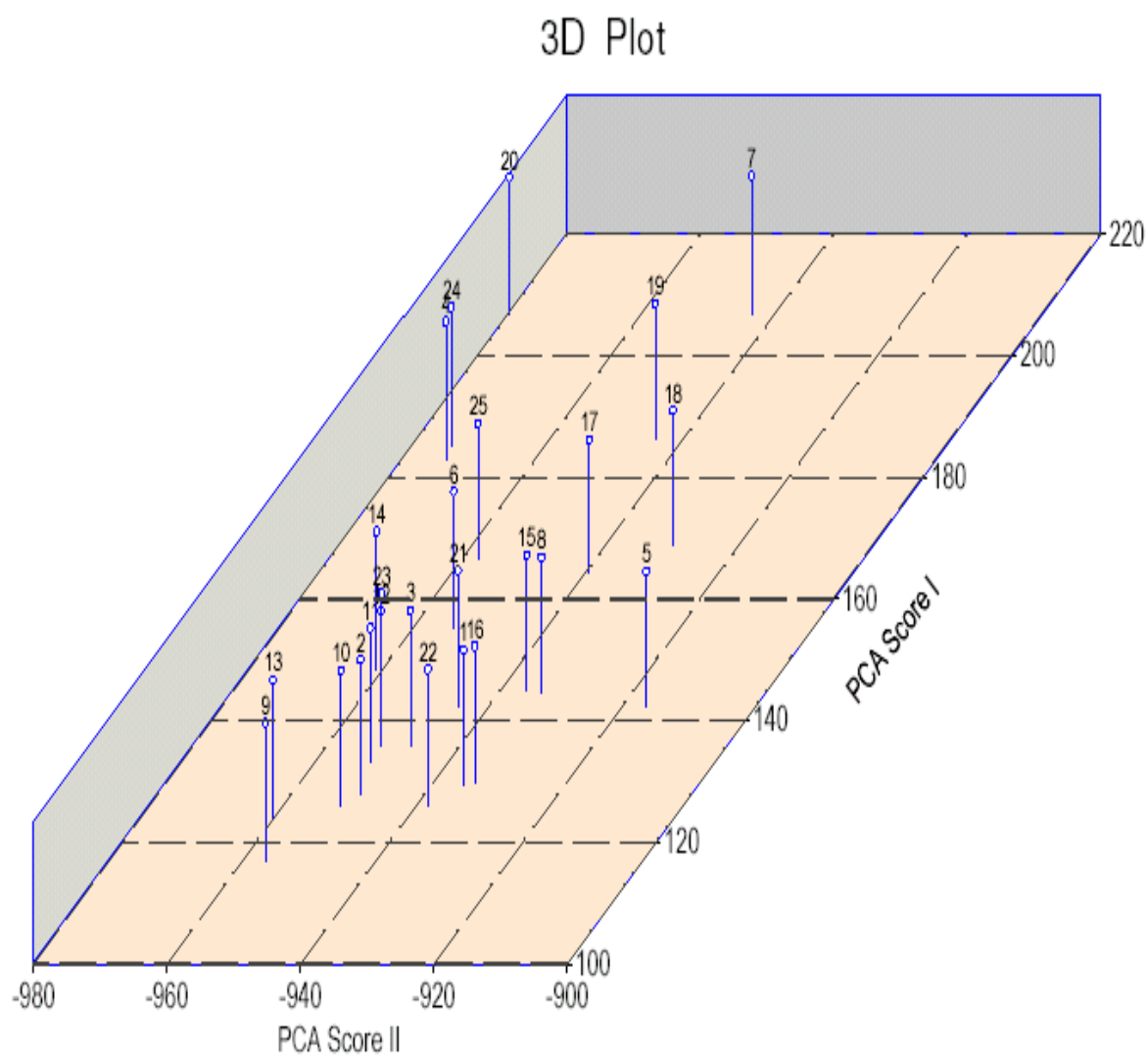


Fig. 14 Three dimensional scatter diagram showing the relative position of sapota genotypes based on PCA scores of tree morphological characters

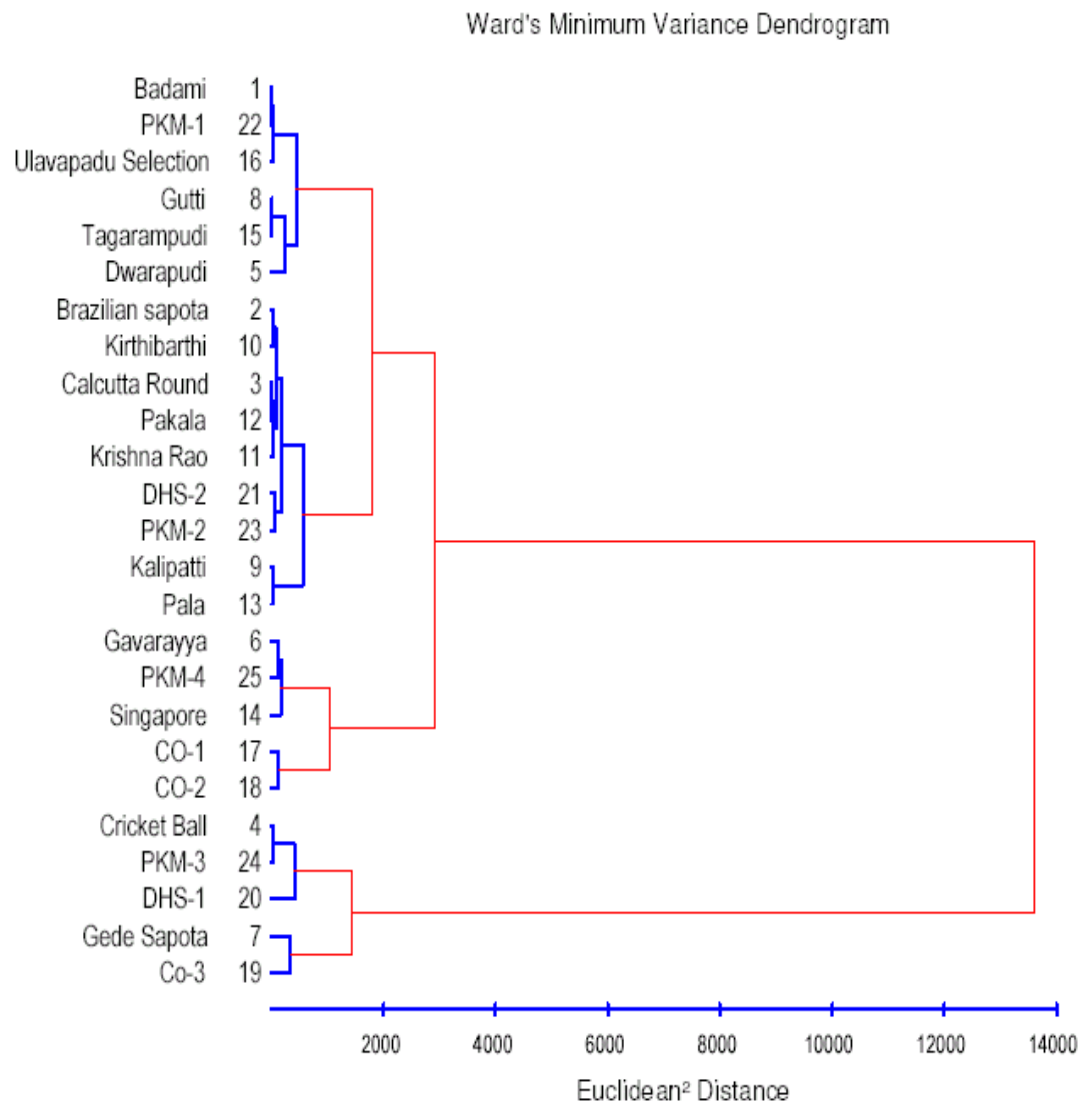
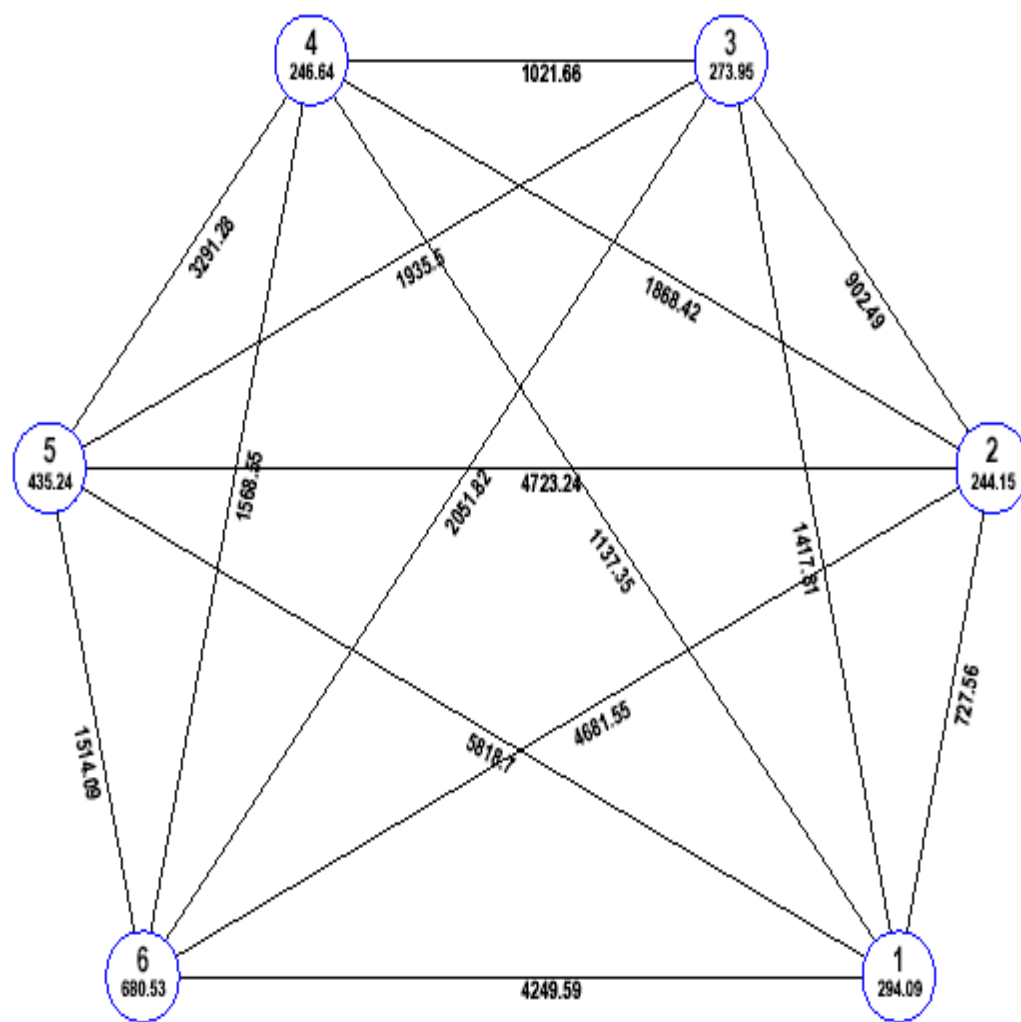


Fig. 15 Diagram illustrating the clustering pattern in sapota genotypes by Ward's minimum variance method for tree morphological characters



Euclidean² Distance (Not to the Scale)

Fig. 16 Cluster diagram showing intra and inter cluster distances for tree morphological characters in sapota genotypes

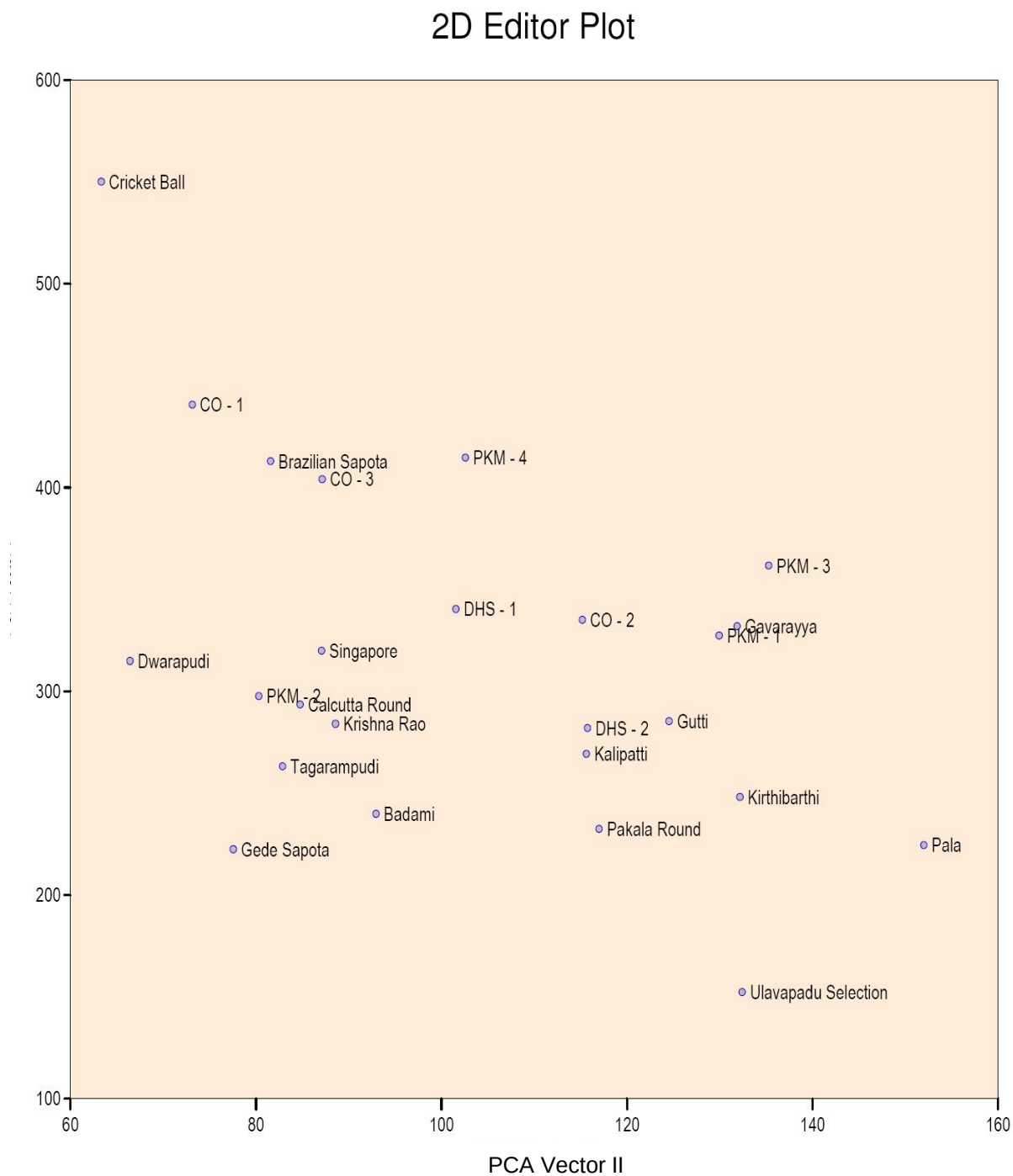


Fig. 17 Two dimensional scatter diagram showing the relative position of sapota genotypes based on PCA scores of fruit morphological and biochemical characters

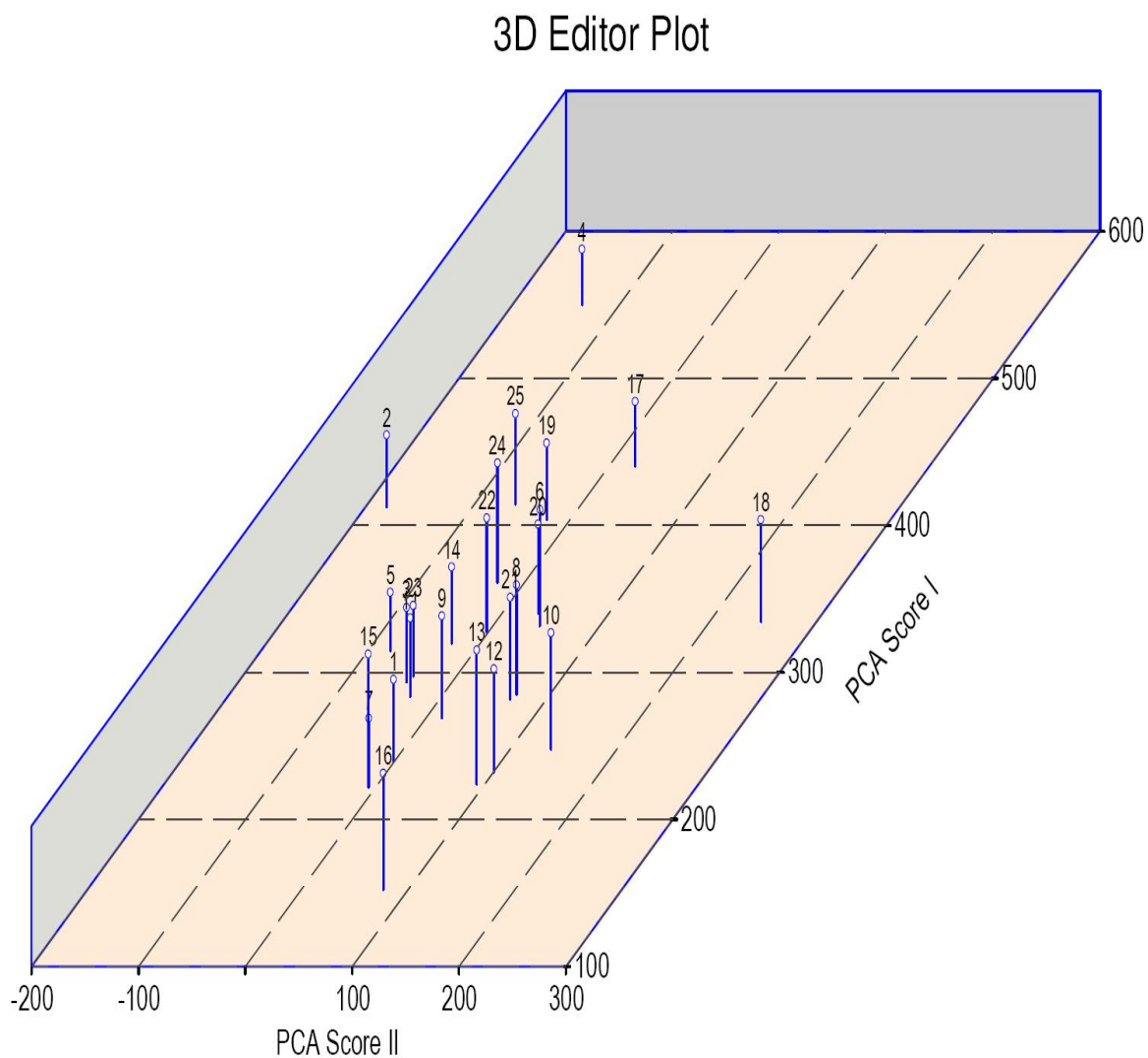
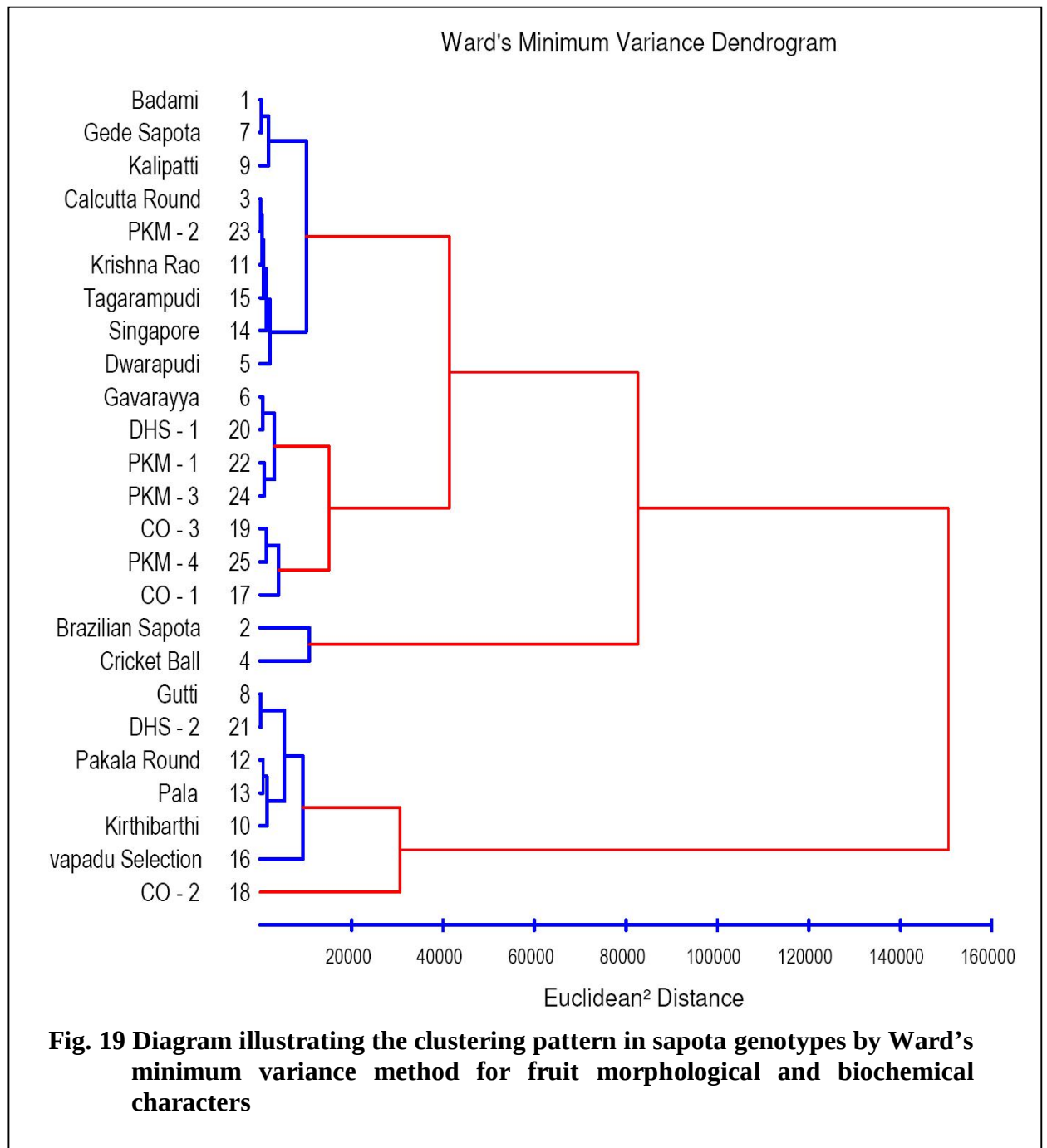
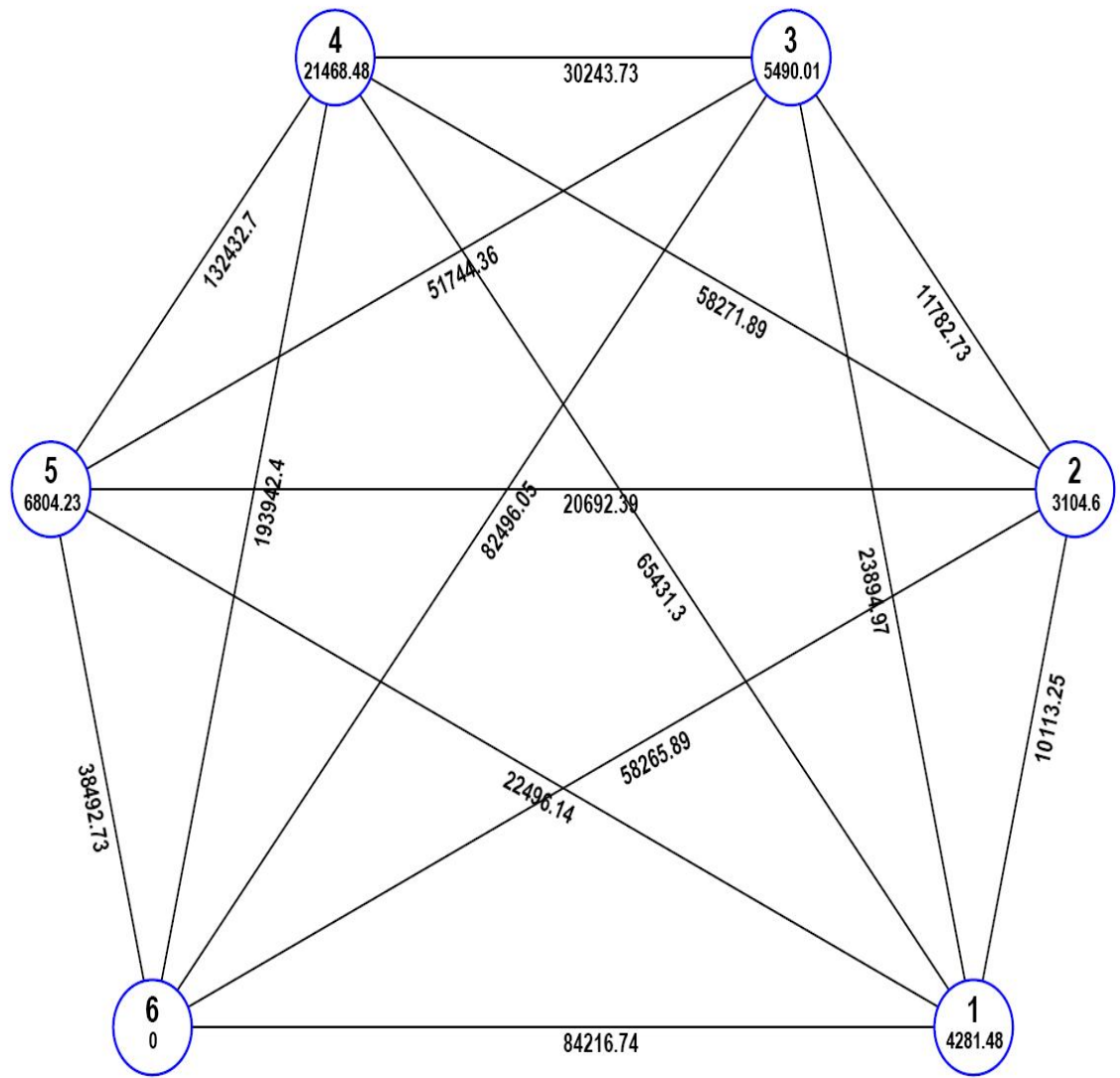


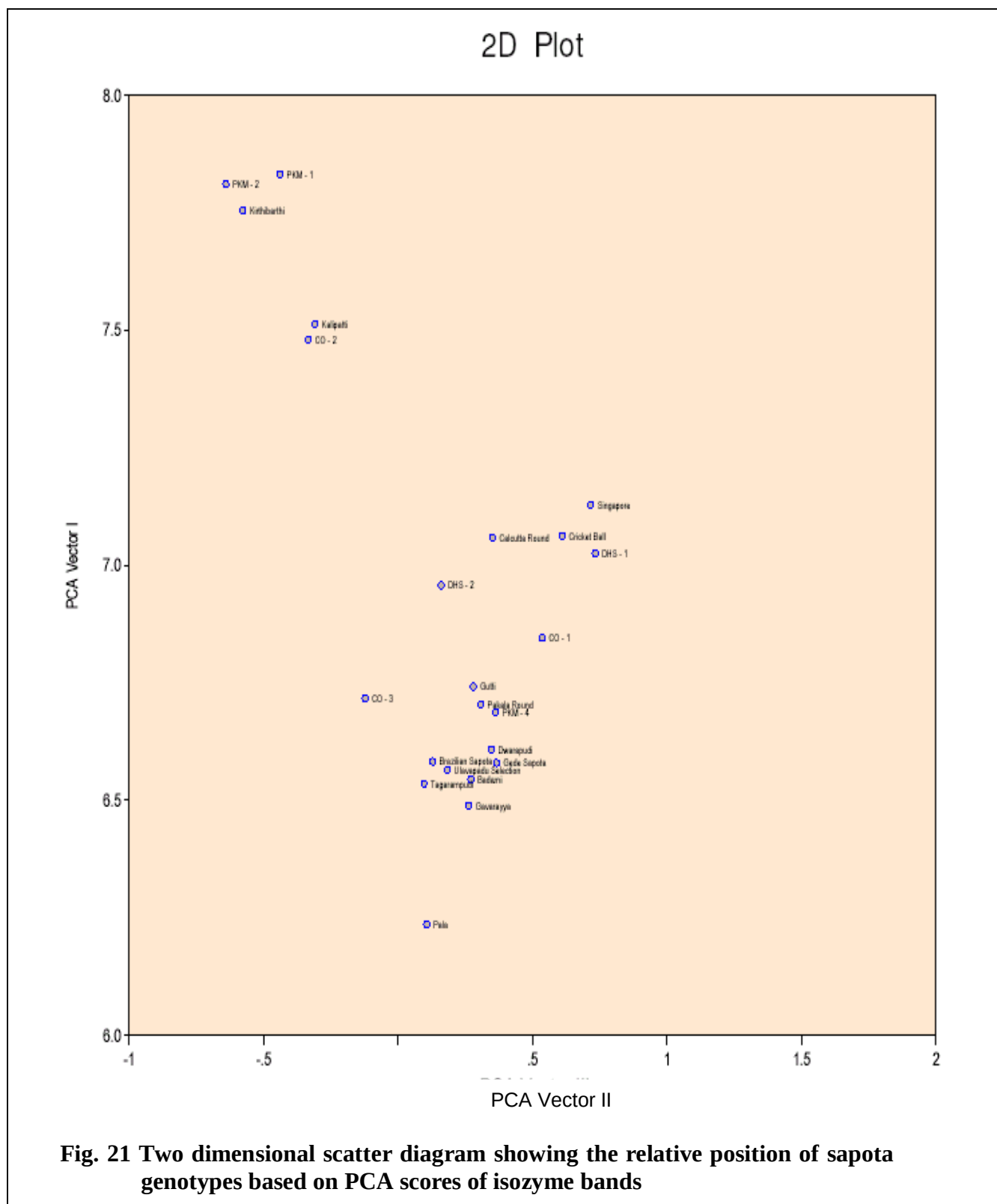
Fig. 18 Three dimensional scatter diagram showing the relative position of sapota genotypes based on PCA scores of fruit morphological and biochemical characters

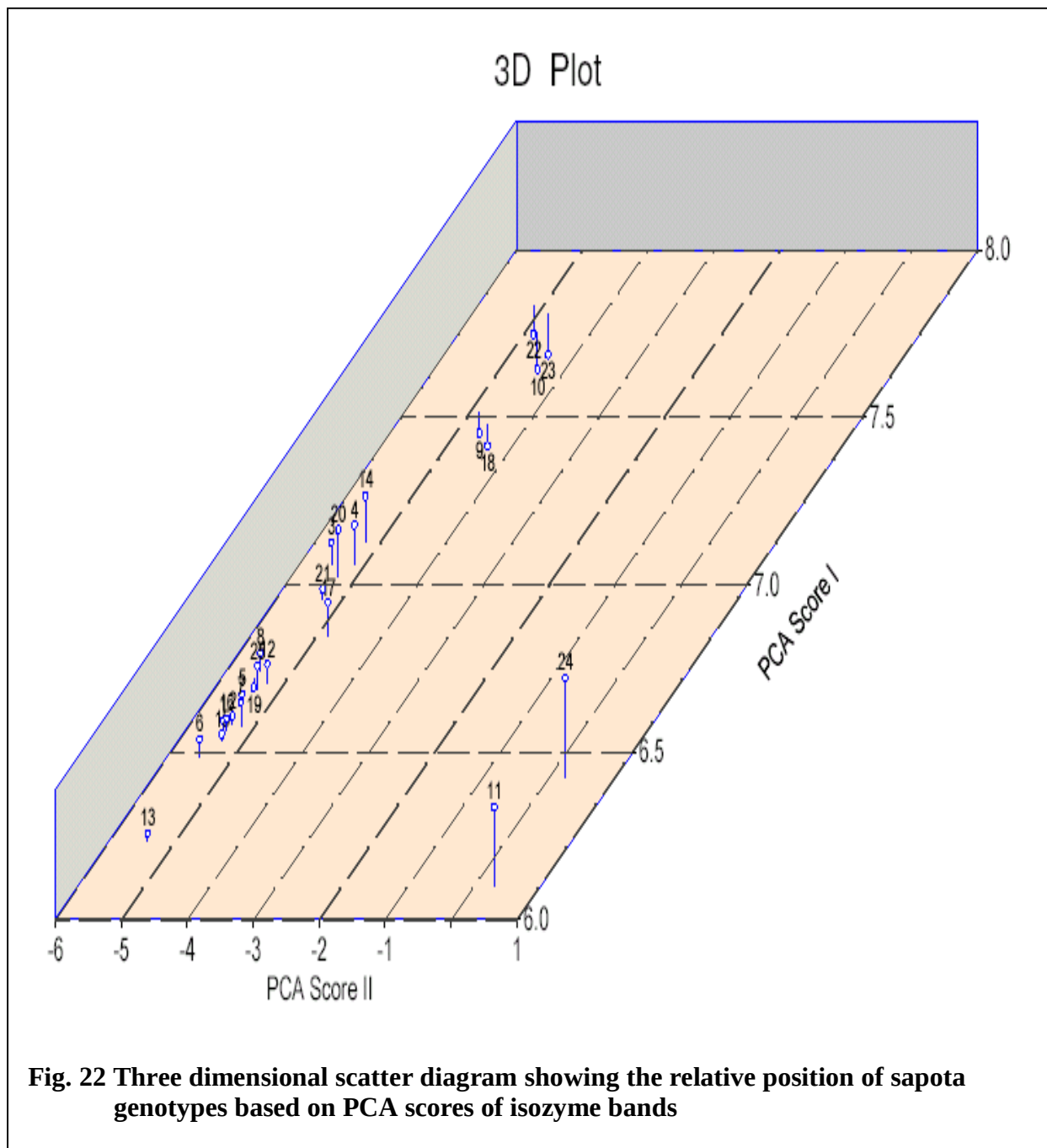




Euclidean² Distance (Not to the Scale)

Fig. 20 Cluster diagram showing intra and inter cluster distances for fruit morphological and biochemical characters in sapota genotypes





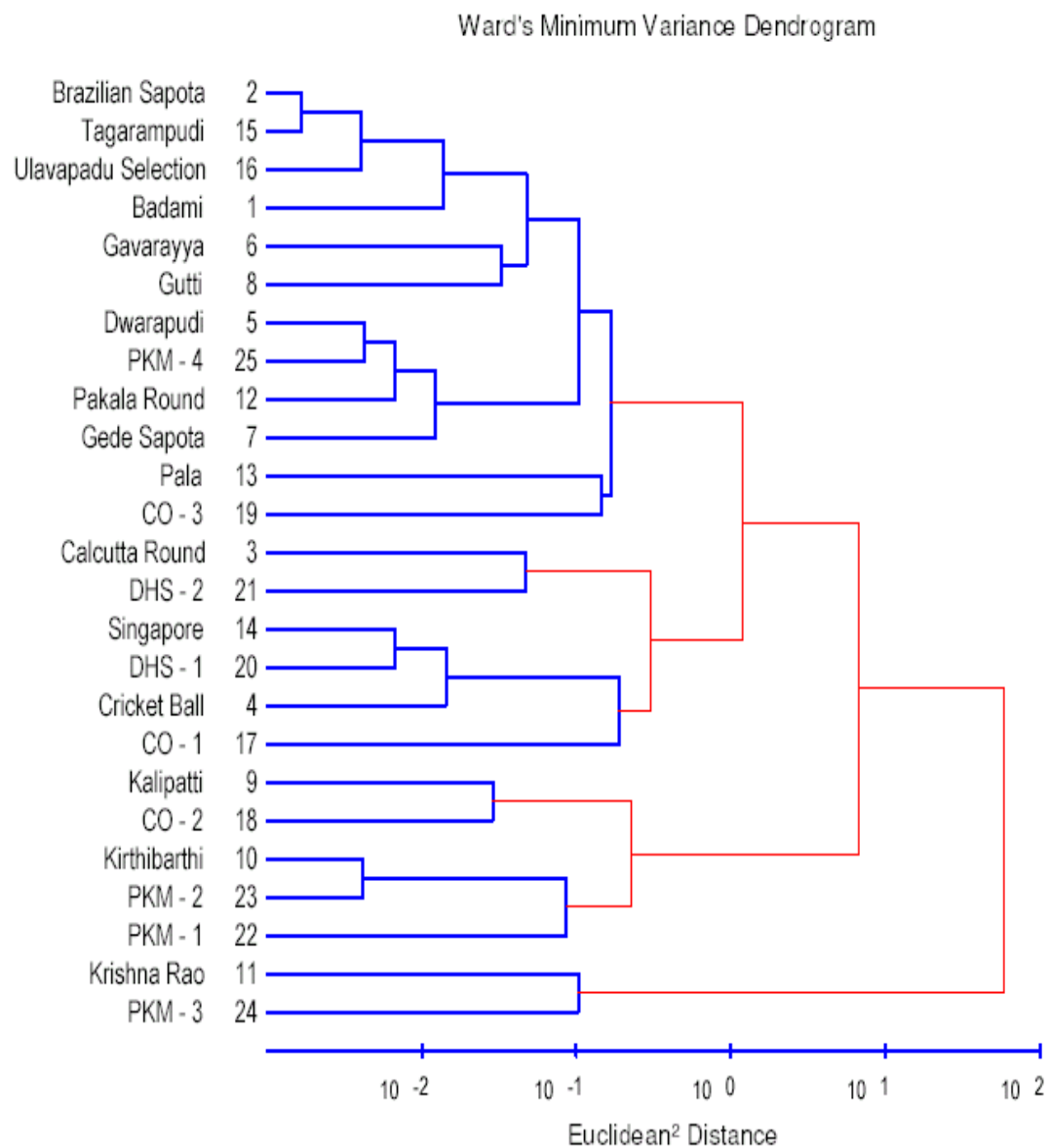


Fig. 23 Diagram illustrating the clustering pattern in sapota genotypes by Ward's minimum variance method for isozyme bands

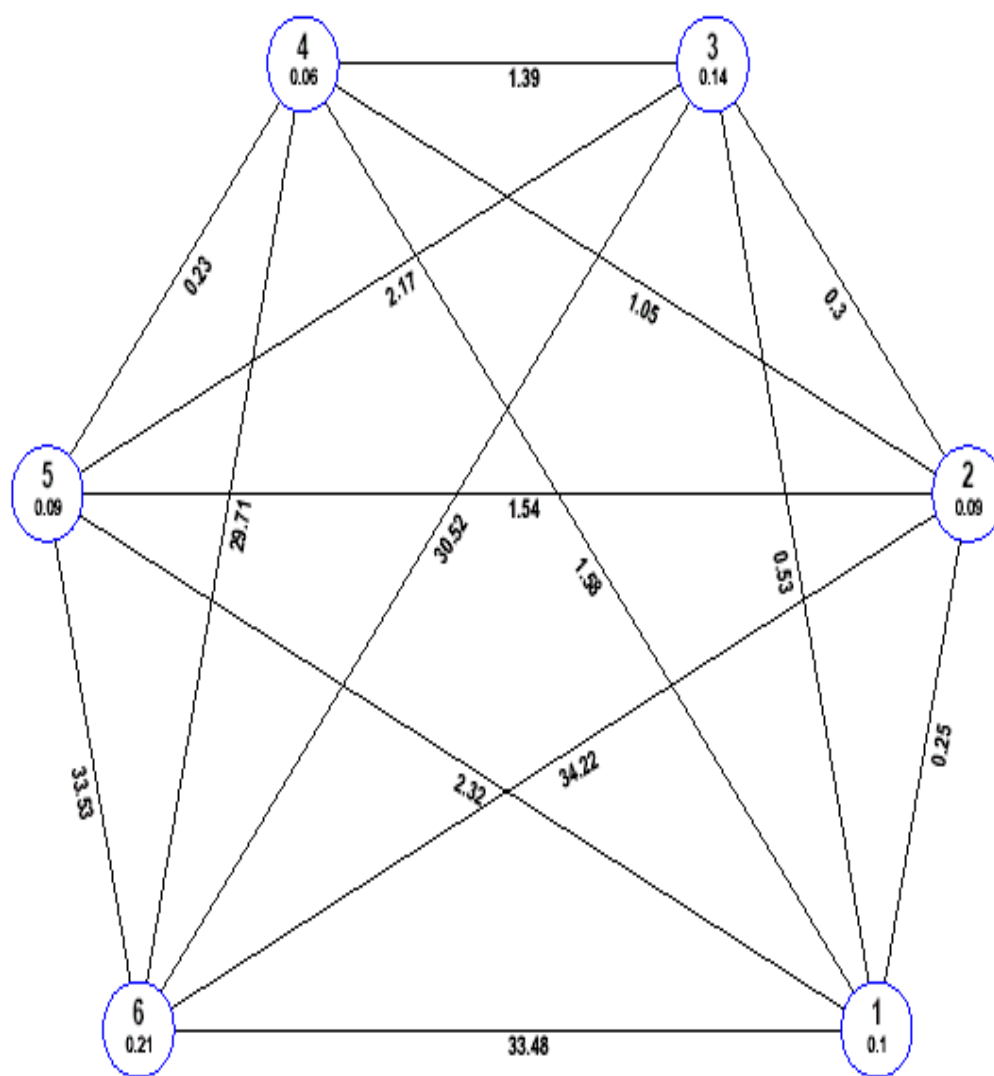
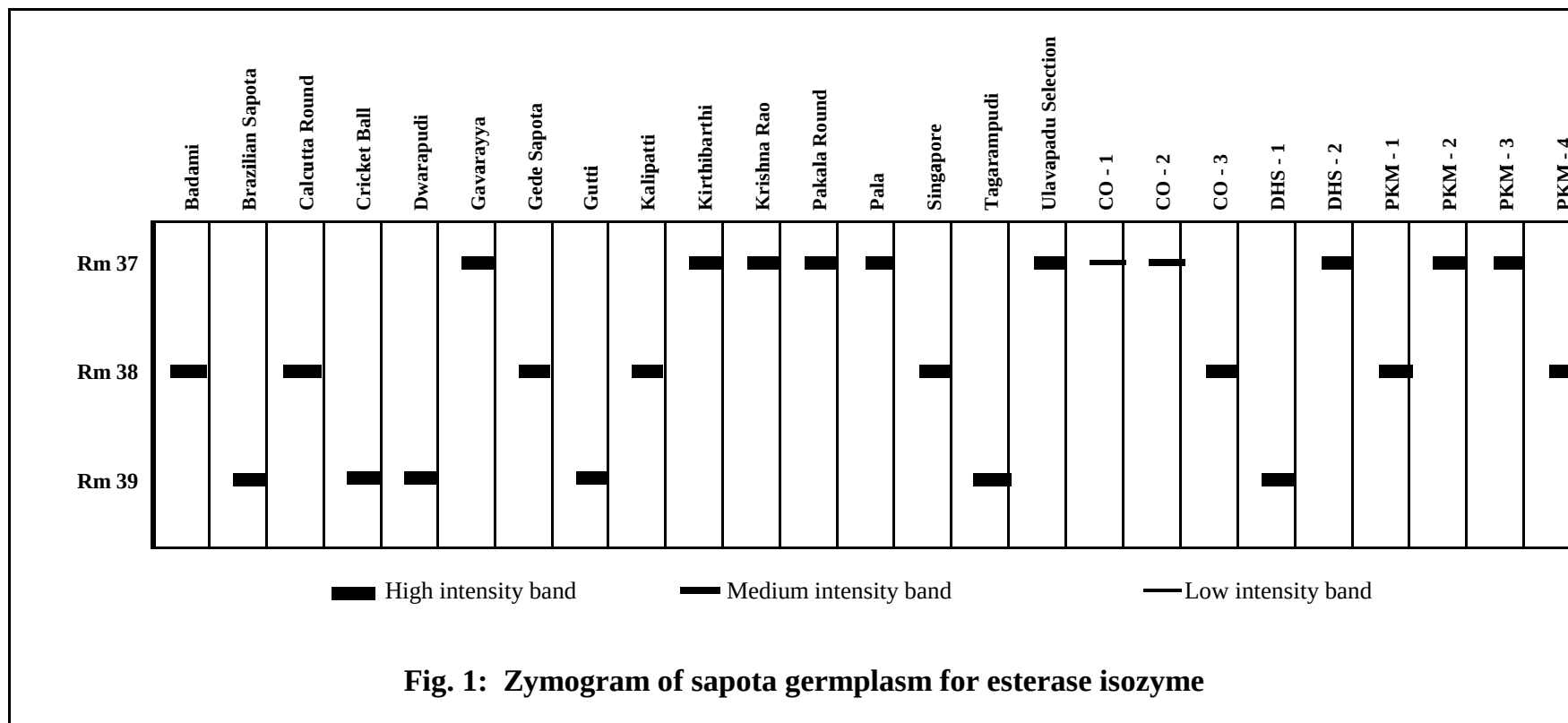


Fig. 24 Cluster diagram showing intra and inter cluster distances for isozyme bands in sapota genotypes



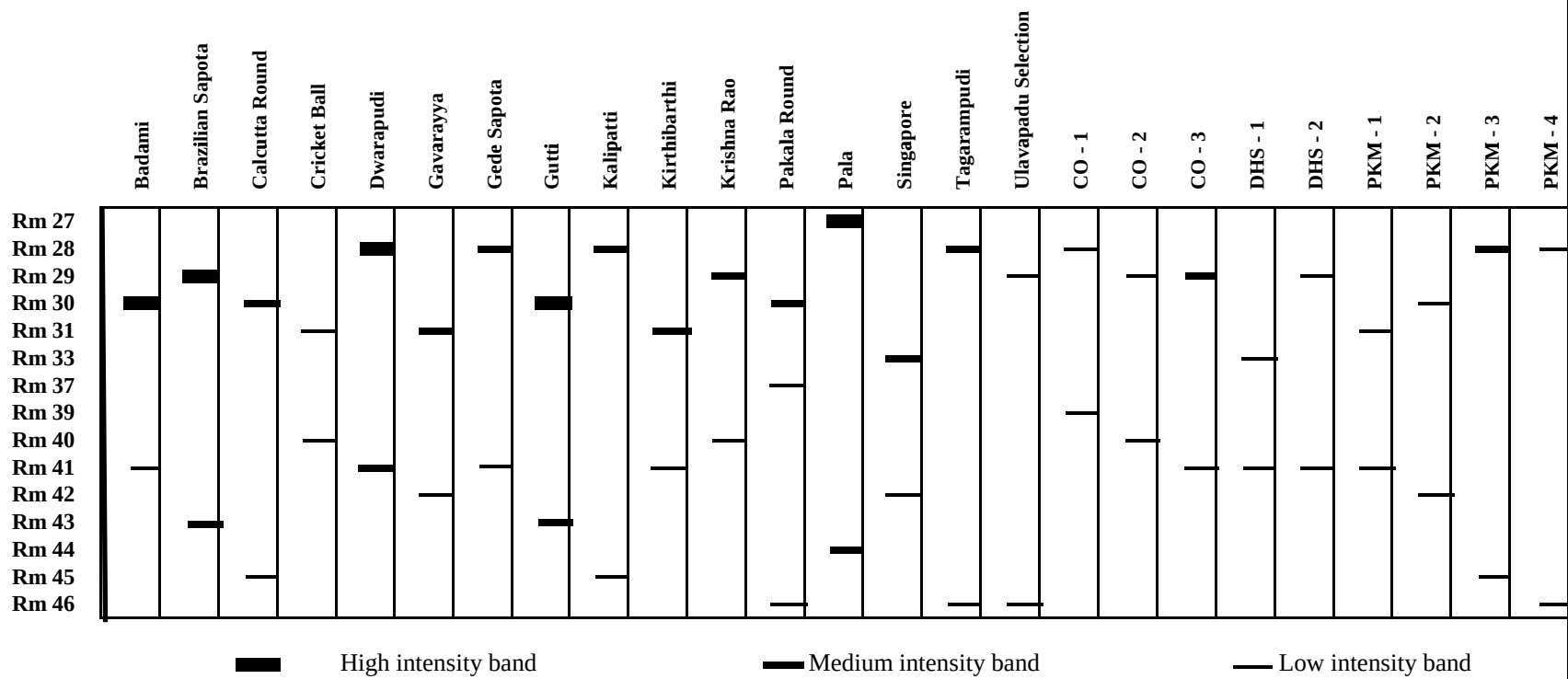


Fig. 2: Zymogram of sapota germplasm for peroxidase isozyme

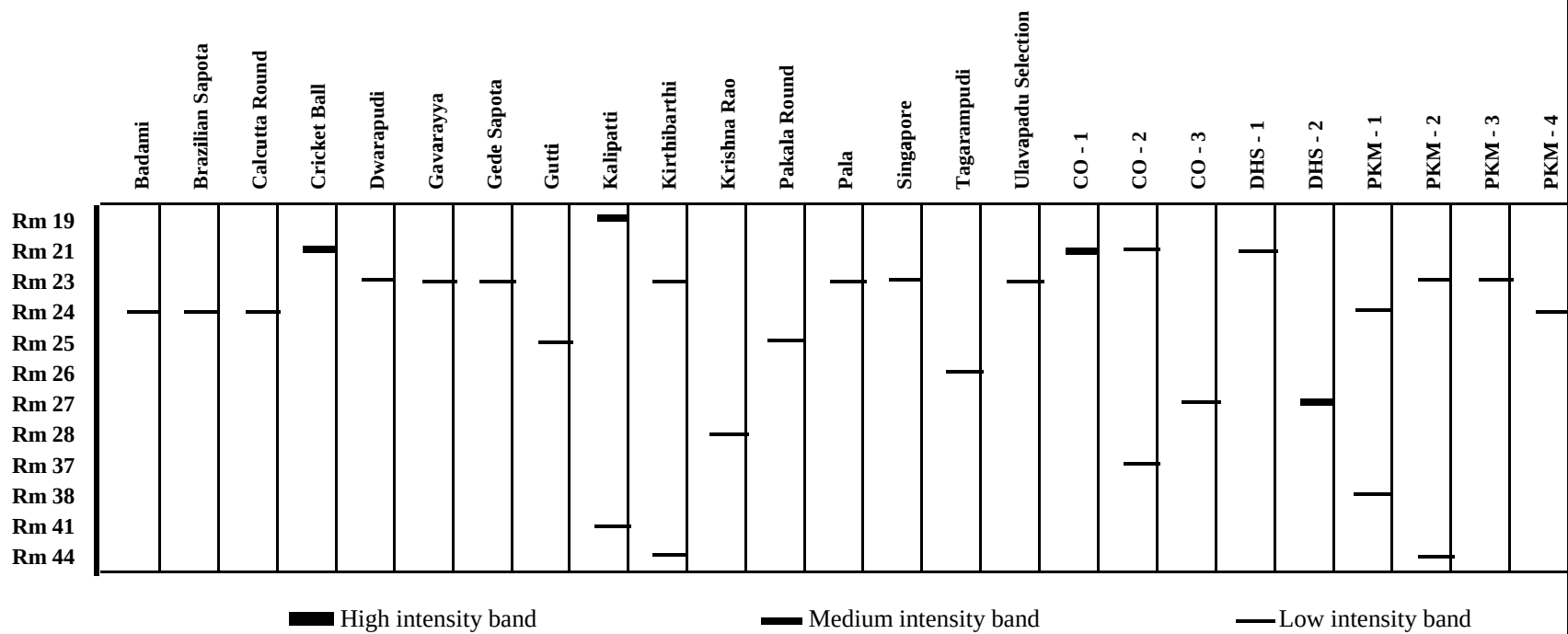


Fig. 3: Zymogram of sapota germplasm for polyphenol oxidase isozyme

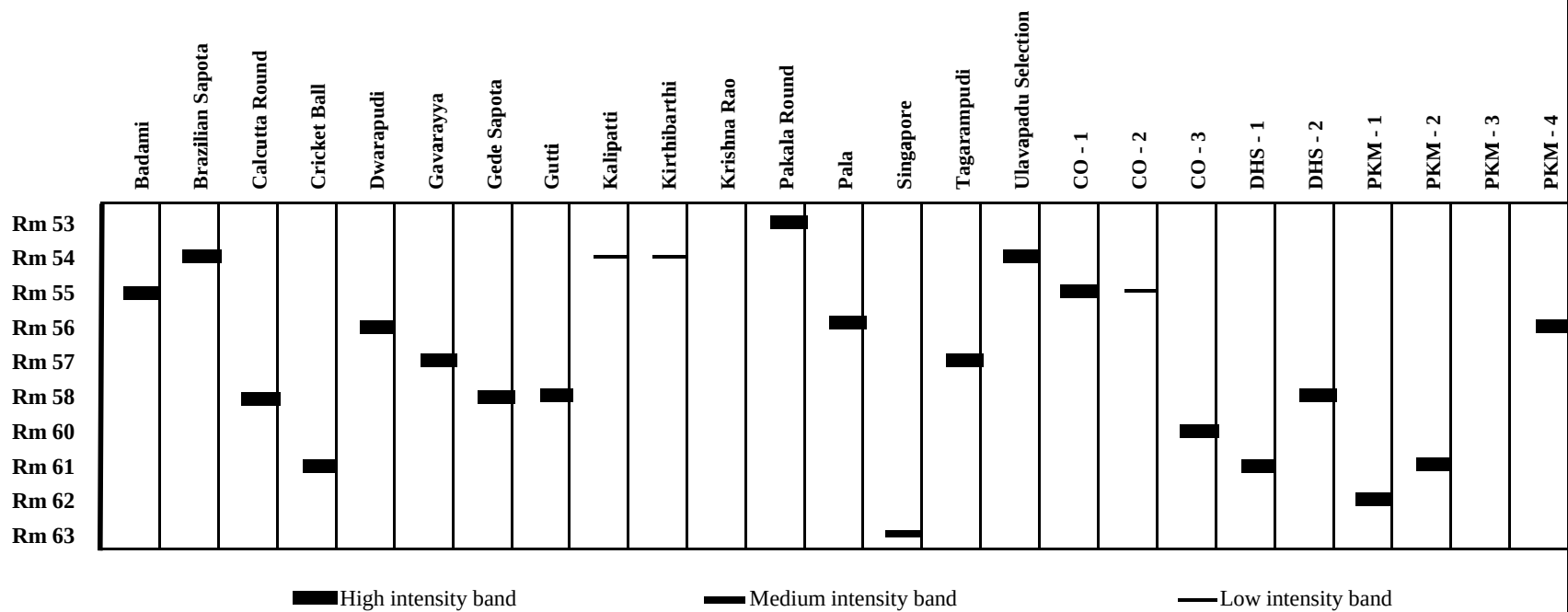


Fig. 4: Zymogram of sapota germplasm for catalase isozyme

CHAPTER V

DISCUSSION

Sapota (*Manilkara achras* (Mill.) Fosberg) is one of the important fruits grown in the country. Off late, the crop has attained the status of an important fruit crop in Andhra Pradesh after mango, citrus, banana and grape, where it is being grown in an area of about 15,400 ha yielding about 1,53,600 tonnes of fruits (www.indiastat.com). In Andhra Pradesh sapota is grown as mono-crop as well occasionally as intercrop in mango and coconut gardens and thus suits well into the cropping systems of Andhra Pradesh. Farmers are attracted to grow this crop because of its pest and disease free nature, drought resistant nature and occurrence of regular income throughout the year.

There are very few varieties of sapota being cultivated in India in the states, namely, Gujarat, Maharashtra and Karnataka and the genetic base is very narrow. However, work on the characterization of the varieties was limited though certain reports and monographs have appeared on sapota since the last few decades. Further, most of these publications have descriptions of only fruit characters but quantitative assessment of the variability available in the accessions is lacking in most of the studies. However, within this narrow genetic base, there exists certain ambiguity in nomenclature of the varieties since varieties are known by local names. More over, characterization of sapota genotypes using molecular markers, which are more reliable than morphological characters, was not attempted so far in sapota. Use of molecular markers such as isozymes facilitate establishment of genetic purity, location of duplicate accessions and classification

of the genotypes into distinct clusters (Moore and Durham, 1992). Thus only limited efforts seem to have been made for systematic characterization of genetic resources of sapota.

Hence, in the present investigation, studies have been carried out on various tree morphological characters, fruit and biochemical characters and isozyme banding patterns of four isozymes to characterize the 25 sapota genotypes being maintained at Horticultural Research Station, Venkataramannagudem (West Godavari District, Andhra Pradesh). Further, principal component analysis technique was also employed for classification of the 25 sapota genotypes into distinct clusters based on tree morphological characters, fruit and biochemical characters and isozyme banding pattern of leaf tissues. The results obtained are discussed hereunder in the light of the recent literature.

5.1 TREE MORPHOLOGICAL CHARACTERS

The analysis of variance had revealed significant differences among the 25 genotypes studied for all the morphological characters, except pollen size, indicating the existence of sufficient variability in the germplasm for the characters studied (Tables 1-3, Plate 1).

A perusal of the results on mean performance of 25 sapota genotypes studied for growth habit of the tree revealed that 64 per cent of the genotypes (Badami, Brazilian Sapota, Calcutta Round, Cricket Ball, Dwarapudi, Gavarayya, Gutti, Krishna Rao, Pakala Round, Tagarampudi, CO – 3, DHS – 1, DHS – 2, PKM – 2, PKM – 3, PKM - 4), were found to be semi-erect (Table 1) and ideal

for accommodation of greater population per unit area, resulting in higher yields and enhanced returns per unit area. Further, 32 per cent of the genotypes were observed to possess spreading growth habit (Gede Sapota, Kalipatti, Kirthibarthi, Pala, Singapore, Ulavapadu Selection, CO – 2 and PKM – 1), while only one genotype, namely, CO – 1 recorded erect growth habit. Sulladhmam and Narayana Reddy (1990) had reported similar trends in growth habit of sapota cultivars based on the growth habit of the tree into four types, namely, erect, drooping, spreading with light green foliage and spreading with dark green foliage. They had further reported that in trees with erect and drooping habit the branches appeared in whorls, while in trees with spreading habit the branching was irregular. However, no such relation between growth habit of the tree and branching pattern was observed in the present investigation.

The results pertaining to tree shape revealed 56 per cent of the genotypes, namely, Badami, Calcutta Round, Cricket Ball, Kalipatti, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Singapore, Tagarampudi, PKM – 1, PKM – 2, PKM – 3 and PKM – 4 were found to be broadly pyramidal and (Table 1) seemed beneficial indicating greater light interception and thereby resulting in enhanced photosynthesis. Further, 16 per cent of the genotypes (Dwarapudi, Gede Sapota, Ulavapadu Selection and CO – 1) studied had recorded elliptical shape. The genotypes, CO – 2 and CO – 3, however recorded irregular tree shape, while the rest of the genotypes were found to possess spherical tree shape.

Majority (72 %) of the genotypes (Badami, Brazilian Sapota, Calcutta Round, Dwarapudi, Gede Sapota, Gutti, Krishna Rao, Pakala Round, Ulavapadu Selection, CO – 1, CO – 2, CO – 3, DHS – 1, DHS – 2, PKM – 1, PKM – 2,

PKM – 3 and PKM - 4) studied were found to possess verticillate branching (Table 1) resulting in compact trees ideal for high density planting. Further, few (24 %) genotypes, namely, Cricket Ball, Kalipatti, Kirthibarthi, Pala, Singapore and Tagarampudi had exhibited horizontal branching pattern. The genotype, Gavarayya alone had recorded irregular branching pattern. Mathew *et al.* (2001) had reported spreading type of branching for cultivar Cricket Ball. Similar branching pattern was also noticed for Cricket Ball in the present investigation.

Medium dense branching was noticed in the present study for 52 per cent of the genotypes (Brazilian Sapota, Calcutta Round, Cricket Ball, Gavarayya, Gede Sapota, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Ulavapadu Selection, DHS – 1, PKM – 3 and PKM - 4). Thirty six per cent of the genotypes, namely, Badami, Dwarapudi, Gutti, Kalipatti, Singapore, Tagarampudi, DHS – 2, PKM – 1 and PKM – 2, however had recorded dense branching (Table 1) desirable for realizing greater fruit yields.

A perusal of the results on leaf characters revealed greater leaf area (more than mean, 21.06 sq. cm) for the genotypes Cricket Ball, Gavarayya, Gede Sapota, Singapore, CO – 1, CO – 2, CO – 3, PKM – 3 and PKM – 4 indicating their potential for enhanced photosynthesis compared to other genotypes studied in the present investigation (Table 2, Plate 1).

Sapota being a cross pollinated crop greater pollen size coupled with higher number of pollen grains per anther, a higher pollen fertility and style length would be ideal for realizing increased fruit set and thereby greater yields (Chundawat, 1998). In the present study DHS – 1 exhibited all the above floral characters and hence can be considered to be an ideal genotype with regards to floral characters.

The genotypes Badami, Calcutta Round, Kalipatti and Tagarampudi had also recorded greater style length coupled with desirable characters for majority of the pollen traits studied and are promising (Table 3).

5.2 FRUIT MORPHOLOGICAL AND BIOCHEMICAL CHARACTERS

Most of the fruit produced in the country is consumed as fresh fruit. For a good eating quality dessert type of sapota, thin fruit skin, attractive pulp colour, texture, sweet taste, good flavor, medium fruit weight, and lower seed content would be desirable. In a nutritional sense, the fruit shall possess higher TSS, sugar, ascorbic acid content and lower concentration of total phenols and acid content (Chadha, 1992).

The results obtained in respect of fruit morphological and biochemical characters are discussed below.

5.2.1 Fruit morphological characters

A perusal of the results on fruit quantitative characters revealed prevalence of wide variation among the different genotypes studied (Tables 4-5, Plate 2). The mean fruit length observed in the present study was 5.24 cm, while the range was observed to be 3.71 cm to 7.75 cm. Greater fruit length (>6.0 cm) was recorded in 28 per cent of the sapota genotypes studied (Brazilian Sapota (6.01 cm), Gavarayya (6.16 cm), Cricket Ball (6.48 cm), CO – 3 (6.85 cm), CO – 2 (6.87 cm), CO – 1 (7.28 cm) and PKM – 4 (7.75 cm), while lesser fruit length was recorded (<4.0 cm) in 12 per cent of the sapota genotypes studied (Ulavapadu

Selection (3.71 cm), Gede Sapota (3.82 cm) and Tagarampudi (3.99 cm). The other genotypes (60 %) had recorded moderate fruit length (4.0 – 6.0 cm).

The mean fruit width observed for the genotypes studied was 4.69 cm, while the range was found to be 2.37 cm to 6.79 cm. Further, greater fruit width (> 5.0 cm) was noticed for 32 per cent of the sapota genotypes studied, namely Gutti (5.10 cm), DHS – 2 (5.14 cm), Brazilian Sapota (5.15 cm), CO -1 (5.77 cm), Krishna Rao (5.80 cm), CO – 3 (5.82 cm), CO – 2 (5.89 cm) and Cricket Ball (6.79 cm) while minimum fruit width of 2.37 cm was recorded for Ulavapadu Selection. The other sapota genotypes studied (64 %) had recorded moderate fruit width (3.5 – 5.0 cm) (Table 4, Plate 2).

The average fruit weight of sapota genotypes studied in the present investigation was 96.63 g, while the range was 44.83 g to 198.74 g. Greater fruit weight (> 96.5 g) was recorded for 40 per cent of the sapota genotypes studied in the present investigation, namely, Brazilian Sapota (97.57 g), Gutti (102.01 g), Gavarayya (104.03 g), PKM – 3 (105.08 g), DHS – 1 (120.21 g), PKM – 4 (127.04 g), CO – 3 (127.29 g), CO – 2 (150.65 g), CO – 1 (170.67 g) and Cricket Ball (198.74 g). The genotype Cricket Ball had recorded highest fruit weight (198.74 g). Similar higher fruit weight for Cricket Ball variety of sapota was reported by Mathew *et al.* (2001) in their studies on physico-chemical characteristics of sapota cultivars grown in West Bengal. Further, Ulavapadu Selection had recorded minimum fruit weight of 44.83 g, while other genotypes (56 %) had recorded moderate fruit weight (60.00 – 96.5 g) in the present investigation (Table 4, Plate 2).

The average fruit yield per tree was noticed to be 57.66 kg/tree, while the range was 26.20 kg/tree to 93.35 kg/tree indicating wide variation in the yield among the genotypes. Further, a perusal of the results revealed that the yields were higher than general mean in 48 per cent of the genotypes, namely, PKM – 3 (93.35 kg/tree) followed by PKM – 1 (89.95 kg/tree), Kirthibarthi (86.45 kg/tree), Singapore (85.35 kg/tree), Kalipatti (83.40 kg/tree), DHS – 1 (81.55 kg/tree), DHS – 2 (75.80 kg/tree), PKM – 4 (70.50 kg/tree), PKM – 2 (66.75 kg/tree), Pala (65.75 kg/tree), Ulavapadu Selection (63.50 kg/tree) and Tagarampudi (60.45 kg/tree). Lower fruit yield/tree were recorded in 28 per cent of the other genotypes, namely, CO – 2 (26.20 kg/tree), CO – 1 (26.40 kg/tree), Gede Sapota (31.70 kg/tree), Krishna Rao (31.45 kg/tree), Badami (32.35 kg/tree), CO – 3 (32.95 kg/tree) and Gutti (36.15 kg/tree), while moderate fruit yields per tree were recorded in 24 per cent of the genotypes, namely, Dwarapudi (37.65 kg/tree), Calcutta Round (45.15 kg/tree), Brazilian Sapota (52.80 kg/tree), Gavarayya (55.15 kg/tree), Pakala Round (55.25 kg/tree) and Cricket Ball (55.40 kg/tree) (Table 4).

A perusal of the above fruit quantitative characters revealed greater fruit length, width and fruit weight for Brazilian Sapota, Cricket Ball, Gavarayya, Gutti, CO – 1, CO – 2, CO – 3, PKM – 3 and PKM - 4 genotypes studied in the present investigation. Fruit yields per tree were greater for PKM – 3, PKM – 1, Kirthibarthi, Singapore, Kalipatti, DHS – 1, DHS - 2 and PKM – 4. Further, the fruits of Cricket Ball were observed to be globose, heavy and large. Similar observations were also noticed by Chundawat and Bhuva (1982) and Mathew *et al.* (2001).

In the present investigation, fruit shape of sapota genotypes studied ranged from turbinate to oval shape, while the flesh colour ranged from greenish brown to reddish brown and flesh texture from sandy to soft. Similarly, the flesh taste of the sapota genotypes studied ranged from starchy to sweet and our observations are in tune with the reports of Chadha (1992).

The genotype, Kalipatti had recorded smooth fruit surface texture, brown flesh colour, firm fruit texture and sweet flesh taste (Table 5), while CO – 1 and CO – 2 genotypes had recorded smooth fruit surface texture, yellowish brown flesh colour, sandy flesh texture and sweet flesh taste. Further, the genotypes, namely, Gavarayya and Tagarampudi had recorded rough fruit surface texture, brown to reddish brown flesh colour, firm flesh texture and starchy flesh taste, while Kirthibarthi had recorded smooth fruit surface texture, brown flesh colour, soft flesh texture and sweet flesh taste. Similar observations were reported for the genotypes by Mone *et al.* (1991) in his studies on qualitative aspects of sapota varieties.

In general higher seed content is considered undesirable and the genotypes Brazilian Sapota and Cricket Ball had recorded maximum seed number/fruit in addition to high seed weight. Similar results in respect of seed number/fruit for Cricket Ball variety were reported earlier in sapota by Sundararajan and Madhava Rao (1967) and Mathew *et al.* (2001).

5.2.2 Fruit biochemical characters

A perusal of the results on fruit biochemical characters also revealed considerable variation among the genotypes studied (Table 6). Total soluble solids

content was observed to range from 20.20^o brix (Cricket Ball) to 24.30^o brix (Pala). Further, high TSS values compared to mean value was recorded for 48 per cent of the genotypes studied, namely, Kirthibarthi (22.60^o brix), Calcutta Round (22.90^o brix), DHS – 1 (22.90^o brix), Gutti (23.00^o brix), Singapore (23.00^o brix), Brazilian Sapota (23.10^o brix), Ulavapadu Selection (23.40^o brix), PKM – 4 (23.50^o brix), PKM – 3 (23.70^o brix), Kalipatti (23.90^o brix), PKM – 1 (24.10^o brix) and Pala (24.30^o brix). Similar trend in TSS content of sapota varieties was earlier reported by several workers (Shanmugavelu and Srinivasan, 1973, Ponnuswamy and Irulappan, 1987 and 1989, Paralkar *et al.*, 1987, Shanmugavelu *et al.*, 1987, Mone *et al.*, 1991, Waskar and Nikam, 1996 and Mathew *et al.*, 2001) and strengthen these results.

The content of reducing sugars (Table 6) was observed to range from 5.88 per cent (Dwarapudi) to 10.65 per cent (Kalipatti). Higher content of reducing sugars was recorded for 48 per cent of the genotypes studied in the present investigation, namely, DHS – 1 (8.11 %), Ulavapadu Selection (8.15 %), DHS – 2 (8.20 %), PKM – 4 (8.31 %), Kirthibarthi (8.47 %), Calcutta Round (8.48 %), PKM – 1 (8.81 %), Singapore (8.50 %), CO – 2 (8.89 %), PKM – 3 (9.21 %), Pala (10.05 %) and Kalipatti (10.65 %). Similar results with regard to reducing sugars were reported earlier for Kirthibarthi (Shanmugavelu and Srinivasan, 1973) and Kalipatti (Paralkar *et al.*, 1987, Shanmugavelu and Srinivasan, 1973, Paralkar *et al.*, 1987, Ponnuswamy and Irulappan, 1987 and Mathew *et al.*, 2001).

A high content of non-reducing sugars was recorded for 44 per cent of the genotypes studied, namely, Pala (1.88 %), Pakala Round (1.99 %), CO – 3 (1.99

%), Gutti (2.02 %), Kirthibarthi (2.23 %), Singapore (2.32 %), Gede Sapota (2.34 %), CO – 1 (2.39 %), Gavarayya (2.76 %), Cricket Ball (3.27 %) and Dwarapudi (4.21 %). A variation in the content of non-reducing sugars (Table 6) with the genotypes studied was observed in the present investigation and similar findings were reported for the trait in sapota by earlier workers (Shanmugavelu and Srinivasan, 1973 and Mathew *et al.*, 2001).

In the present study, the total sugars content (Table 6) of sapota genotypes ranged from 8.21 per cent (Badami) to 11.93 per cent (Pala). In this study a high content of total sugars was recorded for 44 per cent of the genotypes, namely, Pakala Round (9.90 %), Cricket Ball (9.80 %), PKM – 1 (9.83 %), Gutti (9.96 %), Dwarapudi (10.09 %), CO – 2 (10.14 %), PKM – 3 (10.65 %), Kirthibarthi (10.71 %), Singapore (10.82 %), Kalipatti (11.78 %) and Pala (11.93 %). Shanmugavelu and Srinivasan (1973) reported total sugar content of sapota varieties to range from 7.0 per cent to 12.3 per cent, while Ponnuswamy and Irulappan (1987) observed the total sugars content to range from 6.94 per cent to 14.04 per cent. Mathew *et al.* (2001) also reported that the total sugars content in different cultivars of sapota ranged from 7.7 per cent to 11.1 per cent. Similar high content of total sugars in Kalipatti (Paralkar *et al.*, 1987) and Cricket Ball (Mathew *et al.*, 2001) genotypes was reported earlier.

The titrable acidity content (Table 6) of sapota genotypes studied in the present investigation varied from 0.01 per cent (DHS – 1) to 0.07 (Gavarayya). Titrable acidity was found to be high in 20 per cent of the genotypes studied, namely, Gavarayya (0.07 %); Cricket Ball, CO – 1, PKM – 1 and PKM - 2 (0.05

%). Moderate values for titrable acidity (0.04 %) were observed for 44 per cent of the genotypes studied, namely, Badami, Calcutta Round, Dwarapudi, Gede Sapota, Gutti, Kalipatti, Tagarampudi, CO – 2, CO – 3, DHS – 2 and PKM – 4. However, low titrable acidity (<0.04 %) was recorded for the genotypes studied, namely, Brazilian Sapota, Kirthibarthi, Krishna Rao, Pala, Singapore, Ulavapadu Selection and PKM – 3 (0.03 %), Pakala Round (0.02 %) and DHS – 1 (0.01 %). Similar low content of titrable acidity was also reported earlier (Shanmugavelu and Srinivasan, 1973) for Gutti genotype/cultivar (0.03 %). Similar variation in titrable acidity content of sapota cultivars was reported by Shanmugavelu and Srinivasan (1973), Ponnuswamy and Irulappan (1987) and Mathew *et al.* (2001).

The sugar:acid ratio (Table 6) was found to vary from 142.01 (Gavarayya) to 582.08 (DHS -1) among the sapota genotypes studied. Higher sugar:acid ratio was observed in 32 per cent of the genotypes studied, namely, Gutti (311.03), Ulavapadu Selection (347.28), Pala (399.00), Brazilian Sapota (399.14), PKM – 3 (461.73), Kirthibarthi (469.25), Pakala Round (492.73) and DHS – 1 (582.08). Further, low sugar:acid ratio compared to general mean (304.31) was observed in the present investigation for 68 per cent of the sapota genotypes studied, namely, Calcutta Round (303.20), Krishna Rao (298.40), Singapore (295.30), Kalipatti (293.17), CO – 2 (276.04), Badami (275.23), DHS – 2 (257.68), Dwarapudi (256.32), Tagarampudi (246.06), PKM – 4 (245.51), PKM – 1 (227.20), Gede Sapota (221.87), CO – 3 (216.05), CO – 1 (214.074), Cricket Ball (201.59), PKM – 2 (175.10) and Gavarayya (142.01). Similar low levels of sugar:acid ratio

were reported earlier for PKM – 1 (222.50), Tagarampudi (229.03) and Gavarayya (186.09) by Ponnuswamy and Irulappan (1987).

The ascorbic acid content (Table 6) of the sapota genotypes studied in the present investigation varied from 5.12 mg/100g (Dwarapudi) to 11.37 mg/100g (Kalipatti) (Table 6). The ascorbic acid content was observed to be high in 48 per cent of the genotypes, namely, Gutti (9.00 mg/100g), DHS – 1 (9.00 mg/100g), Pala (9.50 mg/100g), PKM – 4 (9.62 mg/100g), Ulavapadu Selection (9.75 mg/100g), CO – 3 (9.75 mg/100g), CO – 2 (10.25 mg/100g), PKM – 3 (10.25 mg/100g), PKM – 1 (10.37 mg/100g), CO – 1 (10.75 mg/100g), Singapore (11.00 mg/100g) and Kalipatti (11.37 mg/100g). Similar variation in the ascorbic acid content of the sapota genotypes/cultivars was reported earlier (Shanmugavelu and Srinivasan, 1973 and Ponnuswamy and Irulappan, 1987 and Paralkar *et al.*, 1987).

In the present investigation, total phenol content (Table 6) ranged from 0.23 mg/100g (Pala) to 0.78 mg/100g (Dwarapudi). A lower total phenol content was recorded in 52 per cent of the genotypes studied namely, Pala (0.23 mg/100g), Ulavapadu Selection (0.25 mg/100g), CO – 1 (0.26 mg/100g), Cricket Ball (0.33 mg/100g), CO – 2 (0.33 mg/100g), Singapore (0.34 mg/100g), Gavarayya (0.36 mg/100g), Kalipatti (0.36 mg/100g), Calcutta Round (0.40 mg/100g), PKM – 1 (0.40 mg/100g), Tagarampudi (0.41 mg/100g), PKM – 4 (0.42 mg/100g) and DHS – 1 (0.43 mg/100g). Identical results in respect of total phenols content were reported earlier by Selvaraj and Pal (1984).

When the 25 genotypes were grouped into three categories, namely, local cultivars, improved cultivars and hybrids (Table 32), the results revealed greater

fruit weight, yield per tree, pulp:seed ratio, reducing sugars and ascorbic acid content coupled with low seed number and total phenols in hybrids, namely, CO – 1, CO – 3, DHS – 1, DHS – 2, PKM – 2 and PKM – 3 and can be considered superior compared to local genotypes (Badami, Calcutta Round, Cricket Ball, Dwarapudi, Gavarayya, Gede Sapota, Gutti, Kalipatti, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Singapore and Tagarampudi) and other selections (Brazilian Sapota, Ulavapadu Selection, CO – 2, PKM – 1 and PKM – 4) studied.

5.2.3 Other morphological characters

The other morphological characters studied exhibited considerable variability in characters like leaf margin, shape of the leaf, leaf tip, fruit base, fruit apex and seed shape (Table 7) and may be used as indices for characterizing the sapota genotypes.

5.3 ISOZYME ANALYSIS

Multiple forms of enzymes with similar catalytic activity are popularly called isozymes (Markert and Moller, 1959). Isozymes or molecular markers have been used extensively to estimate genetic diversity and trueness to type in several horticultural and agricultural crops. The utility of isozymes as genetic markers is generally attributed to their polymorphism, simple inheritance, rapid and simple assay (Weeden and Lamb, 1985), codominance, and ubiquity in plant tissues (Arulsekhar and Parfitt, 1986, Moore and Durham, 1992). The identification of cultivars and germplasm accessions through isozyme analysis is far more precise

than using morphological descriptors alone. It facilitates establishment of genetic purity, location of duplicate accessions and identification of somaclonal variants and especially the technique is not influenced by environmental factors and hence is being used in precise identification of varieties/genotypes/germplasm accessions in crops (Asiedu, 1992 and Moore and Durham, 1992).

In the present study, four isozymes, namely, esterase, peroxidase, polyphenol oxidase and catalase were assayed by native polyacrylamide gel electrophoresis (PAGE technique) to identify the genetic diversity among different sapota genotypes. The available literature was thoroughly scanned before selection of the isozymes to be studied and after careful search it was observed that use of the isozymes esterase, peroxidase, polyphenol oxidase and catalase was reported in rubber (Sreelatha *et al.*, 1993, Krishnakumar *et al.*, 1999 and Sobhana *et al.*, 2001), a latex containing crop similar to sapota. Hence, these isozymes were chosen for the present study and before hand, the electrophoretic procedures have been standardized to suit the tissues of sapota leaves (details furnished in materials and methods chapter).

The banding patterns of all the four isozymes studied revealed polymorphism for the isozymes but with different intensities (Tables 8-11, Fig. 1-4 and Plates 3-6). Based on the relative mobility (R_m) value of the bands for each genotype, an attempt was made to characterize or identify the sapota genotypes. Such characterization of the genotypes based on the R_m values was also made earlier by Degani *et al.*, (1990), Degani *et al.* (1992), Mustaffa (1993) and Subedi *et al.* (2005) in mango; Jarret and Litz (1986a and b) and Bhat *et al.* (1992a and b) in Banana, Torres *et al.* (1978 and 1982) in citrus, Khan and Roose (1988) in

citrus, Azad *et al.* (2007) in jackfruit, Torres and Bergh (1980) in avocado, Degani and Blumenfeld (1986) in loquat, Atkinson *et al.* (1986) in cacao, Al-Jibouri and Adham (1990) in date palm, DeWald *et al.* (1988) in pineapple Messina *et al.* (1991) in Kiwi fruit, Weeden and Lamb (1985) in apple, Cousineau and Donnelly (1992) in raspberry, Weeden *et al.*, (1988) in grape, Arulsekhar *et al.* (1985) in walnut and Granger *et al.* (1992) in sweet cherry. The banding pattern of the isozymes esterase, peroxidase, polyphenol oxidase and catalase were explained with reference to their band position and intensity.

5.3.1 Peroxidase

In the present investigation, studies were under taken with peroxidase isozyme for characterization of 25 sapota genotypes being maintained. A perusal of the results revealed the existence of two or three bands for each genotype under study (Table 9, Fig.2, and Plate 4). The band position and intensity, however, varied with the genotype indicating polymorphism of the isozyme for the genotypes studied. High, medium and low intensity bands were observed for a wide range of Rm values starting from Rm 27 to Rm 46, at 15 different Rm positions, indicating greater resolution and higher variability for band molecular weight in the isozyme. Further, the most frequent peroxidase bands were noticed at Rm 41 and Rm 28 in eight and seven genotypes, respectively.

The characterization of the 25 sapota genotypes studied in the present investigation based on peroxidase banding pattern is presented in Table 33. A perusal of these results revealed unambiguous identification of 15 genotypes studied, based on Rm value, (Pakala Round, Singapore, Krishna Rao, Pala,

CO – 1, Brazilian Sapota, CO – 2, Ulavapadu Selection, Badami, Gutti, Calcutta Round, PKM – 2, Gavarayya, DHS – 1 and PKM – 4), except Kalipatti, PKM – 3, Cricket Ball, Dwarapudi, Gede Sapota, Kirthibarthi, Tagarampudi, DHS – 2, CO – 3 and PKM – 1.

The suitability of peroxidase isozyme for characterisation of banana cultivars was reported by Jarret and Litz (1986b), while suitability of the isozyme for identification of temperate fruit tree species from the highlands of Thailand was reported by Iamtham *et al.* (1998). Further, identification of several crop cultivars with the help of polymorphism in peroxidase isozyme was reported in rubber by Sreelatha *et al.* (1993) and Sobhana *et al.* (2001); in muskmelon by Sujatha *et al.* (1991); in arecanut by Swaminathan and George (2007); in woody perennials by Pandeya *et al.* (2007); in wild apple by Petrokas and Stanys (2008); and in *Lillium* species by Arzate-Fernandez *et al.* (2005). The above references lend support to these findings of sapota with peroxidase isozyme.

5.3.2 Polyphenol oxidase

A perusal of the results on banding pattern of polyphenol oxidase isozyme recorded for the different sapota genotypes revealed the existence of one or two bands for each genotype (Table 10, Fig.3, and Plate 5). The band position and intensity however varied with the genotype under study indicating polymorphism of the isozyme for the genotypes. High, medium and low intensity bands were observed for a wide range of R_m values starting from R_m 19 to R_m 44 at 12 different positions. The most frequent polyphenol oxidase band was noticed at R_m 23.

Characterisation of the sapota genotypes studied based on banding pattern of the isozyme is presented in Table 34. The results revealed a scope for distinction of genotypes, namely, Kalipatti, CO – 2, Kirthibarthi, PKM - 2, PKM – 1, Krishna Rao and Tagarampudi genotypes. Similar results with polyphenol oxidase were also reported in arecanut by Swaminathan and George (2007) and in coconut by Niral *et al.* (2007).

5.3.3 Catalase

Studies on banding pattern of the isozyme revealed the occurrence of single band per genotype with the exception of PKM – 3 and Krishna Rao in which no bands were observed (Table 11, Fig. 4, and Plate 6). However, the band position and intensity varied with the genotype indicating polymorphism of the isozyme for the genotypes studied.

High, medium and low intensity bands were observed for Rm values starting from Rm 53 to Rm 65 at ten different positions. The most frequent catalase bands were recorded at Rm 54 and Rm 58 in six genotypes (Brazilian Sapota, Ulavapadu Selection, Gede Sapota, Gutti, DHS – 2 and Calcutta Round) studied in the present investigation.

Identification of the sapota genotypes based on banding pattern of catalase isozyme is detailed in Table 35. The results indicated scope for distinction of genotypes, namely, Pakala Round, CO – 3, PKM – 1 and Singapore genotypes studied. Similar polymorphism with catalase isozyme and identification of genotypes was earlier reported in banana by Bhat *et al.* (1992a); in *Lillium* species by Arzate-Fernandez *et al.* (2005); and in rubber by Sobhana *et al.* (2001).

5.3.4 Esterase

A perusal of the results on banding pattern of the isozyme revealed the occurrence of a single band for all the sapota genotypes studied in the present investigation (Table 8, Fig. 1 and Plate 3). However, the band position and intensity varied with the genotype under study indicating polymorphism of the isozyme for the genotypes studied. It was observed that high and medium intensity bands could be observed at Rm 37, Rm 38 and Rm 39 positions. The most frequent esterase band was noticed at Rm 37 in 11 genotypes studied in the present investigation.

However, the occurrence of bands at three different Rm values for the sapota genotypes studied indicated the different mobility pattern suggesting the existence of different molecular weights for the bands. But because of the low variability and prevalence of a low polymorphism, the results with esterase isozyme alone cannot be utilized for identification of genotypes of sapota. However, evidence of genetic variation among genotypes with esterase isozyme was reported in mango by Subedi *et al.* (2005); in cashew by Maranan and Mendioro (2008); in rubber by Sreelatha *et al.* (1993) and Sobhana *et al.* (2001); in muskmelon by Sujatha *et al.* (1991); in *Cymbopogan* species by Ganjewala and Luthra (2007); and in arecanut by Swaminathan and George (2007). Further, the characterization of genotypes to distinguish them, based on esterase banding pattern is presented in Table 36.

A summation of the results on banding pattern of isozymes, namely, peroxidase, polyphenol oxidase, catalase and esterase for the 25 sapota genotypes studied in the present investigation indicated polymorphism for all the isozymes

with regards to genotypes except esterase isozyme. However, peroxidase isozyme was found to be most useful and efficacious, since it had resulted in clear distinction of 15 genotypes studied and a combination of peroxidase with either polyphenol oxidase or catalase isozymes was found to efficiently distinguish all the 25 sapota genotypes studied in the present investigation. Further, the ease of availability and low cost of the chemicals for assay of peroxidase isozyme, makes it ideal for use as effective molecular marker for sapota.

Characterization of sapota genotypes based on specific isozyme markers identified:

The present study revealed that peroxidase, polyphenol oxidase and catalase isozymes may be used as marker (single isozyme specific marker) for identification of 19 genotypes out of 25 sapota genotypes studied, the exceptional genotypes being Cricket Ball, Dwarapudi, Gede Sapota, PKM – 3, Kirthibarthi and DHS – 2.

Isozyme analysis revealed (Table 37 and Appendix II) presence of triple band peroxidase (at Rm 50, Rm 37, Rm 46) and single band catalase isozyme (at Rm 53) for the genotype Pakala Round, which is identified as specific molecular marker. Similarly two isozyme markers were also identified for the genotypes, namely, Singapore (peroxidase at Rm 33, Rm 42 and catalase at Rm 63) and Krishna Rao (peroxidase at Rm 29, Rm 40 and polyphenol oxidase at Rm 28).

Further, peroxidase isozyme was very effective to discriminate different genotypes, namely, Pala (Rm 27, Rm 44), CO – 1 (Rm 38, Rm 39), Brazilian Sapota (Rm 29, Rm 43), CO – 2 (Rm 29, Rm 42), Ulavapadu Selection (Rm 29, Rm 46), Badami (Rm 30, Rm 41), Gutti (Rm 30, Rm 43), Calcutta Round (Rm

30, Rm 45), PKM – 2 (Rm 30, Rm 42), Gavarayya (Rm 31, Rm 42), DHS – 1 (Rm 33, Rm 41) and PKM – 4 (Rm 28, Rm 46). Further, polyphenol oxidase could be identified as a marker for the genotypes Kalipatti (Rm 19, Rm 41), Tagarampudi (Rm 26), CO – 2 (Rm 21, Rm 37), Kirthibarthi (Rm 23, Rm 44), PKM – 2 (Rm 23, Rm 38), PKM – 1 (Rm 24, Rm 38), Krishna Rao (Rm 28), while catalase was identified as a definite marker for the genotypes CO – 3 (Rm 60), PKM -1 (Rm 62), Singapore (Rm 63) and Pakala Round (Rm 53).

A combination of more than one isozyme markers was also evident from the present study to distinguish the sapota genotypes where a single isozyme marker cannot be employed for the identification of genotypes. The genotype Cricket Ball could be identified by the presence of a combination of peroxidase (Rm 31, Rm 41) and polyphenol oxidase (Rm 21), while the genotype Dwarapudi can be identified by the presence of a combination of peroxidase (Rm 28, Rm 41) and catalase (Rm 56). Further, the genotype Gede Sapota can be identified by peroxidase (Rm 28, Rm 41) and catalase (Rm 58), the genotype Kirthibarthi by peroxidase (Rm 31, Rm 41) and polyphenol oxidase (Rm 23, Rm 44), the genotype DHS – 2 by peroxidase (Rm 29, Rm 41) and polyphenol oxidase (Rm 27) and the genotype PKM - 3 by peroxidase (Rm 28, Rm 45) and catalase (no band). However, the isozyme esterase was found to be non-specific isozyme suggesting its limited utility for use as isozyme marker for sapota genotypes.

5.4 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 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. Hence, the total variance needs to be partitioned into heritable and non-heritable components to assess the inheritance pattern into particular character under study (Siva Subramanian and Menon, 1973). Higher phenotypic coefficient of variation over genotypic coefficient of variation indicates the influence of environment over the character.

Heritability indicates the relative degree at which a character is transmitted from parent to offspring. High heritability values indicate that the character under study is less influenced by environment in their expression and such characters could be improved by adopting simple selection methods (Uma *et al.*, 2000). Further, the information on genetic variation, heritability and genetic advance helps to predict the genetic gain that could be obtained in later generations, if selection is to be exercised for improving the particular trait under study. A relative comparison of heritability and expected genetic advance expressed as per cent mean also gives an idea about the nature of gene action governing a particular character (Singh and Narayanam, 2006).

Selection would be the most important and easier and time saving method in sapota for improvement of existing cultivars and development of new ones. However, for an effective selection programme, information on genetic parameters of variability for different traits is a prerequisite for fruit breeding programmes. The phenotypic and genotypic coefficients of variation provide a

measure of the variability available in the crop and also help in comparison of the variability present for different characters. Further, heritability has to be considered in conjunction with genetic advance to get an idea about expected genetic gain in the next generation.

In an important fruit crop like sapota very few attempts were made to study the extent of variability and heritability. Hence, genetic studies were also undertaken in respect of 25 genotypes of sapota to estimate variability, heritability and genetic advance.

The range of phenotypic coefficient of variation estimated was 0.67 to 37.96 among the tree morphological characters, while it ranged from 4.52 to 46.75 among the fruit and biochemical characters. Further, the range of genotypic coefficient of variation among the tree morphological characters was 0.44 to 37.59 (Table 12) and 4.12 to 40.69 among the fruit and biochemical characters studied (Table 13).

High variability (PCV and GCV) values were recorded for the tree morphological characters, namely, shape of the tree, branch density and leaf area and for fruit morphological and biochemical characters, namely, fruit weight, fruit yield, fruit shape, flesh texture, fruit dessert quality, seed number/fruit, seed weight, pulp:seed ratio, total soluble solids, titrable acidity, sugar:acid ratio, ascorbic acid and total phenols indicating the effectiveness of selection for these traits. Similar trends regarding variability of PCV and GCV were obtained in litchi (Ghanta *et al.*, 1994), in banana (Rosamma and Namboodiri 1990, Rekha and Prasad, 1993); in mango (Karibasappa, 1995); and in acid lime (Ranpise and

Desai, 1994) by different researchers, these reports support the present findings in sapota.

However, low values for variability was observed for pollen size, pollen fertility, fruit length, fruit width and non-reducing sugars in the present investigation. Further, moderate variability was recorded for leaf width, style length, fruit surface texture, flesh colour and reducing sugars in the present investigation (Tables 12 and 13).

In addition, the values of genotypic coefficient of variation were slightly lower than phenotypic coefficient of variation for all the traits studied clearly indicating the influence of environment for different traits investigated. Similar results were reported earlier by Reddy *et al.* (2002) in cashew.

In the present investigation, heritability values for the different tree morphological traits ranged from 41.87 to 99.03 per cent; and from 55.94 to 97.97 per cent for fruit and biochemical characters (Tables 12 and 13). Higher heritability values were noticed for all the traits studied, namely, fruit weight, fruit yield, fruit shape, total sugars and ascorbic acid barring pollen size and titrable acidity, which had recorded moderate heritability. Identical results on heritability were reported earlier for fruit length, fruit width, fruit weight, and seed weight in certain fruit crops, namely, litchi (Ghanta *et al.*, 1994); for fruit yield in banana (Rosamma and Namboodiri 1990; Rekha and Prasad, 1993 and Uma *et al.*, 2000); and in acid lime (Ranpise and Desai, 1994); for fruit length, fruit width, fruit weight, total soluble solids, reducing sugars, total sugars and ascorbic acid in mango (Attri *et al.*, 1999); and for sugar:acid ratio, skin colour and pulp colour in mango by Karibasappa (1995). In the present investigation, moderate heritability

values for titrable acidity were noticed, which are in accordance with the results obtained in banana by Rekha and Prasad (1993).

The genetic advance as per cent mean (Tables 12 and 13) ranged from 0.58 to 77.14 for tree morphological characters and 8.05 to 81.51 for fruit morphological and biochemical characters. High genetic advance as per cent mean was recorded for the tree morphological characters, namely, growth habit of the tree, shape of the tree, branching pattern, branch density, leaf area and style length; and for all the fruit morphological and biochemical characters studied with the exception of fruit length, fruit width, peel colour and flesh taste in the present investigation indicating that most of the traits are heritable in nature, therefore the breeder can rely on these characters. Similar results of high values of genetic advance as per cent mean was reported in litchi for fruit weight and seed weight (Ghanta *et al.*, 1994), while for titrable acidity and total soluble solids in banana by Rekha and Prasad (1993) which support the results in sapota.

The overall perusal of the above results on variability, heritability and genetic advance as per cent mean together in sapota genotypes in general indicated high variability, heritability and genetic advance noticed for characters, namely, shape of the tree, branch density, leaf area, fruit weight, fruit shape, flesh texture, fruit dessert quality, seed number /fruit, seed weight, pulp:seed ratio, total soluble solids, sugar:acid ratio, ascorbic acid and total phenols indicating that these characters are clearly governed by additive gene action, hence the breeder can rely on these characters and in passing generations simple selection process for improvement of the above characters in sapota will be rewarding.

From these genetic studies it can be inferred that there is prevalence of high variability (as evident from high PCV and GCV values) in the sapota germplasm and still there is scope for enriching the germplasm with more accessions. Characters, namely, shape of the tree, branch density, leaf area, fruit weight, fruit shape, flesh texture, fruit dessert quality, seed number /fruit, seed weight, pulp:seed ratio, total soluble solids, sugar:acid ratio, ascorbic acid and total phenols exhibited high values for heritability and genetic advance and these indicate excellent scope for use of these characters as selection indices in sapota improvement.

5.5 PRINCIPAL COMPONENT ANALYSIS

Principal component analysis is a useful data reduction biometrical technique which works by removing inter-correlations among variables. By using principal component analysis not only is the number of comparisons between treatment means reduced, but the meaningfulness of these comparisons is enhanced (Broschat, 1979). The method provides information on the degree of relatedness among individuals or genotypes and results in formation of clusters of morphologically similar phenotypes and is highly helpful in characterization of germplasm (Iezzoni and Pritts, 1991). There are instances where principal component analysis and clustering technique has been extensively employed for classification of the genotypes in large germplasm pools in several horticultural crops, such as banana (Mandal *et al.*, 2007), guava (Hernandez-Delgado *et al.*, 2007), mango (Mitra *et al.*, 2008 and Subedi *et al.*, 2005), grape (Roytchev, 2007), cacao (Bekele and Bekele, 1996), coconut (Vargas and Blanco, 2000),

soursop (Evangelista-Lozano *et al.* , 2003), peach (Perez *et al.*, 1993), cherry (Hillig and Iezzoni, 1988), apple (Moore, 1965 and Currie *et al.*, 2000), raspberry (Jennings, 1979 and Dale, 1988), quince (Silva *et al.*, 2005), walnut (Kodad and Company, 2006 and Pandey *et al.*, 2007), apricot (Byrne, 1989), date palms (Khan *et al.*, 2008) and garlic (Matus *et al.*, 1999).

In the present study principal component analysis was carried out for tree morphological characters, fruit and biochemical characters and banding pattern of four leaf isozymes in 25 genotypes of sapota and the results obtained are discussed hereunder (Tables 14-31, Fig. 13-24).

5.5.1 Principal component analysis of tree morphological characters

In the present study, the first three principal components with eigen values more than one were observed to contribute 74.23 per cent of cumulative variability among the genotypes for the 11 different tree morphological characters studied (Tables 14). The other principal components had eigen values less than one and hence were considered non-significant and ignored, as they were unlikely to have any practical significance (Legendre and Legendre, 1984). The character loading values for principal components represented the weights defining the contribution of different characters for the respective principal components. Further, the loadings signs (+ / -) are indicative of the direction of contribution, similar to that of regression coefficients.

The total tree morphological characters were sorted out into three major principal components. The contribution of the main characters for variance can be easily identified by the characters loaded on the first principal component as it

explained maximum variance. The first principal component contributed for 40.81 per cent of variability. Further, characters, namely, leaf area (0.41), leaf length (0.38) and pollen production per anther (0.34), were observed to be loaded with positive correlation, while, pollen size (-0.44), was noticed to be loaded with negative correlation for the first principal component. These results suggest that this principal component represents leaf and pollen characters of all the genotype under study and maximum variability is present in these characters (Table 15).

The second principal component accounting for 21.72 per cent of the total variance reflected high loadings for style length (0.49), branch density (0.48) and branching pattern (0.30) which were positively correlated; and pollen fertility (-0.47) and shape of the tree (-0.38) which were negatively correlated. This reveals that this principal component reflects floral characters of each genotype.

The third principal component described 11.71 per cent of variability and showed high positive correlation for growth habit of tree (0.72) coupled with negative correlation for branch density (-0.40) and pollen fertility (-0.31). Hence, it can be suggested that this principal component reflects growth habit of the tree of all the genotypes under study.

In the two dimensional and three dimensional scattered diagrams (Fig. 13 and 14) the genotypes of divergent clusters were observed to be scattered far apart, while genotypes of similar clusters were placed close to each other in the centre.

The dendrogram constructed adopting Ward's minimum variance method grouped the 25 sapota genotypes studied into six clusters (Table 17, Fig. 15). Maximum number of genotypes (nine) were grouped in Cluster – II, followed by

six genotypes in Cluster I, while minimum number (two) were recorded for Cluster IV and VI. Clustering of the genotypes in the present study for tree morphological traits did not exhibit any relevance to geographical diversity.

An analysis of the results on inter-cluster distance revealed maximum distance between Cluster I and Cluster V, followed by Cluster II and Cluster V indicating the existence of wide genetic diversity between genotypes of these clusters (Table 18, Fig. 16). Further, Cluster V had recorded high mean values for most of the important attributes, namely, leaf area, pollen size, pollen production/ anther, pollen fertility and style length indicating desirability of the genotypes in the cluster for use in the crop improvement programmes.

A perusal of the results on intra-cluster distance revealed maximum distance for Cluster VI followed by Cluster V, indicating the existence of variability among the genotypes within the clusters for the tree morphological characters studied (Table 18, Fig. 16). However, Cluster II and Cluster IV had recorded minimum intra-cluster distance indicating greater similarity of the genotypes in these clusters for the tree morphological traits studied.

5.5.2 Principal component analysis of fruit and biochemical characters

A perusal of the results on principal component analysis for fruit morphological and biochemical characters revealed the first three principal components with eigen values more than one to contributed for 89.79 per cent of the total variability among the 25 sapota genotypes studied for 22 fruit morphological and biochemical characters (Table 20). The other principal components with eigen values less than one were ignored. The total fruit

characters could be sorted out into three major principal components. The contribution of the main characters for variance can be easily identified by the characters loaded on the first principal component as it explained maximum variance.

The first principal component (PC I) accounted for 59.07 per cent of the variability and was characterized by high and positive loadings for fruit surface texture (0.27), titrable acidity (0.27), fruit weight (0.26) and peel colour (0.25) coupled with negative loadings for reducing sugars (-0.27), TSS (-0.27), ascorbic acid (-0.26), flesh colour (-0.25) and flesh taste (-0.25) (Table 21). These results suggest that this principal component represents fruit quality component of all genotypes under study (Table 21).

The second principal component (PC II) explained prevalence of 18.93 per cent of the variability and showed high positive loadings for total sugars (0.47), pulp:seed ratio (0.46), sugar:acid ratio (0.38) and non-reducing sugars (0.26) coupled with negative loadings of total phenols (0.42). It may be inferred that this principal component reflects sugars content and phenol content of all the genotypes.

The third principal component (PC III) described for 11.78 per cent of the variability and showed high positive correlation for fruit shape (0.52), flesh texture (0.51), fruit dessert quality (0.31) and yield per tree (0.32); and negative loadings for seed number per fruit (-0.28). These results suggest that this principal component reflects the fruit shape and fruit yield components of all the genotypes under study.

In the two dimensional and three dimensional scattered diagrams (Fig. 17 and 18) genotypes of divergent clusters were noticed to be scattered far apart, while genotypes of similar clusters were placed close to each other.

Dendrogram of the 25 sapota genotypes for the different fruit morphological and biochemical characters studied in the present investigation, resulted in hierarchical clustering of the genotypes into six different clusters (Table 23, Fig. 19). Cluster I had recorded maximum number of genotypes (nine), followed by six in Cluster V, while minimum number of genotypes (one) were recorded for Cluster VI. Further, clustering of the genotypes for the fruit morphological and biochemical traits was observed to have no relevance to geographical diversity.

A perusal of the results on inter-cluster distances (Table 24, Fig. 20) revealed maximum distance between Cluster IV and VI followed by Cluster IV and V, Cluster I and VI and Cluster III and VI, indicating the existence of wide genetic distance between the genotypes of these clusters. Based on these results, it can be inferred that crosses may be affected between the genotypes of these clusters for development of improved varieties with regards to fruit morphological and biochemical characters.

Analysis of the intra-cluster distance revealed maximum distance for Cluster IV followed by Cluster V and Cluster III, indicating the existence of variability among the genotypes within the clusters. Further, Cluster VI had recorded high mean values for fruit weight, ascorbic acid and pulp:seed ratio, Cluster II for fruit yield/tree, while Cluster V had recorded high means for total sugars and sugar:acid ratio. Hence, selection of genotypes from these clusters

accordingly is suggested for use in breeding programmes aimed at specific improvement of the above traits.

5.5.3 Principal component analysis of isozyme banding pattern

The technique of principal component analysis of isozyme banding pattern was studied by several workers in various horticultural crops to classify and group the genotypes (Subedi *et al.*, 2005 in mango; Mowrey and Werner, 1990 in *Prunus* species; Revilla and Tracy, 1995 in sweet corn; Dias *et al.*, 1994 in Cabbage and Lefevre and Charrier, 1993 in *Manihot*).

In the present study, the principal component analysis of leaf isozyme banding pattern for four different enzyme systems, namely, esterase, peroxidase, polyphenol oxidase and catalase revealed constitution of two major principal components with eigen values more than one contributing to 73.04 per cent of cumulative variability among the 25 sapota genotypes evaluated (Table 26). The contribution of the isozymes for variance can be easily identified by the characters loaded on the first principal component as it explained maximum variance.

The percentage variation accounted for by PC I and PC II were 45.40 and 27.64 per cent respectively. The most important variables integrated in PC I were esterase (0.60), peroxidase (0.58) and polyphenol oxidase (0.54). This suggests that this principal component represents esterase, peroxidase and polyphenol oxidase isozymes of all the genotypes under study. Further, high negative loadings of catalase (-0.90) isozyme were recorded for the second principal component, suggesting that this principal component represents catalase isozyme of all the genotypes (Table 27).

The dendrograms constructed adopting Ward's minimum variance method grouped the 25 sapota genotypes into six clusters, wherein maximum number of genotypes (12) were grouped in Cluster I, while minimum number of genotypes (two) were grouped in Clusters II, IV and VI (Table 29, Fig. 23).

Analysis of the results on inter-cluster distances (Table 30, Fig. 24) revealed maximum distance between Cluster II and VI followed by Cluster V and Cluster VI, Cluster I and VI and Cluster III and VI indicating the existence of wide genetic diversity between genotypes of these clusters. Further, maximum intra-cluster distance was recorded for Cluster VI followed by Cluster III indicating the existence of variability among the genotypes within the cluster for isozyme banding patterns.

From the summation of results of the aforesaid principal component analysis on tree morphological, fruit and biochemical characters and isozyme banding patterns, it is evident that three major principal components (PCs) could be identified in respect of tree morphological characters, which accounted for 74.23 per cent of cumulative variance. PC I explained 40.81 per cent of variance and negatively correlated with pollen size and positively correlated with leaf area, leaf length and pollen production per anther. PC II had high positive correlation for style length, branch density, pollen fertility and branching pattern and high negative correlation for shape of the tree while PC III had high positive correlation for growth habit of tree and negative correlation for branch density and pollen fertility.

When fruit morphological characters were subjected to principal component analysis, three major PC's could be formed, which accounted for

89.79 per cent of cumulative variance. PC I accounted for 59.07 per cent of variance and reflected the fruit quality components. PC II accounted for 18.93 per cent of variance and had high positive loadings of total sugars, pulp:seed ratio, sugar:acid ratio, non-reducing sugars and high negative loadings of total phenols. PC III explained 11.78 per cent of the variance and reflected fruit shape, fruit texture and fruit yield/tree. Principal component analysis of isozyme banding resulted in two major PC's, both accounting for 73.04 per cent of cumulative variance. PC I accounted for 45.40 per cent of variance and reflected esterase, peroxidase and polyphenol oxidase isozymes, while PC II, accounting for 27.64 per cent of variance, had high loadings of catalase isozyme. By principal component analysis the 25 genotypes could be grouped into six clusters. It is also evident that the inter-cluster distance between Cluster I and Cluster VI was found to be the highest indicating the prevalence of wider variability and genetic divergence among these clusters and this can be utilized by the fruit breeders by choosing them as parents for crossing programme.

Among the PCs obtained from principal component analysis PC I explained maximum variance and hence important traits for characterization of the genotypes were identified from PC I, which are leaf length, leaf area, pollen size, pollen production per anther, fruit weight, fruit surface texture, peel colour, flesh colour, flesh taste, reducing sugars, titratable acidity and ascorbic acid.

A comparison of genetic variability studies and principal component analysis revealed that the characters with high variability, heritability and genetic advance as per cent mean, namely, shape of the tree, branch density, leaf area, fruit weight, fruit yield, fruit shape, flesh texture, fruit dessert quality, seed

number, seed weight, pulp:seed ratio, TSS, sugar:acid ratio, ascorbic acid and total phenols exhibited high variability in principal component analysis as well. These characters contained high loadings in PC I and PC II or PC III confirming the extent of high variability for these characters.

By employing principal component analysis technique the genetic relatedness of diversity existing in 25 genotypes of sapota could be precisely assessed and established and such information would be extremely useful to the fruit breeders.

Characterization of sapota genotypes

In the present study on characterization of sapota genotypes (Table 38 and Appendix III), it can be noticed that considerable variability could be noticed in characters, namely, tree shape, branching pattern, leaf characters, fruit shape, fruit weight, fruit yield/tree, TSS, sugar:acid ratio, pulp:seed ratio, flesh texture and colour and banding pattern of (intensity and position) of isozymes, particularly, peroxidase and hence these characters can be used to characterize the genotypes. The 25 sapota genotypes evaluated consisted of local cultivars, hybrids, and improved cultivars. Among these genotypes the extent of PCV varied from 0.67 to 37.96 among the tree morphological characters and from 4.52 to 46.75 among the fruit and biochemical characters. Whereas, GCV ranged from 0.44 to 37.59 and 4.12 to 40.69 respectively among tree morphological and fruit and biochemical characters studied.

Based on a comparison of cluster means as well as general means the genotypes, namely, Gavarayya, DHS – 1, PKM – 1 and PKM – 3 could be adjudged superior in terms of higher tree yield, fruit size, TSS, sugar content,

ascorbic acid content, low total phenols and other fruit characters and the genotypes Gutti, DHS – 2, Pakala Round, Pala, Kirthibarthi and Ulavapadu Selection, CO – 2, Co – 3, PKM – 4, CO – 1, Badami, Gede Sapota, Kalipatti, PKM – 2, Tagarampudi, Singapore, Krishna Rao, Calcutta Round and Dwarapudi were found to be medium in performance.

FUTURE LINE OF WORK

1. In the present study morphological and fruit biochemical characters were chosen for characterization of the sapota genotypes. However, chemotaxonomic studies may also be undertaken for further characterization and classification of the genotypes.
2. Certain isozymes, namely, peroxidase, polyphenol oxidase, catalase and esterase were analysed for distinguishing the genotypes in this study. However, other isozymes systems may also be tried for a precise identification of the genotypes.
3. Biometric studies may be continued involving large number of accessions/genotypes and some more biometric techniques may be employed to understand adequately about basic genetics, namely, character association and their direct and indirect effects and the extent of total diversity in germplasm of sapota.
4. There is scope for precise estimation of genetic diversity in sapota germplasm using DNA markers (RAPD or SSR markers) in conjunction with specific morphological and fruit characters and isozyme markers as diagnostic tools to have complete genome coverage of the species involved.

Table 33: Characterisation of sapota genotypes based on peroxidase banding pattern

Genotype	Characteristic band intensity and position
Pala	High at Rm 27 and Medium at Rm 44
Dwarapudi	High at Rm 28 and Medium at Rm 41
Gede Sapota	Medium at Rm 28 and Low at Rm 41
Kalipatti and PKM - 3	Medium at Rm 28 and Low at Rm 45
Tagarampudi	Medium at Rm 28 and Low at Rm 46
CO - 1	Low at Rm 28 and Low at Rm 39
PKM - 4	Low at Rm 28 and Low at Rm 46
Brazilian Sapota	High at Rm 29 and Medium at Rm 43
Krishna Rao	Medium at Rm 29 and Low at Rm 40
CO - 3	Medium at Rm 29 and Low at Rm 41
DHS - 2	Low at Rm 29 and Low at Rm 41
CO - 2	Low at Rm 29 and, Low at Rm 42
Ulavapadu Selection	Low at Rm 29 and Low at Rm 46
Badami	High at Rm 30 and Low at Rm 41
Gutti	High at Rm 30 and Medium at Rm 43
Pakala Round	Medium at Rm 30 and Low at Rm 37, Low at Rm 46
Calcutta Round	Medium at Rm 30 and Low at Rm 45
PKM – 2	Low at Rm 30 and Low at Rm 42
Kirthibarthi	Medium at Rm 31 and Low at Rm 41
Gavarayya	Medium at Rm 31 and Low at Rm 42
Cricket Ball and PKM - 1	Low at Rm 31 and Low at Rm 41
Singapore	Medium at Rm 33 and Low at Rm 42
DHS - 1	Low at Rm 33 and Low at Rm 41

Table 34: Characterisation of sapota genotypes based on polyphenol oxidase banding pattern

Genotype	Characteristic band intensity and position
Kalipatti	Medium at Rm 19 and Low at Rm 41
CO - 1 and Cricket ball	Medium at Rm 21
CO - 2	Low at Rm 21 and Low at Rm 37
DHS - 1	Low at Rm 21
Dwarapudi, Gavarayya, Gede Sapota, PKM - 3, Ulavapadu Selection, Pala and Singapore	Low at Rm 23
Kirthibarthi	Low at Rm 23 and Low at Rm 44
PKM – 2	Low at Rm 23 and Low at Rm 38
Badami, Brazilian Sapota, Calcutta Round and PKM - 4	Low at Rm 24
Pakala Round and Gutti	Low at Rm 25
Tagarampudi	Low at Rm 26
DHS - 2	Medium at Rm 27
CO - 3	Low at Rm 27
PKM – 1	Low at Rm 24 and Low at Rm 38
Krishna Rao	Low at Rm 28

Table 35: Characterisation of sapota genotypes based on catalase banding pattern

Genotype	Characteristic band intensity and position
Pakala Round	High at Rm 53
Brazilian Sapota and Ulavapadu Selection	High at Rm 54
Kalipatti and Kirthibarthi	Low at Rm 54
Badami and CO - 1	High at Rm 55
CO - 2	Low at Rm 55
Dwarapudi, Pala and PKM -4	High at Rm 56
Gavarayya and Tagarampudi	High at Rm 57
Gede Sapota, Gutti, DHS - 2 and Calcutta Round	High at Rm 58
CO - 3	High at Rm 60
DHS - 1, PKM - 2 and Cricket Ball	High at Rm 61
PKM – 1	High at Rm 62
Singapore	Medium at Rm 63
PKM – 3 and Krishna Rao	--

Table 36: Characterisation of sapota genotypes based on esterase banding pattern

Genotypes	Characteristic band intensity and position
Gavarayya, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Ulavapadu Selection, DHS - 2, PKM - 2 and PKM - 3	High at Rm 37
CO - 1, CO - 2	Medium at Rm 37
Badami, Calcutta Round, Gede Sapota, Kalipatti, Singapore, CO - 3, PKM - 1 and PKM - 4	High at Rm 38
Brazilian Sapota, Cricket Ball, Dwarapudi, Gutti, Tagarampudi and DHS - 1	High at Rm 39

Table 37: Identified specific isozyme markers in sapota genotypes

S. No.	Sapota genotype	Identified specific isozyme marker
A.	Two isozymes specific marker:	
i)	Pakala Round	Peroxidase (Rm 30, Rm 37 and Rm 46) Catalase (Rm 53)
ii)	Singapore	Peroxidase (Rm 33, Rm 42) Catalase (Rm 63)
iii)	Krishna Rao	Peroxidase (Rm 29, Rm 40) Polyphenol oxidase (Rm 28)
B.	Single isozyme specific marker:	
a.	<u>Peroxidase:</u>	
i)	Pala	Rm 27, Rm 44
ii)	CO – 1	Rm 28, Rm 39
iii)	PKM - 4	Rm 28, Rm 46
iv)	Brazilian Sapota	Rm 29, Rm 43
v)	CO – 2	Rm 29, Rm 42
vi)	Ulavapadu Selection	Rm 29, Rm 46
vii)	Badami	Rm 30, Rm 41
viii)	Gutti	Rm 30, Rm 43
ix)	Calcutta Round	Rm 30, Rm 45
x)	PKM – 2	Rm 30, Rm 42
xi)	Gavarayya	Rm 31, Rm 42
xii)	DHS - 1	Rm 33, Rm 41
b.	<u>Polyphenol oxidase:</u>	
i)	Kalipatti	Rm 19, Rm 41
ii)	Tagarampudi	Rm 26
iii)	CO – 2	Rm 21, Rm 37
iv)	Kirthibarthi	Rm 23, Rm 44
v)	PKM - 2	Rm 23, Rm 38
vi)	PKM - 1	Rm 24, Rm 38
c.	<u>Catalase:</u>	
i)	CO – 3	Rm 60
ii)	PKM – 1	Rm 62

Table 32: Performance of local cultivars, improved cultivars and hybrids of sapota based on important fruit morphological and biochemical characters

S. No.	Character	Pedigree	Fruit Weight (g)	Fruit yield tree ⁻¹ (kg)	Seed Number/ fruit	Pulp:seed ratio	Reducing sugars (%)	Ascorbic acid (mg 100g ⁻¹)	Total phenols (mg 100g ⁻¹)
Local Cultivars									
1.	Badami	--	64.35	32.35	3	25.07	6.75	6.50	0.69
2.	Calcutta Round	--	77.75	45.15	5	20.50	8.48	6.75	0.40
3.	Cricket Ball	--	198.74	55.40	8	30.88	6.53	5.87	0.33
4.	Dwarapudi	--	83.63	37.65	7	18.02	5.88	5.12	0.78
5.	Gavarayya	--	104.03	55.15	3	43.88	6.95	7.87	0.36
6.	Gede Sapota	--	63.13	31.70	3	21.99	6.29	6.75	0.67
7.	Gutti	--	102.01	36.15	3	45.25	7.94	9.00	0.61
8.	Kalipatti	--	64.42	83.40	3	25.10	10.65	11.37	0.36
9.	Kirthibarthi	--	86.72	86.45	4	53.49	8.47	7.50	0.53
10.	Krishna Rao	--	67.74	31.45	5	21.67	7.87	7.50	0.64
11.	Pakala Round	--	76.37	55.25	4	45.29	7.91	6.50	0.49
12.	Pala	--	63.38	65.75	3	39.60	10.05	9.50	0.23
13.	Singapore	--	92.44	85.35	6	25.42	8.50	11.00	0.34
14.	Tagarampudi	--	63.27	60.45	5	14.68	7.77	8.00	0.41
Mean			86.29	54.40	4.43	30.77	7.86	7.80	0.49
Range			63.13	31.45	3	14.66	5.88	5.12	0.23
Maximum			198.74	86.45	8	53.49	10.65	11.37	0.79
Improved cultivars									
1.	Brazilian Sapota	Selection from Brazil	97.57	52.80	8	11.35	7.71	7.38	0.50
2.	Ulavapadu Selection	Selection from Andhra Pradesh	44.83	63.50	2	27.09	8.15	9.75	0.25
3.	CO – 2	Selection from cv. Baramasi	150.65	26.20	3	100.80	8.90	10.25	0.33
4.	PKM – 1	Selection from cv. Gutti	93.31	89.95	4	29.39	8.81	10.38	0.41
5.	PKM – 4	Selection from cv. PKM – 1	127.04	70.50	4	31.49	8.31	9.63	0.42
Mean			94.19	56.88	4.57	32.93	8.05	8.65	0.46
Range			44.83	26.20	2	11.35	7.71	7.38	0.25
Maximum			150.65	89.95	8	100.80	8.90	10.38	0.50
Hybrids									
1.	CO – 1	Cricket Ball x Oval	170.67	26.40	4	56.11	6.88	10.75	0.26
2.	CO – 3	Cricket Ball x Vavivalasa	127.29	32.95	4	39.79	6.39	9.75	0.51
3.	DHS - 1	Kalipatti x Cricket Ball	120.21	81.55	3	40.58	8.11	9.00	0.43
4.	DHS – 2	Kalipatti x Cricket Ball	89.01	75.80	3	40.63	8.20	8.13	0.66
5.	PKM – 2	Gutti x Kirthibarthi	81.89	66.75	5	21.10	7.68	7.50	0.61
6.	PKM - 3	Gutti x Cricket Ball	105.08	93.35	4	27.89	9.21	10.25	0.49
Mean			111.37	61.54	3.89	43.07	8.10	9.35	0.45
Range			81.89	26.40	3	21.10	6.88	7.50	0.26
Maximum			170.67	93.35	5	56.11	9.21	10.75	0.66

Table 38: Characterization of sapota genotypes based on morphological, biochemical and isozyme studies (Abstract)

S. No.	Genotype	Tree morphological characters	Fruit morphological and biochemical characters	Isozyme markers
1	Badami	Tree shape broadly pyramidal, leaf elliptic, branching verticillate	Fruit shape globose, flesh brown, flesh texture soft, seeds 3, fruit weight 64.35 g, yield tree ⁻¹ 32.35 kg, TSS 21.20 ° brix, sugar acid ratio 275.23, pulp:seed ratio 25.07	Peroxidase Rm 30, Rm 41
	Clustering pattern	Cluster I	Cluster I	Cluster I
2	Brazilian Sapota	Tree shape spherical, leaf broadly elliptic, branching verticillate	Fruit shape oval, flesh greenish brown, flesh texture sandy, seeds 8, fruit weight 97.57 g, yield tree ⁻¹ 52.80 kg, TSS 23.10 ° brix, sugar acid ratio 399.14, pulp:seed ratio 11.34	Peroxidase Rm 29, Rm 43
	Clustering pattern	Cluster II	Cluster IV	Cluster I
3	Calcutta Round	Tree shape broadly pyramidal, leaf sub-ovate, branching verticillate	Fruit shape globose, flesh reddish brown, flesh texture firm, seeds 5, fruit weight 77.75 g, yield tree ⁻¹ 45.15 kg, TSS 22.90 ° brix, sugar acid ratio 303.20, pulp:seed ratio 20.50	Peroxidase Rm 30, Rm 45
	Clustering pattern	Cluster II	Cluster I	Cluster II
4	Cricket Ball	Tree shape broadly pyramidal, leaf elliptic, branching horizontal	Fruit shape globose, flesh yellowish brown, flesh texture soft, seeds 8, fruit weight 198.74 g, yield tree ⁻¹ 55.40 kg, TSS 20.20 ° brix, sugar acid ratio 201.59, pulp:seed ratio 30.86	Peroxidase Rm 31, Rm 41 + Polyphenol oxidase Rm 21
	Clustering pattern	Cluster V	Cluster IV	Cluster III
5	Dwarapudi	Tree shape elliptic, leaf broadly elliptic, branching verticillate	Fruit shape acron, flesh brown, flesh texture firm, seeds 7, fruit weight 83.63 g, yield tree ⁻¹ 37.65 kg, TSS 21.30 ° brix, sugar acid ratio 256.32, pulp:seed ratio 18.02	Peroxidase Rm 28, Rm 41 + Catalase Rm 56
	Clustering pattern	Cluster I	Cluster I	Cluster I

(Contd....)

Table 38: contd....

S. No.	Genotype	Tree morphological characters	Fruit morphological and biochemical characters	Isozyme markers
6	Gavarayya	Tree shape spherical, leaf broadly elliptic, branching irregular	Fruit shape oblong, flesh brown, flesh texture firm, seeds 3, fruit weight 104.03 g, yield tree ⁻¹ 55.15 kg, TSS 21.60 ° brix, sugar acid ratio 142.01, pulp:seed ratio 43.89	Peroxidase Rm 31, Rm 42
	Clustering pattern	Cluster III	Cluster II	Cluster I
7	Gede Sapota	Tree shape elliptic, leaf elliptic, branching verticillate	Fruit shape globose, flesh brown, flesh texture sandy, seeds 3, fruit weight 63.13 g, yield/ tree 31.70 kg, TSS 21.10 ° brix, sugar acid ratio 221.87, pulp:seed ratio 21.99	Peroxidase Rm 28, Rm 41 + Catalase Rm 58
	Clustering pattern	Cluster VI	Cluster I	Cluster I
8	Gutti	Tree shape spherical, leaf broadly elliptic, branching verticillate	Fruit shape oblong, flesh brown, flesh texture soft, seeds 3, fruit weight 102.01 g, yield tree ⁻¹ 36.15 kg, TSS 23.00 ° brix, sugar acid ratio 311.03, pulp:seed ratio 45.25	Peroxidase Rm 30, Rm 43
	Clustering pattern	Cluster I	Cluster V	Cluster I
9	Kalipatti	Tree shape broadly pyramidal, leaf elliptic, branching horizontal	Fruit shape acron, flesh brown, flesh texture firm, seeds 3, fruit weight 64.42 g, yield tree ⁻¹ 83.40 kg, TSS 23.90 ° brix, sugar acid ratio 293.17, pulp:seed ratio 25.10	Polyphenol oxidase Rm 19, Rm 41
	Clustering pattern	Cluster II	Cluster I	Cluster IV
10	Kirthibarthi	Tree shape broadly pyramidal, leaf broadly elliptic, branching horizontal	Fruit shape globose, flesh brown, flesh texture soft, seeds 4, fruit weight 86.72 g, yield tree ⁻¹ 86.45 kg, TSS 22.60 ° brix, sugar acid ratio 469.25, pulp:seed ratio 53.49	Peroxidase Rm 31, Rm 41 + Polyphenol oxidase Rm 23, Rm 44
	Clustering pattern	Cluster II	Cluster V	Cluster V

Table 38: contd....

S. No.	Genotype	Tree morphological characters	Fruit morphological and biochemical characters	Isozyme markers
11	Krishna Rao	Tree shape broadly pyramidal, leaf broadly elliptic, branching verticillate	Fruit shape acron, flesh reddish brown, flesh texture firm, seeds 5, fruit weight 67.74 g, yield tree ⁻¹ 31.45 kg, TSS 22.10 ° brix, sugar acid ratio 298.40, pulp:seed ratio 21.67	Peroxidase Rm 30, Rm 43 Polyphenol oxidase Rm 28
	Clustering pattern	Cluster II	Cluster I	Cluster VI
12	Pakala Round	Tree shape broadly pyramidal, leaf elliptic, branching verticillate	Fruit shape globose, flesh brown, flesh texture soft, seeds 4, fruit weight 76.37 g, yield tree ⁻¹ 55.25 kg, TSS 21.70 ° brix, sugar acid ratio 492.70, pulp:seed ratio 45.29	Peroxidase Rm 30, Rm 37, Rm 43 Catalase Rm 53
	Clustering pattern	Cluster II	Cluster V	Cluster I
13	Pala	Tree shape broadly pyramidal, leaf elliptic, branching horizontal	Fruit shape oval, flesh brown, flesh texture soft, seeds 3, fruit weight 63.38 g, yield tree ⁻¹ 65.75 kg, TSS 24.30 ° brix, sugar acid ratio 399.00, pulp:seed ratio 39.60	Peroxidase Rm 27, Rm 44
	Clustering pattern	Cluster II	Cluster V	Cluster I
14	Singapore	Tree shape broadly pyramidal shape of the tree, leaf broadly elliptic, branching horizontal	Fruit shape acron, flesh brown, flesh texture sandy, seeds 6, fruit weight 92.44 g, yield tree ⁻¹ 85.35 kg, TSS 23.00 ° brix, sugar acid ratio 295.30, pulp:seed ratio 25.42	Peroxidase Rm 33, Rm 42 Catalase Rm 63
	Clustering pattern	Cluster III	Cluster I	Cluster III
15	Tagarampudi	Tree shape broadly pyramidal shape of the tree, leaf elliptic, branching horizontal	Fruit shape globose, flesh reddish brown, flesh texture firm, seeds 5, fruit weight 63.27 g, yield tree ⁻¹ 60.45 kg, TSS 22.10 ° brix, sugar acid ratio 246.06, pulp:seed ratio 14.66	Polyphenol oxidase Rm 26
	Clustering pattern	Cluster I	Cluster I	Cluster I

Table 38: contd.....

S. No.	Genotype	Tree morphological characters	Fruit morphological and biochemical characters	Isozyme markers
16	Ulavapadu Selection	Tree shape elliptic, leaf elliptic, branching verticillate	Fruit shape oval, flesh brown, flesh texture soft, seeds 2, fruit weight 44.83 g, yield tree ⁻¹ 63.50 kg, TSS 23.40 ° brix, sugar acid ratio 347.28, pulp:seed ratio 27.09	Peroxidase Rm 29, Rm 46
	Clustering pattern	Cluster I	Cluster V	Cluster I
17	CO - 1	Tree shape elliptic, leaf broadly elliptic, branching verticillate	Fruit shape turbinate, flesh yellowish brown, flesh texture sandy, seeds 4, fruit weight 170.67 g, yield tree ⁻¹ 26.40 kg, TSS 21.30 ° brix, sugar acid ratio 214.74, pulp:seed ratio 56.11	Peroxidase Rm 28, Rm 49
	Clustering pattern	Cluster IV	Cluster III	Cluster III
18	CO - 2	Tree shape irregular, leaf broadly elliptic, branching verticillate	Fruit shape turbinate, flesh yellowish brown, flesh texture sandy, seeds 3, fruit weight 150.65 g, yield tree ⁻¹ 26.20 kg, TSS 22.30 ° brix, sugar acid ratio 276.04, pulp:seed ratio 100.80	Peroxidase Rm 29, Rm 42
	Clustering pattern	Cluster IV	Cluster VI	Cluster IV
19	CO - 3	Tree shape irregular, leaf broadly elliptic, branching verticillate	Fruit shape acron, flesh brown, flesh texture sandy, seeds 4, fruit weight 127.29 g, yield tree ⁻¹ 32.95 kg, TSS 20.70 ° brix, sugar acid ratio 216.05, pulp:seed ratio 39.79	Catalase Rm 60
	Clustering pattern	Cluster VI	Cluster III	Cluster I
20	DHS - 1	Tree shape semi-circular, leaf elliptic, branching verticillate	Fruit shape acron, flesh brown, flesh texture soft, seeds 3, fruit weight 120.21 g, yield tree ⁻¹ 81.55 kg, TSS 22.90 ° brix, sugar acid ratio 582.08, pulp:seed ratio 40.57	Peroxidase Rm 33, Rm 41
	Clustering pattern	Cluster V	Cluster II	Cluster III

Table 38: contd.....

S. No.	Genotype	Tree morphological characters	Fruit morphological and biochemical characters	Isozyme markers
21	DHS - 2	Tree shape spherical, leaf broadly elliptic, branching verticillate	Fruit shape globose, flesh reddish brown, flesh texture firm, seeds 3, fruit weight 89.06 g, yield tree ⁻¹ 75.80 kg, TSS 21.70 ° brix, sugar acid ratio 257.68, pulp:seed ratio 40.63	Peroxidase Rm 29, Rm 41 + Polyphenol oxidase Rm 27
	Clustering pattern	Cluster II	Cluster V	Cluster II
22	PKM - 1	Tree shape broadly pyramidal, leaf broadly elliptic, branching verticillate	Fruit shape elliptic, flesh brown, flesh texture soft, seeds 4, fruit weight 93.30 g, yield tree ⁻¹ 89.95 kg, TSS 24.10 ° brix, sugar acid ratio 227.20, pulp:seed ratio 29.39	Catalase Rm 62
	Clustering pattern	Cluster I	Cluster II	Cluster V
23	PKM - 2	Tree shape broadly pyramidal, leaf broadly elliptic, branching verticillate	Fruit shape acron, flesh brown, flesh texture firm, seeds 5, fruit weight 81.89 g, yield tree ⁻¹ 66.75 kg, TSS 21.50 ° brix, sugar acid ratio 175.10, pulp:seed ratio 21.10	Peroxidase Rm 30, Rm 42
	Clustering pattern	Cluster II	Cluster I	Cluster V
24	PKM - 3	Tree shape broadly pyramidal, leaf broadly elliptic, branching verticillate	Fruit shape oval, flesh yellowish brown, flesh texture firm, seeds 4, fruit weight 105.08 g, yield tree ⁻¹ 93.35 kg, TSS 23.70 ° brix, sugar acid ratio 461.73, pulp:seed ratio 27.88	Peroxidase Rm 28, Rm 45 + Catalase no band
	Clustering pattern	Cluster V	Cluster II	Cluster VI
25	PKM - 4	Tree shape broadly pyramidal, leaf elliptic, branching verticillate	Fruit shape elliptic, flesh brown, flesh texture sandy, seeds 4, fruit weight 127.04 g, yield tree ⁻¹ 70.50 kg, TSS 23.50 ° brix, sugar acid ratio 245.51, pulp:seed ratio 31.49	Peroxidase Rm 28, Rm 46
	Clustering pattern	Cluster III	Cluster III	Cluster I

CHAPTER VI

SUMMARY

Sapota (*Manilkara achras* (Mill.) Fosberg) is one of the important fruits grown in the country. Systematic work regarding characterization of sapota germplasm and study of genetic diversity for different traits of economic importance has not been carried out in India. Hence, the present investigation on “Morphological, biochemical and isozyme characterization in sapota (*Manilkara achras* (Mill.) Fosberg) germplasm accessions using principal component analysis” was planned. The research work was carried out at Horticultural Research Station, Venkataramannagudem (West Godavari district of Andhra Pradesh). The study was conducted during the years 2006-2008 on 25 genotypes of sapota, most of them planted during 1996.

Studies have been carried out on various tree morphological characters, fruit and biochemical characters and isozyme banding patterns of four isozymes to characterize the 25 sapota genotypes and principal component analysis technique was adopted for classification of the 25 sapota genotypes into distinct clusters. The results of the investigation are summarized below.

1. The analysis of variance of various characters had revealed significant differences among the 25 genotypes studied for all characters, except pollen size, indicating the existence of sufficient variability in the germplasm.
2. The genotypes, namely, Badami, Brazilian Sapota, Calcutta Round, Cricket Ball, Dwarapudi, Gavarayya, Gutti, Krishna Rao, Pakala Round,

Tagarampudi, CO – 3, DHS – 1, DHS – 2, PKM – 3, PKM – 4 were found to be semi-erect in growth habit, facilitating accommodation of greater plant population per unit area. Further, the genotypes, namely, Gede Sapota, Kalipatti, Kirthibarthi, Pala, Singapore, Ulavapadu Selection, CO – 2 and PKM – 1 possessed spreading growth habit.

3. The genotypes, namely, Badami, Calcutta Round, Cricket Ball, Kalipatti, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Singapore, Tagarampudi, PKM – 1, PKM – 2, PKM – 3 and PKM – 4 possessed broadly pyramidal tree shape indicating greater light interception and thereby resulting in enhanced photosynthesis. The genotypes, namely, Dwarapudi, Gede Sapota, Ulavapadu Selection and CO – 1 recorded elliptical shape.
4. Medium dense branching pattern was noticed in the genotypes, namely, Brazilian Sapota, Calcutta Round, Cricket Ball, Gavarayya, Gede Sapota, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Ulavapadu Selection, DHS – 1, PKM – 3 and PKM – 4, while the genotypes, namely, Badami, Dwarapudi, Gutti, Kalipatti, Singapore, Tagarampudi, DHS – 2, PKM – 1 and PKM – 2, however had recorded dense branching pattern desirable for realizing greater fruit yields, while the genotypes CO – 1, CO – 2 and CO – 3 had recorded sparse branching.
5. The average fruit weight of sapota genotypes studied in the present investigation was 96.63 g, while the range was 44.83 g (Ulavapadu Selection) to 198.74 g (Cricket Ball). It was found that higher fruit weight was recorded by the genotypes Cricket Ball, Brazilian Sapota, Gutti, Gavarayya, PKM – 3, DHS – 1, PKM – 4, CO – 3, CO – 2 and CO – 1.

6. It was found that higher fruit yield/tree was recorded for the genotypes PKM – 3, PKM – 1, Kirthibarthi, Singapore, Kalipatti, DHS – 1, DHS – 2, PKM – 4, PKM – 2, Pala, Ulavapadu Selection and Tagarampudi. Among the 25 genotypes, the average fruit yield/tree was observed to be 57.66 kg/tree, while the range comprised of 26.20 kg/tree (CO – 2) to 93.35 kg/tree (PKM – 3) indicating wide variation in the yield potentials among the genotypes.
7. Fruit shape of the genotypes, CO – 1 and CO – 2 was turbinate, while in Dwarapudi, Kalipatti, Krishna Rao, Singapore, CO – 3, DHS – 1 and PKM – 2 the shape was acron. Further, Badami, Calcutta Round, Cricket Ball, Gede Sapota, Kirthibarthi, Pakala Round, Tagarampudi and DHS – 2 recorded globose fruit shape, while it was oblong in the genotypes, Gavarayya and Gutti, oval in the genotypes Brazilian Sapota, Pala, Ulavapadu Selection and PKM – 3, elliptic in the genotypes, PKM – 1 and PKM – 4.
8. The flesh colour of the sapota genotypes studied varied from yellowish brown to reddish brown. It was greenish brown in Brazilian Sapota; brown in Badami, Dwarapudi, Gavarayya, Gede Sapota, Gutti, Kalipatti, Kirthibarthi, Pakala Round, Pala, Singapore, CO – 3, DHS – 1, PKM – 1, PKM – 2 and PKM – 4; yellowish brown in Cricket Ball, Ulavapadu Selection, CO – 1, CO - 2 and PKM -3; and reddish brown in Calcutta Round, Krishna Rao, Tagarampudi and DHS – 2.
9. The range of TSS was observed to be 20.20⁰ brix (Cricket Ball) to 24.30⁰ brix (Pala), while the content of reducing sugars was observed to range

from 5.88 per cent (Dwarapudi) to 10.65 per cent (Kalipatti). Further, the total sugars content of sapota genotypes ranged from 8.21 per cent (Badami) to 11.93 per cent (Pala). The titrable acidity content of sapota genotypes studied in the present investigation varied from 0.01 per cent (DHS – 1) to 0.07 (Gavarayya), while the sugar:acid ratio was found to vary from 142.01 (Gavarayya) to 582.08 (DHS -1). Further, the ascorbic acid content of the sapota genotypes studied in the present investigation varied from 5.12 mg/100g (Dwarapudi) to 11.37 mg/100g (Kalipatti), while the total phenols content ranged from 0.2 mg/100g (Pala) to 0.78 mg/100g (Dwarapudi).

10. In the present study, four isozymes, namely, esterase, peroxidase, polyphenol oxidase and catalase were analysed by polyacrylamide gel electrophoresis technique to identify the genetic diversity among different sapota genotypes. The banding patterns of all the four isozymes studied revealed polymorphism to a varying degree.
11. Peroxidase isozyme revealed high, medium and low intensity bands for a wide range of R_m values starting from R_m 27 to R_m 46, at 15 different R_m positions, indicating greater polymorphism, resolution and higher variability for band molecular weight in the isozyme. The characterization of the 25 sapota genotypes based on peroxidase banding pattern revealed unambiguous identification of 15 genotypes studied (Pakala Round, Singapore, Krishna Rao, Pala, CO – 1, Brazilian Sapota, CO – 2, Ulavapadu Selection, Badami, Gutti, Calcutta Round, PKM – 2,

Gavarayya, DHS – 1 and PKM – 4) and thus this enzyme was found useful for sapota.

12. Characterization based on banding pattern of polyphenol oxidase isozyme revealed a scope for identification of Kalipatti, Kirthibarthi, Krishna Rao, Tagarampudi, CO – 2, PKM – 1 and PKM – 2, genotypes.
13. Studies on banding pattern of catalase isozyme revealed the existence of single band per genotype with the exception of PKM – 3 and Krishna Rao in which no bands were observed. There was a possibility for identification of Krishna Rao, Pakala Round, Singapore, CO – 3, PKM – 1 and PKM – 3 genotypes with catalase isozyme.
14. The banding pattern of esterase isozyme exhibited low level of polymorphism compared to the other isozymes studied and hence cannot be utilized for identification of genotypes of sapota.
15. Genetic studies revealed that the range of phenotypic coefficient of variation comprised 0.67 to 37.96 among the tree morphological characters, while it ranged from 4.52 to 46.75 among the fruit and biochemical characters. Further, the range of genotypic coefficient of variation among the tree morphological characters consisted of 0.44 to 37.59 and 4.12 to 40.69 among the fruit and biochemical characters studied.
16. Heritability values for the different tree morphological traits ranged from 41.87 to 99.03 per cent; and from 55.94 to 97.97 per cent for fruit and biochemical characters. The genetic advance as per cent mean ranged from 0.58 to 77.14 for tree morphological characters and 8.05 to 81.51 for fruit

morphological and biochemical characters indicating the heritable nature of certain characters in sapota.

17. Principal component analysis of tree morphological characters resulted in three major principal components (PC), which contributed to 74.23 per cent of cumulative variability. The 25 sapota genotypes studied were grouped into six clusters, with maximum number of genotypes (nine) grouped into Cluster – II (Brazilian Sapota, Kirthibarthi, Calcutta Round, Pakala Round, Krishna Rao, DHS - 2, PKM – 2, Kalipatti and Pala), followed by six genotypes in Cluster I (Badami, PKM – 1, Ulavapadu Selection, Gutti, Tagarampudi and Dwarapudi), while minimum number (two) were recorded for Cluster IV (CO – 1 and CO – 2) and VI (Gede Sapota and CO - 3).
18. Principal component analysis for fruit morphological and biochemical characters revealed construction of three major PC's, which contributed for 89.79 per cent of the total variability. The first principal component accounted for 59.07 per cent of the variability and was characterized by high and positive loadings for fruit surface texture, titrable acidity, fruit weight and peel colour coupled with negative loadings for reducing sugars, TSS, ascorbic acid, flesh colour and flesh taste. This suggests that this principal component represents fruit quality component of all genotypes.
19. The second principal component explained prevalence of 18.93 per cent of the variability and showed high positive loadings for total sugars, pulp:seed ratio, sugar:acid ratio and non-reducing sugars coupled with negative loadings of total phenols. It may be inferred that this principal component

reflects sugars content and phenol content of all the genotypes. The third principal component described for 11.78 per cent of the variability and showed high positive correlation for fruit shape, flesh texture, fruit dessert quality and yield per tree; and negative loadings for seed number per fruit. These results suggest that this principal component reflects the fruit shape and fruit yield components of all the genotypes.

20. Principal component analysis of leaf isozyme banding pattern revealed prevalence of two principal components contributing to 73.04 per cent of cumulative variability. The percentage variation accounted for by PC I and PC II were 45.40 and 27.64 per cent respectively.

21. The most important variables integrated in PC I, were esterase, peroxidase and polyphenol oxidase. This suggests that this principal component represents esterase, peroxidase and polyphenol oxidase isozymes of all the genotypes under study. The 25 sapota genotypes were grouped into six clusters, wherein maximum number of genotypes (12) were grouped in Cluster I (Brazilian Sapota, Badami, Ulavapadu Selection, Gede Sapota, Gavarayya, Gutti, PKM – 4, Tagarampudi, Pala, Pakala Round, CO – 3 and Dwarapudi).

22. Based on comparison of cluster means and general means the genotypes, namely, Gavarayya, DHS – 1, PKM – 1 and PKM – 3 could be adjudged superior in terms of tree yield, fruit size, TSS, sugar content, ascorbic acid content, low total phenols and other fruit characters and the genotypes Gutti, DHS – 2, Pakala Round, Pala, Kirthibarthi and Ulavapadu Selection, CO – 2, Co – 3, PKM – 4, CO – 1, Badami, Gede Sapota, Kalipatti,

PKM – 2, Tagarampudi, Singapore, Krishna Rao, Calcutta Round and Dwarapudi were found to be medium in performance.

CONCLUSIONS

The following conclusions can be drawn based on the aforesaid discussion of results obtained in this investigation.

1. The analysis of variance for tree morphological and fruit biochemical characters revealed existence of considerable variation among the genotypes for the characters studied.
2. An overall perusal of results revealed greater fruit weight, yield per tree, pulp:seed ratio, reducing sugars and ascorbic acid content coupled with low seed number and total phenols in hybrids, namely, CO – 1, CO – 3, DHS – 1, DHS – 2, PKM – 2 and PKM – 3 compared to local genotypes (Badami, Calcutta Round, Cricket Ball, Dwarapudi, Gavarayya, Gede Sapota, Gutti, Kalipatti, Kirthibarthi, Krishna Rao, Pakala Round, Pala, Singapore and Tagarampudi) and other selections (Brazilian Sapota, Ulavapadu Selection, CO – 2, PKM – 1 and PKM – 4) studied.
3. In this study, polymorphism was observed for three leaf isozymes studied except for esterase. Peroxidase was found to be the most suitable for distinction of maximum number of sapota genotypes studied. But a combination of peroxidase with either polyphenol oxidase or catalase isozyme was found to be effective in distinguishing all the 25 sapota genotypes studied and thus can be used as molecular marker.

4. Genetic studies carried out revealed prevalence of high variability among the genotypes studied. The phenotypic coefficient of variation ranged from 0.67 to 37.96 among the tree morphological characters, while it ranged from 4.52 to 46.75 among the fruit and biochemical characters. The genotypic coefficient of variation ranged from 0.44 to 37.59 and 4.12 to 40.69 among the tree morphological and fruit and biochemical characters studied respectively.
5. Heritability and genetic advance values were found to be high for the characters, namely, tree shape, branch density, leaf area, fruit weight, fruit yield/tree, fruit shape, flesh texture, fruit dessert quality, seed number/fruit, seed weight, pulp:seed ratio, total soluble solids, sugar:acid ratio, ascorbic acid and total phenols indicating the effectiveness of direct phenotypic selection for genetic improvement of these traits.
6. Principal component analysis for tree morphological characters, fruit morphological and biochemical characters and leaf isozyme banding patterns resulted in grouping of the 25 sapota genotypes into six different clusters based on their relatedness and principal components in all the three sets.
7. From the results obtained on morphological and biochemical characterization of sapota genotypes in this study, a vision is obtained about the level of diversity. Certain qualitative and quantitative traits of fruit could be employed in determining the level of variation.

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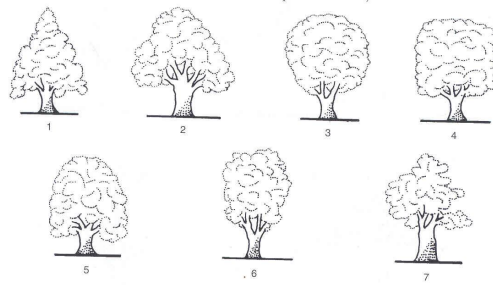
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Note: The pattern of literature cited presented above is in accordance with the guidelines for thesis presentation, Acharya N G Ranga Agricultural University, Hyderabad.

APPENDIX I

Sapota descriptor: (Singh, 1991)

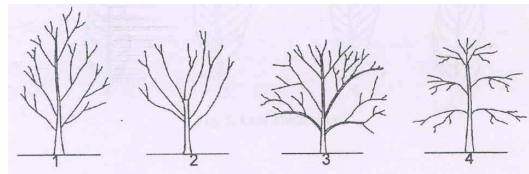
1 Tree shape (Crown)



- 1 Pyramidal
- 2 Broadly pyramidal
- 3 Spherical
- 4 Oblong
- 5 Semi circular
- 6 Elliptical
- 7 Irregular

Fig.3

2 Tree growth habit



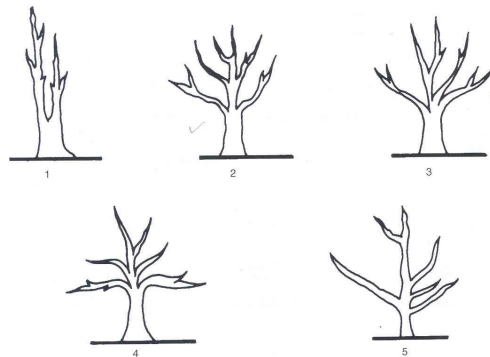
- 1 Erect/upright
- 2 Semi-erect
- 3 Spreading
- 4 Drooping

Fig.4

3 Branching density

- 1 Sparse
- 2 Medium
- 3 Dense

4 Branching pattern



- 1 Erect
- 2 Opposite
- 3 Verticillate
- 4 Horizontal
- 5 Irregular

Fig.5

- | | | |
|---|------------------------|---|
| 5 | Young shoot pubescence | 1 Glabrous
2 Pubescent
3 Sparingly pubescent
4 Densely pubescent |
|---|------------------------|---|

- | | | |
|---|------------------------------|--|
| 6 | Colour of fully matured leaf | 1.Light green
2.Green
3.Dark green
4.Purple |
|---|------------------------------|--|

- | | | |
|---|---|--|
| 7 | Leaf blade length (cm)
(Measured from the base to the tip of the leaf blade) | |
|---|---|--|

- | | | |
|---|---|--|
| 8 | Leaf blade width (cm)
(Measured at the widest portion) | |
|---|---|--|

- | | | |
|---|------------------|---|
| 9 | Leaf blade shape | 1. Elliptic
2.Sub obovate
3. Broadly elliptic
4.Narrowly elliptic
99.others |
|---|------------------|---|

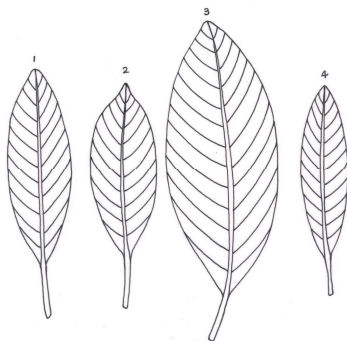


Fig.6

- | | | |
|----|-----------------|---|
| 10 | Leaf apex shape | 1. Acute
2. Retuse
3.Sub acuminate
99.others |
|----|-----------------|---|

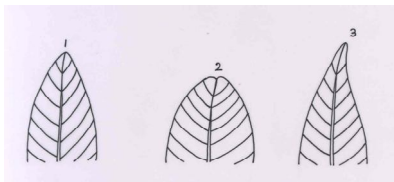


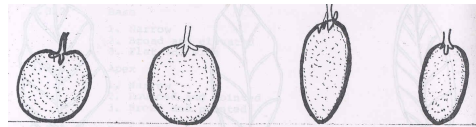
Fig.7

11	Leaf blade margin	1.Entire 2.Crinckled 3.Wavy
12	Arrangement of flowers	1.Solitary 2.Cluster 3.Both
13	Position of flower	1.Axillary 2.Terminal 3. Both
14	Flower bud colour	1.Light green 2.Yellowish green 3.White 4.Brown and its shades
15	Number of sepals	
16	Calyx colour	1.Greenish green 2.Green with yellow 3.Silvery white 4.Yellow
17	Number of petals	
18	Corolla colour	1.Whitish 2.Yellowish 3.Cream
19	Number of stamen	
20	Anther number	
21	Style length	mm
22	Colour of mature fruit skin	1.Brown 2.Yellowish brown 3.Greenish Brown

4.Brown with streaks

5.Scurfy brown

23 Fruit shape



1.Globose

2.Sub globose

3.Elliptic

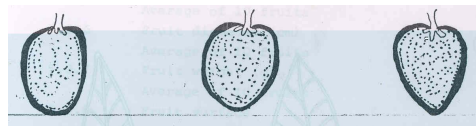
4.Oval

1

2

3

4



5.Oblong

6.Acron

7.Turbinate

5

6

7

Fig.9

24 Fruit weight (g)

25 Fruit surface

1.Smooth

2.Scurfy

3.Web pattern on surface

26 Fruit base



1.Depressed

2.Flattened

3.Inflated

1

2

3

4

4.Pointed

Fig. 10

27 Fruit apex

1.Obtuse

2.Acute

28 Flesh colour

1.Reddish brown

2.Yellowish brown

3.Greenish brown

4.Pale green

		5.Reddish green
29	Flesh content	
	Ratio of pulp to skin + seed	
30	Flesh texture	1.Firm
		2.Sandy
		3.Soft
		4.Melting
		5.Unpleasant
31	Flesh taste	1.Sweet
		2.Sandy
		3.Starchy
		4. Astringent
32	Fruit length (L.S. of fruit)	 cm
33	Fruit width (C.S. of fruit)	 cm
34	Dessert quality	1. Acceptable
		2.Good
		3.Very good
		4.Excellent
35	Number of seeds/fruit	
36	Seed shape	
		1.Semi deltoid with spine
		2.Semi ovate
		3.Fusiform
		4.Broadly clavate with pointed spine
		5.Broadly clavate with spine

Fig. 11

37	Seed weight (g)/fruit
38	Yield per tree (kg per year)
39	TSS (°Brix)
40	Fruit acidity (%)
41	Vit 'C' (mg/100g pulp)
42	Sugar content (%)

Appendix II

Binary matrix of esterase and peroxidase isozymes in sapota genotypes

[illegible]

Appendix II contd...

Binary matrix of polyphenol oxidase and catalase isozymes in sapota genotypes

Genotype	Rm value	Polyphenol oxidase												Catalase									
		19	21	23	24	25	26	27	28	37	38	41	44	53	54	55	56	57	58	60	61	62	63
Badami	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Brazilian Sapota	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Calcutta Round	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Cricket Ball	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Dwarapudi	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Gavarayya	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Gede Sapota	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Gutti	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Kalipatti	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Kirthibarthi	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Krishna Rao	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pakala Round	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pala	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Singapore	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Tagarampudi	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Ulavapadu Selection	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
CO - 1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
CO - 2	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
CO - 3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
DHS - 1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
DHS - 2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
PKM - 1	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0
PKM - 2	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
PKM - 3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PKM - 4	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

APPENDIX - III

Morphological, fruit and biochemical characterization of Sapota genotypes:

S. No.	Character	Badami	Brazilian Sapota	Calcutta Round
1	Growth habit	Semi-erect	Semi-erect	Semi-erect
2	Shape of the tree	Broadly Pyramidal	Spherical	Broadly pyramidal
3	Branching pattern	Verticillate	Verticillate	Verticillate
4	Branch density	Dense	Medium Dense	Medium dense
5	Colour of shoot tip	Brown	Brown	Brown
6	Surface of shoot tip	Pubescent	Pubescent	Pubescent
7	Shape of the leaf tip	Retuse	Retuse	Retuse
8	Leaf margin	Entire	Entire	Entire
9	Leaf colour	Green	Green	Green
10	Leaf shape	Elliptic	Broadly Elliptic	Sub-obovate
11	Leaf area (cm ²)	17.44	17.65	17.84
12	Leaf length (cm)	9.03	8.85	8.93
13	Leaf width (cm)	2.89	3.18	3.20
14	Leaf area constant	0.67	0.63	0.63
15	Inflorescence type	Cymose	Cymose	Cymose
16	Flowers per cluster	Solitary	Solitary	Solitary
17	Position of inflorescence	Axillary	Axillary	Axillary
18	Colour of flower bud	Brown	Brown	Brown
19	Sepal colour	Greenish Brown	Greenish brown	Greenish brown
20	Petal colour	White	White	White
21	Number of sepals	6	6	6
22	Number of petals	6	6	6
23	Length of style (cm)	0.89	0.60	0.81
24	Number of anthers	6	6	6
25	Pollen prod. per anther	692.89	494.11	694.93
26	Pollen size (μ)	16.44	15.67	15.76
27	Pollen fertility (%)	96.80	97.17	97.53
28	Fruit shape	Globose	Oval	Globose
29	Fruit length (cm)	4.70	6.01	4.72
30	Fruit width (cm)	3.71	5.15	4.72
31	Fruit weight (g)	64.35	97.57	77.75
32	Fruit yield/ tree (kg)	32.35	52.80	45.15
33	Peel colour	Brown	Greenish brown	Brown
34	Fruit surface texture	Scurfy	Scurfy	Scurfy
35	Fruit base	Flattened	Depressed	Depressed
36	Fruit apex	Obtuse	Acute	Acute
37	Flesh taste	Sweet	Starchy	Starchy
38	Flesh texture	Soft	Sandy	Firm
39	Flesh colour	Brown	Greenish brown	Reddish brown
40	Flesh weight (g)	61.98	89.67	74.6
41	Fruit Dessert quality	Fair	Fair	Good
42	TSS (° Brix)	21.20	23.10	22.90
43	Titration Acidity (%)	0.03	0.02	0.03
44	Reducing Sugars (%)	6.75	7.71	8.48
45	Total Sugars (%)	8.21	9.15	9.72
46	Non-Reducing Sugars (%)	1.46	1.44	1.24
47	Sugar:Acid Ratio	275.23	399.14	303.20
48	Ascorbic Acid	6.50	7.37	6.75
49	Total Phenols	0.69	0.50	0.40
50	Seed number	3	8	5
51	Seed shape	Broadly clavate with spine	Broadly clavate with spine	Broadly clavate with spine
52	Seed weight (g)	2.49	7.78	3.63
53	Pulp:Seed Ratio	25.07	11.34	20.50

Morphological, fruit and biochemical characterization of Sapota genotypes:

S. No.	Character	Cricket Ball	Dwarapudi	Gavarayya
1	Growth habit	Semi-erect	Semi-erect	Semi-erect
2	Shape of the tree	Broadly pyramidal	Elliptical	Spherical
3	Branching pattern	Horizontal	Verticillate	Irregular
4	Branch density	Medium dense	Dense	Medium dense
5	Colour of shoot tip	Brown	Brown	Brown
6	Surface of shoot tip	Pubescent	Pubescent	Pubescent
7	Shape of the leaf tip	Retuse	Acute	Retuse
8	Leaf margin	Wavy	Entire	Entire
9	Leaf colour	Green	Green	Green
10	Leaf shape	Elliptic	Broadly elliptic	Broadly Elliptic
11	Leaf area (cm ²)	29.10	20.37	22.34
12	Leaf length (cm)	10.98	9.09	9.35
13	Leaf width (cm)	3.98	3.69	3.70
14	Leaf area constant	0.66	0.61	0.66
15	Inflorescence type	Cymose	Cymose	Cymose
16	Flowers per cluster	Solitary	Solitary	Solitary
17	Position of inflorescence	Axillary	Axillary	Axillary
18	Colour of flower bud	Brown	Brown	Brown
19	Sepal colour	Greenish brown	Greenish brown	Greenish brown
20	Petal colour	White	White	White
21	Number of sepals	6	6	6
22	Number of petals	6	6	6
23	Length of style (cm)	0.70	0.90	0.70
24	Number of anthers	6	6	6
25	Pollen prod. per anther	758.11	614.92	700.80
26	Pollen size (μ)	16.65	16.04	15.71
27	Pollen fertility (%)	98.41	96.21	97.50
28	Fruit shape	Globose	Acron	Oblong
29	Fruit length (cm)	6.48	4.56	6.16
30	Fruit width (cm)	6.79	4.70	4.90
31	Fruit weight (g)	198.74	83.63	104.03
32	Fruit yield/ tee (kg)	55.40	37.65	55.15
33	Peel colour	Yellowish Brown	Brown	Brown
34	Fruit surface texture	Smooth	Smooth	Scurfy
35	Fruit base	Depressed	Inflated	Depressed
36	Fruit apex	Obtuse	Acute	Obtuse
37	Flesh taste	Sweet	Sweet	Starchy
38	Flesh texture	Soft	Firm	Firm
39	Flesh colour	Yellowish brown	Brown	Brown
40	Flesh weight (g)	191.97	80.14	101.99
41	Fruit Dessert quality	Good	Fair	Good
42	TSS (° Brix)	20.20	21.30	21.60
43	Titration Acidity (%)	0.05	0.04	0.07
44	Reducing Sugars (%)	6.53	5.88	6.95
45	Total Sugars (%)	9.81	10.09	9.71
46	Non-Reducing Sugars (%)	3.27	4.21	2.76
47	Sugar:Acid Ratio	201.59	256.32	142.01
48	Ascorbic Acid	5.87	5.12	7.87
49	Total Phenols	0.33	0.78	0.36
50	Seed number	8	7	3
51	Seed shape	Semi-deltoid with spine	Broadly clavate with spine	Broadly Clavate with pointed spine
52	Seed weight (g)	6.27	4.55	2.30
53	Pulp:Seed Ratio	30.86	18.02	43.89

Morphological, fruit and biochemical characterization of Sapota genotypes:

S. No.	Character	Gede Sapota	Gutti	Kalipatti
1	Growth habit	Spreading	Semi-erect	Spreading
2	Shape of the tree	Elliptical	Spherical	Broadly pyramidal
3	Branching pattern	Verticillate	Verticillate	Horizontal
4	Branch density	Medium dense	Dense	Dense
5	Colour of shoot tip	Brown	Brown	Brown
6	Surface of shoot tip	Pubescent	Pubescent	Pubescent
7	Shape of the leaf tip	Acute	Retuse	Acute
8	Leaf margin	Wavy	Wavy	Entire
9	Leaf colour	Green	Green	Green
10	Leaf shape	Elliptic	Broadly elliptic	Elliptic
11	Leaf area (cm ²)	35.98	18.00	14.72
12	Leaf length (cm)	12.99	8.95	8.46
13	Leaf width (cm)	5.00	3.15	2.82
14	Leaf area constant	0.55	0.65	0.63
15	Inflorescence type	Cymose	Cymose	Cymose
16	Flowers per cluster	Solitary	Solitary	Solitary
17	Position of inflorescence	Axillary	Axillary	Axillary
18	Colour of flower bud	Brown	Brown	Brown
19	Sepal colour	Greenish brown	Greenish brown	Greenish brown
20	Petal colour	White	White	White
21	Number of sepals	6	6	6
22	Number of petals	6	6	6
23	Length of style (cm)	0.83	0.80	0.81
24	Number of anthers	6	6	6
25	Pollen prod. per anther	617.10	902.84	643.62
26	Pollen size (μ)	16.76	15.82	16.32
27	Pollen fertility (%)	96.61	97.18	98.86
28	Fruit shape	Globose	Oblong	Acron
29	Fruit length (cm)	3.82	4.03	5.19
30	Fruit width (cm)	3.35	5.10	4.10
31	Fruit weight (g)	63.13	102.01	64.42
32	Fruit yield/ tree (kg)	31.70	36.15	83.40
33	Peel colour	Brown	Brown	Yellowish Brown
34	Fruit surface texture	Smooth	Smooth	Smooth
35	Fruit base	Flattened	Depressed	Flattened
36	Fruit apex	Acute	Acute	Acute
37	Flesh taste	Sweet	Starchy	Sweet
38	Flesh texture	Sandy	Soft	Firm
39	Flesh colour	Brown	Brown	Brown
40	Flesh weight (g)	60.00	100.38	61.52
41	Fruit Dessert quality	Fair	Fair	Good
42	TSS (° Brix)	21.10	23.00	23.90
43	Titration Acidity (%)	0.04	0.03	0.04
44	Reducing Sugars (%)	6.29	7.94	10.65
45	Total Sugars (%)	8.63	9.96	11.78
46	Non-Reducing Sugars (%)	2.34	2.02	1.13
47	Sugar:Acid Ratio	221.87	311.03	293.17
48	Ascorbic Acid	6.75	9.00	11.37
49	Total Phenols	0.67	0.61	0.36
50	Seed number	3	3	3
51	Seed shape	Broadly Clavate with spine	Broadly Clavate with spine	Broadly Clavate with pointed spine
52	Seed weight (g)	2.72	2.19	2.42
53	Pulp:Seed Ratio	21.99	45.25	25.10

Morphological, fruit and biochemical characterization of Sapota genotypes:

S. No.	Character	Kirthibarthi	Krishna Rao	Pakala Round
1	Growth habit	Spreading	Semi-erect	Semi-erect
2	Shape of the tree	Broadly pyramidal	Broadly pyramidal	Broadly pyramidal
3	Branching pattern	Horizontal	Verticillate	Verticillate
4	Branch density	Medium dense	Medium dense	Medium dense
5	Colour of shoot tip	Brown	Brown	Brown
6	Surface of shoot tip	Pubescent	Pubescent	Pubescent
7	Shape of the leaf tip	Acute	Retuse	Sub-acuminate
8	Leaf margin	Entire	Entire	Entire
9	Leaf colour	Green	Green	Green
10	Leaf shape	Broadly elliptic	Broadly elliptic	Elliptic
11	Leaf area (cm ²)	15.93	19.17	17.50
12	Leaf length (cm)	8.68	8.71	9.24
13	Leaf width (cm)	3.14	3.28	3.24
14	Leaf area constant	0.59	0.65	0.59
15	Inflorescence type	Cymose	Cymose	Cymose
16	Flowers per cluster	Solitary	Solitary	Solitary
17	Position of inflorescence	Axillary	Axillary	Axillary
18	Colour of flower bud	Brown	Brown	Brown
19	Sepal colour	Greenish brown	Greenish brown	Greenish brown
20	Petal colour	White	White	White
21	Number of sepals	6	6	6
22	Number of petals	6	6	6
23	Length of style (cm)	0.59	0.57	0.72
24	Number of anthers	6	6	6
25	Pollen prod. per anther	648.06	544.72	661.66
26	Pollen size (μ)	16.24	16.86	15.93
27	Pollen fertility (%)	97.46	96.79	97.71
28	Fruit shape	Globose	Acron	Globose
29	Fruit length (cm)	4.72	4.53	4.21
30	Fruit width (cm)	4.10	5.80	4.57
31	Fruit weight (g)	86.72	67.74	76.37
32	Fruit yield/ tree (kg)	86.45	31.45	55.25
33	Peel colour	Yellowish brown	Brown	Brown
34	Fruit surface texture	Smooth	Scurfy	Scurfy
35	Fruit base	Depressed	Depressed	Depressed
36	Fruit apex	Obtuse	Acute	Obtuse
37	Flesh taste	Sweet	Sweet	Sweet
38	Flesh texture	Soft	Firm	Soft
39	Flesh colour	Brown	Reddish brown	Brown
40	Flesh weight (g)	85.42	64.38	74.25
41	Fruit Dessert quality	Good	Fair	Good
42	TSS (° Brix)	22.60	22.10	21.70
43	Titration Acidity (%)	0.02	0.03	0.02
44	Reducing Sugars (%)	8.47	7.87	7.91
45	Total Sugars (%)	10.70	8.89	9.90
46	Non-Reducing Sugars (%)	2.23	1.01	1.99
47	Sugar:Acid Ratio	469.25	298.40	492.73
48	Ascorbic Acid	7.50	7.50	6.50
49	Total Phenols	0.53	0.64	0.49
50	Seed number	4	5	4
51	Seed shape	Broadly clavate with pointed spine	Broadly clavate with spine	Semi-ovate
52	Seed weight (g)	1.57	3.06	1.68
53	Pulp:Seed Ratio	53.49	21.67	45.29

Morphological, fruit and biochemical characterization of Sapota genotypes:

S. No.	Character	Pala	Singapore	Tagarampudi
1	Growth habit	Spreading	Spreading	Semi-erect
2	Shape of the tree	Broadly pyramidal	Broadly pyramidal	Broadly pyramidal
3	Branching pattern	Horizontal	Horizontal	Horizontal
4	Branch density	Medium dense	Dense	Medium dense
5	Colour of shoot tip	Brown	Brown	Brown
6	Surface of shoot tip	Pubescent	Pubescent	Pubescent
7	Shape of the leaf tip	Acute	Sub-acuminate	Acute
8	Leaf margin	Entire	Entire	Entire
9	Leaf colour	Green	Green	Green
10	Leaf shape	Elliptic	Broadly elliptic	Elliptic
11	Leaf area (cm ²)	16.43	21.37	20.84
12	Leaf length (cm)	7.67	9.93	9.48
13	Leaf width (cm)	3.21	3.41	3.43
14	Leaf area constant	0.69	0.63	0.64
15	Inflorescence type	Cymose	Cymose	Cymose
16	Flowers per cluster	Solitary	Solitary	Solitary
17	Position of inflorescence	Axillary	Axillary	Axillary
18	Colour of flower bud	Brown	Brown	Brown
19	Sepal colour	Greenish brown	Greenish brown	Greenish brown
20	Petal colour	White	White	White
21	Number of sepals	6	6	6
22	Number of petals	6	6	6
23	Length of style (cm)	0.69	0.51	0.90
24	Number of anthers	6	6	6
25	Pollen prod. per anther	658.26	714.69	698.04
26	Pollen size (μ)	15.54	16.43	16.68
27	Pollen fertility (%)	98.44	97.97	96.72
28	Fruit shape	Oval	Acron	Globose
29	Fruit length (cm)	4.48	5.10	3.99
30	Fruit width (cm)	4.17	4.35	4.21
31	Fruit weight (g)	63.38	92.44	63.27
32	Fruit yield/ tree (kg)	65.75	85.35	60.45
33	Peel colour	Brown	Brown	Brown
34	Fruit surface texture	Scurfy	Smooth	Scurfy
35	Fruit base	Flattened	Inflated	Depressed
36	Fruit apex	Acute	Acute	Acute
37	Flesh taste	Sweet	Sweet	Starchy
38	Flesh texture	Soft	Sandy	Firm
39	Flesh colour	Brown	Brown	Reddish brown
40	Flesh weight (g)	62.37	89.09	58.80
41	Fruit Dessert quality	Excellent	Good	Good
42	TSS (° Brix)	24.30	23.00	22.10
43	Titration Acidity (%)	0.03	0.03	0.03
44	Reducing Sugars (%)	10.05	8.50	7.77
45	Total Sugars (%)	11.93	10.82	9.00
46	Non-Reducing Sugars (%)	1.88	2.32	1.23
47	Sugar:Acid Ratio	399.00	295.30	246.06
48	Ascorbic Acid	9.50	11.00	8.00
49	Total Phenols	0.23	0.34	0.41
50	Seed number	3	6	5
51	Seed shape	Broadly clavate with spine	Broadly clavate with spine	Broadly clavate with spine
52	Seed weight (g)	1.64	3.53	4.23
53	Pulp:Seed Ratio	39.60	25.42	14.66

Morphological, fruit and biochemical characterization of Sapota genotypes:

S. No.	Character	Ulavapadu Selection	CO-1	CO-2
1	Growth habit	Spreading	Erect	Spreading
2	Shape of the tree	Elliptical	Elliptical	Irregular
3	Branching pattern	Verticillate	Verticillate	Verticillate
4	Branch density	Medium dense	Sparse	Sparse
5	Colour of shoot tip	Brown	Brown	Brown
6	Surface of shoot tip	Pubescent	Pubescent	Pubescent
7	Shape of the leaf tip	Acute	Acute	Sub-acuminate
8	Leaf margin	Wavy	Wavy	Wavy
9	Leaf colour	Green	Green	Green
10	Leaf shape	Elliptic	Broadly elliptic	Broadly elliptic
11	Leaf area (cm ²)	15.35	24.06	24.32
12	Leaf length (cm)	10.42	10.30	10.63
13	Leaf width (cm)	2.66	3.30	3.63
14	Leaf area constant	0.55	0.72	0.62
15	Inflorescence type	Cymose	Cymose	Cymose
16	Flowers per cluster	Solitary	Solitary	Solitary
17	Position of inflorescence	Axillary	Axillary	Axillary
18	Colour of flower bud	Brown	Brown	Brown
19	Sepal colour	Greenish brown	Greenish brown	Greenish brown
20	Petal colour	White	White	White
21	Number of sepals	6	6	6
22	Number of petals	6	6	6
23	Length of style (cm)	0.69	0.68	0.78
24	Number of anthers	6	6	6
25	Pollen prod. per anther	673.85	662.30	781.91
26	Pollen size (μ)	16.20	16.44	15.63
27	Pollen fertility (%)	97.72	96.58	96.65
28	Fruit shape	Oval	Turbinata	Turbinata
29	Fruit length (cm)	3.71	7.28	6.87
30	Fruit width (cm)	2.37	5.77	5.89
31	Fruit weight (g)	44.83	170.67	150.65
32	Fruit yield/ tree (kg)	63.50	26.40	26.20
33	Peel colour	Brown	Yellowish Brown	Brown
34	Fruit surface texture	Smooth	Smooth	Scurfy
35	Fruit base	Flattened	Depressed	Depressed
36	Fruit apex	Acute	Acute	Acute
37	Flesh taste	Sweet	Sweet	Sweet
38	Flesh texture	Soft	Sandy	Sandy
39	Flesh colour	Yellowish Brown	Yellowish brown	Yellowish brown
40	Flesh weight (g)	43.00	167.58	148.69
41	Fruit Dessert quality	Good	Good	Good
42	TSS (° Brix)	23.40	21.30	22.30
43	Titrate Acidity (%)	0.03	0.04	0.03
44	Reducing Sugars (%)	8.15	6.88	8.89
45	Total Sugars (%)	9.31	9.27	10.14
46	Non-Reducing Sugars (%)	1.16	2.39	1.24
47	Sugar:Acid Ratio	347.28	214.74	276.04
48	Ascorbic Acid	9.75	10.75	10.25
49	Total Phenols	0.25	0.26	0.33
50	Seed number	2	4	3
51	Seed shape	Broadly clavate with spine	Broadly clavate with spine	Semi-deltoid with spine
52	Seed weight (g)	1.59	2.97	1.48
53	Pulp:Seed Ratio	27.09	56.11	100.80

Morphological, fruit and biochemical characterization of Sapota genotypes:

S. No.	Character	CO-3	DHS-1	DHS-2
1	Growth habit	Semi-erect	Semi-erect	Semi-erect
2	Shape of the tree	Irregular	Semi-circular	Spherical
3	Branching pattern	Verticillate	Verticillate	Verticillate
4	Branch density	Sparse	Medium dense	Dense
5	Colour of shoot tip	Brown	Brown	Brown
6	Surface of shoot tip	Pubescent	Pubescent	Pubescent
7	Shape of the leaf tip	Retuse	Acute	Sub-acuminate
8	Leaf margin	Wavy	Entire	Wavy
9	Leaf colour	Green	Green	Green
10	Leaf shape	Broadly elliptic	Elliptic	Broadly elliptic
11	Leaf area (cm ²)	29.30	14.54	19.93
12	Leaf length (cm)	10.59	9.13	8.99
13	Leaf width (cm)	4.09	2.53	3.62
14	Leaf area constant	0.67	0.62	0.61
15	Inflorescence type	Cymose	Cymose	Cymose
16	Flowers per cluster	Solitary	Solitary	Solitary
17	Position of inflorescence	Axillary	Axillary	Axillary
18	Colour of flower bud	Brown	Brown	Brown
19	Sepal colour	Greenish brown	Greenish brown	Greenish brown
20	Petal colour	White	White	White
21	Number of sepals	6	6	6
22	Number of petals	6	6	6
23	Length of style (cm)	0.78	0.90	0.69
24	Number of anthers	6	6	6
25	Pollen prod. per anther	702.78	795.14	652.66
26	Pollen size (μ)	15.46	16.12	15.80
27	Pollen fertility (%)	97.29	97.59	97.34
28	Fruit shape	Acron	Acron	Globose
29	Fruit length (cm)	6.85	5.29	4.51
30	Fruit width (cm)	5.82	4.78	5.14
31	Fruit weight (g)	127.29	120.21	89.06
32	Fruit yield/ tree (kg)	32.95	81.55	75.80
33	Peel colour	Brown	Brown	Brown
34	Fruit surface texture	Smooth	Scurfy	Scurfy
35	Fruit base	Depressed	Flattened	Depressed
36	Fruit apex	Acute	Obtuse	Obtuse
37	Flesh taste	Sweet	Sweet	Sweet
38	Flesh texture	Sandy	Soft	Firm
39	Flesh colour	Brown	Brown	Reddish brown
40	Flesh weight (g)	125.33	117.99	88.15
41	Fruit Dessert quality	Good	Good	Good
42	TSS (° Brix)	20.70	22.90	21.70
43	Titration Acidity (%)	0.04	0.01	0.03
44	Reducing Sugars (%)	6.39	8.11	8.20
45	Total Sugars (%)	8.38	9.28	9.40
46	Non-Reducing Sugars (%)	1.99	1.17	1.20
47	Sugar:Acid Ratio	216.05	582.08	257.68
48	Ascorbic Acid	9.75	9.00	8.12
49	Total Phenols	0.51	0.43	0.66
50	Seed number	4	3	3
51	Seed shape	Broadly clavate with spine	Semi-ovate	Semi-ovate
52	Seed weight (g)	3.12	2.89	2.20
53	Pulp:Seed Ratio	39.79	40.57	40.63

Morphological, fruit and biochemical characterization of Sapota genotypes:

S. No.	Character	PKM-1	PKM-2	PKM-3
1	Growth habit	Spreading	Semi-erect	Semi-erect
2	Shape of the tree	Broadly pyramidal	Broadly pyramidal	Broadly pyramidal
3	Branching pattern	Verticillate	Verticillate	Verticillate
4	Branch density	Dense	Dense	Medium dense
5	Colour of shoot tip	Brown	Brown	Brown
6	Surface of shoot tip	Pubescent	Pubescent	Pubescent
7	Shape of the leaf tip	Acute	Acute	Retuse
8	Leaf margin	Entire	Wavy	Wavy
9	Leaf colour	Green	Green	Green
10	Leaf shape	Broadly elliptic	Broadly elliptic	Broadly elliptic
11	Leaf area (cm ²)	15.10	20.49	32.12
12	Leaf length (cm)	8.54	8.67	10.15
13	Leaf width (cm)	3.07	3.42	4.23
14	Leaf area constant	0.57	0.70	0.74
15	Inflorescence type	Cymose	Cymose	Cymose
16	Flowers per cluster	Solitary	Solitary	Solitary
17	Position of inflorescence	Axillary	Axillary	Axillary
18	Colour of flower bud	Brown	Brown	Brown
19	Sepal colour	Greenish brown	Greenish brown	Greenish brown
20	Petal colour	White	White	White
21	Number of sepals	6	6	6
22	Number of petals	6	6	6
23	Length of style (cm)	0.60	0.70	0.82
24	Number of anthers	6	6	6
25	Pollen prod. per anther	768.22	617.08	679.28
26	Pollen size (μ)	15.66	15.33	15.66
27	Pollen fertility (%)	96.91	97.53	98.17
28	Fruit shape	Elliptic	Acron	Oval
29	Fruit length (cm)	5.38	4.68	5.92
30	Fruit width (cm)	4.71	4.30	4.65
31	Fruit weight (g)	93.30	81.89	105.08
32	Fruit yield/ tree (kg)	89.95	66.75	93.35
33	Peel colour	Brown	Brown	Brown
34	Fruit surface texture	Smooth	Smooth	Smooth
35	Fruit base	Inflated	Flattened	Depressed
36	Fruit apex	Acute	Obtuse	Obtuse
37	Flesh taste	Sweet	Sweet	Sweet
38	Flesh texture	Soft	Firm	Firm
39	Flesh colour	Brown	Brown	Yellowish brown
40	Flesh weight (g)	90.12	78.99	101.40
41	Fruit Dessert quality	Good	Fair	Good
42	TSS (° Brix)	24.10	21.50	23.70
43	Titration Acidity (%)	0.04	0.05	0.02
44	Reducing Sugars (%)	8.81	7.68	9.21
45	Total Sugars (%)	9.83	8.78	10.66
46	Non-Reducing Sugars (%)	1.01	1.09	1.45
47	Sugar:Acid Ratio	227.20	175.10	461.73
48	Ascorbic Acid	10.37	7.50	10.25
49	Total Phenols	0.40	0.60	0.48
50	Seed number	4	5	4
51	Seed shape	Broadly clavate with pointed spine	Semi-ovate	Broadly clavate with spine
52	Seed weight (g)	3.02	3.55	3.52
53	Pulp:Seed Ratio	29.39	21.10	27.88

**Morphological, fruit and biochemical characterization
of Sapota genotypes:**

S. No.	Character	PKM-4
1	Growth habit	Semi-erect
2	Shape of the tree	Broadly pyramidal
3	Branching pattern	Verticillate
4	Branch density	Medium dense
5	Colour of shoot tip	Brown
6	Surface of shoot tip	Pubescent
7	Shape of the leaf tip	Sub-acuminate
8	Leaf margin	Wavy
9	Leaf colour	Green
10	Leaf shape	Elliptic
11	Leaf area (cm ²)	26.43
12	Leaf length (cm)	10.11
13	Leaf width (cm)	3.78
14	Leaf area constant	0.69
15	Inflorescence type	Cymose
16	Flowers per cluster	Solitary
17	Position of inflorescence	Axillary
18	Colour of flower bud	Brown
19	Sepal colour	Greenish brown
20	Petal colour	White
21	Number of sepals	6
22	Number of petals	6
23	Length of style (cm)	0.81
24	Number of anthers	6
25	Pollen prod. per anther	671.87
26	Pollen size (μ)	15.64
27	Pollen fertility (%)	97.29
28	Fruit shape	Elliptic
29	Fruit length (cm)	7.75
30	Fruit width (cm)	4.17
31	Fruit weight (g)	127.04
32	Fruit yield/ tree (kg)	70.50
33	Peel colour	Brown
34	Fruit surface texture	Smooth
35	Fruit base	Flattened
36	Fruit apex	Obtuse
37	Flesh taste	Sweet
38	Flesh texture	Sandy
39	Flesh colour	Brown
40	Flesh weight (g)	124.48
41	Fruit Dessert quality	Good
42	TSS (° Brix)	23.50
43	Titrate Acid (%)	0.04
44	Reducing Sugars (%)	8.31
45	Total Sugars (%)	9.68
46	Non-Reducing Sugars (%)	1.37
47	Sugar:Acid Ratio	245.51
48	Ascorbic Acid	9.62
49	Total Phenols	0.42
50	Seed number	4
51	Seed shape	Broadly clavate with spine
52	Seed weight (g)	3.84
53	Pulp:Seed Ratio	31.49