MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF SAPOTA (*Achras zapota* L.) USING RAPD MARKERS

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BY

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CERTIFICATE

This is to certify that the thesis entitled **"MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF SAPOTA (*Achras zapota* L.) USING RAPD MARKERS"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **FRUIT SCIENCE** to the University of Horticultural Sciences, Bagalkot, is a record of bonafide research work carried out by **Miss. SUHASINI JALAWADI** under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.

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Affectionately dedicated
To
Jalawadi family & friends

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SI. No.	Abbreviations	
1.	m	Meter
2.	cm	Centimetre
3.	%	Per cent
4.	g	Gram
5.	kg	Kilo gram
6.	°B	°Brix

INTRODUCTION

1. INTRODUCTION

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

The cultivation of sapota has attracted many farmers on account of its hardy nature and better adaptability to diverse soil and climatic conditions. Though the area under sapota is increasing day by day, the increase is not up to the expected level. One among the several causes attributed for this is the absence of number of good choice varieties. Sapota is a hardy crop and fairly less susceptible to pest and disease which may be due to the presence of endogenous phenolics substances (Lakshminarayana and Subramanyam, 1966). India is a leading producer of sapota with an area and production of 1, 58,000 ha and 13, 46,000 metric tonnes respectively (Anon., 2010). It is commercially grown in the Maharashtra, Gujarat, Karnataka, Andra Pradesh, Tamil Nadu and West Bengal states. Karnataka is the leading producer of sapota, which is mainly distributed in districts of Bagalkot, Bijapur, Belgum, Dharawad, Kolar, Shimoga, Haveri, Uttar Kannada, Chitradurga, *etc.* In Karnataka, it is grown in an area of 2, 93,000 hectares with a production of 3, 60,000 metric tonnes (Anon., 2010).

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

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

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

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

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

DNA can be typified by wide range assortment of fingerprinting techniques. Generally they can be classified into restriction (Restriction), amplification (Randomly Polymorphic DNA) or both restriction and amplification based (Amplified Fragment Length Polymorphism) marker. Among different types of molecular markers available RAPD (Randomly Amplified Polymorphic DNA) markers are attractive because of their simplicity, versatility, modest cost and ability to detect even the relatively small amount of variation (Ragot and Hoisington, 1993). Such precise and refined techniques appear to have not been utilized in the genus *Achras.*

In the light of above information, an investigation entitled "Morphological and molecular characterization of sapota (*Achras zapota* L.) using RAPD markers" was carried out with the following objectives:

1. Morphological characterization of varieties and hybrids of sapota.
2. Molecular characterization of varieties and hybrids of sapota using RAPD markers.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

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

2.1 Vegetative growth parameters

2.1.1 Plant height

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

Hegde (1997) noticed that the hybrid 3/13, DHS-1 (10/25) and Cricket ball exhibited the orthotropic nature (5.06 m, 4.76 m and 4.65 m respectively), while Kalipatti showed plagiotropic nature by recording the lowest tree height (3.06 m) among the varieties and hybrids evaluated under Dharwad conditions.

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

Under Gandevi conditions, the maximum plant height (480.00 cm) was recorded in sapota cv. Bhuripatti in nine years old trees. Among the 14 cultivars of sapota evaluated under Arabhavi conditions, the cultivar CO-1 recorded significantly maximum plant height (365.30 cm), which was on par with DHS-1 (363.70 cm) and DHS-2 (360.00 cm) in nine years old trees (Anon., 2002).

Under Kovvur conditions, the cv. Kalipatti recorded the higher plant height (3.37 m) followed by PKM-3 (3.27 m) and DHS-1 (3.26 m) in seven years old trees of sapota (Anon., 2004). Siddaramayya (2005) reported that plant height was maximum in cv. CO-1 (615.00 cm) followed by cv. Long Oval (491.50 cm) and cv. DHS-1 (585.00 cm) and least in cv. Kirthbarthi (306.50 cm).

Shirol *et al.* (2006) reported that maximum plant height was obtained in cv. CO-1 (517.00 cm) followed by cv. Gavarayya (463.00 cm) and cv.DHS-1 (457.00 cm) and minimum was in cv. Tagarampudi (250.00 cm).

2.1.2 Stem circumference or girth

Maximum stem girth (23.46 cm) was recorded in sapota cv. Kalipatti under Gandevi condition in six years old trees (Anon., 1997).

Under Arabhavi conditions, the sapota cv. DHS-2 recorded maximum stem girth (5.76 cm) during 1998-99 in four years old trees (Anon., 2000). Maximum stem girth (43 cm) was observed in sapota cv. Kalipatti under Kovvur condition in seven years old trees (Anon., 2004 a).

2.1.3 Plant spread

The highest canopy spread was recorded in Hybrid 3/13 (4.30 m east-west and 4.40 m north-south) followed by hybrid 12/8 (3.86 m east-west and 4.03 m north-south) under Dharwad conditions (Hegde, 1997).

Gunaki (1998) recorded the highest canopy spread in cv. DHS-1 (3.85 m east-west and 3.96 m north-south) followed by Kalipatti (3.64 m east-west and 3.46 m north-south) under Dharwad conditions.

The maximum plant spread (81.66 cm east-west and 66.66 cm north-south) was recorded in sapota cv. Kalipatti under Kovvur conditions in two years old trees (Anon., 1997).

Under Kovvur conditions, the maximum canopy spread was recorded in sapota cv. PKM-1 (east-west 203.33 and north-south 206.66 cm) in four years old trees (Anon., 2000).

Maximum north-south canopy spread (570.00 cm) was observed in cv. Kalipatti, whereas maximum east-west canopy (576.67 cm) was recorded in cv. Cricket Ball under Gandevi (Gujarat) conditions in 12 years old trees (Anon., 2004 a).

Siddaramayya (2005) recorded maximum plant spread in cv. DHS-1 (573.33 cm North-South and 588.00 cm East-West) followed by cv. Kalipatti (521.00 cm North-South and 555.00 cm East-West) and cv. CO-1 (520.00 cm North-South and 509.00 cm East-West) and minimum in cv. Kirthbarthi (278.33 cm North-South and 286.66 cm East-West) in 14-15 years old trees.

2.2 Reproductive parameters

2.2.1 Season of flowering

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

Farooqi and Rao (1976) noticed main flowering flush during November-December under Dharwad conditions. Nalawadi *et al.* (1977) reported three main seasons of flowering at Dharwad *viz.,* June to August, October to December and March. They also noticed that flowering was maximum during June to August and minimum during March. Mahadevaiah (1981) also made similar observations under Dharwad conditions. Mone (1989) reported that in sapota there were three main flowering seasons *viz.,* November-January, April-May and July-August with a trace flowering in February-March at Dharwad. Relekar *et al*. (1991) noticed maximum number of sapota flowers opened during November-December.

2.2.2 Per cent fruit

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

Farooqi and Rao (1976) reported that main flowering flush was noticed during November-December under Dharawad conditions. Mahadevaiah (1981) also made similar observations under Dharawad conditions and noticed that fruit set was higher in the flushes of

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

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

Sapota tree bear a number of flowers but the number of fruits that ultimately reach maturity is relatively less, many flowers fail to set fruit and even among set fruits there is some amount of shedding. Madhavarao and Khader (1961) observed that the percentage of fruit set under natural conditions ranged from 0.0 to 11.0 per cent and selfed conditions 2.0 to 10.0 per cent. Farooqi and Rao (1973) reported fruit set in Kalipatti, Cricket Ball, Calcutta Round and oval were of the order 11.4, 23.7, 11.9 and 1.07 per cent respectively under open pollination condition. Mahadevaiah (1981) noticed higher fruit set in the flushes of November and May and the lowest during February-March. Minhas and Sandhu (1985) observed no fruit set in all the cultivars of sapota studied during June flowering under Ludhiana conditions. They obtained a very low fruit set during November flowering under open and self-pollination.

Poor fruit set is a common phenomenon in sapota and has been attributed to several reasons such as presence of self-sterility (Hays, 1960), phenomenon of heterostyly and dichogamy (Madhavarao and Khader, 1961 and Farooqi and Rao, 1976), cross-incompatibility (Farooqi and Rao, 1976), self-incompatibility (Madhavarao and Khader, 1961 and Farooqi and Rao, 1976) and preformed abscission zones in pedicels of dropping flowers (Lakshminarayana and Subramanyam, 1970).

2.3 Yield and quality parameters

2.3.1 Colour of pulp

Rokhade *et al*. (1989) observed that cvs. Kalipatti, Hybrid 2/7, hybrid 2/13, hybrid 7/17, hybrid 10/25 and hybrid 12/8 had light orange coloured pulp. The cv. Cricket Ball exhibited medium brown in coloured pulp. He further reported that Calcutta Round, hybrid 3/13 and hybrid 5/17 produced brown coloured pulp, whereas, Oval, hybrid 10/17, hybrid 11/13 and hybrid 12/24 had orange coloured pulp.

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

2.3.2 Length and diameter of fruit

Lakshminarayana and Subramanyam (1966) reported that length and breadth were continuously increased from fruit set to maturity in Calcutta Round variety of sapota. Sundararajan and Madhavarao (1967) studied the changes in length and diameter of fruit of eleven varieties of sapota at Coimbatore and observed the following pattern of changes.

a. Initial rapid rate of growth
b. An interphase of relatively less rapid to almost static for a short period.
c. A gradual increase in length and diameter till the maturity.

The authors concluded that there was no increase either in length or in diameter after the fruit attained the maturity. There were slight differences among the varieties in the rate of elongation and increase in diameter depending on the shape of the fruit. Sulladamath (1975) observed inconsistent rate of increase in diameter and length during the three stages of growth of fruit. He also reported that the diameter of fruit was always more than that length upto ninth fortnight following fruit set after which the length supercede.

Mahadevaiah (1981) studied the changes in length and diameter of Kalipatti fruits. He observed an increasing trend at the final stage of maturity (14th to 16th fortnight) and also diameter was always more than length upto ninth fortnight following fruit set after which length superceded. Mone (1989) observed that the diameter of different cultivars of sapota fruit superceded the length during the initial period of fruit growth (10th to 11th fortnight) after which the increase in length became more conspicuous.

Gunaki (1998) noted that, in all the cultivars, fruits grew more in diameter than length for about 10th to 11th fortnight and vice-versa thereafter till maturity with some variation among the cultivars. The cv. DHS-2 recorded maximum fruit length (7.25 cm) followed by cvs. Kalipatti (7.10 cm), DHS-1 (6.69 cm) and Cricket Ball (5.79 cm). With respect to diameter of fruit; maximum diameter was observed in cv. Cricket Ball and minimum in cv. Guthi. (Mathew, 2001) reported that fruit length was maximum in hybrid 7/1 and minimum in cv. Guthi. Maximum fruit girth was recorded in cv. DHS-2 (5.63 cm) and cv. DHS-1 (5.53 cm) where as least was recorded in cv. PKM-1 (3.87 cm).

2.3.3 Weight of fruit

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

Lakshminarayana and Subramanyam (1996) observed periodical changes in fresh weight of Calcutta Round Sapota under Mysore conditions and reported that, the weight increased gradually in initial stages followed by a rapid increase from 5th month till 7th months and finally the increase was gradual up to maturity. Sulladmath *et al.* (1978) reported that period-I (first six months) of Sapota (cv. Kalipatti) fruit growth accounted for 23.6 per cent of total weight of fruit attained at maturity, while period-II (6-8 months) accounted for only 14.4 per cent, period-III (8 ½ to 10½ months) accounted for maximum amount of growth (62 %) as measured in terms of fresh weight increase, confirming double sigmoid pattern of growth.

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

2.3.4 Yield attributes

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

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

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

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

2.3.5 Total soluble solids (TSS)

Mone (1989) studied some quality aspects of different sapota varieties and observed the highest TSS (24° Brix) and reducing sugar (12.00 %) in Kalipatti, while non-reducing sugar was highest in Co-2 (6.80 %) which also recorded the lowest starch content (0.22 %).

Rokhade *et al.* (1989) evaluated some promising hybrids in sapota with regard to fruit characters. They observed that TSS varied from 21 to 26 per cent among the hybrid selections and highest TSS (26%) was noticed in hybrid number 10/25.

Chadha (1992) studied quality aspects of different Sapota varieties and hybrids and he observed highest TSS per cent in hybrid 2/4 (27.40° B) where as cv. Dwarapudi recorded lowest (13.00° B) TSS. Hegde (1997) observed that DHS-1 (10/25) recorded significantly higher content of total soluble solids (26.08 °B), while rest of hybrids, it varied from 21.46 to 23.93°B.

Siddaramayya (2005) recorded higher TSS in cv. DHS-1(22.73° B) which was followed by cv. DHS-2 (22.60° B) and cv. Kalipatti (22.33° B), while lowest was noticed in cv. Gavarayya (19.46° B). Mathew (2001) noticed that the TSS of different cultivars ranged between 18-21° B. He recorded highest TSS in cv. Guthi (21° B) and it was lowest in cv. Local Narikeli (18°B).

Shirol *et al.* (2005) reported higher mean TSS in cv. DHS-2 (22.61.00 %) and cv. PKM-1 (22.60 %), where as lower TSS was observed in cv. CO-1 (19.56 %) and cv. Gavarayya (19.84 %).

2.3.6 Acidity

Ponnuswamy and Irulappan (1987) reported that acidity ranged between 0.02-0.108 per cent among the varieties and 0.003 and 0.0930 per cent among the hybrids.

Chundawat and Bhuva (1982) reported acidity varying between 0.019 to 0.216 per cent which was maximum in Cricket Ball (0.018) and minimum in Kalipatti (0.019). Whereas, in Pilipatti acidity were intermediates (0.022 %) and truly speaking, these fruits are acid less.

Avaiya and Singh (1991) reported that the titrable acidity content of fruit varied significantaly from cultivar to cultivar. The highest acidity reported in cv. CO- 1 (0.0171 %) and lowest in Pilipatti (0.0112 %). Gunuki (1998) observed highest acidity in cv. Cricket Ball (0.04 %) and the lowest was in DHS-1 (0.02 %).

Sidaramayya (2005) recorded lowest content of acidity in cv. CO-1 (0.125 %) followed by (0.192 %) and in rest of the varieties and hybrids, acidity was 0.197 per cent. Titrable acidity varied from 0.064 % (cv. Cricket Ball) to 0.128 **%** (cv. Local) (Mathew, 2001).

2.3.7 Number of seeds

Mathew (2001) reported that more number of seeds was found in cv. Cricket Ball than in Local (Narikeli). Shirol *et al.* (2005) recorded less number of seeds in Kalipatti (1.67) and cv. Oval (1.94), whereas, more number of seeds was observed in cv. Gavarayya (4.03). Farooqi and Rao (1976) suggested that variation in the seed number per fruit may be due to self-incompatibility and a natural tendency for cross pollination in Kalipatti sapota.

Sulladamath *et al.* (1978) reported that the round fruits had higher number of seeds (3.99 per fruit) than oval fruits (1.52 per fruit), conversely the shape index was higher for oval fruits than round fruits. They also noticed that seeds were uniformly distributed in round fruits than oval. Mone (1989) reported that when the number of seeds were more, they were arranged in a radial manner which may govern the shape of fruit. He also observed that fruits with round shape (variety Kirthbarathi and CO-2) had more number of seeds than oval shaped once, such as Kalipatti, CO-1, Gavarayya and Thagarampudi. Rokhade *et al.* (1989) reported that the number of seeds varied between 2 to 6 per fruit in different sapota varieties and hybrids.

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

Chundawat and Bhuva (1982) observed more number of seed per fruit in cv. Pilipatti (4.67) followed by cvs. Zumakhia (3.10), Mohangooti (2.70), Cricket Ball (2.63) and Kalipatti (1.72). Avaiah and Singh (1991) reported that, among the different cultivar cv. Cricket Ball showed more number of seed (5.146), while, cv. Pilipatti (1.626) recorded lowest number of seeds.

2.4 Molecular markers

In this chapter efforts have been made to review the existing literature on the importance, origin and distribution, taxonomy, genetic diversity, genetics and inheritance pattern, cytology and morphological markers. Since no work has been reported on the use of molecular markers in sapota in India or elsewhere, exhaustive literature on use of RAPD markers in discriminating cultivars, genetic diversity studies, phylogenetic studies, crop improvement, application and limitations of RAPD markers pertaining to horticultural crops and DNA extraction protocols have been cited.

2.4.1 Genetics and inheritance pattern

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

2.4.2 Cytology

The somatic chromosome number of sapota is 2n = 2x = 26 (Smith, 1976).

2.5 Biotechnological approaches

2.5.1 Morphological markers

Traditionally, cultivars have been identified by morphological, physiological or horticultural descriptions. These descriptions are largely subject to environmental conditions and human judgment. Development of new cultivars that differ little from the existing cultivars also required more reliable methods to discriminate between plant cultivars.

Morphological traits are simple, fast and inexpensive assays that can be studied even from herbarium specimen or other dead tissues. However, these characters are still extremely useful for initial genetic evaluation studies. It is often beset with many problems like influence of environmental conditions and delayed expression of traits, high maintenance cost and errors due to human judgment (Murphy *et al.,* 1990).

Morphological traits are the oldest and most widely used markers and they may still be considered as optimal for identification of certain germplasm and cultivars on the basis of leaf, fruit size, shape, color of flesh and skin and other physical characteristics. However, these characters may change with environmental conditions. Furthermore, the actual identity of some cultivars is still in question, because similar cultivars grown in different areas often have different names (Pathak and Ojha, 1993).

Now it is feasible to fingerprint all the collections present in India using molecular markers to supplement the phenotypic markers which will help to plan breeding programmes.

2.5.2 Molecular markers

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

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

2.5.3 Protein markers

Enzyme polymorphisms have been used successfully to identify various cultivars in fruit crops, through proteins. Protein markers are widely used as genetic markers in horticultural crops (Weeden, 1989). Typically isozymes exhibit simple inheritance, codominance, complete penetrance and consistency of expression under wide range of environmental conditions and their traits make them particularly useful as genetic markers (Weeden and Windel, 1989)

Isozyme polymorphisms have been successfully to identify interspecific hybrids of *Citrus* (Moor, 1984). Cultivars in various fruit species, like papaya (Moor and Litze, 1984), avacado (Goldring *et al.,* 1985), prunus (Drafitt and Arulshekar, 1985), apple (Weeden and Lamb, 1985), loquat (Degani and Blumenfled, 1986), banana (Jarret and Letze, 1986), custard apple and cherimoya (Ellatrand and Lee, 1987) and pine apple (De Wald *et al.,*1988) and also triploids in apple (Chyi and Weeden, 1984). Isozymes, as genetic markers, have been proven to reliable, consistent and essentially unaffected by environmental conditions (Bailey, 1983, Torres and Bergh, 1980). Mango leaf of isozymes of esterases, asperatate aminotransferase, acid phosphatases, and alkaline phosphatases were used to detect possible genetic variation among individuals of so, called clones (Gan *et al.,* 1981). However, enzyme polymorphism in mango has not examined systematically. Although isozyme can be very useful in cultivar identification, they can be tissue specific and the plant tissue must be sampled at comparable physiological stage to obtain uniform and repeatable banding pattern. The limited number of isozyme loci available for examination may be insufficient for identification of plant cultivars (Murphy *et al.,* 1990).

Degani *et al.* (1990) and Schnell and Knight (1992) have demonstrated the feasibility of using isozymes as biochemical markers in mango. Isozyme variation among of the same putative cultivars of mango was described by Gan *et al.* (1981), there by indicating that somatic mutation may occur frequently in certain mango clones. Enzyme polymorphisms were used by Degani *et al*. (1990) to differentiate among mango cultivars and to identify parentage of certain modern mango cultivars. Schnell and Knight (1992) have been able to differentiate zygotic from nucellar seedlings on the basis of enzyme polymorphisms.

Although isozyme markers are relatively simple tools for genetic analysis and linkage studies, it is unlikely that sufficient number of isozymes will be found to saturate the genome completely and uniformly (Tanksley, 1983). The enzymes extracted and subjected to electrophoresis are a tiny and probably non-representative sample of the total array of proteins present in plants. Besides for the mapping purpose, marker loci are useful if different alleles are segregating in the population of interest. Direct DNA based diagnostic assays are considered as powerful and reliable tools for genetic analysis because the numbers of scorable loci generated are greater and expression is similar in all tissues.

2.5.4 DNA isolation

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

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

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

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

2.5.5 DNA based markers

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

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

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

DNA based markers can be classified into:

a. Restriction based (RFLP)

b. PCR based (RAPD)

c. Both restriction and PCR based (AFLP)

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

2.5.5.1 Restriction Fragment Length Polymorphism (RFLP)

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

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

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

2.5.5.2 Amplified Fragment Length Polymorphism (AFLP)

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

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

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

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

2.5.5.3 Randomly Amplified Polymorphic DNA (RAPD)

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

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

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

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

2.5.6 Application of RAPD markers

2.5.6.1 Estimating genetic diversity

Hasnaoui *et al*. (2010) reported that the genetic diversity among Tunisian pomegranate using universal primers, the random amplified polymorphic DNA (RAPD) method was used to generate banding profiles from a set of twelve cultivars. Among the 12 primers tested, only four generated unambiguously reproducible bands after two PCR Runs. These are: OPA04, OPA19, OPH08 and OPH09. The remaining ones all yielded non reproducible bands. There was a total of 29 bands have been generated with a mean of 7.25 per primer. The number of generated bands varied from 3 to 11, with 0.5 to 3 kb size range. OPH07 and OPA19 primers generated nine and eight polymorphic bands, respectively, while amplification with OPH08 and OPA04 gave only four and threepolymorphic bands, respectively. The total number of polymorphic bands produced using four RAPD primers was 24 out of 29. Data was then computed with appropriate programs to construct a dendrogram illustrating the relationships between the studied cultivars. The cluster analysis has exhibited a parsimonious tree branching independent from the geographic origin of the cultivars.

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

Ercisli *et al*. (2011) reported that the genetic variability from 23 wild-grown pomegranate genotypes using eighty-six RAPD primers. Among which 12 gave reliable polymorphic patterns. These primers generated 145 RAPD bands of which 91% were polymorphic. The highest polymorphism ratio was observed with primers OPY06, OPY13, OPBA03, OPBB03, OPBB07, and OPBB09 (100%), while the lowest was with OPBB09 and OPBB10 (75%). The band size was between 250 and 2400 bp. There were five main clusters in the dendrogram, the highest genetic similarity was 0.24 and the lowest was 0.08.

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

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

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

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

Bajpai *et al*. (2008) revealed that the PCR based Random Amplified Polymorphic DNA (RAPD) and Directed Amplification of Minisatellite DNA (DAMD) markers were used to study the genetic diversity and relatedness among 22 guava accessions comprising commercial cultivars, breeding lines and unimproved cultivars. DNA isolated by CTAB method was used for amplification of 96 markers by using 7 RAPD primers and 56 workers generated by 40 DAMAD primers. Genetic distance matrix based on Jaccard's Co-efficient revealed maximum distance between Purple Guava and Allahabad Safed (43%), whereas minimum distance was as low as 5.4 per cent between two breeding lines HPSI-20 and HPSI-26. Interestingly half-sib progenies CISH-G-1 to CISH-G-6 had slightly more distance ranging from 1.8-24.0 per cent. The clustering revealed that most of the cultivars/accessions originated from Indo-Gangetic plains are grouped together.

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

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

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

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

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

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

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

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

RAPD markers were used in cashew to assess the diversity among the varieties and hybrids released in India by Murali (1999). Of the one hundred and twenty random primers screened, ten were chosen to score for 157 bands. The results indicated that the diversity among cashew cultivars and hybrids was moderate. In an attempt to study genetic diversity among fifty mango varieties (Hemanth, 1999), eighty primers were screened, ten of which gave 139 bands. UPGMA analysis based on Jaccard's coefficient revealed a moderate to high degree of genetic diversity among mango cultivars.

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

2.5.6.2 Cultivar identification

Lin *et al.* (2011) made a random amplified polymorphic DNA (RAPD) marker analysis of 68 pear cultivars. All pear genotypes could be distinguished by a combination of eight 11-mer primers. Among the eight primers used, primer Y47 was the first to be screened and used in identification of all the 68 pear cultivars. The electrophoresis results show that primer Y47 generated uniform, clear, and reproducible band patterns with a 1400-bp size in 42 cultivars. Two groups of cultivars including 'Huasu', 'Nanyue', 'Bayuehong', and 'Aiganshui' were randomly chosen to be used for the verification exercise. The primers Y47, Y40 and Y27 were used to separate the four cultivars. PCR results show that the four pear cultivars could initially be separated into two groups by primer Y47, with a band of about 1400 bp. One group had 'Nanyue' and 'Aiganshui', and could further be separated by primer Y27, with the 1900-bp band. The other group was made up of 'Huasu' and 'Bayuehong', and could be divided by use of primer Y40, with a band of about 1200 bp.

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

Gerlach and Stösser (2008) reported that the 200 ten bp-primers were screened for the generation of polymorphic fragments. Twenty-three primers were identified for application to the cultivars and 56 polymorphic fragments were identified as markers to differentiate the cultivars investigated, with respect to reliable presence or absence of a marker. Between 23 and 39 of the markers were realized among the group. Depending on the primer used, one to five polymorphic fragments per primer were amplified, with an average of 2.4 per primer. A few primers targeted DNA regions which were unique for 14 of the cultivars. There was no primer that detects enough genetic variation among the cultivars for a complete differentiation but scoring for the absence and presence of these markers reveals a unique binary code for each cultivar (exceptions mentioned below). Genetic similarity was determined according to Jaccard's Co-efficient (JC) considering only the polymorphic fragments. JC ranged from 0.26 up to 0.89.

Identification of cultivars and validation of genetic relationships in *Mangifera indica* L. was carried out using RAPD markers (Schnell *et al*., 1995). Twenty-five accessions of mango were examined for RAPD genetic markers with 80 decamer random primers. Of the 80 primers screened, 33 did not amplify, 19 were monomorphic and 28 gave reproducible, polymorphic DNA amplification patterns. Eleven primers were selected from 28 for the study. The number of bands generated was primer and genotype dependent, and ranged from one to ten. RAPD data were used to generate simple matching coefficients, which were analyzed phenetically and by means of principal co-ordinate analysis (PCA). The MHS clustered together in both the phenetic and the PCA, while, the randomly selected accessions were scattered with no apparent pattern.

Cheng *et al*. (1996) identified co-dominant RAPD markers tightly linked to fruit skin clolour in apple. Bulked segregate analysis identified one 10-mer base oligomer that generated different fragments in each of the bulks. After testing the primer in four populations, two fragments were associated with red skin colour and another two fragments associated with yellow skin colour. Three of the fragments (1160, 1180 and 1230 bp) were partly sequenced and found to share high sequence homology, suggesting that they generated from the same locus. A pair of universal primers was designed to amplify the fragments. In the cross ‘Rome Beauty’ X ‘White Angel’ population, two fragments were associated with red skin colour: one fragment designated as A1 (1160bp) was from ‘Rome Beauty’ and another fragment A2 (1180bp) was from ‘White Angel’. Progeny possessing both fragments, and one of them, had red fruits. Both parents displayed an alternate fragment, a1 (1230 bp) was associated with yellow-skinned fruit. In three other crosses tested, only fragment A1 co-segregated with red skin colour and two fragments, A1 and A2 (1230 and 1320 bp, respectively) were associated with yellow skin colour.

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

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

Damasco *et al*. (1996) detected dwarf off-types in micro propagated Cavendish (*Musa* spp. AAA) bananas using RAPD markers. A marker specific to the dwarf off-type from micro propagation of Cavendish group cultivars ‘New Guinea Cavendish’ and ‘Williams’ was identified following an analysis of 57 normal and 59 dwarf plants generated from several micropropagation events. Of 66 random decamer primers used in the initial screen, 28.8 per cent revealed polymorphisms between normal and dwarf plants. Primer OPJ – 4 (5'-CCGAACACGG-3') amplified a 1.5 kb band which was consistently present in normal but absent in all dwarf plants of both cultivars. Reliable detection of dwarf plants was achieved using the marker, providing a suitable means of *in vitro* detection. However, micropropagation- induced RAPD polymorphisms were not associated with the dwarf trait. The effectiveness of RAPD analysis for cultivar identification of persimmons (*Diospyros kaki*) was evaluated by using 10 base primers. Among 20 primers, two primers (OPA-6 and OPA-8) were most effective for cultivar identification and fifteen cultivars tested were completely distinguished(Rong et al., 1995), From these primers, two bud mutants of cv. 'Hiratenenashi', *i.e.*' Tonewase' and 'Sugitawase', showed different DNA patterns with a few additional minor bands using the OPA-6 primer.

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

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

2.5.6.3 Crop improvement

Warburton *et al.* (1996) utilized RAPD markers in identifying genetic linkages to genes of economic interest in peach. Some 360 RAPD primers were used with bulked segregant analysis to identify markers linked to loci of specific interest in peach (*Prunus persica*) and peach x almond (*Prunus dulcis*) crosses. The traits analyzed included flesh colour, adhesion, and texture; pollen fertility; plant stature; and the isozyme loci. The Mendelian behaviour of the RAPD loci was established, and RAPD markers were mapped relative to the loci controlling flesh colour, adhesion, and texture, and the isozyme loci *Mdh-1* (malate dehydrogenase), 6*Pgd-2* (phosphogluconate dehydrogenase) and *Aat-1* (aspartate aminotransferase), as well as the existing RFLP genetic linkage map constructed previously using a peach x almond F_2 population. Loci controlling these traits mapped predominantly to linkage groups two and three of the peach genetic linkage map. Linkages to genes with both dominant and co-dominant alleles were identified, but linkages to dominant genes were more difficult to find. In several crosses, RAPD marker bands proved to be allelic. One co-dominant RAPD formed a heteroduplex band in heterozygous individuals and in mixtures of alternate homozygotes. The Mendelian behaviour of the RAPD loci studied was established and the results suggest that RAPD markers will be useful for plant improvement in peach.

2.5.6.4 Phylogenetic relationship

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

Mishra (2009) reported that the phylogenetic relationships of 36 locally grown apricot (*Prunus armeniaca* L.) genotypes using 31 PCR markers (20 RAPDs). RAPD analysis yielded

139 fragments, of which 136 were polymorphic, with an average of 6.8 polymorphic fragments per primer. Clustering of genotypes within groups was not similar when RAPD derived dendrogram were compared, whereas the pattern of clustering of the genotypes remained more or less the same in RAPD.

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

RAPD analysis was applied to 10 ornamental *Allium* species to assess the degree of polymorphism within the genus. Wide variations in banding profiles among the species were observed with each of the nine-decamer primers tested. These were assessed for use in systematic studies within the genus. Some 265 bands were scored for all the species studied. Genetic similarities between each of the species were calculated and cluster analysis was used to generate a dendrogram showing phylogenetic relationships among them. The resulting analysis, to a certain degree, agreed with the previous classification of the species, although it showed a closer relationship between *A. albopilosum* [*A. christophii*] and *A. giganteum* (Hong *et al.,* 1996).

2.5.7 Other application of RAPD

RAPD markers are also used for assessment of phylogenetic relationships by various workers in mango (Schnell *et al.,* 1993)**,** kalanchoe (Gehrig *et al.,* 1997)**,** citrus (Federici *et al.,* 1998) and ptizer junipers (Due *et al.,* 1999).

Ye *et al.* (1998) employed RAPD to investigate the relationship among 16 grape cultivars and sports thought to have arisen from these cultivars. From 53 primers, a total of 464 bands were generated, of which 29 per cent were common to all genotypes tested. Cluster analysis classified all tested cultivars into two main groups (*Vitis vinifera* L. and *V. Labruscana* Bailey) as expected. No polymorphism was detected among known clones of Chardonnay (Ch. Clone 7, Ch. Clone 78 and Ch. Geneva clone) or Pinot noir (P. n. clone 29, P. n. Geneva clone and P. n. Pernand).

A genetic linkage map of avocado (*Persea americana* Mill.) recently generated consists of simple sequence repeat (SSR) markers as well as DNA fingerprint (DFP) and randomly amplified polymorphic DNA (RAPD) markers. These markers were used to detect putative quantitative trait loci (QTLs) of eight avocado fruit traits. Two statistical methods were used: one-way analysis of variance and interval mapping. Six traits were found to be associated with at least one of the 90 DNA markers. Based on the two statistical approaches, a putative QTL associated with the presence of fibers in the flesh, was found to be located on linkage group 3. This putative QTL was found to be associated with the SSR marker AVA04 having a high significant value (P = 4.4 x 10(-8)). The haplotype analysis of linkage group 3 showed a putative dominant interaction between the alleles of this locus. (Sharon *et al.,* 1998).

Pooler and Scorza (1995) are of the opinion that, genetic analysis of RAPD markers can be complicated by the sensitivity of the RAPD-PCR procedure and the dominant nature of the markers. Using a unique system of six doubled haploid peach parents and their seven resulting F1 hybrid populations. They found that 18 per cent of the 50 scorable bands from 25 primers did not follow expected inheritance patterns. Bands present in parents were not transmitted to the progeny, or non-parental bands appeared in the progeny. Differences in RAPD patterns were also observed between haploids and spontaneous diploid bud sports on the same tree. Because peach is a long-lived clonally propagated crop, it is possible that somatic rearrangements would have lead to this unexpected inheritance of RAPD markers. Such anomalous patterns indicate that RAPD markers should be used cautiously in peach mapping and genetic diversity studies.

Gallego and Martinez (1998) observed that, the genomic DNA from 25 cultivars of rose was amplified using twenty 10 mer primers RAPD markers. The analysis reveals no variability within cultivars and high degree of variation between the cultivars. The patterns obtained with two of the primers (OPA-11 and OPA-17) all the rose cultivars were unequivocally identified. The investigation suggested that RAPD profile provide a single and efficient way to identify rose cultivars. Meir and Vainstein (1993) reported that, DNA fingerprint analysis of genetic relatedness between rose genotypes, using human-derived minisatellite probes, correctly reflected relationships within and between categories of rose plants. Nearly identical DNA fingerprint patterns were observed for cultivars ‘Dick Koster’ and its two sports. A high level of similarity was detected between genotypes of the hybrid tea category while a low level of similarity was detected within the miniature category. Between categories, the highest level of similarity was detected between hybrid tea and floribunda categories, the lowest between hybrid tea and miniature categories. Comparisons of a wild species, *R. cannina*, with all cultivated hybrid categories revealed very large differences.

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

Nucleic acid extracts from 23 *Alstroemeria* cultivars were amplified with eight random decamers by PCR. The primers OPC02, OPC03, OPD02, OPD05, OPD08, OPD11, OPD13 and OPD18 produced 24, 19, 21, 20, 10, 17, 25 and 29 RAPD bands, respectively. The distinctive RAPD patterns generated from these cultivars could be used as genomic `fingerprints' to establish the identity of a given genotype. The `Orchid' and `Butterfly' types were clearly separated in distinct subclusters in a phyllogram obtained by unweighted pair group method analysis (UPGMA) of the genetic distances. The `Hybrid' types were distributed in two major subclusters, reflecting the diversity of the parental species used to generate the population. This phyllogram conformed to expectations based on the available pedigree data (Joseph *et al.,* 1997).

Iqbal *et al.* (1995) used RAPD profiles of thirteen rhododendron hybrids, species and cultivars to study their genetic relationships. Two varieties of *Rhododendron yakushimanum*, var. Mist Maiden and var. Ken Janeck, clustered together while *R. yakushimanum* var. Pink Parasol clustered with a hybrid of Pink Parasol X *R. smirnowii*. These three varieties and the hybrid then clustered with *R. smirnowii*. Similarly, the other hybrids with common parents showed a closer genetic relatedness with each other than with other rhododendrons (Rayburn *et al.,* 1993). The sequence homology of two amplification products was studied by Southern hybridization. The amplification fragment OPL07660 of four Rhododendron species, *R.anna Baldsiefens, R. arborescens, R. atlanticum*, and *R. poukhanense* were homologous to each other. In contrast to this, amplification product OPL03900 of *R. anna Baldsiefens* has no homology to any other amplification product. The genetic relationship revealed from cluster analysis on the basis of RAPD profiles was similar to their known genetic makeup.

Starman *et al.* (1999) investigations revealed that, the DNA fingerprinting of 11 cultivars of poinsettia. Thirty one per cent polymorphisms and distinguished all cultivars.

2.5.8 Errors encountered in RAPDs

The study conducted by Yae *et al*. (1995 b) indicated that there can be errors while going for RAPD fingerprinting in apples. They are of the opinion that errors crept in when template DNA concentration was higher, with 1 unit of *Taq* polymerase, dNTP's and reaction buffer. These problems were overcome by optimizing PCR conditions**.** Staub *et al.* (1996) reported the sources of potential errors in the application of RAPD s in cucumber. The influence of tissue age, pathogen infestation, intrapopulation contamination and PCR conditions were probable sources of error in RAPD analysis. DNA from young, uninfected tissue from cucumber (lines G421 and H-19) leaves provided the most consistent results. Cucumber plants infected with *Sphaerotheca fuliginea* showed variation in RAPD banding patterns compared with those of uninfected plants. Differences In banding patterns were detectable when DNA from the lines was mixed at dilution ratios of 20:1 but not 50:1. Differing lots of commercially available 10X reaction buffer, $MgCl_2$ stock solutions and *Taq* DNA polymerase affected RAPD banding patterns and overall yield. For reproducibility of RAPD assays, it may be necessary to optimize reactions for specific lots of PCR reagents from either commercial or in-house sources.

MATERIAL AND METHODS

3. MATERIAL AND METHODS

The present investigation on Morphological and Molecular Characterization of Sapota using RAPD markers was carried out at All India Co-ordinated Research Project on Tropical Fruits, Kittur Rani Channamma College of Horticulture, Arabhavi (University of Horticultural Sciences, Bagalkot), Gokak taluk of Belgum district, Karnataka during 2010-2011. The material used and techniques adopted and observations recorded during the course of the investigations are furnished in this chapter.

3.1 Geographical location of the experimental site

Arabhavi is situated in Northern dry tract of Karnataka State at 16°15' north latitude and 74° 45' east longitude and at an altitude of 612.03 m above the mean sea level.

3.2 Climate

Arabhavi lies on zone-3 of region-2 of agro-climatic zones of Karnataka and is considered to have the benefit of both South-West and North-East monsoons. The average rainfall of this area is 522 mm distributed over a period of five to six months (June to November) with peak during October (Appendix I). The command area receives water from Ghataprabha Left Bank Canal (GLBC) from mid-July to mid-February.

3.3 Type of soil

The soil of the experimental site was medium deep black soils having a depth of 90 to 100 cm.

3.4 Details of treatments

Experiment No. 1 Morphological characterization of varieties and hybrids of sapota

Morphological study was carried out during August to December

The following genotypes were included for the study, *i,e.,* 24 accessions.

T_1- DHS-1
T_2- DHS-2
T_3- PKM- 1
T_4- PKM-2
T_5- PKM-3

T_{17}- Murabba
T_{18}- CO-3
T_{19}- Pala
T_{20}- Mohangootee
T_{21}- PKM-4

T_6- CO-1
T_7- CO-2
T_8- Cricket Ball
T_9- Long Oval
T_{10}- Kalipatti
T_{11}- Guthi
T_{12}- Gavarayya
T_{13}- Oval
T_{14}- Kirthbharti
T_{15}- Cricket Ball (ARSA)
T_{16}- Tagarmpudi
T_{22}- Virudhanagar
T_{23}- Singapore
T_{24}- Cricket Ball (Udupi)

3.4.1 Experimental design

The design adopted for the experiment was completely randomised block design (RBD) with three replications (three trees per replication).

3.5 Sailent features of the genotypes

3.5.1 Experiment No. 1- 24 genotypes and Experiment No. 2- 31 genotypes are used

3.5.1.1 DHS- 1 (10/25) (Dharwad Hybrid Sapota-1)

This hybrid was released by University of Agricultural Sciences, Dharwad during 1991 for growing in Karnataka. It is a cross between Kalipatti x Cricket ball. It gives about 30 per cent higher yield than its parents. The fruits are oval in shape and pulp is very sweet with light orange soft and mellow with total soluble solids (TSS) of 25 per cent. Fruits are very large size (150 g) and also having good shelf life.

3.5.1.2 DHS-2 (10/17) (Dharwad Hybrid Sapota-2)

This hybrid was released by University of Agricultural Sciences, Dharwad during 1991 for growing in Karnataka. It is also a cross between Kalipatti x Cricket ball. It gives about 30 to 35 per cent higher yield than its parents. The fruits have round shape with bigger size than Cricket ball (180 g). The pulp texture is granular soft and mellow having light orange colour with TSS of 23 per cent.

3.5.1.3 PKM-1 (Periyakulam-1)

It is a popular variety of Tamil Nadu Agricultural University, which is selection from Guthi. Trees are short stature with dense crown, fruits are medium in size, elliptic to oblong in shape with thin skin, rich in sugars, high TSS of 24°B, profuse bearing habit.

3.5.1.4 PKM-2 (Periyakulam-2)

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

3.5.1.5 PKM-3 (Periyakulam-3)

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

3.5.1.6 CO-1 (Coimbatore-1)

It is grown in Tamil Nadu. This is a hybrid between Cricket Ball and Oval. The combination was designed to get together the large fruit size of the female parent and superior taste of the male parent. The hybrid came to bearing after four years of planting. Fruits are bigger in size whish are oval in shape with reddish brown flesh and 18 per cent TSS.

3.5.1.7 CO-2 (Coimbatore-2)

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

3.5.1.8 Cricket Ball

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

3.5.1.9 Long Oval

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

DHS -1

DHS - 2

PKM - 1

PKM - 2

PKM - 3

CO - 1

Plate 1a. Sapota cultivars

CO - 2

Cricket Ball

Cricket Ball (ARSA)

Kalipatti

Gavarayya

Oval

Plate 1b. Sapota cultivars

3.5.1.10 Kalipatti

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

3.5.1.11 Guthi

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

3.5.1.12 Gavarayya

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

3.5.1.13 Oval

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

3.5.1.14 Kirthbarti

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

3.5.1.15 Cricket Ball (ARSA)

It is a selection from Cricket Ball variety from Agricultural Research Station Arabhavi, Karnataka. Located at All India Co-ordinated Research Project on Tropical Fruits, Kittur Rani Channamma College of Horticulture, Arabhavi. The fruits are round with medium size and prolific bearer.

3.5.1.16 Tagarampudi

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

3.5.1.17 Murabba

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

3.5.1.18 CO-3 (Coimbatore-3)

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

3.5.1.19 Pala

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

3.5.1.20 Mohangootee

It is grown in Maharashtra and Gujarat. Leaves are light green with medium sized fruit having good taste.

3.5.1.21 Variegated Sapota

It is medium growing tree. Leaves are variegated having yellowish patch on green leaves hence the name variegated sapota. The white or cream coloured patches are also seen on fruit surface.

3.5.1.22 PKM-4

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

3.5.1.23 French Indochina

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

3.5.1.24 Dwarapudi

It is grown in Andhra Pradesh. Large sized fruit, round, sweet and much in demand. The fruits resemble those of Cricket Ball but smaller in size.

3.5.1.25 Gholwad Sapota

It is a medium growing tree and grown in Maharashtra. Plant vigour is medium, tree is compact. Gholwad is a village in Thane district of Maharashtra where for the first time the sapota was introduced in India.

3.5.1.26 PKM-5

It is a selection from open pollinated seedlings of Virudhunagar local at Periyakulam (Tamil Nadu Agriculture University). The tree canopy is compact with narrow clusters of leaves. Fruits are borne in clusters with oval to oblong shape.

3.5.1.27 Calcutta Round

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

3.5.1.28 Virudhanagar

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

3.5.1.29 Singapore

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

3.5.1.30 Cricket Ball (Udupi)

It is selection from Cricket Ball. Tree is medium growing, foliage is light green. Fruits are bigger in size with buff coloured fruit surface.

3.5.1.31 Cricket Ball (Sirsi)

It is selection from Cricket Ball. Tree is compact. The fruits are round and bigger in size.

3.6 Details of the experiment

3.6.1 Selection of tree for growth parameters

In each accession uniform sized trees spaced at 10 X 10 m and exposed to identical conditions of weather, soil and cultural operations were selected.

3.6.2 Observations recorded on growth parameters

3.6.2.1 Plant height (m)

The height of the plant was measured in meters from ground level to the tip of growing plant. It was expressed in meters.

3.6.2.2 Stem circumference (cm)

The stem circumference was measured by measuring circumference of the trunk at the marked area (just above the graft union) with the help of measuring tape and was expressed in centimetres.

3.6.2.3 Plant spread (m)

The measurement was made in meters in north-south and east-west direction at the widest point of the plant.

3.6.2.4 Shoot length (cm)

The shoot length of ten randomly selected shoots was measured with the help of measuring tape and expressed in centimeters.

3.6.2.5 Shoot girth (cm)

The shoot girth of ten randomly selected shoots was measured with the help of measuring tape and expressed in centimeters.

3.6.2.6 Number of leaves

The ten random shoots were selected and leaves were counted.

3.6.3 Observations recorded on reproductive parameters

3.6.3.1 Number of flowers/shoot

The uniformly developed flower buds were taken for these observations. The fully opened flowers were counted from randomly selected ten shoots.

3.6.3.2 Days for flower opening

The fully opened flowers were considered from day of flower bud initiation to flower open. The days were counted from flower bud initiation to fully opened flowers.

3.6.3.3 Days taken for fruit set

The peanut stage fruits were considered for this observation. Days were counted from flower open to peanut stage fruits.

3.6.3.4 Per cent fruit set (%)

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

3.6.4 Quality parameters

3.6.4.1 Fruit colour/pulp colour

Colour of the fruit pulp was recorded by visual observation.

3.6.4.2 Fruit diameter (cm)

Fruit diameter was measured with the help of digital vernier callipers at widest middle point where maximum girth was noticed and it was expressed in centimeters.

3.6.4.3 Fruit length (cm)

The distance between the stalk end and floral end of the fruit was measured with the help of vernier callipers and was expressed in centimeters.

3.6.4.4 Number of fruits per tree

Fruits are harvested when they are fully matured and number of fruits are recorded. The total number of fruits are calculated by adding the values obtained in different harvests of year and expressed number of fruits per tree per year.

3.6.4.5 Weight of the fruit (g)

Weight of fruit was recorded by using digital balance and expressed in grams (g).

3.6.4.6 Yield (kg)

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

3.6.4.7 Total soluble solids (° B)

Total Soluble Solids (TSS) of the pulp was recorded with the help of Erma (0-32 %) hand refractometer and expressed in ° Brix.

3.6.4.8 Titrable acidity

A known weight of sample (5 g) was macerated in distilled water, filtered through muslin cloth and made up the volume to 50 ml with distilled water. This was titrated against standard NaOH using phenolphthalein indicator. The appearance of light pink colour was marked as the end point. The value was expressed in terms of malic acid as per cent titrable acidity of pulp (Anon., 1984).

3.6.4.9 Number of seeds

The seeds from 5 ripe fruits were counted and recorded after dissecting the fruit and average number of seed present in five fruits was recorded.

3.7 Statistical interpretation of the results

The data on growth parameters, reproductive parameters and quality parameters were subjected to statistical interpretations as per the methods suggested by Panse and Sukhatme (1967).

Experiment No. 2 Molecular characterization of varieties and hybrids of sapota using RAPD markers

The following genotypes were included for the study, *i,e.,* 31 accessions.

T_1- DHS-1
T_2- DHS-2
T_3- PKM- 1
T_4- PKM-2
T_5- PKM-3
T_6- CO-1
T_7- CO-2
T_8- Cricket Ball
T_9- Long Oval
T_{10}- Kalipatti
T_{11}- Guthi
T_{12}- Gavarayya
T_{13}- Oval
T_{14}- Kirthbharti
T_{15}- Cricket Ball (ARSA)
T_{16}- Tagarmpudi
T_{17}- Murabba
T_{18}- CO-3
T_{19}- Pala
T_{20}- Mohangootee
T_{21}- Varigated Sapota
T_{22}- PKM-4
T_{23}- French Indochina
T_{24}- Dwarapudi
T_{25}- Gholwad Sapota
T_{26}- PKM-5
T_{27}- Culcutta Round
T_{28}- Virudhnagar
T_{29}- Singapore
T_{30}- Cricket Ball (Udupi)
T_{31}- Cricket Ball (Sirsi)

3.8 Protocol for isolation of DNA

3.8.1 DNA isolation and purification

3.8.1.1 Collection of plant materials

Totally 31 sapota individuals have been analysed in the present investigation consisting of 19 cultivars, 6 landraces and 6 hybrids. The leaf samples of both hybrids and varieties were collected from sapota germplasm of All India Co-ordinated Research Project on Tropical Fruits, Kittur Rani Channamma College of Horticulture, Arabhavi (University of Horticultural Sciences). Recently matured leaves that were free from damage caused by pests or disease and available throught the year were used for isolation of DNA. Leaf samples were collected in brown paper covers and stored in poly bags at -20 °C.

3.8.1.2 Processing of sample

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

3.8.1.3 DNA isolation protocol

Stock solutions

CTAB : 6 per cent

NaCl : 5 M

Tris-Base : 1 M

EDTA : 0.5 M

RNA'se : 10 mg/ml

Isopropanol

Sodium Acetate : 3 M

PVP : 2 per cent

Chloroform: Isoamyl alcohol (24:1 v/v)

β-Mercaptoethanol: 2 per cent

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

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

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

b) The mixture was cooled to room temperature and 200 µl of cold chloroform: Isoamyl alcohol (24:1 v/v) was added and the contents were gently vortexed and spun at 6000 rpm at 10 °C for 15 minutes.

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

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

e) It was shakened for 2-3min and spun at 6000 rpm at 10 °C for 15 minutes and supernatant was discarded.

f) The pellet was washed with 70 % ethanol and dried at room temperature and it was spun at 6000 rpm at 10 °C for 15 minutes.

g) The ethanol was discarded and DNA pellet was dried till ethanol evaporates.

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

3.8.2 DNA Quantification

The amount of DNA of all the samples was measured by Spectrophotometer. The assay solution contained 2985µl of sterile water + 15µl of DNA. The transmittance/absorbance is red at $A_{260}/A_{280.}$ The quantity of DNA was calculated by using formula:

DNA mg/ml = $A_{260} \times 50 \times$ Dilution factor/1000 Dilution factor=200

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

3.9.1 PCR ingredients (Stock solutions)

Template DNA : 20 ng/µl

dNTPs : 1 mM (2.5 mM each)

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

Primers : 3 µM (Operon technologies Ltd.)

***Taq* DNA polymerase :** 3unit/µl

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

3.9.2 PCR programme

PCR was carried out in Thermo cycler of [Thermo Electro Corporation Company (USA)] using programme profile consisting of an initial hot start at 94°C for 5 minutes, followed by 38 cycles of one minute denaturation at 94°C, one minute annealing at 38°C and 1.5 minutes extension at 72°C, with final extension step of ten minutes at 72°C and 4°C hold.

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

3.9.3 Primer selection

Primer screening was carried out using the above-mentioned PCR protocol utilizing DNA from 4 genotypes namely, Mohangootee, Gholwad Sapota, PKM-5 and Virudhanagar. Out of 20 primers screened *viz.,* A, B, C, D, F, G and K series, 15 primers amplified at least one band. From these 15 primers, 7 primers yielding strong, intense and unambiguous bands were selected for RAPD analysis of all the selected genotypes.

3.9.4 Agarose gel electrophoresis

Materials

Agarose : 0.8per cent

Running buffer : TBE (Tris base 54g, 27 g Boric acid, 20 ml EDTA; pH 8.0 per litre of DDW)

Dye : (0.25% Bromophenol blue)

Ethidium bromide : 10mg/ml

Method

Amplified DNA fragments were resolved on 0.8 per cent agarose gel stained with ethidium bromide. Agarose is a polysaccharide derivative of agar, contains micro pores and hence acts as a molecular sieve. Agarose was melted in 1x running buffer till it forms a clear solution. Then it was cooled to 55°C, ethidium bromide at 0.4 mg/ml was added and casted in a mould with combs. After solidification the combs were removed (Sambrook *et al.,* 1989). The reaction volume 20 µl along with 5 µl loading buffer was loaded in the wells and the gel was fully submerged in 1x running buffer. Electrophoresis was conducted at 80-90 volts for 3 to 4 hours and gel was photographed under UV light by using Bio-Vis gel doc system.

3.10 Data analysis, estimation of genetic distance and clustering analysis

3.10.1 Data scoring and analysis

Fragments amplified by the primer used and molecular weights in base pairs (bp) were scored for their presence or absence (Echt *et al.,* 1992) and a matrix of different RAPD phenotypes was assembled. Further, a fragment was counted only if it was intense, clear and strong. Diffuse and/or very weak fragments were not scored as such fragments have been reported to possess the greatest propensity for poor reproducibility (Heun and Helentjaris, 1993). The band sizes were estimated by using a 1000 bp ladder marker, which was run along with the amplified products.

3.10.2 Statistical analysis and estimating genetic distances

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

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

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

3.10.3 Cluster analysis

The agglomerative method of clustering using UPGMA (Unweighted Pair Group Method with Arithmetical averages) for developing dendrogram was adopted. This calculates the congruence between assays of values typically densitometric assays. As it compares curves as a whole, it is independent of band definitions and is thus ideally suited for a quick comparison of pattern without first having to edit the bands. It is largely intensive to relative concentrations, but is sensitive to differences in background. Jaccard's Co-efficient considers only the presence of band as similarity and hence, is more conservative in declaring genetic diversity. The subset of data involving 31 genotypes, cultivars, landraces and hybrids were analyzed separately.

EXPERIMENTAL RESULTS

4. EXPERIMENTAL RESULTS

The studies on “Morphological and Molecular Characterization of Sapota using RAPD markers” were conducted at the Kittur Rani Channamma College of Horticulture, Arabhavi (University of Horticultural Sciences, Bagalkot) during 2009 to 2011 and results of the study are presented in this chapter.

Experiment No.1 Morphological characterization of sapota genotypes.

4.1 Vegetative growth parameters

4.1.1 Plant height (m)

The data pertaining to plant height was recorded in different age group (15-16 years and 10-11 years old) of sapota cultivars and are presented Table - 1 and 2.

The observations indicated that, there was significant difference among the cultivars with respect to plant height of 15-16 years old plants (Table 1). Significantly maximum plant height was observed in cv. CO-1 (6.80 m) which was on par with cvs. Gavarayya (6.77 m), Cricket Ball (6.67 m), DHS-1 (6.00 m) and PKM – 3 (5.83 m) and it was it was least in cv. Kalipatti (3.80 m).

In 10-11 years old trees cv. Cricket Ball (ARSA) recorded significantly longer plant height (5.10 m) (Table 2). Which was on par with cv. Guthi (4.50 m).While, significantly shorter plant height was observed in cv.CO-3 (2.67 m).

4.1.2 Stem circumference (cm)

There was significant difference among the cultivars with respect to stem circumference in 15-16 years and 10-11 years old trees. Significantly maximum stem circumference was observed in cv. DHS-1 (88.67 cm), which was on par with DHS-2 (74.33 cm), under the age group 15-16 years (Table 1) and minimum stem circumference was noticed in cv. Tagarampudi (39.67 cm). In 10-11 years old trees (Table 2), significantly maximum stem circumference was noticed in cv. Cricket Ball (ARSA) (40.00 cm) followed by cv. Guthi (35.00 cm) and the minimum was observed in cv. Murabba (28.67 cm).

4.1.3 Tree spread (m)

The data on tree spread (North-South and East-West) in different age group of sapota cultivars are presented in Table- 1and 2.

The results revealed that in 15-16 years age group of plants there was a significant difference among the cultivars with respect to tree spread (North-South and East-West) (Table 1). Significantly maximum plant spread was recorded in cv. DHS-2 (8.73 m North-South) which was on par with cvs. Long oval (8.10 m North-South) and DHS-1 (8.00 m North-South) and minimum spread was observed in cv. Kirthbharti (4.60 m North-South). Significantly maximum East-West plant spread was recorded in cv. DHS-1 (8.50 m) which was on par with cv. DHS-2 (8.37 m) and minimum spread was noticed in cv. Tagarampudi (4.47 m).

Among the 10-11 years age group of plants significantly maximum North-South plant spread was observed in cv. Cricket Ball (ARSA) (4.53 m) (Table 2) which was on par with cv. Guthi (4.27 m) and least tree spread was recorded in cv. Singapore (1.93 m). Maximum East-West plant spread was observed in cv. Guthi (5.60 m) which was on par with Virudhanagar (5.20 m) and Mohangootee (4.17 m) minimum plant spread was recorded in cv. Singapore (2.60 m).

4.1.4 Shoot length (cm)

The data on shoot length was recorded in different age group of sapota cultivars and presented in Table -1and 2.

The data on the shoot length of 15-16 years old plant showed significant difference among different accessions (Table 1). Significantly maximum shoot length was recorded in cv. Cricket Ball (25.60 cm). This was found statistically on par with cvs. CO-1 (25.17 cm), Kirthbharti (24.03 cm), CO-2 (23.87 cm) and DHS-2 (23.87 cm). Minimum shoot length was noticed in cv. Oval (20.20 cm). While under the age group of 10-11 years old plants (Table 2), significantly maximum shoot length was observed in cv. Cricket Ball (ARSA) (24.43 cm) which was on par with cv. Virudhanagar (23.00 cm), where as minimum shoot length was recorded in cv. Singapore (19.60 cm).

4.1.5 Shoot girth (cm)

The data on shoot girth was recorded in different age group of sapota cultivars are presented in Table- 1and 2.

There was a significant difference among the accessions was noticed for shoot girth under age group of 15-16 years old (Table 1). Maximum shoot girth was recorded in cv. DHS-1

Table 1. Vegetative parameters of different cultivars of sapota

Cultivars*	Plant height (m)	Stem circumference (cm)	Tree spread (m)		Shoot length (cm)	Shoot girth (cm)	Number of leaves per shoot
			N-S	E-W			
DHS-1	6.00	88.67	8.00	8.50	22.43	4.17	20.33
DHS-2	5.50	74.33	8.73	8.37	23.87	3.57	17.97
PKM-1	4.33	55.33	6.20	6.13	22.07	4.12	15.00
PKM-2	5.50	68.67	5.90	6.90	21.10	3.00	16.67
PKM-3	5.83	66.00	6.33	6.63	23.47	3.51	11.87
CO-1	6.80	60.67	6.83	7.10	25.17	3.60	13.80
CO-2	5.50	47.33	7.80	7.80	23.87	2.50	12.67
Cricket Ball	6.67	62.33	7.70	7.90	25.60	3.38	15.27
Long oval	5.47	59.00	8.10	7.60	22.83	3.60	14.77
Kalipati	3.80	66.67	6.30	6.40	21.63	2.46	16.17
Gavarayya	6.77	75.33	6.90	7.53	20.90	2.36	11.60
Oval	4.33	65.67	6.43	6.70	20.20	2.64	14.80
Kirthbharti	4.67	42.67	4.60	5.23	24.03	3.57	13.47
Tagarampudi	4.57	39.67	4.73	4.47	20.97	2.90	11.60
S. Em±	0.38	7.89	0.56	0.56	1.13	0.29	1.71
C.D. @ 5%	1.11	22.94	1.64	1.63	3.29	0.84	4.97
CV (%)	12.28	21.94	14.51	14.05	8.65	15.55	20.13

***15-16 years old trees**

Table 2. Vegetative parameters of different cultivars of sapota

Cultivars*	Plant height (m)	Stem circumference (cm)	Tree spread (m)		Shoot length (cm)	Shoot girth (cm)	Number of leaves per shoot
			N-S	E-W			
Guthi	4.50	35.00	4.27	5.60	20.00	3.03	13.63
Cricket Ball (ARSA)	5.10	40.00	4.53	4.57	24.43	2.96	15.00
Murabba	3.00	28.67	3.63	4.13	21.50	2.31	13.87
CO-3	2.67	29.33	2.73	4.77	22.20	3.21	10.20
Pala	2.73	30.67	3.00	3.13	20.80	3.28	12.80
Mohangoote	2.83	29.33	3.97	4.17	20.47	3.17	13.03
PKM-4	3.33	29.33	3.07	3.33	21.63	2.50	12.07
Virudhanagar	3.67	30.00	2.43	5.20	23.00	3.15	11.87
Singapore	3.50	29.67	1.93	2.60	19.60	3.30	10.13
Cricket Ball (Udupi)	3.27	31.00	2.60	5.00	21.60	3.28	12.37
S. Em±	0.35	2.16	0.38	0.48	0.73	0.18	0.93
C. D. @ 5%	1.06	6.42	1.15	1.44	2.17	0.54	2.76
CV (%)	17.87	11.97	20.89	19.84	5.87	10.45	12.90

***10-11 years old trees**

(4.17cm) which was on par with cvs. PKM-1 (4.12 cm), CO-1 (3.60 cm) and Long oval (3.60 cm). While, it was least in cv. Gavarayya (2.36 cm).

Among the 10-11 years old tress (Table 2), significantly maximum shoot girth was observed in cv. Singapore (3.30 cm) which was on par with cvs. Cricket Ball (Udupi) (3.28 cm), Pala (3.28 cm) and Guthi (3.03 cm) and minimum shoot girth was recorded in cv. Murabba (2.31 cm).

4.1.6 Number of leaves per shoot

The data on number of leaves per shoot of different age group of sapota cultivars are presented in Table-1 and 2.

Among the 15-16 year old trees (Table 1) cv. DHS-1 recorded maximum number of leaves per shoot (20.33), which was on par with DHS-2 (17.97) and PKM-2 (16.67). Whereas, minimum number of leaves were recorded in cv. Tagarampudi and Gavarayya (11.60).

Among the 10-11 years old trees (Table 2) cv. Cricket Ball (ARSA) recorded maximum number of leaves per shoot (15.00). This was found statistically on par with cv. Murabba (13.87) and cv. Guthi (13.63). While, minimum numbers of leaves were recorded in cv. Singapore (10.13).

4.2 Reproductive parameters

4.2.1 Number of flowers per shoot

The observation on number of flowers per shoots recorded in sapota cultivars are presented in Table - 3.

There was significant difference among the cultivars with respect to number of flowers per shoot. Cv. CO-1 recorded highest number of flowers per shoot (15.33), which was on par with cv. Guthi (13.00) whereas less number of flowers was recorded in cv. Murabba (5.93).

4.2.2 Days to flower open

The data on days to flower open was recorded in different cultivars are presented in Table - 3.

Table 3. Reproductive parameters of different cultivars of sapota

Cultivars	Number of flowers/shoot	Days to flower opening (Days)	Days to fruit set (Days)	Per cent fruit set (%)
DHS-1	9.20	37.13	28.33	21.80
DHS-2	9.80	36.77	25.00	20.97
PKM-1	7.50	37.27	26.67	29.50
PKM-2	7.20	36.47	24.67	24.15
PKM-3	6.93	39.10	25.00	20.15
CO-1	15.33	39.13	25.67	11.27
CO-2	7.43	38.33	26.67	26.20
Cricket Ball	9.87	37.73	29.33	11.11
Long oval	6.87	38.53	26.67	15.96
Kalipatti	10.90	36.27	30.00	17.64
Guthi	13.00	34.57	28.33	14.60
Gavarayya	11.27	37.53	25.33	11.76
Oval	7.30	37.03	29.33	13.69
Kirthbharti	11.20	34.27	25.67	12.61
Cricket Ball (ARSA)	7.67	34.73	24.00	16.71
Tagarampudi	11.00	37.87	25.00	10.73
Murabba	5.93	34.50	28.67	14.62
CO-3	9.80	36.50	27.67	10.49
Pala	7.47	34.13	28.33	17.98
Mohangootee	8.67	34.37	29.67	14.50
PKM-4	8.20	35.43	25.00	11.86
Virudhanagar	7.53	36.73	26.67	12.62
Singapore	6.30	36.67	30.00	9.82
Cricket Ball (udupi)	6.80	38.80	28.33	12.92
S. Em±	0.822	0.816	1.410	1.827
C.D. @ 5%	2.342	2.324	4.015	5.201
CV (%)	16.636	3.858	9.021	19.798

Among all the cultivars cv. CO-1 took maximum days (39.13) for flower opening, which was on par with cvs. PKM-3 (39.10), Cricket Ball (Udupi) (38.80), Long oval (38.53) and Tagarampudi (37.87) and cv. Pala took significantly minimum days (34.13) for flower opening.

4.2.3 Days to fruit set

The data on days to fruit set was recorded in different cultivars are presented in Table - 3.

The observations revealed that the cv. Kalipatti and cv. Singapore have taken maximum days for fruit set (30.00 and 30.00 respectively), which was on par with cvs. Mohangootee (29.67), Cricket Ball (29.33), Cricket Ball (Udupi) (28.33) and DHS-1 (28.33) and significantly minimum days for fruit set was recorded in cv. Cricket Ball (ARSA) (24.00).

4.2.4 Per cent fruit set (%)

The data on per cent fruit set was recorded in different cultivars are presented in Table - 3.

There was significant differences with respect to per cent fruit set among the different cultivars of sapota.

Highest per cent fruit set was observed in cv. PKM-1 (29.50%), which was on par with cv. CO-2 (26.20%), whereas least was registered in cv. Singapore (9.82%).

4.3 Yield and quality parameters (plate 2a, 2b)

4.3.1 Colour of pulp

Perusal of the data given in Table- 6 indicated that the cvs. DHS-1, Long Oval, Oval and Gholwad Sapota have orange brown coloured fruits and cv. DHS-2 have light orange fruits and cvs. PKM-1, PKM-2, Cricket Ball, Cricket Ball (ARSA), Murabba, PKM-4, and Singapore have light brown coloured fruits and cvs. PKM-3, Guthi, Mohangootee have buff coloured fruits and cv. CO-1 has pinkish yellow fruits and cvs. CO-2, Kirtbharti, CO-3 and Virudhanagar have brown coloured fruits and cv. Gavarayya have yellowish brown fruits and cv. Pala have medium brown fruits.

4.3.2 Fruit diameter (cm)

There was significant difference among the cultivars with respect to fruit diameter of different cultivars (Table 4). Maximum diameter of fruit was noticed in cv. CO-2 (5.96 cm), which was on par with cvs. DHS-2 (5.68 cm), DHS-1 (5.46 cm), CO-1 (5.40 cm) and Gavarayya (5.33 cm) whereas least diameter was recorded in cv. Singapore (3.60 cm).

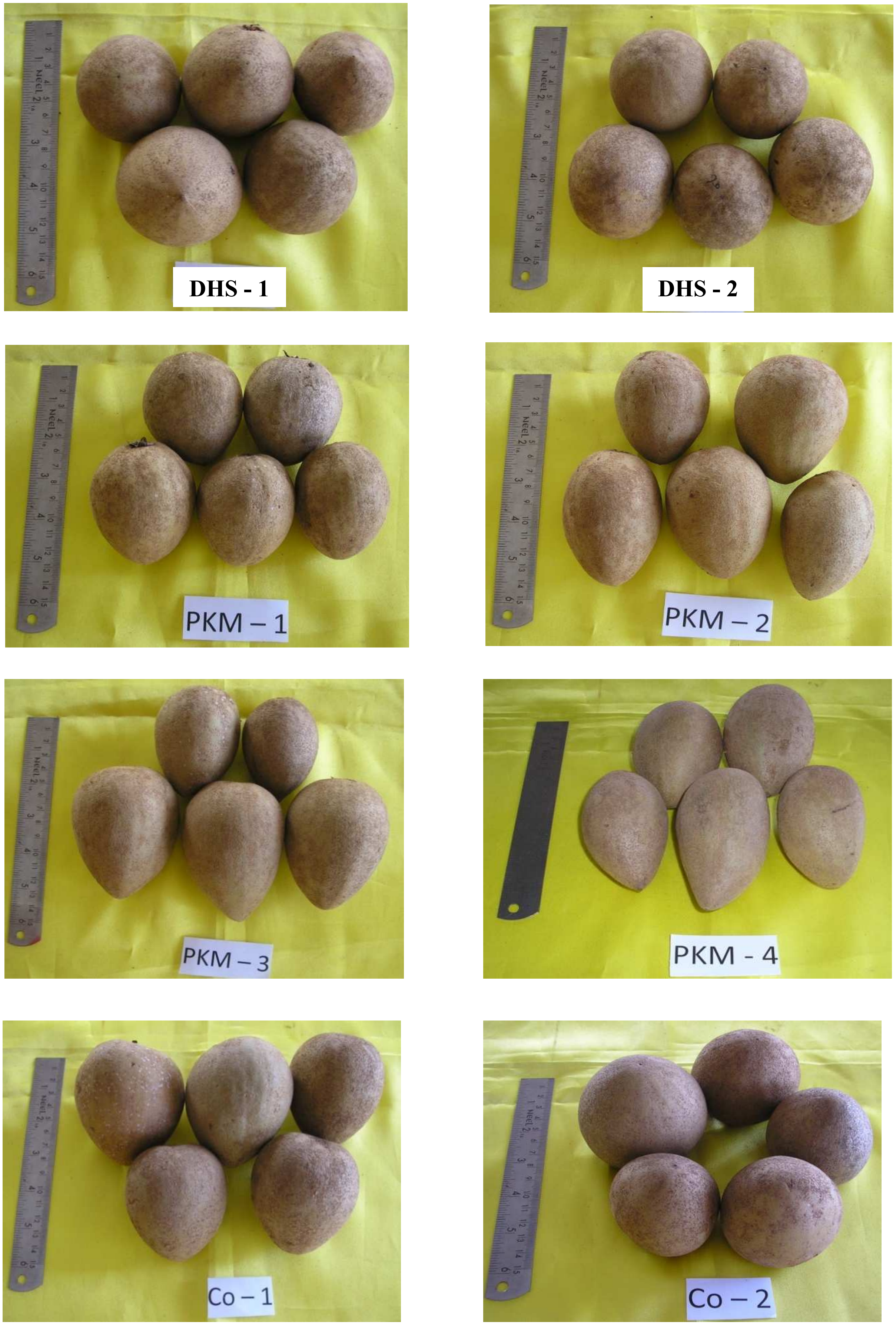

Plate 2a. View of sapota varieties at Arabhavi


Co – 3


Cricket Ball


Kalipatti


Long oval


Gavarayya


Kirthibarthi


Tagarampudi


Virudhanagar

4.3.3 Fruit length (cm)

There was significant difference among different cultivars (Table 4). Maximum fruit length was recorded in cv. CO-1 (6.64 cm) which was on par with cvs. PKM-3 (6.60 cm), PKM-1 (6.57 cm) and Oval (6.17 cm) whereas minimum fruit length was recorded in cv. Murabba (4.40 cm).

4.3.4 Fruit weight (g)

There was significant difference among the cultivars (Table 4). Significantly maximum fruit weight was recorded in cv. CO-1 (133.41 g) followed by cv. DHS-2 (122.00 g), cv. CO-2 (119.27 g), cv. DHS-1 (101.13 g) and cv. Gavarayya (99.15 g) whereas minimum fruit weight was noticed in cv. Virudhanagar (59.67 g).

4.3.5 Yield attributes

The data pertaining to number of fruits per tree and fruit yield (kg/tree) of different cultivars are presented in Table 4.

Significantly highest number of fruits per tree was recorded in cv. PKM-1 (2450) which was on par with cvs. Kalipatti (2400) and DHS-1 (2350), whereas less number of fruits per tree was recorded in cv. PKM-4 (180). Significantaly maximum yield was observed in cv. DHS-1 (169.2 kg/tree) followed by cv. Kalipatti (158.8 kg/tree) and DHS-2 (153.5 kg/tree), whereas minimum yield was observed in cv. CO-3 (13.5 kg/tree).

4.3.6 Total soluble solids (°B)

There was significant difference among the cultivars with respect to total soluble solids of different cultivars (Table 4).

The cv. DHS-1 recorded higher content of total soluble solids (23.90 °B), which was on par with cvs. DHS-2 (23.00 °B), Kalipatti (22.70 °B) , PKM-1 (22.67 °B), PKM-3 (22.33 °B), Oval (22.17 °B), Cricket Ball (ARSA (21.97 °B), Cricket Ball (21.97 °B), Long Oval (21.83) and CO-2 (21.83 °B) while lower content of TSS was noticed in cv. Guthi (19.00 °B).

Table 4. Yield and quality parameters of different cultivars of sapota

Cultivars	Colour of pulp	Fruit diameter (cm)	Fruit length (cm)	Fruit weight (g)	No. of fruits per tree	Yield (Kg/tree)	TSS (°B)	Acidity (%)	Number of seeds per fruit
DHS-1	Orange brown	5.46	5.74	101.13	2350	169.2	23.90	0.196	2.40
DHS-2	Light orange	5.68	5.47	122.00	1975	153.5	23.00	0.193	4.14
PKM-1	Light brown	3.93	6.57	63.33	2450	117.6	22.67	0.192	3.58
PKM-2	Light brown	4.43	7.57	86.73	1560	105.0	21.33	0.196	3.27
PKM-3	Buff colour	4.94	6.60	95.78	1610	112.7	22.33	0.194	3.50
CO-1	Pinkish yellow	5.40	6.64	133.41	1090	115.5	20.70	0.126	3.33
CO-2	Brown	5.96	4.87	119.27	2010	145.0	21.83	0.198	4.13
Cricket Ball	Light brown	5.06	5.15	75.33	1150	87.5	21.97	0.196	2.98
Long oval	Orange brown	4.73	5.95	72.80	859	60.5	21.83	0.198	1.74
Kalipatti	Light brown	4.60	5.00	72.67	2400	158.8	22.70	0.195	2.17
Guthi	Buff colour	4.87	5.67	66.18	1190	63.5	19.00	0.193	2.53
Gavarayya	Yellowish brown	5.33	5.92	99.15	1050	75.6	20.23	0.193	2.53
Oval	Orange brown	4.67	6.17	65.20	890	59.7	22.17	0.196	3.40
Kirthbharti	Brown	4.97	4.88	75.70	520	37.5	20.00	0.193	2.43
Cricket Ball (ARSA)	Light brown	4.10	4.71	71.53	628	44.7	21.97	0.196	2.50
Tagarampudi	Brown	4.98	5.18	66.80	475	34.5	21.00	0.193	4.07
Murabba	Light brown	3.77	4.40	62.67	325	21.0	21.67	0.196	3.50
CO-3	Brown	4.73	5.29	89.43	350	13.5	21.70	0.183	3.07
Pala	Medium brown	4.64	5.32	65.67	780	15.0	20.57	0.193	2.67
Mohangoote	Buff colour	4.49	4.89	69.67	475	24.5	21.20	0.196	2.53
PKM-4	Light brown	4.03	5.58	76.70	180	13.9	21.42	0.193	2.75
Virudhanagar	Brown	5.15	5.63	59.67	1250	87.0	21.57	0.196	1.90
Singapore	Medium brown	3.60	4.47	75.23	1200	84.0	20.32	0.196	2.07
Cricket Ball (Udupi)	Light brown	4.13	4.99	74.33	265	19.9	20.23	0.193	3.80
S. Em±		0.244	0.276	4.257	37.57	0.58	0.805	0.001	0.288
C. D. @ 5%		0.694	0.786	12.119	106.94	1.67	2.291	0.004	0.821
CV (%)		8.924	8.662	8.833	5.77	1.346	6.498	1.268	16.559

4.3.7 Acidity (%)

There was a significant difference for acidity among the different accessions of sapota (Table 4).

The lower content of acidity was recorded in cv. CO-1 (0.126%) followed by cvs. Co-3 (0.183%), PKM-1 (0.192%), DHS-2 (0.193%), Guthi (0.193%), Gavarayya (0.193%), Kirthbharti (0.193%), Tagarampudi (0.193%), Pala (0.193%), PKM-4 (0.193%) and Cricket Ball (Udupi) (0.193%) wherein highest content of acidity was noticed in cvs. CO-2 and Long Oval (0.198%).

4.3.8 Number of seeds per fruit

The cultivars differed significantly for number of seed per fruit (Table 4). Significantly more number of seeds per fruit was recorded in cv. DHS-2 (4.14), which was on par with cvs. CO-2 (4.13), Tagarampudi (4.07) and Cricket Ball (Udupi) (3.80) whereas cv. Long Oval records less number of seeds per fruit (1.74).

Experiment No. 2 Molecular characterization of varieties and hybrids of sapota using RAPD markers

5.1 DNA isolation protocol

5.1.1 Sample collection

The leaf samples of both hybrids and varieties were collected from All India Co-ordinated Research Project on Tropical Fruits, Kittur Rani Channamma College of Horticulture, Arabhavi (University of Horticultural Sciences, Bhaglkot). Recently matured leaves that were free from damage caused by pests or disease and available throught the year were collected from each individuals. Mature leaves were avoided since they are fibrous and contained high phenols and polysaccharides.

5.1.2 DNA isolation and purification

DNA isolation done by following modified CTAB method as described in material and methods chapter (3.1.1.2). It was carried out using different amounts of leaf samples (100 mg, 150 mg, 200 mg and 250 mg), extraction buffer and PVP at various concentrations (350 µl, 450 µl, 600 µl and 750 µl and 2 %, 3 %, 4% and 5 %, respectively). The good quality and high molecular weight DNA was obtained with 200 mg of leaf sample using 750 µl extraction buffer and 2 per cent PVP. This protocol was also resulted in dull transparent pellets, which easily dissolved in TE buffer.

5.1.3 DNA Quantification

The isolated DNA was analyzed quantitatively by using UV Spectrophotometer. DNA had a A_{260}/A_{280} ratio of 1.8 to 1.9 showing that it was relatively pure and free from impurities. The quality of DNA isolated was very good.

5.2 PCR amplification

5.2.1 Amplification conditions

For the diversity study, experimental conditions for PCR amplification were optimized based on the protocol outlined by Williams *et al.* (1990) and Welsh and McClelland (1990) with slight modifications.

PCR amplification conditions: Each cycle of PCR consisted of the three main steps.

1. Initial strand separation or hot-start at 94°C for 5 minute.
2. Denaturation at 94°C for 1 minute.

   Annealing at 38°C for 1 minute. } 38 cycles

   Extension at 72°C for 1.5 minute.
3. Final extension at 72°C for 10 minutes.

Different concentrations of template DNA (15 ng, 20 ng, 25 ng and 50 ng) and dNTPs (100 µM, 150 µM, 200 µM and 250 µM) were tired with similar amplification conditions. A concentration of 40 ng of template DNA and 100 µM of dNTPs was found optimum for obtaining intense, clear and repeatable banding patterns. In all the cases, 0.3 µM of primer and one unit of *Taq* DNA polymerase were used per sample. However, large changes in concentration (*i.e.* in order of magnitude) of template DNA did affect the amplification, with too little DNA causing either reduced or no amplification of small bands and too much DNA producing a smear effect. Therefore, accurate quantification of the DNA was essential for optimal amplification.

5.2.2 Primer selection

Nearly twenty primers were screened among these seven selected primers (OPA 04, OPB 03, OPB 06, OPB 08, OPC 05, OPC 02 and OPC 08) amplified unambiguous, readable and reproducible polymorphic bands. A total of 48 amplification products were produced from the selected 7 primers and the number of bands varied from 5-12 with an average of 7.5 bands per primer and the size ranged from 0.1 to 1 kb. The Bands which are more than 100 bp are selected for scoring. The details are presented in Table 5. Each and every individual could be identified using the gel profiles.

5.3 Analysis and estimating genetic distance

5.3.1 Estimation of genetic distance

In the analysis, all 48 polymorphic fragments were used to generate the similarity matrix and construct a dendrogram. The pair wise genetic similarity matrix was obtained based on Jaccard's Co-efficient and is presented in Table 8. In this matrix highest genetic similarity of 100 % was observed between the 'DHS-1' and 'DHS-2', while least (23 %) was between 'PKM-3' and 'Culcutta Round'.

Table 5. Synthetic deoxyribonuclotide 10-mer random primers used for genetic analysis of sapota

Primers	Nucleotide sequence (5' to 3')	Number of fragments amplified
OPA 04	AATCGGGCTC	04
OPB 03	CATCCCCCTG	05
OPB 06	TGCTCTGCCC	05
OPB 08	GTCCACACGG	07
OPC 02	GTGAGGCGTC	06
OPC 08	TGGACCGGTG	12
OPC 05	GATGACCGCC	09
Total		48

Source: MWG. Biotech AG

Legend: Lane 1 to Lane 31 is as follows (Fig. 1)

1- DHS-1
2- DHS-2
3- PKM- 1
4- PKM-2
5- PKM-3
6- CO-1
7- CO-2
8- Cricket Ball
9- Long Oval
10- Kalipatti
11- Guthi
12- Gavarayya
13- Oval
14- Kirthbharti
15- Cricket Ball (ARSA)
16- Tagarmpudi
17- Murabba
18- CO-3
19- Pala
20- Mohangootee
21- Varigated Sapota
22- PKM-4
23- French Indochina
24- Dwarapudi
25- Gholwad Sapota
26- PKM-5
27- Culcutta Round
28- Virudhanagar
29- Singapore
30- Cricket Ball (Udupi)
31- Cricket Ball (Sirsi)

NOTE: The above legend remains same for the remaining gel profiles (Fig. 2 to Fig. 7)

5.3.2 Cluster analysis

5.3.2.1 Variation among all the 31 genotypes

UPGMA (Unweighted Pair Group Method with Arithmetical averages) cluster analysis using Jaccard's Co-efficient of similarity of 31 genotypes showed medium to high diversity, which are distributed between the ranges of 35-100 per cent. Genotypes are categorized into two distinctive groups based on fruit characteristics (shape) and canopy shape. Where oval and round shaped fruit bearing genotypes are grouped in first cluster and oblong fruit bearing genotypes are grouped in second cluster. Among oval and round shape fruit bearing genotypes, the round shape fruit bearing genotypes has been grouped in separate sub clusters which includes, 'DHS-1', 'DHS-2', 'Cricket Ball', 'PKM-1', 'PKM-2', 'Gavarayya', 'Guthi', 'Oval', 'Murabba', 'CO-1', 'Kirthbharti', 'French Indochina', 'CO-2',

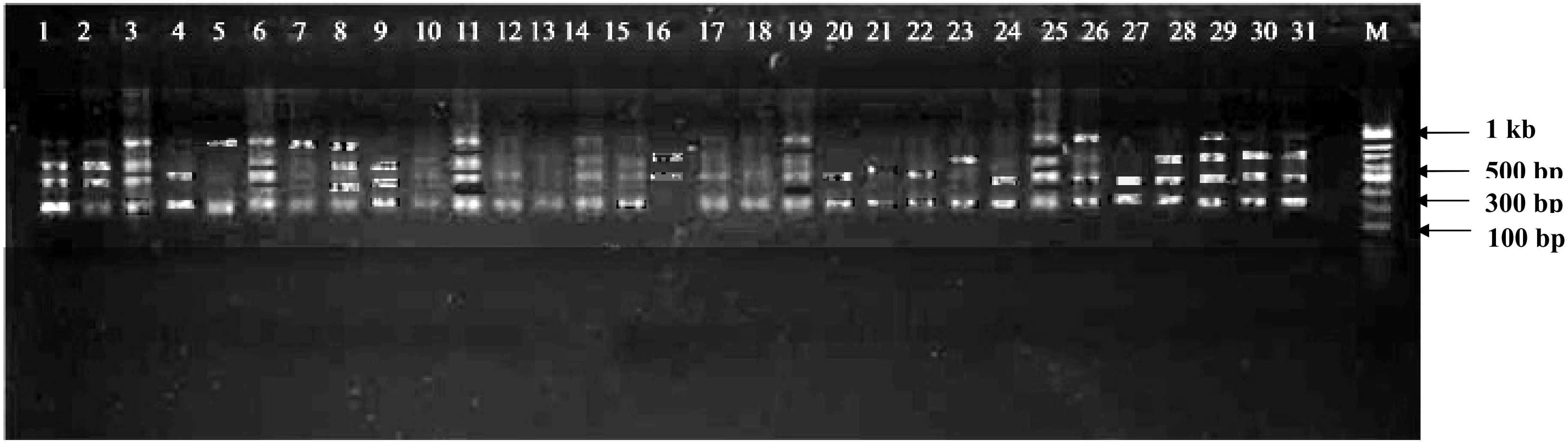

Fig. 1. RAPD gel profile of 31 sapota genotypes according to Operon Primer A - 04

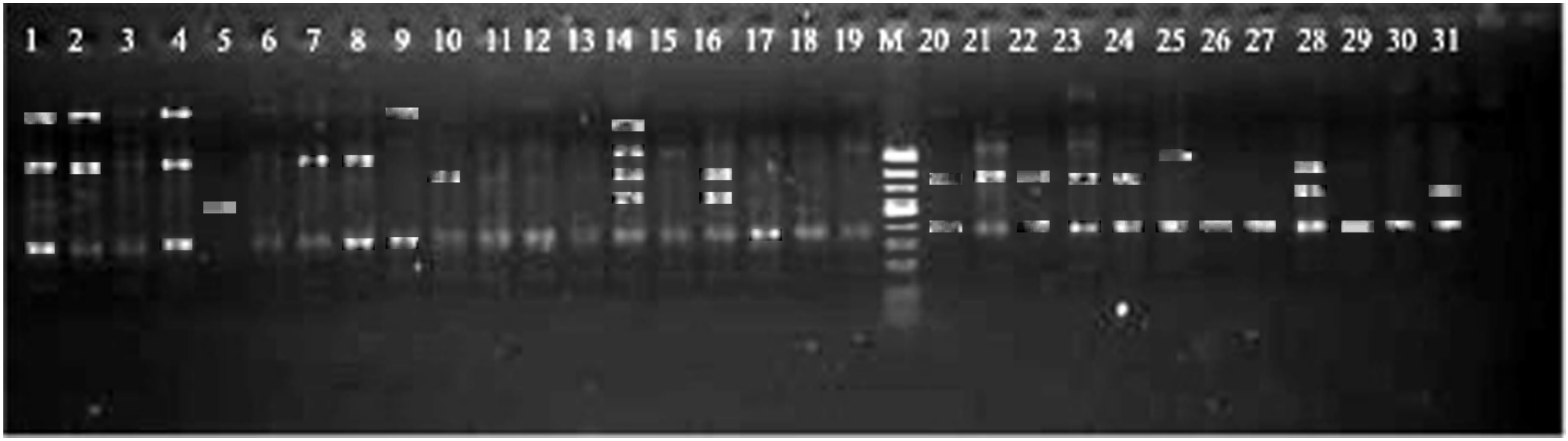

Fig. 2. RAPD gel profile of 31 sapota genotypes according to Operon Primer B - 03

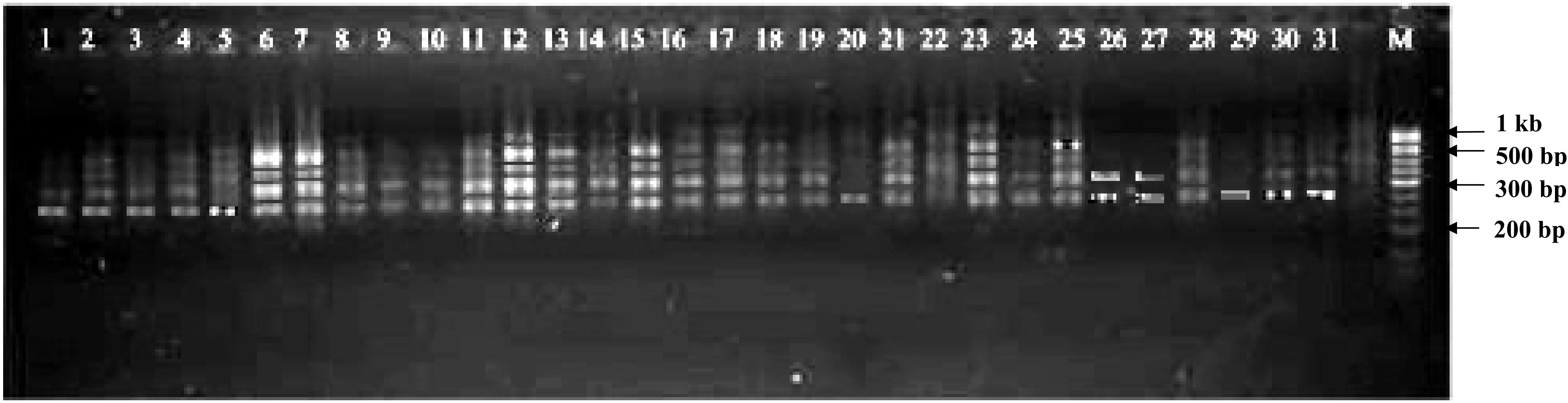

Fig. 3. RAPD gel profile of 31 sapota genotypes according to Operon Primer B - 06

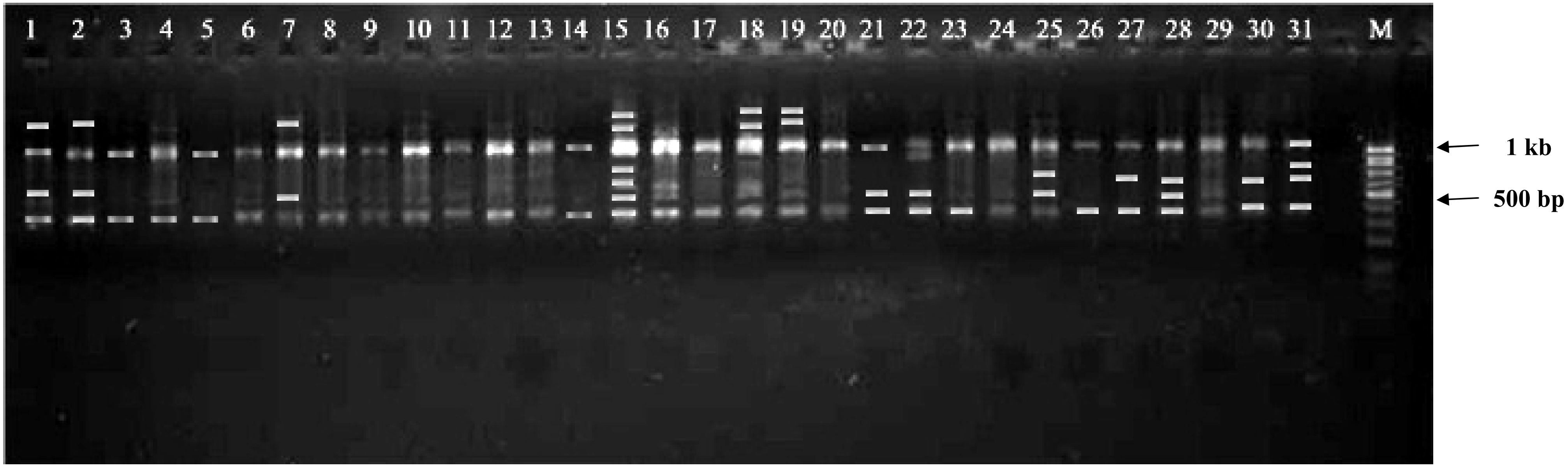

Fig. 4. RAPD gel profile of 31 sapota genotypes according to Operon Primer B - 08

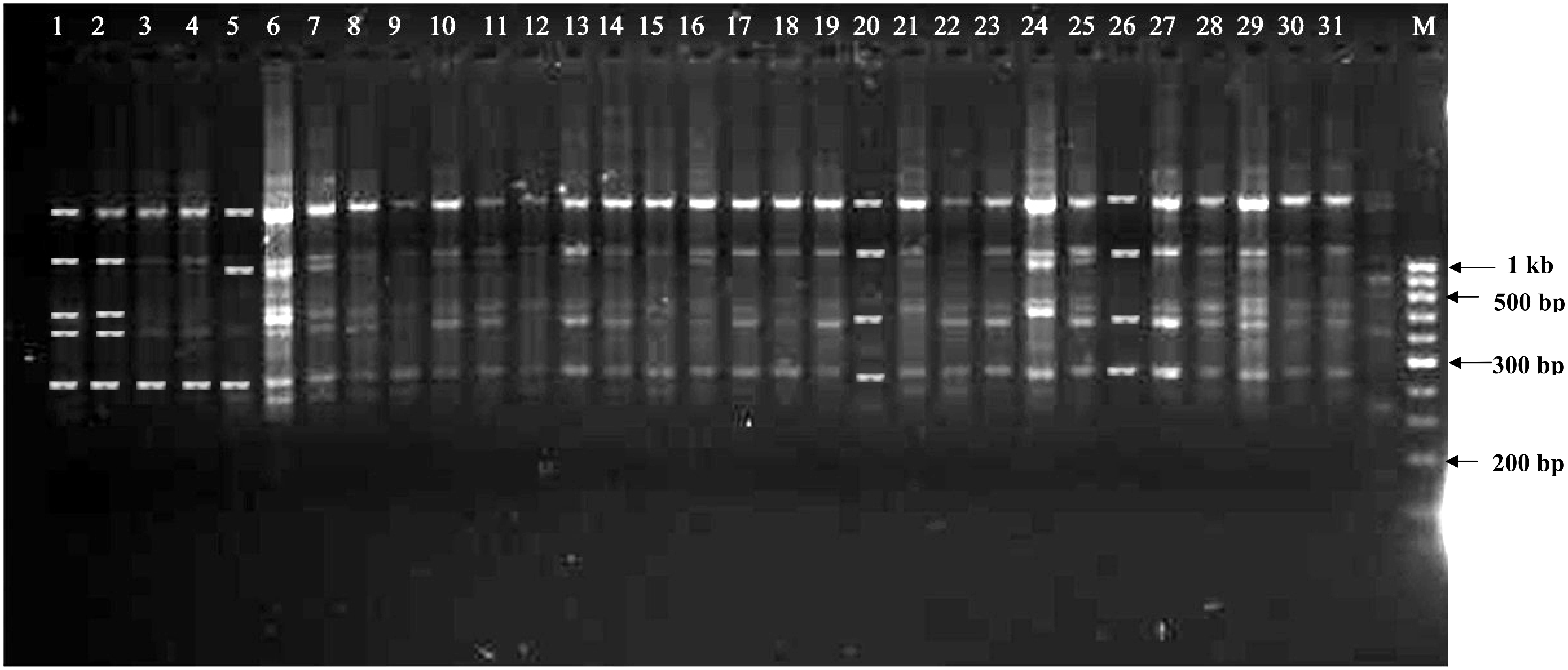

Fig. 5. RAPD gel profile of 31 sapota genotypes according to Operon Primer C - 02

Fig. 6. RAPD gel profile of 31 sapota genotypes according to Operon Primer C - 08

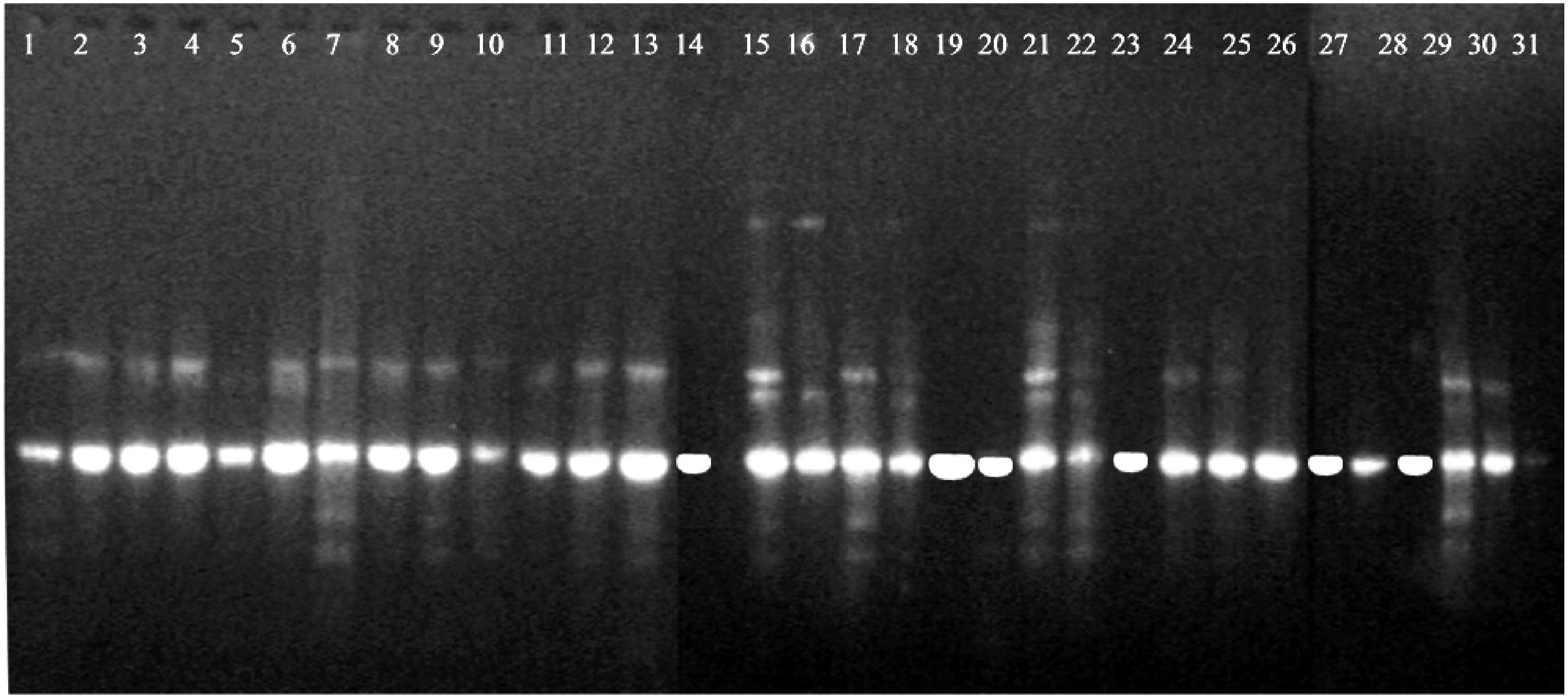

Fig. 7. RAPD gel profile of 31 sapota genotypes according to Operon Primer C - 05

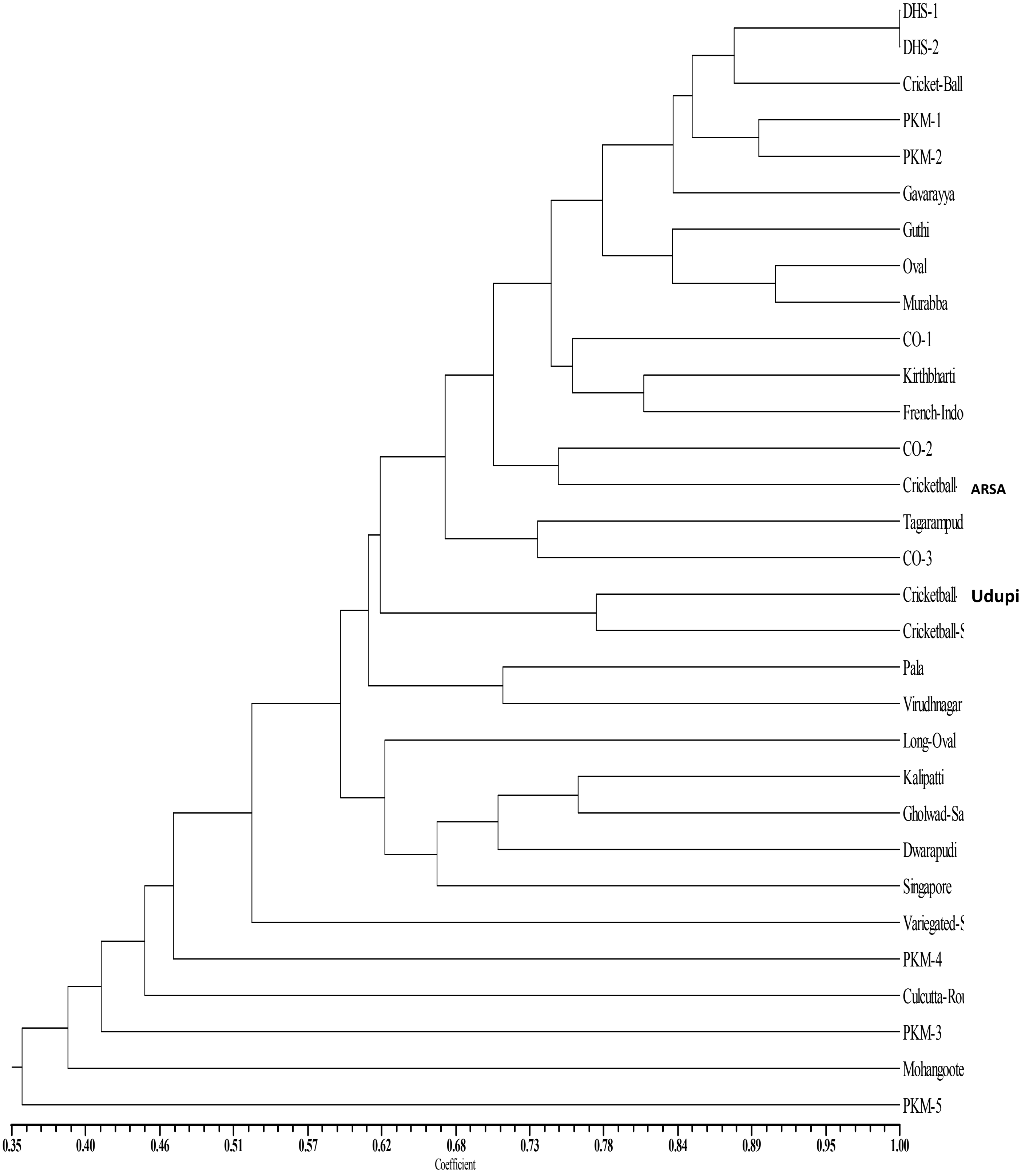

Fig.8. Dendrogram of different sapota genotypes (31) according to Jaccard's Co-efficie

Table 6. Jaccard's similarity Co-efficient of 31 genotypes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	1.00																														
2	1.00	1.00																													
3	0.87	0.87	1.00																												
4	0.84	0.84	0.90	1.00																											
5	0.48	0.48	0.50	0.48	1.00																										
6	0.74	0.74	0.73	0.67	0.41	1.00																									
7	0.67	0.67	0.65	0.63	0.36	0.73	1.00																								
8	0.88	0.88	0.87	0.79	0.48	0.84	0.76	1.00																							
9	0.56	0.56	0.58	0.56	0.37	0.51	0.56	0.61	1.00																						
10	0.58	0.58	0.56	0.58	0.38	0.61	0.75	0.67	0.76	1.00																					
11	0.82	0.82	0.81	0.73	0.47	0.78	0.70	0.88	0.59	0.66	1.00																				
12	0.84	0.84	0.83	0.81	0.43	0.71	0.68	0.84	0.61	0.63	0.78	1.00																			
13	0.79	0.79	0.78	0.76	0.50	0.72	0.73	0.79	0.68	0.74	0.84	0.76	1.00																		
14	0.82	0.82	0.75	0.68	0.47	0.74	0.66	0.82	0.46	0.57	0.76	0.73	0.74	1.00																	
15	0.76	0.76	0.70	0.73	0.42	0.66	0.75	0.76	0.61	0.71	0.71	0.73	0.74	0.63	1.00																
16	0.71	0.71	0.64	0.67	0.45	0.60	0.61	0.71	0.48	0.51	0.70	0.62	0.68	0.60	0.70	1.00															
17	0.78	0.78	0.76	0.69	0.45	0.79	0.76	0.83	0.62	0.72	0.82	0.74	0.91	0.77	0.73	0.62	1.00														
18	0.70	0.70	0.64	0.67	0.42	0.68	0.65	0.75	0.50	0.61	0.69	0.71	0.63	0.61	0.74	0.74	0.71	1.00													
19	0.63	0.63	0.56	0.59	0.32	0.62	0.54	0.63	0.50	0.57	0.62	0.64	0.56	0.57	0.59	0.56	0.59	0.71	1.00												
20	0.41	0.41	0.38	0.36	0.39	0.42	0.38	0.45	0.34	0.31	0.44	0.41	0.38	0.35	0.39	0.42	0.39	0.40	0.30	1.00											
21	0.53	0.53	0.46	0.45	0.32	0.60	0.64	0.53	0.49	0.51	0.51	0.49	0.62	0.55	0.53	0.54	0.66	0.59	0.40	0.34	1.00										
22	0.47	0.47	0.44	0.43	0.42	0.44	0.47	0.51	0.57	0.55	0.50	0.52	0.58	0.38	0.57	0.48	0.53	0.50	0.33	0.44	0.41	1.00									
23	0.71	0.71	0.70	0.68	0.47	0.78	0.66	0.82	0.55	0.66	0.76	0.68	0.74	0.81	0.67	0.65	0.72	0.61	0.62	0.44	0.48	0.46	1.00								
24	0.55	0.55	0.53	0.51	0.34	0.71	0.59	0.64	0.61	0.68	0.68	0.56	0.61	0.54	0.52	0.49	0.65	0.58	0.50	0.41	0.57	0.43	0.63	1.00							
25	0.68	0.68	0.66	0.64	0.44	0.74	0.67	0.77	0.61	0.76	0.76	0.74	0.69	0.67	0.68	0.57	0.78	0.75	0.63	0.41	0.49	0.56	0.71	0.74	1.00						
26	0.39	0.39	0.44	0.38	0.35	0.32	0.28	0.39	0.48	0.37	0.41	0.43	0.35	0.32	0.33	0.26	0.36	0.33	0.36	0.32	0.24	0.31	0.32	0.38	0.39	1.00					
27	0.41	0.41	0.38	0.36	0.23	0.46	0.50	0.50	0.44	0.53	0.48	0.41	0.42	0.44	0.39	0.52	0.43	0.44	0.43	0.36	0.34	0.39	0.53	0.55	0.50	0.26	1.00				
28	0.66	0.66	0.59	0.62	0.40	0.68	0.56	0.66	0.53	0.60	0.65	0.57	0.68	0.65	0.54	0.59	0.71	0.64	0.71	0.38	0.58	0.32	0.70	0.62	0.61	0.39	0.42	1.00			
29	0.48	0.48	0.45	0.48	0.24	0.55	0.63	0.51	0.51	0.71	0.54	0.51	0.61	0.46	0.56	0.38	0.64	0.50	0.46	0.33	0.49	0.47	0.50	0.64	0.63	0.30	0.50	0.53	1.00		
30	0.67	0.67	0.70	0.68	0.44	0.65	0.57	0.67	0.59	0.61	0.61	0.68	0.64	0.56	0.58	0.50	0.63	0.60	0.56	0.41	0.50	0.44	0.61	0.68	0.67	0.44	0.46	0.59	0.53	1.00	
31	0.67	0.67	0.70	0.63	0.44	0.65	0.53	0.67	0.44	0.47	0.66	0.63	0.59	0.61	0.58	0.50	0.63	0.56	0.52	0.46	0.42	0.35	0.61	0.58	0.62	0.44	0.41	0.55	0.45	0.78	1.00

1-DHS-1
2-DHS-2
3-PKM-1
4-PKM-2
5-PKM-3
6-CO-1
7-CO-2
8-Cricket-Ball
9-Long-Oval
10-Kalipatti
11-Guthi
12-Gavarayya
13-Oval
14-Kirthbharti
15-Cricketball-ARSA
16-Tagarampudi
17-Murabba
18-CO-3
19-Pala
20-Mohangootee
21-Variegated-Sapota
22-PKM-4
23-French-Indochina
24-Dwarapudi
25-Gholwad-Sapota
26-PKM-5
27-Culcutta Round
28-Virudhanagar
29-Singapore
30-Cricketball-Udupi
31-Cricketball-Sirsi

‘Cricket Ball (ARSA)’, ‘Tagarampudi’, ‘CO-3’, ‘Cricket Ball (Udupi)’ and ‘Cricket-Ball (Sirsi)’. In this round shaped fruit cluster ‘DHS-1’ and ‘DHS-2’ are closed together because of their same parent, again these two are closed with one of their parent ‘Cricket-ball’. ‘Oval’ and ‘Murabba’ are closed together and they are 91 per cent similar due to their canopy shape. Guthi and Oval are closely associated due to oval shaped fruits. ‘CO-1’ and ‘French-Indochina’ are closely associated with each other as they are having pinkish pulp. ‘Tagarampudi’ and ‘CO-3’ are closely grouped each other due to round shaped fruits and ‘Cricket Ball (Udupi)’, ‘Cricket Ball (Sirsi)’ are grouped together due to their round shaped fruits.

Sub cluster of oval shaped fruit bearing genotypes are ‘Pala’, ‘Virudhanagar’, ‘Long Oval’, ‘Kalipatti’, Gholwad Sapota’, ‘Dwarapudi’ and ‘Singapore’, they are all clustered together with same fruit shape (oval) except ‘Dwarapudi’, (round) but it is similar in canopy shape.

The oblong shape fruit bearing genotypes are narrowly associated with the round and oval shaped fruit bearing genotypes, which includes are ‘PKM-4’, ‘Culcutta Round’, PKM-3’and ‘Mohangootee’, all these genotypes are having oblong shaped fruit except ‘Culcutta Round’, whereas ‘PKM-5’ has been made distinctive cluster in the dendrogram because of their distinctive oblong shaped fruit which is closely associated with ‘PKM-4’, ‘PKM-3’ and ‘Mohangootee’ (Fig. 8).

5.3.2.2 Variation among cultivars

Jaccard’s Co-efficient of similarity analysis has been carried out for cultivars separately. In our investigations cultivars are categorized into two groups based on fruit characteristics and canopy shape. Among all cultivars round and oval shaped fruit bearing cultivars are grouped in first cluster which includes, ‘PKM-1’, Cricket Ball’, ‘Guthi’, ‘Gavarayya’, ‘Oval’, ‘Murabba’, ‘Kirthbharthi’, ‘CO-2’, ‘Kalipatti’, ‘Gholwad Sapota’ and ‘Tagarampudi’, ‘Pala’, ‘Virudhanagar’, ‘Long Oval’, ‘Dwarapudi’ and ‘Singapore’, within this ‘PKM-1’, Cricket Ball’, ‘Guthi’, ‘Gavarayya’, ‘Oval’, ‘Murabba’, ‘Kirthbharthi’ are grouped together, and ‘CO-2’, ‘Kalipatti’ and ‘Gholwad Sapota’, are grouped together and ‘Kalipatti’ and ‘Gholwad Sapota’ are closely associated with each other as they are having oval shaped fruits. ‘Oval’ and ‘Murabba’ are closely placed due to their canopy shape and they are 91 per cent similar. ‘PKM-1’ is closely associated with ‘Guthi’ because ‘PKM-1’ is selected from ‘Guthi’. In sub-cluster, the oval shaped fruit bearing genotypes are ‘Pala’, ‘Virudhanagar’, ‘Long Oval’, ‘Dwarapudi’ and ‘Singapore’

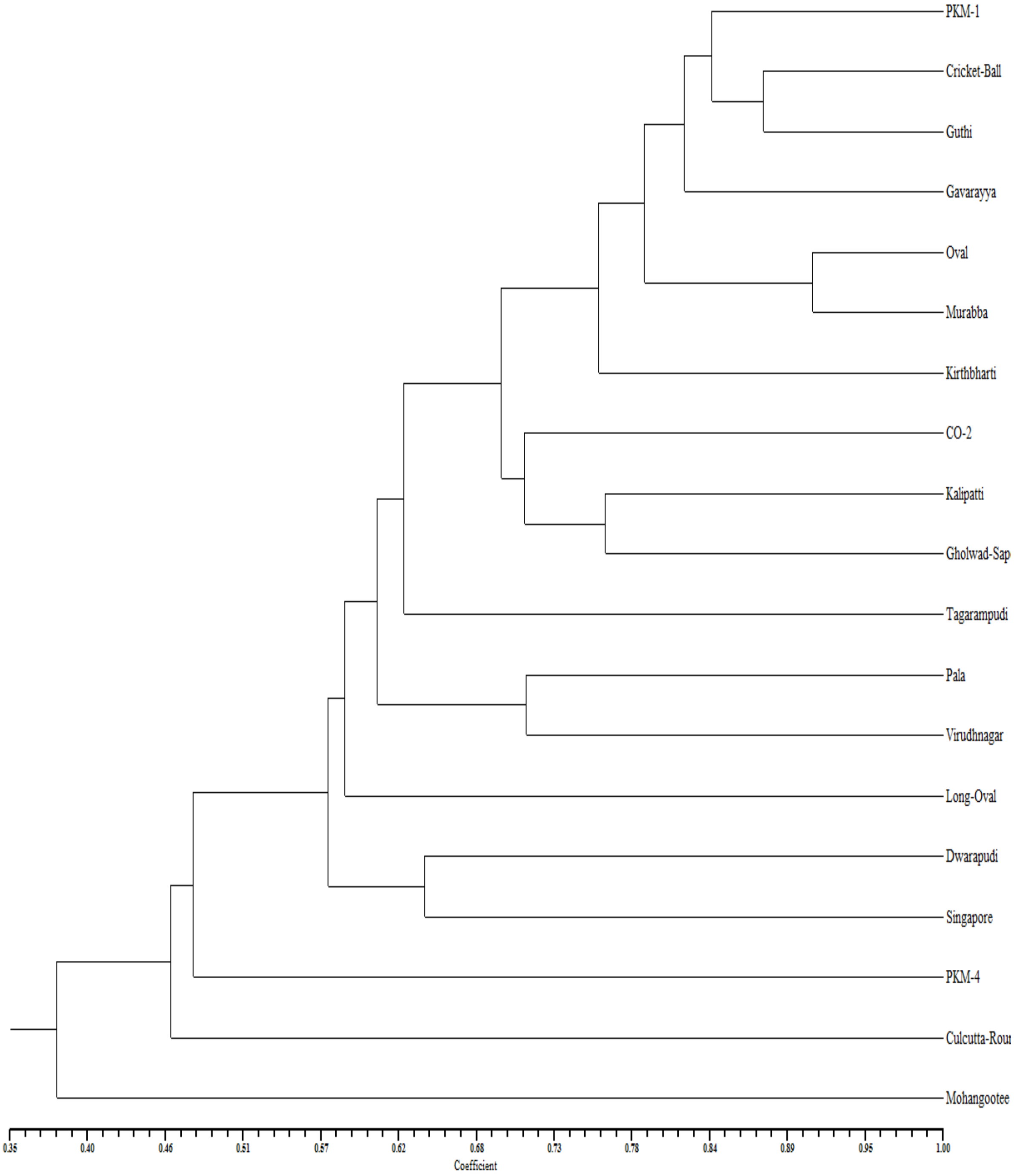

Fig.9. Dendrogram of different sapota cultivars (19) according to Jaccard's Co-efficient

Table 7. Jaccard's similarity Co-efficient of 19 sapota cultivars

	PKM-1	CO-2	CB	L-Oval	Kalipatti	Guthi	Gavarayya	Oval	Kirthbharti	Tagarampudi	Murabba	Pala	Mohangootee	PKM-4	Dwarapudi	G-Sapota	CR	Virudhnagar	Singapore
PKM-1	1.00																		
CO-2	0.65	1.00																	
CB	0.87	0.76	1.00																
L-Oval	0.58	0.56	0.61	1.00															
Kalipatti	0.56	0.75	0.67	0.76	1.00														
Guthi	0.81	0.70	0.88	0.59	0.66	1.00													
Gavarayya	0.83	0.68	0.84	0.61	0.63	0.78	1.00												
Oval	0.78	0.73	0.79	0.68	0.74	0.84	0.76	1.00											
Kirthbharti	0.75	0.66	0.82	0.46	0.57	0.76	0.73	0.74	1.00										
Tagarampudi	0.64	0.61	0.71	0.48	0.51	0.70	0.62	0.68	0.60	1.00									
Murabba	0.76	0.76	0.83	0.62	0.72	0.82	0.74	0.91	0.77	0.62	1.00								
Pala	0.56	0.54	0.63	0.50	0.57	0.62	0.64	0.56	0.57	0.56	0.59	1.00							
Mohangootee	0.38	0.38	0.45	0.34	0.31	0.44	0.41	0.38	0.35	0.42	0.39	0.30	1.00						
PKM-4	0.44	0.47	0.51	0.57	0.55	0.50	0.52	0.58	0.38	0.48	0.53	0.33	0.44	1.00					
Dwarapudi	0.53	0.59	0.64	0.61	0.68	0.68	0.56	0.61	0.54	0.49	0.65	0.50	0.41	0.43	1.00				
G-Sapota	0.66	0.67	0.77	0.61	0.76	0.76	0.74	0.69	0.67	0.57	0.78	0.63	0.41	0.56	0.74	1.00			
CR	0.38	0.50	0.50	0.44	0.53	0.48	0.41	0.42	0.44	0.52	0.43	0.43	0.36	0.39	0.55	0.50	1.00		
Virudhanagar	0.59	0.56	0.66	0.53	0.60	0.65	0.57	0.68	0.65	0.59	0.71	0.71	0.38	0.32	0.62	0.61	0.42	1.00	
Singapore	0.45	0.63	0.51	0.51	0.71	0.54	0.51	0.61	0.46	0.38	0.64	0.46	0.33	0.47	0.64	0.63	0.50	0.53	1.00

CB- Cricket Ball, L- Long Oval, G- Gholwad, CR- Culcutta Rounr these are all grouped together as they are having oval shaped fruit bearing genotypes, except 'Dwarapudi' but it is similar in canopy shape. 'Pala' and 'Virudhnagar' are closely placed due to 71 per cent oval shaped fruits.

In second cluster includes, 'PKM-5', 'Culcutta Round' and 'Mohangootee' as they are having oblong shaped fruits, except 'Culcutta Round'. These oblong shaped fruit bearing genotypes are narrowly distributed to round and oval shaped fruit bearing genotypes (Fig. 9).

5.3.2.3 Variation among landraces

Jaccard's Co-efficient of similarity analysis has been carried out for landraces also; land races are also distributed based on fruit characteristics. Among landraces the round shaped fruit bearing landraces are grouped in cluster-I, which includes 'Cricket Ball (ARSA)', 'French Indochina', 'Cricket Ball (Udupi)', 'Cricket Ball (Sirsi)' and 'Variegated Sapota', within this cluster 'Cricket Ball (Udupi)', 'Cricket Ball (Sirsi)' are closely associated with each other as they are having distinctively round shaped fruits and they are 78 per cent similar. Cricket Ball (ARSA)', 'French Indochina', 'Cricket Ball (Udupi)', 'Cricket Ball (Sirsi)' are closely associated with 'Variegated Sapota' as it is having round shaped fruits. 'PKM-5' placed separately in cluster-II, as it is having oblong shaped fruits (Fig. 10).

5.3.2.4 Variation among hybrids

Separate Jaccard's Co-efficient of similarity has been analyzed for development of dendrogram of hybrids. Hybrids are distributed based on fruit shape, growth habit, Parentage and bearing habit, which made two distinctive groups. Group-I contains 'DHS-1', 'DHS-2', 'PKM-2', CO-1' and 'CO-3' which are grouped together because of their oval and round shaped fruits with spreading and single bearing habit. Within this group 'DHS-1' and 'DHS-2' are 100 per cent similar as they are having same parentage ('Kalipatti' X 'Cricket Ball'). 'PKM-2' is closely related with 'DHS-1' and 'DHS-2' as it is having oval shape fruits as that of 'DHS-1'. 'CO-1' and 'CO-2' are closely linked with 'DHS-2' due to round shaped fruits. 'PKM-3' has been placed in separate cluster (group-II), which have oblong shaped fruits, vertical growth and cluster bearing (Fig. 11).

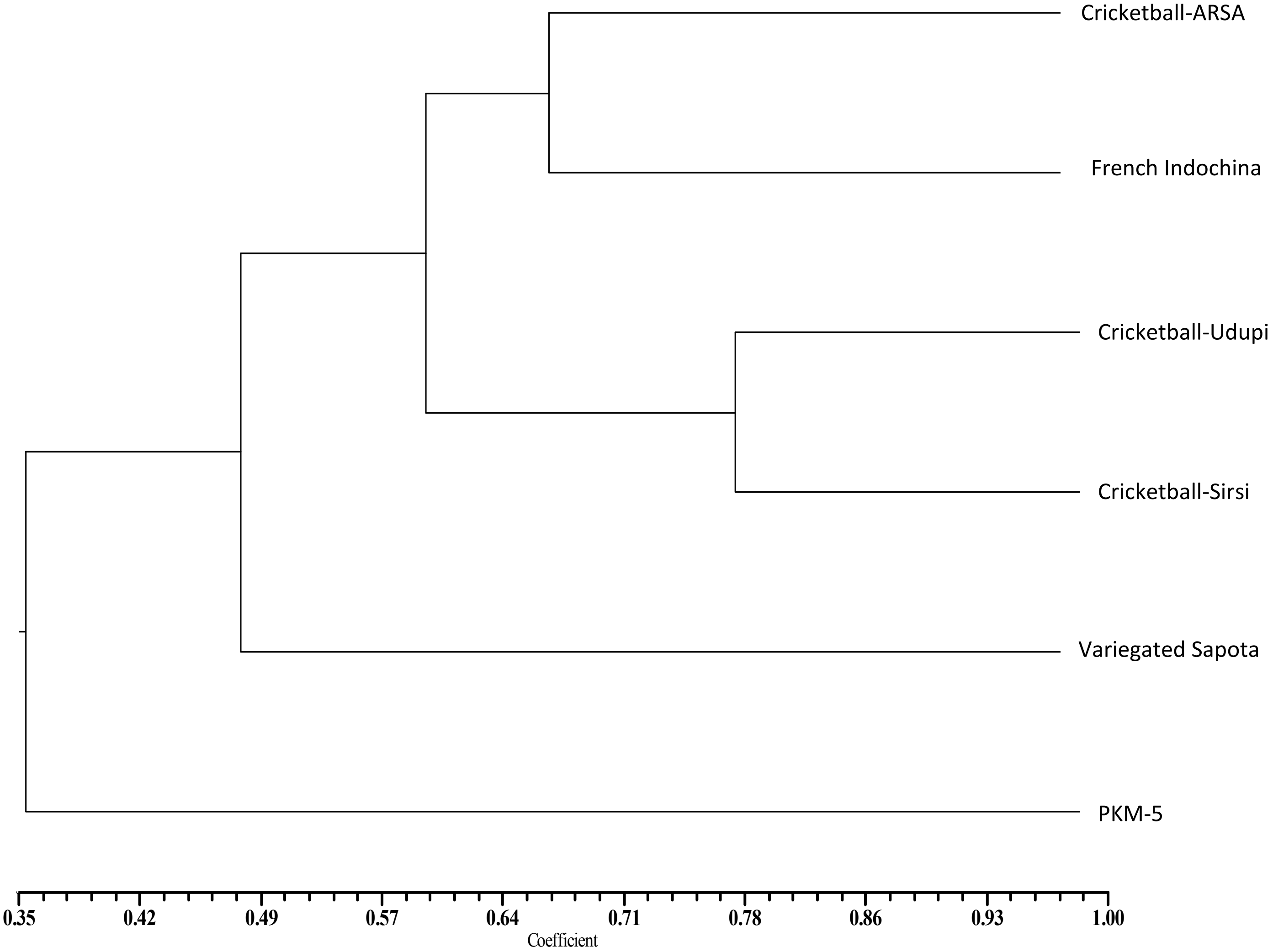

Fig.10. Dendrogram of different sapota landraces (6) according to Jaccard’s Co-efficient

Table 8. Jaccard's similarity Co-efficient of 6 landraces

	CB-ARSA	F-Indochina	CB-Udupi	CB-Sirsi	V-Sapota	PKM-5
CB-ARSA	1.00					
F-Indochina	0.67	1.00				
CB-Udupi	0.58	0.61	1.00			
CB-Sirsi	0.58	0.61	0.78	1.00		
V-Sapota	0.53	0.48	0.50	0.42	1.00	
PKM-5	0.33	0.32	0.44	0.44	0.24	1.00

CB- Cricket Ball, F- French, V- Variegated

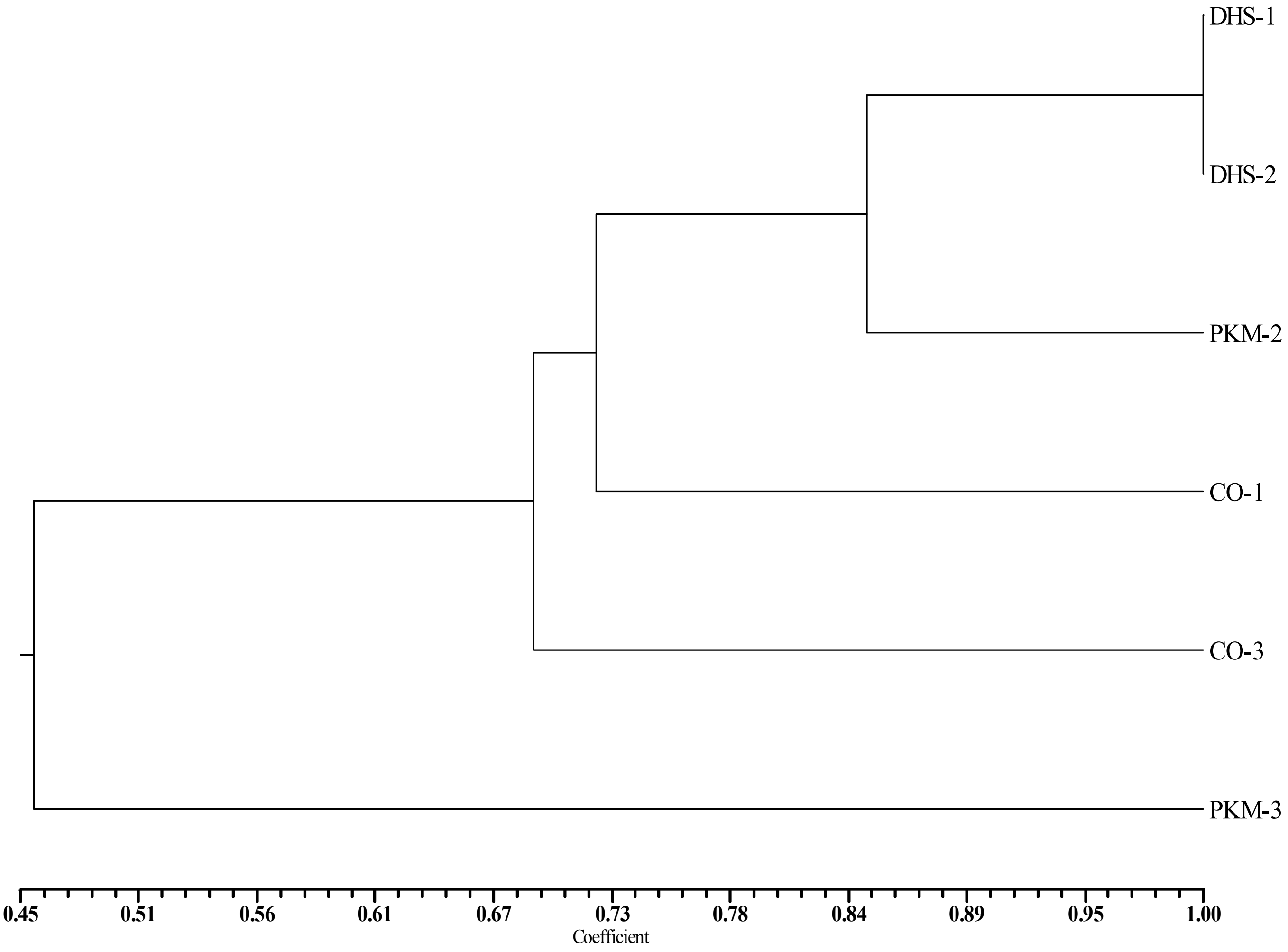

Fig.11. Dendrogram of different sapota hybrids (6) according to Jaccard's Co-efficient

Table 9. Jaccard's similarity Co-efficient of 6 hybrids

	DHS-1	DHS-2	PKM-2	PKM-3	CO-1	CO-3
DHS-1	1.00					
DHS-2	1.00	1.00				
PKM-2	0.84	0.84	1.00			
PKM-3	0.48	0.48	0.48	1.00		
CO-1	0.74	0.74	0.67	0.41	1.00	
CO-3	0.70	0.70	0.67	0.42	0.68	1.00

DISCUSSION

5. DISCUSSION

Sapota, an important fruit of tropical area, is commonly known as chikku and sapota in India. It is originated in tropical America (Mexico). It was taken to Philippines by Spanish explores and from there, it was spread to other countries. In India, sapota cultivation was taken up for the first time in Gholwad village of Thane district of Maharashtra in 1898. Thereafter, it was spread to other states and now it occupies a significant position among the fruit crops. In India, it is mainly grown in Gujarat, Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh states, wherein Karnataka stands first in area and productivity (Chadha, 1992).

It is becoming a commercial fruit crop in many parts of India due to its hardy nature and wider adoptability to diverse agro climatic condition. Trees are medium sized, erect to spreading evergreen plant with milky latex, growing to a height of 20 m. Growth parameters such as plant height, stem circumference, plant spread, shoot length, shoot girth and number of leaves were observed to have genetic association with fruit characters and these associations would be valuable in predicting the quality parameters (Shikhamany *et al.,* 1978). Such information especially on bearing stage of the tree could be more useful in forecasting its future yielding ability well in advance.

Sapota bears flower almost throughout the year with three to four peak flowering flushes. The time taken for flower bud to open is very slow, it takes 32 to 40 days under South-Indian conditions (Singh *et al.,* 1991) and 39 days in North-Indian conditions (Ludhiana).

The studies on the genetic polymorphisms and phenotypic relationships can provide a scientific basis for the utilization of these phenotypes for the efficient crop improvement. Hence, characterization of varieties at phenotypic levels based on morphological characters supplemented with molecular characterization at genetic level is first step towards efficient utilization, conservation and maintenance of the existing genetic diversity. Considering these facts, an investigation on "Morphological and molecular characterization of sapota using RAPD markers" was carried out Kittur Rani Channamma College of Horticulture, Arabhavi during 2009-2011. The results of the present studies are discussed in this chapter.

5.1 Vegetative growth

It is clear from results of the present study (Tables 1and 2) that maximum plant growth, stem circumference, tree spread, shoot length, shoot girth and number of leaves are recorded in cvs. CO-1, DHS-1, DHS-2, Cricket Ball, DHS-1 and DHS-1 respectively in 15-16 years old trees. Among the 10-11 years old trees maximum plant height, stem circumference, tree spread, shoot length and number of leaves was recorded in cv. Cricket Ball (ARSA) and maximum shoot girth was observed in cv. Singapore. This might be probably due to differences among cultivars for genetic constitution (Anon., 1998).

5.2 Reproductive parameters

The observation on reproductive parameters indicated that maximum number of flowers per shoot was observed in cv. CO-1 (15.33). Maximum days to flower opening was observed in cv. CO-1 (39.13 days) and maximum days to fruit set was recorded in cv. Kalipatti and Singapore (30 days). This may be attributed to different genetic constitution of cultivars (Anon., 1998). Among all the cultivars studied, cv. PKM-1 recorded higher percentage of fruit set (29.50%) followed by cv. PKM-2 (24.15%) and it was least in cv. Singapore (9.82%). The variation in fruit set among the cultivars could be mainly due to their genotypic differences. Madhavarao and Khader (1961) also observed variation in fruit set (0 to 11 per cent) among seven varieties under different seasons. Present investigation is in confirmormaty with the earlier reports (Patil and Narwadkar, 1974; Mone, 1989 and Mulla and Desle, 1990).

5.3 Quality parameters

The observations on quality parameters such as fruit length, fruit weight, TSS, acidity, colour of pulp and number of seeds per fruit indicated that sapota fruit follows a characteristic double sigmoid pattern of growth. These findings are in accordance with the reports of Sundararajan and Madhavarao (1967), Sulladamath (1975), Mahadevaiah (1981), Mone (1989) and Gunaki (1999). The present investigation also revealed that maximum fruit diameter and maximum fruit length was observed in cv. CO-2 (5.96 cm) and CO-1 (6.64 cm) respectively. These observations are in close conformity with the findings of Rhokade (1989), Shirol (2005) and Siddaramayya (2005).

Among all cultivars, average fruit weight was higher in cv. CO-1 (133.41 g) followed by cv. DHS-2 and DHS-1 and least was noticed in cv.Virudhanagar. These results are in line with the observations reported by Mone (1989). The varietal variation in the quality parameters might be due to differences in their genotypic characteristics (Anon., 1998).

It is evident from the present study that the total soluble solids content ranged from 19.00°B to 23.90 °B. The difference of variation in the TSS content among the cultivars probably due to differences in their varietal character and prevailing climatic condition of the area. Similar observations were also reported by Mone (1989) and Rokhade *et al.* (1989) who reported that the different varieties of sapota exhibited varying level of TSS.

There was significant difference among the cultivars with respect to acidity of fruits which ranged between 0.126 to 0.198 per cent. These findings are in agreement with the results of Chundwat and Bhuva (1982) and Avaiya and Singh (1991). This might be due to characteristic feature of the genotype.

The results presented in Table - 4 revealed that the cv. DHS-2 and CO-2 recorded more number of seeds. It is, therefore, evident from the results that the number of seeds in a fruit determines the fruit shape. These reports are in close agreement with the findings of Sulladamath (1975) and Mone (1989) which showed that the direct relationship exists between the number of seeds and fruit shape.

Estimation of diversity among existing populations and authenticated identification of cultivars are necessary both for breeders to ensure protection of intellectual property rights and for end users like farmers, processors and consumers. The traditional method of identifying cultivars by morphological characters is now gradually being replaced by more reliable protein or DNA profiling because of several limitations. In recent years DNA based fingerprinting techniques are being favoured because protein profiles are often influenced by environmental factors and tissue type (Murphy *et al.,* 1990). DNA based analysis of cultivars exploits the inherent polymorphism in the base sequences of genomic DNA between two individuals. RAPD are the most favoured method largely because of data can be generated with relative ease and faster than RFLP and microsatellites (Williams *et al.,* 1990) and this technique can be practiced in any molecular biology laboratory. However, use of RAPDs to asses genetic diversity and cultivar identification has been criticised for revealing unreliable phylogenics because of lack of reproducibility. These shortcomings can be minimized by accurate standardization of the amplification protocol and using the same amplification conditions and avoiding contamination (Warburton and Bliss, 1996).

Hence, an attempt has been made to molecular characterization some of the commercial varieties, landraces and hybrids of sapota and to study the genetic diversity and relationships among them by using RAPD markers, which is very valuable for further crop improvement. The results of the investigation on "Molecular characterisation of sapota using RAPD markers" are discussed here under.

5.4 DNA isolation

5.4.1 Sample collection

Sapota is an evergreen perennial fruit crop. Although DNA can be isolated from leaves at any stage**,** DNA from matured material has been shown to be difficult to isolate and unstable for long term storage (Lodhi *et al.,* 1994). Porebski *et al.,* (1997) and Lefort and Douglas (1999) opined that with maturity, leaves accumulate increased quantities of polyphenols, polysaccharides, tannins and phenols, which pose a major problem in extraction because they are difficult to separate from DNA. Hattori *et al.* (1987) reported that extraction of highly purified genomic DNA from plant tissues is a difficult task partly due to their rigid-cell wall composed of large amounts of complex carbohydrates. Contamination by polysaccharides has been reported as the most common problem affecting plant DNA purity (Demeke and Adams, 1992; Murray and Thompson, 1980). Rogers *et al.* (1996) found that it was very difficult to isolate PCR amplifiable DNA from non-leaf tissues has they pose particular problems. It is, therefore, essential to isolate contamination free DNA using right plant material and with a suitable protocol. In the present study sampling was done with most recently matured leaves, which are having comparatively low carbohydrates and other secondary metabolites. Similarly, recently matured leaves have been used to isolate good quality of DNA from cashew (Murali, 1999), mango (Hemanth, 1999) and jasmine (Mukandan, 2000).

5.4.2 Sample preparation

No doubt, use of liquid nitrogen (Franklin and Arkesh, 1991; Porebski *et al.,* 1997) yields good quantities of high quality DNA but the use of liquid nitrogen presented some problems. Once exposed to liquid nitrogen, it is imperative that the tissue not be allowed to thaw before extraction. If large populations of plants were to be extracted the tissue would have to be stored frozen or processed and then stored in a freezer (Jennifer and Paul, 1990). Storage of tissues in freezers may lead to problems involving lack of space. In addition, plant material, which is only available from distant sources, must be shipped frozen at high cost. To circumvent these problems with liquid nitrogen, it has become standard practice to lyophilize plant tissue before extraction. In this freeze dried state, it is not necessary to keep the tissue in a freezer. The tissue lends itself to mechanical processing and shipping of such material would be relatively easy and inexpensive. Unfortunately, lyophilizers are quite expensive and have only a limited capacity. Moreover, lyophilisation requires several days to complete and errors in operation may result in degradation of DNA.

The present protocol developed by utilizing recently matured leaves and grinding them for 5-10 minutes by using 2 per cent PVP and 2 per cent CTAB in the extraction buffer (750 µl) consistently gave adequate amounts of high quality DNA suitable for RAPD analysis.

5.4.3 Extraction protocol

In the present study the modified CTAB method (Porebski *et al.,* 1997) was used with some modifications. The modified CTAB extraction procedure in sapota includes PVP to remove polyphenols (Maliyakal, 1992). An extended RNA'ase treatment was fallowed for the DNA product to be RNA free and PCR amplifiable. The DNA obtained from the protocol was found suitable for PCR amplification. Similar protocol was followed for extraction of high quality DNA in mango (Hemanth, 2000; Schnell *et al.,* 1995), cashew (Murali, 1999) and grapes (Wang *et al.,* 1999).

The present protocol developed by utilizing recently matured leaves and grinding them for 5-10 minutes by using two per cent PVP and two per cent CTAB in the extraction buffer consistently gave adequate amounts of high quality DNA suitable for RAPD analysis. DNA was cleaned further with RNAase treatment and did not produce any visible ground smears. Similar results were obtained in *Heliconia* (Kumar *et al.,* 1998) and *Abelmoschs* (Tai and Tankelsy, 1990). The variation in DNA yield is perhaps partly due to the subtle differences in the leaf maturity and leaf constituents, despite using young leaves.

DNA quantification using spectrophotometer revealed that the 260/280 ratios were in the range of 1.8 - 1.9 indicating that the DNA obtained from this protocol was free of contaminants. Further, DNA was amplified by using *Taq* polymerase, which also is an indicate that DNA obtained was of good quality.

5.5 Amplification conditions

Since even a single molecule of DNA can be amplified by PCR it is important to prevent contamination. To avoid contamination it is necessary to use sterile materials (pipettes, eppendorf tubes, tips and reagent bottles), autoclaved reagents and clean work places for both DNA isolation and PCR reaction. The two factors that mainly affect the stringency of PCR amplification are the magnesium chloride concentration and the annealing temperature. It was found that magnesium concentration of 1.5 mM was optimum, since lower concentrations resulted in less intense bands and higher concentrations resulted in either a smear or no amplification. An annealing temperature of 38°C was selected, since at this temperature reliable amplification of DNA was obtained utilising Operon random primers of 10 base pairs length. Annealing time for a minute was effective for good banding pattern than longer time (2 minutes). The annealing temperature for longer periods decreased the number of bands and increased band size. A concentration of 1mM dNTPs was found optimum. The higher concentrations of dNTPs resulted in smears and increased mispriming and misincorporation of nucleotides and low concentrations gave faint bands or no fragments.

Very low concentration of dNTPs below 100 μM adversely affects the processivity of *Taq* polymerase (Asim and Meena, 1994). Faints bands were observed at lesser dNTPs concentrations, whereas, at higher concentrations of the same resulted in larger unseperated bands with smear background. However, the accurate reproduction of all selected parameters in the present studies assures a satisfactory reproducibility of all results.

5.6 Genetic diversity

The present analysis on genetic diversity/genetic relatedness among sapota varieties, landraces and hybrids were based on 48 bands. Sapota belongs to family Sapotaceae with chromosome number 2n = 26. This is a first attempt of molecular characterization in sapota. Therefore, there is no reliable information on genetic background, genome size and on other related genera of this crop. *Argania spinosa* (L.) Skeel is an endemic species from Southwest Morocco being the unique representative of the tropical Sapotaceae in this area. A karyotype constituted for ten pairs of chromosomes ($2n = 2x = 20$) and the putative karyotype proposed has been of four submetacentric and six metacentric pairs. The four submetacentric pairs were the longest with a mean total length between 1.14 and 1.69 µm and the total length of six metacentric pairs were between 0.59 and 1.03 µm (Majourhat *et al*., 2007).

Perusal of the dendrogram of different cultivars, landraces and hybrids clearly indicated moderate to high genetic diversity. Sapota is a native of Mexico and Central America and it is not known when it was first introduced into India (Singh *et al.,* 1963). Since it is open-pollinated crop, a great deal of variability was thrown up in the population. In any introduced crop such as sapota, genetic variation depends on the number of introduced cultivars or genotypes. Generally it is expected to be very narrow, due to small introductions. Since sapota is an introduced crop to India and no information is available on the number of cultivars introduced or the origin of these cultivars, it was expected that the variability in genotypes would be less. However, results of the present study Revealed moderate to higher genetic diversity due to open pollination habit of the crop. Genotypes/landraces/hybrids are grouped based fruit shape/canopy shape/parentage/location. As it is evident from the dendrogram that there were no clear closely formed groups.

The dendrogram with UPGMA (Unweighted Pair Group Method with Arithmetical averages) drawn based on 48 markers on 31 sapota cultivars, landraces and hybrids clearly indicated that the genetic diversity is moderate to high. The measure of similarity namely Jaccard's Co-efficient showed different levels of diversity. The similarity for 31 sapota genotypes ranged from 35 to 100 per cent. The similarity of cultivars ranged from 36 to 91 per cent, for landraces it was 24 to 67 per cent and it was 41 to 100 per cent in hybrids.

Sapota is a introduced crop to India and hence is believed to have narrow genetic base/ diversity. But our analysis clearly suggests that the diversity is not very narrow instead it is moderate to high, because of open pollination habit of the crop

The cluster analysis based on UPGMA using Jaccard's Co-efficient with seven random primers (48 markers) allowed discriminating individual cultivars, landraces and hybrids effectively. The distinctive discriminative profiles from each cluster are very useful in identifying cultivars, landraces and hybrids. This information is also very useful to document each variety and for Plant Varietal Rights purposes. Similar discriminative ability of RAPD markers in identifying cultivars and hybrids has been demonstrated in many other crops like avocado (Lewis, 1992), avocado (Torres *et al.,* 1980), chrysanthemum (Wolff and Peters-Van Rijin, 1993), rhododendron (Rayburn *et al.,* 1993), apple (Koller *et al.,* 1993), raspberry (Parent *et al.,* 1993), buffolograss (Wu and Lin, 1994) and mango (Schnell *et al.,* 1995; Hemanth *et al.,* 2000).

In addition to providing a measure of genetic diversity, the RAPD analysis could also provide a genetic profile, characteristic to a cluster in which the individuals share a common morphological trait and place of origin. In the following paragraphs an attempt has been made to describe the relationship between genotypes based on RAPD analysis with respect to morphological characters and location.

The dendrogram of 31 genotypes (Fig.8) of sapota indicated that, all round and oval shaped fruit bearing genotypes are grouped together (Group I) are 'DHS-1', 'DHS-2', 'Cricket Ball', 'PKM-1', 'PKM-2', 'Gavarayya', 'Guthi', 'Oval', 'Murabba', 'CO-1', 'Kirthbharti', 'French Indochina', 'CO-2', 'Cricket Ball (ARSA)', 'Tagarampudi', 'CO-3', 'Cricket Ball (Udupi)' and 'Cricket Ball (Sirsi)', although were oval shaped genotypes are grouped under round shaped fruit bearing genotypes might be due to the fact that, probably one of the parents is round shape.'PKM-1' and 'PKM-2' are closely associated to each other may be due to they are from same place of origin. 'DHS-1', 'DHS-2', are closely associated with one of its parent 'Cricket Ball'. 'CO-1' and 'French Indochina' are grouped near due to their pinkish colour pulp. Further, the closer relationship observed from RAPD markers between 'Tagarampudi' and 'CO-3' may explain the round shape fruits. Similarly, 'Cricket Ball (Udupi)', and 'Cricket Ball (Sirsi)', 'Pala' and 'Virudhanagar', 'CO-2' and 'Cricket Ball (ARSA)'. 'DHS-1', 'DHS-2' are cent per cent closely grouped in a dendrogram due to their same parentage ('Kalipatti' x 'Cricket Ball'). 'Guthi', 'Oval' and 'Murabba' are grouped in same cluster may be due to 'Oval' and 'Murabba' having the same canopy shape and 'Guthi' and 'Oval' having round to slight oval shape fruits. In sub group of cluster I 'Long Oval', 'Kalipatti', 'Gholwad Sapota', Dwarapudi' and 'Singapore' all are grouped together due to they bear oval shape fruit except 'Dwarapudi' which is having same canopy shape. Within this group 'Kalipatti' and 'Gholwad Sapota are closely associated due to distinct oval shape fruit and morphologically same growth habit. Cluster II 'Variegated Sapota', 'PKM-4', Culcutta Round', 'PKM-3' and 'Mohangootee' are grouped together may be due to oblong shape fruits, except 'Culcutta Round' which is similar in growth habit. Oblong shape fruit bearing genotypes are narrowly distributed with oval and round shape fruit bearing genotypes. 'PKM-5' has been placed in Group 2 as due to distinctive oblong shape fruits which are closely associated with 'PKM-3' and 'PKM-4. Similar discriminative ability of

RAPD markers in identifying species, cultivars and hybrids has been demonstrated in many other crops like avocado (Lewis, 1992), apple (Koller *et al.,*1993), mango (Schnell *et al.,* 1995; Hemanth *et al.,*2000), Passion fruit (Cerqueirasilva *et al*.,2010), Pomegranate (Ercisli *et al*., 2011), Pear (Yildirim *et al.,* 2010), Jack fruit (Anburaj and Sudarmani, 2010), Guava (Bajpai *et al*., 2008), Citrus (Baig et *al*., 2008), Citrus (Machado *et al*., 1996).

5.6.1 Variation among cultivars

The dendrogram generated by UPGMA based on Jaccard's Co-efficient only for the 19 cultivars (Fig.9) showed that round, oval and oblong fruit bearing genotypes are grouped in separate clusters. The maximum similarity of 91 units was found between 'Murabba' and 'Oval' (Table 9). From this it is evident that the magnitude of genetic diversity is higher in cultivars. The ova and round shape fruit bearing genotypes are 'PKM-1', 'Cricket Ball', 'Guthi', 'Gavarayya', 'Oval', 'Murabba', 'Kirthbharthi', 'CO-2', 'Kalipatti', 'Gholwad Sapota', 'Tagarampudi', 'Pala', 'Virudhanagar', 'Long Oval', 'Dwarapudi' and 'Singapore', are clustered in first group within this group 'PKM-1' and 'Guthi' are closely related due to 'PKM-1 is a selection from 'Guthi'. 'Oval' and 'Murabba' are nearly placed in group due to same fruit characteristics, similarly, 'Kalipatti' and 'Gholwad Sapota', 'Pala' and 'Virudhanagar'. 'PKM-4', 'Culcutta round' and 'Mohangootee' are placed in another cluster as they are having oblong shape fruits except 'Culcutta Round which is similar in growth (canopy) habit with 'Mohangootee'. The similar association between morphological characters and RAPD profiles have been reported in different crop like, Fig (Salhi-hannach *et al*.,2006), Apple (Yae *et al*.,1995 a), Pomegranate (Hasnaoui *et al*., 2010), Mango (Hemanthkumar *et al*., 2001), Apricot (Ercisli *et al*., 2009), Mango (Bajpai *et al*., 2008), Strawberry (Graham *et al.,* 1996), Peach (Xiang *et al*.,1996), Pear (Lin *et al*., 2011), Loquat (Hussain *et al*., 2009), Sweet cherry (Gerlach and Stösser, 2008) and (Joseph *et al*., 1997).

5.6.2 Variation among landraces

The dendrogram developed exclusively for landraces based on UPGMA using Jaccard's Co-efficient of similarity clearly revealed that based on fruit characteristics, growth habit the landraces were clustered together (Fig.10). The maximum similarity of 78 per cent between 'Cricket Ball (Udupi)' and 'Cricket Ball (Sirsi)' from this it is evident that the magnitude of genetic diversity is medium in landraces (Table 10). 'Cricket ball (ARSA)', 'French Indochina', ‘Cricket Ball (Udupi)’ and Cricket Ball (Sirsi)’ are grouped together due to round shape fruits. Within this group ‘Cricket Ball (Udupi)’ and Cricket Ball (Sirsi)’ are closely placed due to distinctive round shape fruits and they are from same location. ‘Variegated Sapota’ is closely associated with ‘Cricket Ball (ARSA)’, ‘French Indochina’, ‘Cricket Ball (Udupi)’ and Cricket Ball (Sirsi)’ due to round shape fruits. ‘PKM-5 has kept alone in cluster due to distinctive oblong shape fruits.

5.6.3 Variation among hybrids

The dendrogram developed exclusively for hybrids based on UPGMA using Jaccard’s Co-efficient of similarity clearly revealed that based on fruit characteristics, growth habit and bearing habit the hybrids were clustered together (Fig.11). Maximum similarity of 100 per cent between ‘DHS-1’ and ‘DHS-2’ may be attributed to sharing of same parentage (‘Kalipatti’ x ‘Cricket Ball’). It is evident that the magnitude of genetic diversity is medium in hybrids (Table 11). ‘DHS-1’ and ‘DHS-2’, ‘CO-1’ and ‘CO-3’ are grouped together due to round shape fruit except ‘DHS-2’ and spreading habit and solitary bearing. ‘PKM-3’ has placed singly in the cluster due to oblong shape fruits, vertical growth habit and cluster bearing.

Based on outcome of the present investigation, we conclude that RAPD as molecular markers for fingerprinting as well as estimation of genetic diversity and genetic relatedness in sapota genotypes is effective, precise and more efficient than morphological markers. It offers a general association between RAPD profile and common morphological characters. This helps in identifying suitable parents for effecting crosses and selecting desirable types at early stages.

Future line of work

1. The other DNA based markers like RFLP, AFLP, SSR and SNPs etc., could be used to add more authenticity of these findings.
2. Studies on identification of markers linked to traits of interest may be carried out.
3. Molecular markers could be used in classical breeding programmes to enhance speed and efficiency of sapota breeding programme.

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

Sapota (*Achras zapota* L.), a hardy fruit crop is commercially grown on a large area in Karnataka. Area under this crop is increasing due to its wider adaptability to varied agro-climatic conditions and higher productivity. However, one of the major limiting factors for the expected level of expansion of the area under this crop is the non-availability of grower or consumer, specific variety with ideal characters. There are few cultivars like Kalipatti, Cricket Ball and PKM-1 and hybrids like DHS-1, DHS-2, CO-1, PKM-2 and PKM-3 developed and released by University of Agricultural Sciences, Dharwad and Tamil Nadu Agricultural University, Coimbatore. The present investigations were carried out to study their morphological performance under dry zone of agro-climatic conditions of Karnataka.

Sapota cultivars in general exhibited a high growth rate. Among the cultivars of different age group studied, cv. CO-1 recorded maximum plant height (6.80 m) and minimum was recorded in cv. Kalipatti (3.80 m). With respect to stem circumference cv. DHS-1 showed maximum stem circumference (88.67 cm) and minimum was recorded in cv. Tagarampudi (39.67 cm) but the maximum tree spread was noticed in DHS-1 (8.73 cm north-south and 8.50 cm east-west), while it was minimum in cv. Kirthbharti (4.60 cm north-south) and cv. Tagarampudi (4.47 cm east-west). Maximum shoot length was recorded in cv. Cricket Ball (25.60 cm), while minimum was recorded in cv. Oval (20.20 cm). Maximum shoot girth was recorded in cv. DHS-1 (4.17 cm) and minimum was noticed in cv. Gavarayya (2.36 cm). Maximum number of leaves were observed in cv. DHS-1 (20.33) and minimum number of leaves were noticed in cv. Gavarayyya and cv. Tagarampudi (11.60) in 15-16 years old trees.

Among the 10-11 years old trees, longest plant height was recorded in cv. Cricket Ball (ARSA) (5.10 m), while, it was shortest in cv. CO-3 (2.67 m). Maximum stem circumference was noticed in cv. Cricket Ball (ARSA) (40.00 cm) and minimum was noticed in cv. Murabba (28.67 cm). With respect to tree spread maximum spread was noticed in cv. Cricket Ball (ARSA) (4.53 cm north-south) and cv. Guthi (5.60 cm east-west) minimum spread was recorded in cv. Singapore (1.93 cm north-south and 2.60 cm east-west). Maximum shoot length was observed in cv. Cricket Ball (ARSA) (23.40 cm) and minimum was observed in cv. Singapore (19.60 cm). Maximum shoot girth was recorded in cv. Singapore (3.30 cm) and minimum was recorded in cv. Murabba (2.31 cm) and maximum number of leaves are observed in cv. Guthi (15.00) and minimum number of leaves per shoot was recorded in cv. Singapore (10.13).

Among different 24 sapota cultivars studied, maximum number of flower per shoot was recorded in cv. CO-1 (15.33), while, it was minimum in cv. Murabba (5.93). With respect to number of days to flower open cv. Pala took fewer days to flower open (34.13), while, cv. CO-1 recorded more number of days to flower open (39.10). With respect to number days for fruit set, minimum number of days to fruit set was observed in cv. Cricket Ball (ARSA) (24.00), while, it was maximum in cv. Kalipatti (30.00). Among the all cultivars maximum per cent of fruit set was recorded in cv. PKM-1 (29.15 %) followed by cv. PKM-2 (24.15 %), while, it was minimum in cv. Singapore (9.82 %).

Among the cultivars studied, cv. CO-2 recorded maximum fruit diameter (5.96 cm) and cv. CO-1 recorded maximum fruit length (6.64 cm), whereas cultivars Singapore and Murabba recorded minimum fruit diameter (3.60 cm) and fruit length (4.40 cm) respectively and cv. CO-1 recorded maximum fruit weight (133.41 g) among the different cultivars. More number of fruits per tree was recorded in cv. PKM-1 (2450), whereas less number of fruits per tree was recorded in cv. PKM-4 (180). cv. DHS-1 recorded maximum yield (169.2 kg/tree) and minimum yield was recorded in cv. CO-3 (13.5 kg/tree).

Maximum total soluble solids was recorded in cv. DHS-1(23.90° B), whereas, it was minimum in cv. Guthi (19.00 ° B). Significantly lower acidity was recorded in cv. CO-1 (0.126%). With respect to number of seeds per fruit cv. DHS-2 recorded maximum number of seeds per fruit (4.14), while, it was minimum in cv. Long oval (1.74).

The present study on "Molecular characterization of sapota (*Achras zapota* L.) using RAPD markers" indicated, selection of recently matured leaves and grinding for 5-10 minutes was found suitable for extraction of DNA. Using two per cent PVP and two per cent CTAB in 750µl of extraction buffer for 200 mg of leaf sample yielded high quality and amplifiable genomic DNA.

Protocol for DNA amplification for RAPD analysis using Operon random primers was standardized. A reaction volume of 20 µl with 40 ng of template DNA, 10 mM Tris-HCl, 500 mM KCl, 15 mM $MgCl_2$, 100 µM of dNTP's, one unit of *Taq* polymerase and 0.3 µM of primers was found suitable for PCR amplification.

The cluster analysis with Jaccard's Co-efficient showed a high to moderate level of genetic diversity in sapota. The two major clusters could be identified by UPGMA based on Jaccard's Co-efficient of similarity. The cluster one contained 31 genotypes and it again formed into two sub groups. Cluster two contained 1 genotype (PKM - 5). The dendrogram indicated that the round and oval (Sub cluster I) shape fruit bearing genotypes and oblong (cluster II) shape fruit bearing genotypes were separate groups.

The cluster analysis for 19 cultivars clearly indicated that the genetic diversity between species is moderate. The grouping was occurred based on morphological characters and fruit shape, the 19 cultivars clustered in to two main clusters. The cluster one contained all round and oval shape fruit bearing cultivars where as cluster two contained oblong shape fruit bearing cultivars except one round shape fruit bearing cultivar.

The cluster analysis for 6 landraces indicated that the genetic diversity was moderate. From the cluster analysis it is clear that landraces are clustered based on their fruit characteristics.

The cluster analysis for 6 hybrids indicated that the genetic diversity was moderate to high. From the cluster analysis it is clear indicated that hybrids are clustered based on their fruit characteristics, growth habit, bearing habit and parentage. All spreading, round shape fruit bearing and solitary bearing hybrids were clustered together and vertical growing, oblong shape fruit bearing and cluster bearing hybrid was clustered alone in the dendrogram.

It is evident from the present studies that RAPD markers is simple, less expensive, fast and reliable means of estimating genetic relationships and for discriminating genotypes, cultivars, landraces and hybrids of sapota.

REFERENCES

7. REFERENCES

Anburaj, J. and Sudarmani, D.N.P., 2010, Estimation of Genetic Diversity in Jackfruit Employing PCR Based D-19 RAPD Fingerprinting. *Advanced Biotech*, 34, **10**(6).

Anonymous, 1997, *Research report* 1997, All India Coordinated Research Project, University of Agricultural Sciences, Dharwad, pp. 56-62.

Anonymous, 1984, *Official Methods of Analysis*. Ed. Sioney Williams, Association Official Analytical Inc., Verginia, 14th Ed., pp. 423-462.

Anonymous, 1998, *Text Book of sapota*, Agrotech Publishing Academy, Udaipur.

Anonymous, 2000, *Research Report 2000.* All India Coordinated Research Project and ICAR Ad-hoc Schemes on Tropical Fruits. Mahatma Phule Krishi Vidyapeet, Rahuri, pp. 50-87.

Anonymous, 2010, *www. nhb.gov.in/statics/area-production-statistics.htm/.*

Anonymous, 2002, *Research Report 2002.* All India Coordinated Research Project and ICAR Ad-hoc Schemes on Tropical Fruits. Tamil Nadu Agricultural University, Coimbatore, pp. 54-95.

Anonymous, 2004, *Research Report* 2004. All India Coordinated Research Project and ICAR *Ad-hoc Scheemes on Tropical Fruits. S.V.Agric. College., Tirupati,* pp. 49-92.

Anonymous, 2004a, *Research Report* 2004, All India coordinated Research Project and ICAR Ad-hoc Schemes on Tropical Fruits. S.V. Agricultural College, Tirupati, pp. 49-92.

Asim, K. and Meena, H.M., 1994, Thermostable DNA polymerases for *in vitro* DNA amplifications **In**. PCR Technology: *Current innovations*. (Eds) Hugh. G.G. and Annette, M.G. p 223.

Ausubel, M.F., Brent, R., Kingston, E.R., Moore, D.D., Seidman, J.G., Smith, A.J. and Struhl, K., 1989, Preparation and analysis of DNA. **In.** PCR Technology: *Current innovations*. (Eds) Hugh. G.G. and Annette, M.G.p 223.

Avaiya, Y.V. and Singh, S.P., 1991, Physico-chemical study of mature sapota (*Achras sapota* L.) fruits of different cultivars. *Orissa J. Hort.*, **19**: 83-86.

Baig, M.N.R., Grewa, S. and Dhillon, S., 2008, Molecular characterization and genetic diversity analysis of citrus cultivars by RAPD markers. *Turky J. Agric*., **33**: 375-384.

Bailey, D,C., 1983, Isozymic variation and plant breeders right. **In**: Isozymes in plant genetics and breeding part A, S.D.Tanksley and T.J. Orton (eds), *Elsvier*, New York.pp: 425-440.

Bajpai, A., Chandra, R., Rajan S. and Srivastava, N., 2008, RAPD and minisatellite markers for genetic diversity and relationship in guava varieties. *Indian J. Genet*., **68**(4): 441-445.

Bajpai, A., Srivastava, N., Rajan, S. and Chandra, R., 2008, Genetic diversity and discriminationof mango accessions using RAPD markers. *Indian J. Hort*., **65**(4): 377-382.

Bal, J.S. 1997, *Fruit growing*, Kalyani Publishers, Ludhiana, India.

Bartolozzi, F., Warburton, M.L., Arulsekar, S. and Gracziel,T.M., 1998, Genetic characterization and Relatedness among Calofornia Almond cultivars and Breding lines detected by RAPD analysis. *J. Amer. Soc. Hort. Sci.,* **123**(3): 381-387.

Bowers, J.E., Bradman, B.E., Carole, P. and Meridith, 1993. DNA fingerprinting characterization of some vine grapes. *Amer.J.Enol.Vitic.,* **44**(3): 1-10.

Cerqueirasilva, C.B.M., Cardoso-Silva, C.B., Santos, E.S.L., Conceição, L.D.H.C.S., Pereira, A.S., Oliveira, A.C. and Corrêa, R.X., 2010, Genetic diversity in wild species of passion fruit (*Passiflora trintae*) based on molecular markers. *Genet, Mol Res*., **9** (4): 2123-2130.

Chadha, K.L., 1992, Stratergy for optimization of productivity and utilization of sapota (*Manilkara achras* (Mill.) Fosberg). *Indian J. Hort.,* **49**(1): 1-17.

Chaudhary, S.M., Shete, M.B. and Desai, U.T., 1995, Performance of some sapota [*Manilkara achras* (mill) Fosberg] cultivars under semi-arid region of Maharastra. *Recent Hort.*, **2**(2): 47-51.

Cheng, F.S., Weeden, N.F. and Browan, S.K., 1996, Identification of co-dominant RAPD markers tightly linked to fruit skin color in apple. *Theor. Appl. Genet.,* **93**(1-2): 222-227.

Chundawat, B.S. and Bhuva, H.P., 1982, Performance of some cultivars of sapota (*Achras sapota* L.) in Gujarath. *Haryana J. Hort Sci.,* **11**(3-4): 154-158.

Chyi, Y.S. and Weeden, N.F., 1984, Relative isozymes band intensities permit the identification of the 2n gamete parent for triploid cultivars. *Hort. Sci.,* **19**: 818-819.

Damasco, O.P., Graham, G.C., Henry, R.J., Adkins, S.W., Smith, M.K. and Godwin, I.D., 1996, Randomly Amplified Polymorphic DNA (RAPD) detection of dwarf off- types in micropropagated Cavendish (*Musa* spp. AAA) bananas. *Plant Cell Reports.* **16**(1-2):118-123.

Darftt, D.E. and Arulsekar, S., 1985, Identification of plum x peach hybrid by isozymes analysis. *Hort. Sci.,* 20: 246-248.

De Wald, M.G., Moore, G.A. and Sherman, W.B., 1988, Identification of pineapple cultivars by isozyme genotypes. *J. Amer. Soc. Hort. Sci*., **113**: 935-938.

Degani, C. and Blumenfeld, A., 1986, The use of isozyme analysis for differentiation between loquat cultivars. *Hort. Sci.,* 21: 1457-1458.

Degani, C., El-batsri, R. and Gazit, S., 1990, Enzyme polymorphism in mango. *J. Amer. Soc. Hort. Sci*., **115**: 844-847.

Demeke, T. and Adams, R.P., 1992, The effects of polysaccharides and buffer additives on PCR. *Biotechniques.* **12**: 332-334.

Due, A.L., Adama. P.R. and Zhong, M., 1999, Using Randomly Amplification of Polymorphic DNA for a Taxonomic Reevaluation of Pfitzer Junipers. *Hort. Sci.,* **34**(6): 1123-1125.

Echt, C.S., Erdahl, A.S. and McCoy, T.G., 1992, Genetic segregation of randomly amplified polymorphic DNA in diploid cultivated alfafa. *Genome*, **35**(1): 84-87.

Ellatrand, N.C. and Lee, J.M., 1987, Cultivar identification of cherimoya (*Annona cherimola* Mill.) using isozyme markers. *Scientia Hort.,* **32**: 25-31.

Ercisli, S., Agar, G., Yildirim, N., Esitken, A. and Orhan, E., 2009, Identification of apricot cultivars in Turkey (*Prunus armeniaca* L.) using RAPD markers. *Romanian Biotechnological Letters.* **14**(4): pp. 4582-4588.

Ercisli, S., Gadze, J., Agar, G., Yildirim, N. and Hizarci, Y., 2011, Genetic relationships among wild pomegranate (*Punica granatum*) genotypes from Coruh Valley in Turkey. *Genet, Mol Res.*, **10** (1): 459-464.

Farooqi, A.A. and Rao, M.M., 1976, studied on fruit set in some sapota varieties in relation to intra and inter varietal pollination. *Mysore Journal of Agricultural Sciences*, **10**: 28-34.

Farooqi, A.A., Rao, M.M., Bankapur, V.M. and Kololgi, S.D., 1973, A note on extent of fruiting in relation to flowering in sapota (*Achras sapota* L.). *Mysore J. Agric Sci.*, **7**: 132-134.

Federicl, C.T., Fang, D.Q., Scora, R.W. and Roose, M.L., 1998, Phylogenetic relationships within the genus Citrus (Rutaceae) and related genera as revealed by RFLP and RAPD analysis. *Theor. Appl. Genet.,* **96**: 812-822.

*Frankel, O.H. and Brown, A.H.B., 1984, Current plant genetic resources. A critical approach. **In**: *Genetics*, New Frontiers, Proceedings of the XV international congress of genetics, **IV**. Eds. Chopra V.L., Joshi, B.C., Sharma, R.P. Sharma and Bansal, H.C. Oxford University Press, Cambridge.

Franklin, E.C. and Arkesh, M.M., 1991, Alternative approaches for consistent yields of genomic DNA from cotton (*Gossypium hirsutum*L.). *Plant Mol. Biol. Reporter*. **9** (3): 252-261.

Gallego, F.J. and Martinez, I., 1998, Molecular typing of rose cultivars using RAPDs. *J. Hort. Sci.,* **71**(6): 901-908.

*Gan, Y., Zaini, S. and Idris, A., 1981, Genetic variation in the grafted vegetatively propagated mango (*Mangifera indica* L), *Pertanika*, **4**: 53-62.

Gehrig, H.H., Rosicke, G. and Kuge, M., 1997, Detection of DNA polymorphisms in the genus *Kalanchoe* by RAPD-PCR fingerprint and its relations to infrageneric taxonomic position and ecophysiological photosynthetic behaviour of the species. *Pl. Sci.,* **125**: 41-51.

Gelfand, P.H. and White, T.J., 1990, Thermostable DNA polymerases, pp: 129-141. **In**: PCR protocols: A guide to methods and applications (M.A. Innis, Gelfand. D.H., Sninky, J.J. and White, T.J., eds,). Academic Press, Sand Diego.

Gerlach, H.K. and Stösser, R., 2008, Sweet Cherry cultivar identification using RAPD-derived DNA fingerprints. *Acta Hort.,* 468.

Goldring, A., Zamir, D. and Degani, C., 1985, Duplicated phosphoglucose isomersae genes in avocado. *Theor. Appl. Genet*., **71**: 491-494.

Graham, M.J. and Mcnicol, R.J., 1995, An examination of the ability of RAPD markers to determine the relationships within and between *Rubus* species. *Theor. Appl. Genet*., **90** (7-8): 1128-1132. *Genet*., **93**(3): 402-406.

Grodzicker, T., Williama, J., Sharp, P. and Sambrook, J., 1974, Physical mapping of temperature sensitive mutations of adenovirus. Cold spring Harbour symp. *Quant. Biol.,* **39**:439-446.

Gunuki, K.N., 1998, Studies on growth and development of sapota (*Manilkara achras* (Mill.) Fosberg). *M. Sc. Thesis*, University of Agricultural Sciences, Dharwad.

Gunuki, K.N., Hulamani, N.C., Athani, S.I., Shankargouda, P. and Swamy, G.S.K., 1999, Studies on flowering and fruit set in some sapota (*Manilkara achras* (Mill.) Fosberg) varieties and hybrids. *Advances in Agric.Res.India.*, **12**: 58-60.

Gupta, O.P., Kainsa, R.L., Dhawan, S.S. and Chauhan, K.S., 1981, Post-harvest studies in fruits comparison of sugar for the preparation of candies. *Haryana Agric. Univ. Res. J*., **11**(3): 389-392.

Hasnaoui, N., Mars, M., Chibani, J. and Trifi, M., 2010, Molecular Polymorphisms in Tunisian Pomegranate (*Punica granatum* L.) as Revealed by RAPD Fingerprints. *Diversity,* **2:** 107-114.

Hattori, J., Gottlob-Mchugh, S.G. and Johnson, 1987, The isolation of high molecular weight DNA from plants. *Ani. Biochem*., **165**: 70-74.

Hays, W.B., 1960, *Fruit growing in India*. Kitabistan, Allahabad, India, pp. 389-394.

Hegde, N.S., 1997, Performance of sapota (*Manilkara achras* (Mill.) Fosberg). Varieties and hybrids under transitional tract of Karnataka. *M. Sc. (Agri.) Thesis,* University of Agricultural Sciences, Dharwad.

Hemanth, K.N.V., 1999, Fingerprinting and estimation of genetic diversity of certain commercial mango (*Mangifera indica* L.) varieties using RAPD markers, *M. Sc. Thesis* submitted to UAS, Bangalore.

Hemanth, K.N.V., Narayanaswamy, P., Suresh, N. Sondur. and Theerthaprasad, D., 2000, Paternity analysis of mango (*Mangifera indica* L.) hybrids through RAPD markers. *National Seminar on Hi- Tech Horticulture*, held at Atria Hotel, Bangalore, between 26-28 June, 2000.

Hemanthkumar, N.V., Narayanswamy, P., Theerth Prasad, D., Mukunda, G.K. and Suresh, N.S., 2001, Estimation of genetic diversity of commercial mango (*Mangifera indica* L.) cultivars using RAPD markers. *J. Hort, Sci and Biotech*., **76**(5): 529-533.

Heun, M. and Helentjaris, T. 1993. Inheritance ofRAPDs in F1 hybrids of corn. *Theor Appl Genet*. **85**:961-969.

Hill, M., Witsenboer, H., Zabeau, M., Vos, P., Kesseli, R. and Michelmore, R., 1996, PCR-based fingerprinting using AFLPs as a tool for studying genetic relationships in *Lactuca* spp. *Theor*. *Appl*. *Genet*., **93**: 1202-1210.

*Hong, C., Arissumi, K., Etoh, T. and Hong, C.J., 1996, RAPD analysis of ornamental alliums for phylogenetic relationship. Memoirs of the faculty of Agriculture Kagoshima University. **32**: 51-58.

Hormaza, J.I., 1998, Early selection in cherry combining RAPDs with embryo culture. *Scientia Hort.,* **79** (1-2): 121-126.

Huang, H., Li, Z. And Li, J., 2002, Phylogenetic Relationships in *Actinidia* as Revealed by RAPD Analysis. *J. Amer. Soc. Hort. Sci*. **127**(5):759–766.

Hussain, A., Abbasi, N.A., Hafiz, I.A., Ahmad, Z. and Naqvi, S.M.S., 2009, Molecular characterization and genetic relationship among loquat (*Eriobotrya japonica* Lindl.) genotypes of Pakistan assessed by RAPD markers. *Pakistan. J. Bot*., **41**(5): 2437-2444.

Iqbal, M.J., Paden, D.W. and Lane, R.A., 1995, Assessment of genetic relationships among rhododendron species, varieties and hybrids by RAPD analysis. *Scientia Hort*., **63** (3-4): 215-223.

Jain, P.K., Saini, M.L., Pathak, H. and Gupta, V.K., 2007, Analysis of genetic variation in different banana (*Musa* species) variety using random amplified polymorphic DNAs (RAPDs). *African J. of Biotech.,* **6** (17), pp. 1987-1989.

Jarret, R.I. and Lttze, R.E., 1986, Isoenzyme markers in bananas and plantains. *Euphytica*, **35**: 539-549.

Jennifer, A.C. and Paul, L.F., 1990, Isolation of DNA from plants high in polyphenols. *Plant Mol. Biol. Rep.,* **8**(1): 8-12.

Joseph, G.D., Murata, N. and Shinoda, K., 1997. RAPD analysis of genetic relationships among *Alstroemeria* L. cultivars. *Scientia Hort*., **68** (1-4): 181-189.

Joshi, P.S., Prabhakar, K.R. and Gupta, S.V., 1999, Molecular markers in plant genome analysis. *Curr. Sci.,* **77**(2): 230-238.

Koller, B., Lehmann, A., Mcdermott, J.M. and Gessler, C., 1993, Identification of apple cultivars using RAPDs and RFLPs. *Agronomie*. **17**(6-7): 323-333.

Kumar, P.P., Yau, J.C.K. and Goh, J.C., 1998, Genetics analysis of *Heliconia* species and cultivars with RAPD markers. *J. Amer. Soc. Hort. Sci*., **123**(1): 91-97.

Lakshminarayana, S. and Subramanyam, H., 1966, Physical, chemical and physiological changes in sapota fruit (*Achras sapota* L.) family Sapotaceae, during development and ripening. *J. Food Sci, Tech.,* **3**: 151-154.

Lakshminarayana, S. and Subramanyam, H., 1970, Formation of abscission zones in the pedicels and shedding of flowers in *Achras sapota* L. (Sapotaceae). *Sciences and Culture*, **36:** 227-230.

Lanham, P.G. and Brennan, R.M., 1999, Genetic characterization of goose berry (*Ribes grossularia* sub genus *Grossularia*) germplasm using RAPD, ISSR and AFLP markers. *J. Hort. Sci. Biotech.,* **74**(3): 361-366.

Lefort,F. and Douglas, C.G., 1999, An efficient micromethod of DNA isolation from mature leaves of four hardwood tree species *Acer, Fraxinus, Prunus and Quercus. Ann. Forestry Sci.* **56**: 259-263.

Lenka, P.C., Das, D.K. and Samal, B., 1996, Studies on floral biology and physical characteristics of sapota cultivars, *Orissa. J. Hort*., **24**(1-2): 42-46.

*Lewis, E., 1992, Identification of avocado cultivars with RAPD markers. *Inligtings bulletin-Instituut-vir-Tropiese- Subtropiese- Gewasse*. **241**:7-9.

Lin, J., Wang, X.C., Chang, Y.H. and Fang, J.G., 2011, Development of a novel and efficient strategy for practical identification of *Pyrus* spp (Rosaceae) cultivars using RAPD fingerprints. *Genet, Mol Res*., **10** (2): 932-942.

Lodhi, M.A., Ye, G.N., Weeden, N.F. and Reisch, B.I., 1994, A simple and efficient method for DNA extractions for grape vine cultivars *Vitis* spp. *Plant Mol. Biol. Rept.* **12**: 6-13.

Machado, M.A., Coletta, F.H.D., Targon, M.L.P.N. and Pompeu, J.J., 1996, Genetic relationship of Mediterranean mandrins (*Citrus deliciosa Tenore*) using RAPD markers. *Euphytica*., **92**(3): 321-326.

Madhavarao, V.N. and Abdul Khader, J.B.M.M., 1961, Studies on certain aspects of following and pollination in some varieties of sapota with special reference to self and cross compatibility. *Madras Agric J.,* **48:** 19-20.

Mahadevaiah, G.M., 1981, Studies on flowering, fruit set and fruit development in ‘Kalipatti’ sapota (*Manilkara achras* (Mill.) Fosberg*). M. Sc. (Agri.) Thesis*, University of Agricultural Sciences, Bangalore, pp. 1-77.

Majourhat, K., Jabbar, Y., Araneda, L., Zeinalabedini, M., Hafidi, A. and Martínez-Gómez P., 2007, Argania spinosa (L.) Skeel is an endemic species from Southwest Morocco being the unique representative of the tropical Sapotaceae in this area. *J. Bot*., **73**(4): 661-663.

Maliyakal, E.J., 1992, An efficient method for isolation of RNA and DNA from plants containing polyphenols. *Nucleic Acid Res*. **20**: 2381.

Mathew, B., Ghosh, S.N. and Manna, S. 2001, Studies on physic chemical studies of sapota cultivars grown in West Bengal. *J. Maharashtra Agric. Univ.,* **26**(2): 224-226.

Maughan, P.J., Saghai, Maroof, M.A., Buss, G.R. and Huestis, G.M., 1996, Amplified Fragment Length Polymorphism (AFLP) in soyabean: species diversity inheritance and near isogenic line analysis. *Theor, Appl, Genet.,* **93**: 392-401.

Meir, H. B. and Vainstein, A., 1993, Assessment of genetic relatedness in roses by DNA fingerprint analysis. *Scientia Hort*., **58** (1-2): 115-121.

Minhas, P.P.S. and Sandhu, A.S., 1985, Investigation on floral biology of sapota (*Achras sapota* L.) under Ludhiana conditions II. Ime of anthesis, dehiscence, pollen studies, stigma receptivity and fruit set. *J. Res.,* **22**: 270-276.

Mishra, G.P., 2009, Correspondence of DNA Markers for Genetic Diversity Studies among Different Apricot Genotypes from Cold Arid Deserts of Ladakh. *DRDO Sci. Spectrum*. pp. 151-156.

Mone, S.V., 1989, Studies on growth and productivity of sapota (*Manilkara acharas (Mill.) Fasberg)* varieties. *M. Sc. (Agri.) Thesis,* University of Agricultural Sciences, Dharwad, pp. 1-88.

Moor, G.A. and Litze, R.E., 1984, Biochemical markers for *Carica papaya, C. cauliflora* and plants from somatic embryos of their hybrids. *J. Amer. Soc. Hort. Sci.,* **109**: 213-218.

Moor, G.A., 1984, Isozyme banding pattern in citrus. *Hort. Sc*i., **19**: 66.

Mulla, A.L. and Desle, G.Y., 1990, Pollination studies in sapota cultivars. *J. Maharastra Agric.,* **15**: 266-268.

Mullis, K.B. and Faloona, F.A., 1987, Specific synthesis of DNA *in vitro* via a polymerase catalyzed reaction. *Methods in Enzymology*, **255**: 335-350.

*Mullis, K.B., Faloona, F.A., Scharf, S., Saiki, R., Horn, G. and Erlich, H., 1986, Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. Cold Spring Harbor Symp. *Quantitative Bio.,* **51**: 263-273.

Murali Raghavendra Rao, M.K., 1999, Estimation of genetic diversity in cashew (*Anacardium occidentale* L.) cultivars by using molecular markers. *M. Sc. Thesis* submitted to UAS, Bangalore, pp. 1-98.

*Murphy, R.W. Stiles, J.W.J., Buth, D.G. and Haufler, C.H., 1990, **Proteins I**: Isozyme electrophoresis. In: Hillis, D.M. and Moritz, C. (eds), *Molecular Systematics*. Sinauer Associates, Sunderland, Massachusetts, pp. 45-126.

Murry, M.G. and Thomposon, W.F., 1980, Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res*., **8**(19): 4321-4325.

Mukandan, S., 2000, Characterization of improvement cultivars of *Jasminum* species using molecular markers. *M. Sc (Hort.) Thesis* submitted to UAS, Bangalore, pp. 1-120.

Nalwadi, U.G., Dasappa and Sulikeri, G.S., 1977, Floral biology of some varieties of sapota (*Achras sapota* L.). *Progressive Hort.,* **9**: 27-32.

Novy, R.G., Kobak, C., Goffreda, J. and Vorsa, N., 1994, RAPD's identify varietal misclassification and regional divergence in cranberry (*Vaccinium macrocarpon*(Ait) Pursh). *Theor. Appl. Genet.,* **88**(8): 1004-1010.

Nymbom, H. and Schaal, B.A., 1990, DNA fingerprints application to paternity analysis in apples (*Malus* x *domestica*). *Theor. Appl. Genet.,* **76**: 763-768.

Panse, V.G. and Sukhantne, P.V., 1967, Statistical Methods for agricultural workers, *Indian Council of agric. Res. Publication*., New Delhi, pp, 167-174.

Parent, T.J.G., Fortin, M.G. and Page, D., 1993, Identification of raspberry cultivars by random amplified polymorphic DNA (RAPD) analysis. *Canadian J. Plant Sci.,* **73**(4): 1115-1122.

Paterson, A.H., Lander, E.S., Peterson, S., Hewitt, J.D., Lincolin, S.F. and Tanksley, S.D., 1998, Resolution of quantitative traits into mendelian factors by using a complete linkage map of RFLP. *Nature.* **335**: 721-726.

Pathak, R.K. and Ojha, C.M., 1993, Improvement. of guava. **In**: Advances in Horticulture. **Vol I.** (eds. K.L. Chadha and O.P. Pareek), Malhotra Publishing House, New Delhi, pp: 143.

Patil, V.K. and Narwadkar, P.R., 1974, Studies on flowering, pollination fruit set and fruit-drop in chicku. *Punjab Hort. J.,* **14**: 39-42.

Paul, S., Wachira, F.N., Powell, W. and Waugh, R., 1997, Diversity and genetic differentiation among populations of Indian and Kennnyan tea [*Camellia sinensis* (L) O. Kuntze] revealed by AFLP markers. *Theor. Appl. Genet*., **94**: 225-263.

Ponnuswamy, V. and Irulappan,I., 1987, Evaluation of the chemical composition of fruits of different varieties and hybrids of sapota. *South Indian Hort.*, **35**: 446-447.

Ponnuswamy, V. and Irulappan,I., 1989, A research note on the study of range, mean and co-efficient of variability in sapota varieties (*Achras zapota* L.). *South Indian Hort.,* **37**(2): 112-114.

Pooler, M.R and Scorza, R., 1995, Aberrant transmission of RAPD markers in haploids, doubled haploids, and F1 hybrids of peach: observations and speculation on causes. *Scientia Hort.,* **64** (4): 233-241.

Porebeski, S., Bailey, G. and Baum, B.R., 1997, Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol. Biol. Rep.,* **15**(1): 8-15.

*Rayburn, A.L., Iqbal, M.J. and Paden, D.W., 1993, Positive identification of rhododendron through DNA fingerprinting. *J. American Rhododendendron society*, Summer 137-138.

Relekar, P.P., Desai, A.G., Rajput, J.C. and Salvi, M.J., 1991, Fruit production in sapota cv. Kalipatti. *Current Res.,* **20**: 104-106.

Ragot, K. and Hoisington, D. A., 1993, Molecular markers for Plant Breeding Comaparisons of RFLP and RAPD genotyping cost. *Theor. Appl. Genet.,* **86**

Rogers, S.O. and Bandich, A.J., 1994, Extraction of total cellular DNA from algae and fungi. **In**: plant biology manual. (S.B.Gelvin and R.A.Schilperoot eds.) 2nd eds. Kulwer Academic Publisher. Dordecht. pp.15.

Rogers, J.H., Burns, A.N. and Parks, C.H., 1996, Comparison of small scale methods for rapid extraction of plants DNA suitable for PCR analysis. Plant Mol. Bio. Rep., 14(2): 172-183.

Rohlf, F.J., 1998, NTSYS-pc Numerical taxonomy and multivariate system, version 2.0. Appl. *Biostatistics*. New York, USA.

Rokhade, A.K., Nalawadi, U.G. and Farooqui, A.A., 1989, Evaluation of promising hybrids in sapota (*Manilkara achras* (Mill.). Fosberg) at Dharwad-1-Fruit characters. *Karnataka J. Agric Sci.,* **2**:286-290.

Rong, L.Z., Yonemori, K., Sugiura, A. and Luo, Z.R., 1995, Evaluation of RAPD analysis for cultivar identification of persimmons. *J. Japanese Soc. Hort. Sci.,* **64**(3): 535-541.

Salhi-hannach, A., Chatti, K., Saddoud, O., Mars, M., Rhouma, A., Marrakchi, M. and Trifi, M., 2006, Genetic diversity of different Tunisian fig (*Ficus carica* L.) collections revealed by RAPD fingerprints. *Hereditas* **143**: 15-22.

Sambrook, J., Fristch, E.F. and Maniatis, T., 1989, Molecular cloning: A laboratory manual Second Edn., CSH Laboratory Press, **Vol I**. Section 6.

Schnell, R.J., Knight, J.Jr. and Schaffer, B., 1993, Genetic relationships among *Mangifera* spp. Based on RAPD markers. *Acta hort.,* **341**: 86-92.

Schnell, R.J. and Knight, R.J.Jr., 1992, Frequency of zygotic seedling from five polyembryonic mango rootstock. *Hort. Sci.,* **27**: 174-176.

Schnell, R.J., Ronning, C.M. and Knight, J.Jr., 1995, Identification of cultivars and validation of genetic relationships in Mangifera indica L. using RAPD markers. *Theor. Appl. Genet*., **90** (2): 269-274.

Shanmugavelu, K. G. and Srinivasan, C., 1973, Proximate composition of fruits of sapota cultivars (*Achras sapota* L.). *South Indian Hort*., **21**: 107-108.

Shantha, I.M., Tiwari, N, Sachdev, A., Johari, R.P. and Lodha, M.L., 1998, Laboratory manual: Modern techniques in plant biochemistry and molecular biology, ICAR, centre of Advanced Studies, *Division of Biochemistry*, IARI, New Delhi, pp 4.

Sharon, D., Hillel, J., Mhameed, S., Cregan, P.B., Lahav, E. and Lavi, U., 1998, Association between DNA markers and loci controlling avocado traits. *J. Amer. Soc. Hort. Sci.,* **123**(6): 1016-1022.

Shikhamany, S.D., Iyer, C.P.A., Ramachander, P.R. and Srinivasan, V.R., 1978, Study of relationships of certain growth attributes with fruit yield in guava (*Psidium guanjava* L.) multiple regression analysis. *Vatika*, **1**: 15-25.

Shirol, A.M., Hanamashetti, S.I., Kanamadi, V.C., Thammaiah, N. And Shankargowda Patil., 2006, Performance of some sapota cultivars under ghataprabha command area. *Karnataka J. Agric. Sci.,* **19**(2): 366-370.

Shirol, A.M., Kanamadi, V.C., Tammaiah, N. and Hanamashetti, S.I., 2005, Studied on pre-soaking of sapota root stock khirni, *Karnataka J. Agric. Sci*., **18**(1): 96-100.

Siddaramayya , 2005, Studies on evaluation of sapota germ plasm, *M. Sc thesis*, University of Agricultural Sciences, Dharwad.

Singh, A., Dhaliwal, H.S., Grewal, S.S. and Kaundal, 1991, Studies on varietal performance of chicku (Manilkara achras (Mill.) Fosberg) in Punjab. Paper represented in *National Seminar on Optimization of Productivity and Utilization of Sapota*, 8th October 1991, G.A.U., Navasari.

Singh, A., Negi, M.S., Rajagopal, J., Bhatia, S., Tomar, U.K., Srivastava, P.S. and Lakshmikumaran, M., 1999, Assessment of genetic diversity in *Azadirachta indica* using AFLP markers. *Thero. Appl. Genet*., **99**: 272-279.

Singh, Sham, Krishnamurthy,S. and Katyal, S.L., 1963, *Fruit cultivar* in India. ICAR, New Delhi, pp.192-8.

Smith, P.M., 1976, Minor crops, (In) Evolution of crop plants (Simmonds, N.W. ed.) Longman, London, pp. 301-324.

Starman, T.W., Duan, X. and Abbit, S., 1999, Nucleic Acid Scanning Techniques Distinguish Closely Related Cultivars of Poinseettia. *Hort. Sci*., **34**(6): 1119-1122.

Staub, J., Bacher, J. and Poetter, K., 1996, Sources of potential errors in the application of random amplified polymorphic DNAs in cucumber. *Hort. Sci.,* **31**(2):262-266.

Sulladamath, V.V., 1975, Studies on growth and development in sapota var. Kalipatti. *M. Sc. (Agri.)* Thesis, University of Agricultural Sciences, Bangalore.

Sulladamath, V.V., Rao, M.M., and Farooqi, A.A., 1978, Preliminary studies on diversity in fruit shape in Kalipatti sapota. *South Indian Horticulture*, **26**:16.

Tai, H.T. and Tanksley, D.S., 1990, A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. *Plant Mol. Bio. Rep*., **8**(4): 297-303.

Tanksley, S. and Pichersky, E., 1988, Organization and evaluation of sequences in the plant nuclear genome. **In**: plant evolutionary biology (Eds Gotteleib,L/D. and Jain, S.K.), Chapmann and Hall Ltd. pp:55-79.

Tanksley, S. D., 1983, Molecular markers in Plant breeding. *Plant. Mol. Bio. Rep*., **1**(1): 3-8.

Tanksley, S., Miller, J., Paterson, A. and Bernatzky, R., 1998, Molecular mapping of plant chromosomes. **In** : Chromosome structure and function- impact of new concepts (Eds. Gustafson,J.P and Appels,R.), 18th Stadler genetics symposium. Plenum press, New York.

Torres, A.M. and Bergh, B.O., 1980, Fruit and leaf isozymes as genetic markers in avocado. *J. Amer. Soc. Hort. Sci.,* **105**: 614-619

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T.V.D., Hornes, M., Fritjers, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M., 1995, AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* **23**(21): 4407-4414.

Walton, M., 1993, Molecular markers: which ones to use?. *Seed World*, **131**(8): 22-29.

Wang, Y., Chen, J., Lu, J. and Lamikanra, O., 1999, Randomly Amplified Polymorphic DNA analysis of *Vitis* species and Florida bunch grapes. *Scientia Hort*., **82**: 85-94.

Warburton, M.L. and Bliss, F.A., 1996, Genetic diversity in peach (*Prunus persica* L. Batch) revealed by RAPD markers and compared to inbreeding coefficients. *J. Amer. Soc. Hort. Sci*., **12**(6): 1012-1019.

Warburton, M.L., Bercrra, V.V.L., Goffreda, J.C. and Bliss, F.A., 1996, Utility of RAPD markers in identifying genetic linkages to genes of economic interest in peach. *Theor. Appl. Genet.,* **93** (5-6):920-925.

Weeden, N.F. and Windel, J.F., 1989, Genetics of plant isozymes, P. 46-72. **In**: Isozymes in plant biology (D. E. Soltis and P.S. Soltis, eds.), Dioscorides, Port Land, Core.

Weeden, N.F., 1989, Applications of isozymes in plant breeding. *Plant Breeding Rev*., **6**: 11-54.

Weeden, N.F. and Lamb, R.C.,1985. Identification of apple cultivars by isozyme phenotypes. *J. Amer Soc. Hort. Sci*., **110:** 7213-7218.

Weising, K.H., Nymbom, K., Wolff. and Meyer, W., 1995, DNA fingerprinting in plants and fungi, CRC Press, Baca Roto, Fla.

Welsh, J. and Mc Clelland, M., 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res*., **18**: 7213-7219.

Wiesman, Z., Avidan, N., Lavee, S. and Quebedeaux, 1998, Molecular characterization of common olive varieties in Israel and the West Bank using Randomly Amplified Polymorphic DNA (RAPD) markers. *J. Amer. Soc. Hort. Sci.,* **123**(5): 837-841.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski J.A. and Tingey, S.V., 1990, DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*., **18**: 6531-6535.

Williams, J.G.K., Rafalski, J.A. and Tingey, S.V., 1992, Genetic analysis using RAPD markers. *Methods in Enzymology*. Pp.66

Wolff, K., Peters-van Rijin, J. and Hofstra, H., 1993, RFLP analysis in chrysanthemum. I Probe and primer development. *Theor*. *Appl*. *Genet.,* **88**: 472-478.

Wu, L. and Lin, H., 1994, Identifying buffolograss [*Buchloe dactyloide* (Nutt.) Engelm] cultivar breeding lines using random amolified polymorphic DNA (RAPD) markers. *J. Amer. Soc. Hort. Sci.,* **119**(1): 126-130.

Xiang, L.Z., Reighard, G.L., Baird, W.V., Abobott, A.G., Rajapakse, S. and Lu, Z.X., 1996, Identification of peach rootstock cultivars by RAPD markers. *Hort Sci.,* **31**: 127-129.

Yae, B., Ko, K., Yae, B.W. and Ko, K.C., 1995a, Classification of *Malus domestica* cultivars by random amplified polymorphic DNA markers. *J. Korean Soc. Hort. Sci.,* **36**(6): 824-828.

Yae, B., Park, H., Shin, Y., Lee, D., Kim, J., Ko, K., Yae, B, W., Park, H. Y., Shin, Y.U., Lee, D.K., Kim, J.H. and Ko, K.C., 1995b, Factors influencing RAPD band patterns in *Malus domestica*. *J. Korean Soc. Hort. Sci.,* **36**(5): 649-654.

Yildirim, N., Ercisli, S., Agar, G., Orhan, E. and Hizarci, Y., 2010, Genetic variation among date plum (*Diospyros lotus*) genotypes in Turkey. *Genet, Mol Res*., 9 (2): 981-986.

*Ye, G.N., Soylemezoglu,G., Weeden, N.F., Lamboy,W.F., Pool, R.M. and Reisch,B.I., 1998, Analysis of the relationship between cultivars, sports and clones via DNA fingerprinting. *Vitis*., **37** (1): 33-38.

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*Originals not seen

APPENDICES

Appendix- I

Meteorological data recorded during the period of experimentation (2010) from Agricultural Research Station, Arabhavi.

Month	Temperature (°C)		Relative Humidity (%)		Evaporation (mm)	Rainfall (mm)
	Minimum	Maximum	Morning	Evening		
January 2010	18.76	29.12	88.16	58.68	3.02	5.10
February 2010	22.96	33.07	94.03	58.69	3.79	0.00
March 2010	26.48	36.56	88.41	49.85	4.79	0.00
April 2010	28.53	39.02	84.63	50.30	6.31	0.00
May 2010	23.32	38.80	83.58	50.98	7.40	0.00
June 2010	22.52	32.63	83.42	58.35	4.57	102.80
July 2010	22.30	30.11	88.96	74.48	2.5	110.9
August 2010	21.84	30.63	89.65	80.94	2.72	56.30
September 2010	20.94	31.75	90.9	82.7	2.87	42.80
October 2010	21.18	31.74	91.97	69.52	3.3	119.10
November 2010	17.67	32.43	93.07	71.43	2.96	85.20
December 2010	13.19	29.99	83.87	72.65	2.90	0.00
Total	-	-	-	-	-	522

APPENDIX - II

dNTP	dinitro-triphosphate
µg	microgram
µl	microlitre
µM	micromolar
bp	base pairs
kb	kilo base pairs
M	molar
mg	milligram
mM	millimolar
ng	nanogram
pg	picogram
pmols	picomoles
rpm	rotations per minute
g	gram
ml	mililitre
v/v	volume/volume
CTAB	Cityl Trimethyl Ammonium Bromide
EDTA	Ethylene Diamine Tetraacetic Acid
NaCl	Sodium chloride
PVP	Polyvinyl pyrrolidone
$MgCl_2$	Magnesium chloride
TBE	Tris Boric Acid EDTA buffer
DDW	Double Distilled Water

Morphological and molecular characterization of sapota (*Achras zapota* L.) using RAPD markers

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ABSTRACT

An investigation was carried out on morphological and molecular characterization of sapota using RAPD markers during 2010-2011 at Kittur Rani Channamma College of Horticulture, Arabhavi. Among the different cultivars evaluated cv. CO-1 recorded maximum plant height (6.80 m), whereas, maximum stem circumference (88.67 cm) and shoot girth (4.17 cm), were observed with cv. DHS-1. Significantly maximum plant spread and shoot length was obtained with cv. DHS-2 (8.73 north-south), Cricket Ball (25.60 cm) respectively. More number of flowers per shoot was noticed in cv. CO-1 (15.33). Cultivars pala and Cricket Ball (ARSA) took minimum days for flower opening (34.13 days) and fruit set (24.00), respectively. The cultivar PKM-1 recorded significantly maximum per cent fruit set (29.50%) and number of fruits per tree (2450). Significantly maximum fruit diameter (5.96 cm) was observed with cv. CO-2, while, maximum fruit length (6.64 cm) and fruit weight (133.41 g) was recorded with cv. CO-1. Significantly higher content of total soluble solids was observed with DHS-1 (23.90 ° B), whereas, cv. CO-1 recorded lower content of acidity (0.126%). Maximum number of seeds per fruit was noticed with cv. DHS-2 (4.14).

The study was also conducted to find out the genetic diversity among 31 accessions (comprising of 19 cultivars, 6 landraces and 6 hybrids) of sapota using PCR based Randomly Amplified Polymorphic DNA (RAPD) markers. Among 20 random decamer primers screened, seven gave consistent banding patterns. These primers yielded 48 markers which were selected for the estimation of genetic diversity using UPGMA (Unweighted Pair Group Method with Arithmetical Averages) cluster analysis having Jaccard's Co-efficient of similarity matrix. Results indicated a moderate to high genetic diversity among 31 genotypes, which are distributed between the ranges of 35 to100 per cent. Genotypes are categorized into two distinctive groups based on fruit characteristics (shape) and canopy shape and hybrids are distributed based on fruit shape, growth habit, parentage and bearing habit, which made two distinctive groups. The cluster analysis revealed that all round and oval shape fruit bearing genotypes are genetically closely related, while, oblong shape fruit bearing genotypes are genetically more diverse from them as they grouped together in different cluster.