MORPHOMETRIC AND MOLECULAR DIVERSITY STUDIES IN TAMARIND (*Tamarindus indica* Linn.) POPULATION.

TELEM RATAN SINGH

PAK 8195

DEPARTMENT OF GENETICS AND PLANT BREEDING

UNIVERSITY OF AGRICULTURAL SCIENCES
G.K.V.K., BANGALORE

2010

MORPHOMETRIC AND MOLECULAR DIVERSITY STUDIES IN TAMARIND (*Tamarindus indica* Linn.) POPULATION.

TELEM RATAN SINGH

PAK 8195

Thesis submitted to the

UNIVERSITY OF AGRICULTURAL SCIENCES, BENGALURU

in partial fulfillment of the requirements

for the award of the Degree of

Master of Science (Agri.)

in

GENETICS AND PLANT BREEDING

BENGALURU JULY, 2010

Affectionately Dedicated

to my beloved parents

Shri. T. Prafullo Singh

and

Smt. T. Landhoni Devi

DEPARTMENT OF GENETICS AND PLANT BREEDING
UNIVERSITY OF AGRICULTURAL SCIENCES
G.K.V.K., BENGALURU

CERTIFICATE

This is to certify that the thesis entitled **"Morphometric and molecular diversity studies in Tamarind (*Tamarindus indica* Linn.) population"** submitted by **Mr. TELEM RATAN SINGH, ID No. PAK 8195,** for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **GENETICS AND PLANT BREEDING** of the University of Agricultural Sciences, G.K.V.K., Bengaluru is a record of research work done by him during the period of his study in this university under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

Bengaluru
July , 2010

(R. NANDINI)
Major Advisor
Assistant professor
Department of Genetics and Plant Breeding

Approved By :

Chairman : _____
(R. NANDINI)

Members : _____
(CHIKKADEVAIAH)

_____
(D.L.SAVITHRAMMA)

_____
(BALAKRISHNA GOWDA)

ACKNOWLEDGEMENT

The task of acknowledging the help that was offered to me throughout this study by my teachers and friends is bigger than the study itself. I feel scanty of words to the magnitude of their help. I could not have completed this work without enjoying their endless patience and affection. Under this decorum I would like to recall all of them with utmost gratitude.

First and foremost, I would like to express my deepest gratitude and profound indebtness to ***Dr. R. Nandini****, Assistant Professor, Department of Genetics and Plant Breeding and chairperson of my advisory committee for her support, subtle guidance and supervision throughout the period of my research and during the preparation of the manuscript. I am privileged to have worked under her and honestly confess that it has been a rare privilege for me to be one of her students.*

I feel immense pleasure in expressing my deep sense of gratitude and profound indebtness to the members of my advisory committee ***Dr. Chikkadevaiah****, In-charge Vice Chancellor, U.A.S., Bengaluru for his sincere advice;* ***Dr. D.L. Savithramma****, Professor, Department of Genetics and Plant Breeding, for her unreserved helped, invaluable guidance and giving me the freedom to avail the facilities in her laboratory;* ***Dr. Balakrishna Gowda****, Professor of Forestry and Environmental Science for his invaluable suggestions throughout the period of my study.*

I would also like to thank ***Dr. Shailaja Hittalmani,*** *Professor and Head, Department of Genetics and Plant Breeding, UAS, G.K.V.K, Bengaluru, for her kind co-operation during the course of my study and to the all the staff of the department for their constant help.*

As a personal note I express my heart full regards to my ***father****,* ***mother****,* ***brother****, relatives and well wishers for their love, support, blessings, inspiration and constant encouragement.*

I don't find words to acknowledge my senior ***Mr. Chakpram Birendrajit*** *and junior* ***Mr. Lungthaorei Francis*** *and* ***Mr. Vijayakumar*** *for the assistance rendered by them. I also enjoy the company of friends –* ***Bijen, Donald, Rowndel, Sunil, Julius Uchoi, Tashi Lungthan, Pawan, Sourabh and Ayoob,*** *for their humour and bonds.*

A special note of thanks to ***Mr. Anjaneyulu Jalagam, Mr. Ali Ahmed Algabal, Vinodhini Madam, Mr. Madhu, Mr. Abdul Jawad*** *and* ***Somya Madam*** *for their tireless help, constant encouragement and cooperation throughout the study.*

I also extend my sincere thanks to ***Lakshmi Aunty*** *for her constant help and support during my research work in the laboratory.*

I also acknowledge the help of ***Mrs. Indrakumari, Mr. Ningaiah. Mr. Viswanatha*** *and* ***Mr. Nagana.***

*It would be incomplete in acknowledging, if I don't thank to all my beloved classmates – **Asif, Anita, Basavaprabhu, Harshiya, Harikrishna, Nandini, Naresh, Mrinmoy, Prahalada, Parashuram, Shilpa, Sidramappa and Venkatesh** who wholeheartedly helped and encouraged me during the course of my investigation.*

Last but not the least, I am highly thankful to all those who have extended their helping hands directly or indirectly.

Any omission in this brief acknowledgement does not mean lack of gratitude.

Bengaluru *(**Telem Ratan Singh**)*

July , 2010

MORPHOMETRIC AND MOLECULAR DIVERSITY STUDIES IN TAMARIND (*Tamarindus indica* Linn.) POPULATION.

THESIS ABSTRACT

Tamarind is a highly cross-pollinated crop, hence wide variability is common in this species. The intrapopulation variations between the trees is of paramount importance and it can be used to select the very best trees. The magnitude of variability presented by its morphometric and molecular analysis for each character indicates the potential of each tree and the scope for further improvement through selection. The legendary tamarind grove at Nallur, Devanahalli taluk, derives its importance from the very old and gigantic trees among nearly 300 tamarind trees spread over an area of 53 acres. A range of intrapopulation variation for floral colours in sepals and petals have been reported among the 100 genotypes studied. Similarly, the fruit colour dimorphism observed can also be used as a morphological markers in progeny testing programme. Significant differences among all the metric traits were observed. The genotype 167 was having the highest pod length of 20.04 cm and genotype 197 with the highest fruit weight of 16.41gm. From RAPD profiles, a total of 61 scorable bands were produced with 6 primers ranging from 8 for OPA-R15 to 13 for OPA-Y01. A 120 bp fragment has been amplified exclusively by primer OPA-K06 in the genotype 01 and not in any other samples. This genotype is considered to be one of the oldest trees having 11 clones which are not self sown. Among the 100 genotypes, 5 genotypes *viz.,* 47, 50 65, 244 and 245 identified as elite can serve as the base material to develop a clonal orchard or single progeny trials. The genotype 201 has the highest pulp to seed ratio of 11.2:1 but cannot be considered as an elite tree because of its irregular shaped crown and sparse bearing habit. A considerable amount of intrapopulation diversity observed can be used to identify diverse parents which can be utilize in many hybridization programmes to efficiently introgress the desirable trait of interest.

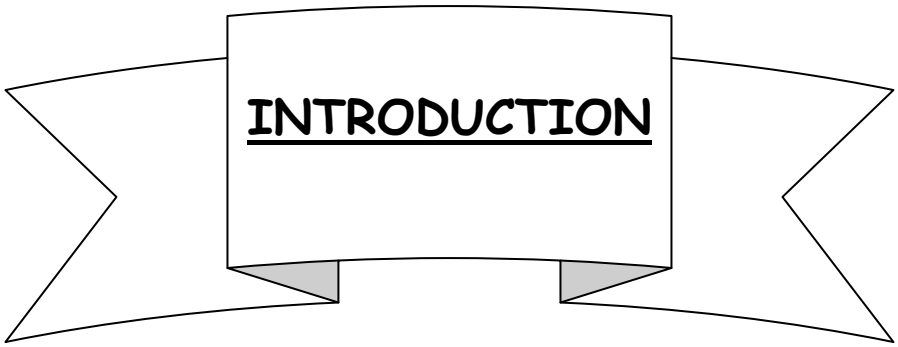
Signature of Major Advisor

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES


INTRODUCTION

I. INTRODUCTION

Tamarind, *Tamarindus indica* L., is a multipurpose tropical fruit tree used primarily for its fruits, which are either eaten fresh or processed. The species has a wide geographical distribution in the subtropics and semiarid tropics and is cultivated in numerous regions. Tamarind belongs to the dicotyledonous family Leguminosae which is the third largest family of flowering plants with a total of 727 genera recognised and the number of species is estimated at 19,327 (Lewis *et al.*, 2005). It has a somatic chromosome number of 2n=24 (Purseglove, 1987). *Tamarindus* itself is a monotypic genus, containing the sole species *T. indica*.

Tamarind is indigenous to tropical Africa and probably also to India. Dry Savannas of tropical Africa is also considered to be the native and believed to have been introduced to Asia in ancient times by Arab traders. It was introduced into India so long ago that it has often been reported as indigenous there, and it was apparently from India that it reached the Persians and the Arabs who called it "tamar hindi" (Indian date, from the date-like appearance of the dried pulp), giving rise to both its common and generic names. However, the specific name, "indica", also perpetuates the illusion of Indian origin.

In India it is chiefly grown in Madhya Pradesh, Andhra Pradesh, Bihar, Tamil Nadu and Karnataka. Sour types of tamarind comprise about 95% of total world production. India is the major sour tamarind producer in Asia. Its annual production is about 300,000 tonnes of which 4,000 tonnes are exported to Europe and North America and the rest is locally consumed. About 20,000 tonnes of Tamarind Kernel Powder (TKP) is also produced annually in India, but this could be doubled through improved post harvest and processing techniques. Annual returns from tamarind seed powder are estimated at Rs 16 -17 million. Tamarind products possess good export potential. The major products for export are the dried Pulp, Seed and Seed Powder. Fresh

tamarind is also a favourite in countries like Italy, Iraq, United Kingdom, USA and Germany (Rao, 1995).

Tamarind is a long-lived, large, evergreen or semi-evergreen tree, 20-30 m tall with a thick trunk up to 1.5-2 m across and up to 8 m in circumference. The tree is not exacting as regards to soil but thrives best in deep alluvium. The tree prefers warm climate but is sensitive to frost. Tamarind is suited to semi-tropical region with low rainfall. The fruit starts bearing 13-14 years after transplanting (Lewis and Neelakantan, 1964). It can be productive for more than 50 years and survives upto 200 years (Hernandez-Unzon and Lakshminarayana, 1982). The most valuable and commonly used part of the tamarind tree is the fruit. The pulp constitutes 30-50% of the ripe fruit (Purseglove, 1987; Shankaracharya, 1998), the shell and fibre account for 11-30% and the seed about 25-40% (Chapman, 1984). Tamarind has a low water content and a high level of protein, carbohydrates (60-72%) and minerals. The most outstanding characteristic of tamarind is its sweet acidic taste, the acid is mostly due to tartaric acid, ranging from 12.2-23.8%, and uncommon in other plant tissues (Ulrich, 1970).

Tamarind is a nutritious fruit with a variety of uses. Tamarind has many valuable properties and virtually every part of the tree has been utilised by both rural and urban dwellers. Tamarind is a good source of carbohydrates and protein. It also has small amounts of vitamins, carotene, Vitamin B and nicotinic acid. Tamarind fruit contains a biologically important source of mineral elements and with a high antioxidant capacity associated with high phenolic content. The acidic pulp is used as a favourite ingredient in culinary preparations (Dalziel, 1937; Eggeling and Dale, 1951; Little and Wadsworth, 1964).The tender leaves and flowers are used as vegetables.

Tamarind is widely grown as a subsistence crop for meeting local demands. It is also grown commercially. Numerous national programmmes have recognised tamarind as an underutilised crop with wider potential since demand for products is substantial and the species can be incorporated into agroforestry systems. In India, many farmers integrate several species, including tamarind, with their agricultural crops and livestock. The increasing integration of tamarind with other trees and crops on farmlands offers a strategy to minimise the risk of crop failure (Relwani, 1993).They are used as ornamental trees and to provide shade on the country roads and highways. There are also well established international trade channels. Further exploitation of tamarind can therefore provide added incomes for poor rural people thereby improving their well-being.

The legendary tamarind grove at Nallur, Devanahalli taluk, is believed to have its origin in the Chola dynasty which reigned over this region during early 13th century AD. Apart from its ancient nature it derives its importance from the very old and gigantic trees among nearly 300 Tamarind trees spread over an area of 53 acres .They are said to be nearly as old as the site itself and developed huge irregularly and crookedly branched crowns picturesquely spread out like eagles wings against a backdrop of glowing blue sky. The obvious geriantricity and peculiar trunk and crown formations not withstanding they amazingly remain active bearing flowers and fruits, though scarcely. Thus they provide wondrous sights and curious objects to onlookers. Additionally, the bulk of the population comprising old, middle and young aged trees along with clones (proxy mother trees), which have generally behaved normally have over the years accumulated a sizable intra-population variation of scientific and commercial significance. Apart from the traditional multipurpose utility of the tree species , it is the unusual combinations of gerianticity and heterogenicity which has made the Karnataka

Biodiversity Board to declare this grove as heritage Tamarind biodiversity site realising the fabulous heritage value of the site.

Tamarind is a highly cross-pollinated crop, hence wide variability is common in this species. The individual variation between the trees within a population is of paramount importance and it may be worthwhile concentrating only to the very best trees in relation to neighbouring ones and plus trees may be selected within ecological zones for increasing their frequencies. The magnitude of variability and its quantitative estimation for each character would indicate the potential of each tree and the scope for improving the desirable and economic characters through selection. It offers more avenues for establishing desirable clones by simple seedling selection. Plus trees or the elite trees selected for stem form, biomass, total yield, size and quality of fruits etc., are the starting point of any tree improvement activity. Selection of a large number of elite trees serves as an immediate source for clonal propagation, with fairly high genetic gain.

Genetic analysis based on phenotype is a function of the heritability of the trait, factors like the environment, multigenic and quantitative inheritance or partial and complete dominance often confound the expression of a genetic trait. Many of the complications of a phenotype-based assay can be mitigated through direct identification of genotype with a DNA- based diagnostic assay. For this reason, DNA – based genetic markers are being integrated into several plant systems and are expected to play an important role in the future of plant improvement. Hence, RAPD (Random Amplified Polymorphic DNA) assay, which detects nucleotide sequence polymorphisms in DNA amplification- based on assay using only a single primer of arbitrary nucleotide sequence if employed can give accurate results. In this reaction, a single species of primer binds to the genomic DNA at two different sites on opposite strands of the DNA templates. If these primers sites are within amplifiable distance thermocyclic

amplification, the presence of each amplification product identifies complete or partial nucleotide sequence homology, between the genomic DNA and the oligonucleotide primer, at each end of the amplified product. On an average , each primer will direct the amplification of several discrete loci in the genome, making the assay an efficient way to screen for nucleotide sequence polymorphisms between individuals. The major advantage of this assay is that there is no requirement for DNA sequence information. The protocol is also relatively quick and easy to perform and uses florescence in leiu 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.

Thus, considering the above information, the present investigations were carried out on this heritage site at the Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru, with the following specific objectives:

1. Estimation of genetic diversity based on morphological differences.
2. Establishing intra population genetic variation using RAPD markers.
3. Identification of elite trees based on the morphometric parameters.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Tamarinds are slow growing, long lived, evergreen trees that under optimum conditions can grow 80 feet high with a spread of 20 to 35 ft. The bright green, pinnate foliage is dense and feathery in appearance, making an attractive shade tree with an open branch structure. The inconspicuous, inch wide, five petaled flowers are borne in small racemes and are yellow with orange or red streaks (Radhamani *et. al.,* 1998). Fruit setting in tamarind is low, a condition quite common in tropical trees and normally it is an indicator of pollinator limitation (Bawa, 1974 ; Calvo, 1990). However, peculiar arrangement of anther makes pollinator attractive and promotes outcrossing under natural conditions (Harder and Barrett, 1993; Nilsson, 1998).

Although tamarind is an ancient domesticated tree, little attempt has been directed to its genetic improvement. This is understandable because tree improvement research that combines developmental and operational phases is time consuming and the large scale cultivation of tamarind is still in its early stages. Indigenous farmers have however selected planting materials from natural populations based on desirable and observable characteristics but such phenotypic selection cannot validate the variability present in the population without the knowledge of its genetic components.

A knowledge of the breeding systems and the inheritance of yield and associated characters is necessary in a successful breeding programme. Tamarind is a highly cross pollinated crop and a significant improvement is possible by selecting the plus trees and futher multiplication by clonal propagation. Wide variation for most of the attributes is observed in tamarind and there is scope for identification of divergent types (Von Mueller, 1881). Description based upon traits that reflect genetic variation can be used to

measure genetic diversity and can, therefore, be used to monitor and promote efficient conservation and utilisation of genetic diversity (Smith and Smith, 1991).

Since research work on variabilities given by qualitative and quantitative parameters and the genetic variation shown by using molecular markers is limited in tamarind, similar work done in other crops is reviewed for better understanding of the problem. The literature pertaining to the present investigation especially on the genetic diversity studies based on morphometric traits and DNA markers has been reviewed and compiled as mentioned below.

2.1 Origin and evolution

The origin and evolution of this species is still a subject of controversy (Lefevre, 1971; El-Siddig *et al.,* 1999; Grollier *et al.,* 1998), however, Coates-Palgrave (1988) claimed that it originated in Africa and was introduced into India at an early date, whereas Wunderlin (1998) and Poupon and Chauvin (1983) assumed that its origin is in Asia particularly in India, because of its appelation "Tamar hindi" which when translated means "Indian date" and from the species epithet *indica*, it was speculated upon the possible Indian origin. It is found throughout the tropics and subtropics and has been naturalized at many places. Troup (1921) placed it in Ethiopia, but others considered it indigenous to the drier savannahs of tropical Africa, from Sudan, Ethiopia, Kenya and Tanzania, westward through sub-Sahelian Africa to Senegal (Brandis, 1921; Ridley, 1922; Dalziel, 1937; Dale and Greenway, 1961; Irvine, 1961; NAS, 1979). Dry Savannas of tropical Africa is also considered to be the native and believed to have been introduced to Asia in ancient times by Arab traders.

2.2 Geographical Distribution

Tamarind occurs widely throughout tropical Africa, where it is frequently planted as a shade tree (Storrs, 1995). It is commonly found in woodlands, and is well adapted to the arid and semi-arid zones (Watt, 1893; Purseglove, 1987; Coronel, 1991; Hong *et al.*, 1996). Tamarind is now widely spread throughout semi-arid South and Southeast Asia (Gamble, 1922; Chaturvedi, 1985). It is presently cultivated in homegardens, farmlands and on roadsides.

In India, it is most commonly grown in the drier warmer areas of the South and Central region, where it thrives best, although it is planted as far North as Punjab, where the fruits do not ripen (NAS, 1979; Sozolnoki, 1985 and Coates-Palgrave, 1988). It is chiefly grown in Madhya Pradesh, Andhra Pradesh, Bihar, Tamil Nadu and Karnataka.

The tree is believed to have been introduced to Sri Lanka in pre-historic times (Watanabe and Dissanayake, 1999). It grows in the dry and intermediate zones, up to an elevation of about 600 m through natural regeneration or sometimes as a planted tree (Gunasena, 1999). Although a few small scale sweet tamarind orchards have been established in Thailand products are mostly collected from wild trees (Feungchan *et al.,* 1996). Tamarind is also well established in the dry areas of Fiji. It is believed to have been introduced by Indian labourers for culinary purposes, but it is presently grown as a shade tree (Jayaweera, 1981).

Patino (1969) states that the first reference to tamarind in the Americas is from Acapulco (Mexico) in 1615, suggesting that it may have arrived from Asia across the Pacific with the Spanish. Tamarind is now produced commercially in Mexico and is widespread in the states of Chiapas, Colima, Guerro, Jalisco, Oaxaca and Veracruz, covering an area of 4440 ha (10,000 acres). Tamarind is also found

growing throughout the Caribbean islands including Jamaica, Cuba, the Greater and Lesser Antilles and the Dominican Republic.

2.3 Taxonomy

Tamarind (*Tamarindus indica* L.) is a hardy evergreen monotypic tree which belongs to the family leguminosae. It belongs to the subfamily Caesalpinioideae which in turn has been variously divided into a number of tribes. Opinions are still divided on how many tribes there are, or indeed to which one *Tamarindus* belongs. Leonard (1957) included it in the Amherstieae (Pettigrew and Watson, 1977) which contains 25 genera. More recently it was included in the tribe Deterieae thought to be close to Amherstieae. Differences of opinion probably stemmed from the fact that morphological trends can occur independently in different groups of the Deterieae as suggested by Polhill and Raven (1981), this fact alone causing them to think that Amherstieae was not monophyletic.

2.4 Cytology

Tamarind is diploid (2n = 24 ; x = 12), belonging to the monotypic genus *Tamarindus* wherein *Tamarindus indica* is sole species (Anon, 1976 ; Purseglove,1987). There are no reports on existence of polyploids in tamarind.

2.5 Economic Importance

Tamarind is a nutritious fruit with a variety of uses. The properties of this species have been extensively studied, particularly with reference to the components of the seed. Tamarind has many valuable properties and virtually every part of the tree has been utilised by both rural and urban dwellers.

The most valuable and commonly used part of the tamarind tree is the fruit. The pulp constitutes 30-50% of the ripe fruit (Purseglove, 1987; Shankaracharya, 1998), the shell and fibre account for 11-30%

and the seed about 25-40% (Chapman, 1984). Tamarind has a low water content and a high level of protein, carbohydrates (60-72%) and minerals. The soluble solids content varies from 54-69.9 Brix (Benero *et al.*, 1974; Baragano de Mosqueda, 1980). The pulp contains oil, which is greenish in colour and liquid at room temperature. The saponification value of the oil is high but the iodine value is low.

Tamarind is used extensively in Tamil Nadu and Andhra Pradesh cuisines, where it is used to prepare Rasam, Sambhar, Puliyogare and Vatha Kuzhambu. It is also used in various types of chutneys as a flavouring agent. The tender pods and flowers are also pickled and used as a side dish. A traditional food plant in Africa, tamarind has potential to improve nutrition, boost food security, foster rural development and support sustainable landcare (Coates-Palgrave, 1988).

The pulp, leaves, and bark also have medical applications. For example, in the Philippines, the leaves have been traditionally used in herbal tea for reducing malaria fever. Tamarind is used as an Ayurvedic Medicine for gastric and/or digestion problems, cardioprotective activity (Jayaweera, 1981; Parrotta, 1990).

In temples, especially in Asian countries, the pulp is used to clean brass shrine furniture, removing dulling and the greenish patina that forms. The wood is a bold red color. Due to its density and durability, tamarind heartwood can be used in making furniture and wood flooring (El-Siddiq *et. al.,* 1999).

2.6 Genetic diversity studies using markers

The basic character of life is its unlimited diversity (Narian, 2000). No two individuals in sexually reproducing population are same. The underlying factor in this is diversity. The estimation of genetic diversity among different genotypes is the first and foremost

process in any plant breeding programme. For a plant breeder, reliable knowledge of the genetic diversity of his breeding material is important in order to select parents for a new breeding cycle. Genetic diversity is desirable for long term crop improvement and reduction to vulnerability to important crop pest and pathogens (Liu *et al.,* 2000).

The assessment of genetic diversity or genetic variation that may exist among a set or sets of germplasm reveals genetic and evolutionary relationships. Classical methods of estimating the genetic diversity among groups of plants have relied upon morphological characters. However , these characters can be influenced by environmental factors. Molecular markers avoid many of these complications by looking directly at the genetic material itself. Molecular markers, therefore, represent a powerful and potential rapid method for characterizing and managing plant germplasm, both *in situ* and *ex situ* (Virk *et al.,* 1995). These markers are appropriate for identifying useful genes within germplasm collections. Lynch (1998) proposed that the technique of DNA fingerprinting could be employed for the estimation of relatedness.

2.6.1 Morphological markers

Polymorphic and highly heritable morphological triats were some of the earliest genetic markers employed in scientific investigations (De Vries, 1912). They are the oldest and mostly wide used genetic marker and they may still be optimal for certain management practices of germplasm and cultivars where the cultivars have been identified on the basis of leaf, panicle, fruit and other physical characteristics. These characters however 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 various names (Lakshminarayana, 1980). The prime advantages of the morphological traits are simplicity and

fast, inexpensive assays, even from herbarium specimens and other dead tissues.

2.6.1.1 Variability studies in Tamarind (*Tamarindus indica*)

Thimmaraju *et al.* (1978) reported that *Tamarindus indica* L. is a highly cross pollinated tree and offers scope for selection of superior clones.

Sanjay *et al.* (2008) reported that in tamarind, a range of colouration of floral parts and the fruit colour dimorphism observed are of immense breeding value and can be effectively used as a morphological marker in progeny testing programmes.

Nagarajan *et al.* (1998) observed considerable vegetative and reproductive phenological variations studied in five Tamarind clones. Flowers showed strong entomophilous adaptations, open pollination, fruit setting was between 1% to 2%. Controlled pollinations indicate that tamarind is a predominantly out crossing species with extremely low level of selfing, apomixes was absent. Fruits showed colour dimorphism.

Samiullah (1984) assessed the variability in tamarind across three hundred genotypes and noticed wide variability for all the characters studied. The variations observed for the characters , viz., trunk length, trunk volume, pod yield, pulp yield, seed yield, etc., were significant.

Divakara (2008) reported improvement in the crop yield of tamarind depends on the progress of variability in the desired characters in the base material vis-a-vis germplasm collection. An evaluation of thirty – five genotypes of tamarind was carried out based on variability and character association in various pod traits as a scope for further productivity improvement.

Shivanandam *et al*. (1988) showed that the length of fruit was positively correlated with weight of fruit , pulp, seeds and number of seeds in four types of tamarind fruits. The fruit thickness was negatively correlated with fibre weight, seed weight and seed number. Since fruit length, weight and thickness are measures of fruit size, the larger the fruit the heavier is its pulp weight. Fruit size, therefore, can be the basis for the preliminary evaluation of a large number of tamarind trees.

Samiullah (1993) employed principal component analysis to study the genetic diversity for twenty yield and yield attributing characters across 300 genotypes of tamarind. The first 12 components accounted for 96.63 per cent of variation, while the first two principal components explained 42.37 per cent of variation. Pod yield per tree, trunk length and number of primary branches were the most important characters that contributed towards divergence.

Shivanandam (1980) recognised four types of tamarind based on fruit shape, viz., fruit straight and bulged, fruits straight and flattened, fruits curved and bulged, and fruits curved and flattened. Straight and bulged type fruits have shown more length (16.35 cm) compared to other types of fruits. Most of the samples studied recorded pulp with light red colour. Similarly, Hanamashetti in 1996 observed three different types of pod shape *viz.,* straight, semicurved and curved. Significant differences in length, width, thickness, weight, pulp weight per pod were also observed.

2.6.1.2 Other tree species

Abraham *et al.* (2006) found extensive range of variability in fruit colour, shape, size and nature of branching and canopy of trees from the fifty-six accessions of Malabar tamarind [*Garcinia cambogia* (Gaertn.) Desr.]. Characterisation of thirteen fruit and five seed characters was done for fifty-one accessions. The variability was found to be maximum for nipple length (74.8%) and minimum for fruit girth

(12.8%). Two promising accessions were identified based on mean fruit weight (161g) in IC 354028 and mean rind thickness (15mm) and mean rind weight (125g) in IC 354019.

Kumaran (1991) found significant variation between 28 (one percent) families of *Pongamia pinnata* in seed parameters in seed parameters and seedling traits. Study also showed, the genotypic coefficients of variation for basal diameter and volume were 21.82 and 49.74 respectively.

Singh and Choudhary (1992) obtained maximum variation for plant height followed by number of branches, base diameter, leaf breadth and leaf length among 28 families of *Prunus armeniaca.*

Srivastava *et al.* (1993) observed that eight years old *Terminalia arjuna* recorded the highest genotypic coefficient of variation for leaf yield followed by length of leaf and number of leaves per branch. On the contrary, maximum phenotypic coefficient of variation in leaf yield was followed by breadth of leaf, number of branches per plant and number of leaves per branch. The GCV values were lower in magnitude than PCV in all the seven characters studied except for length of leaf revealing that environment greatly influenced expression of these characters.

Falkenhages (1991) noticed no provenances differences for growth traits studied, viz., stem form, crown form, height and volume in pinus (*Pinus radiata*).

Teak (*Tectona grandis*) differed significantly for height and diameter of breast height (dbh) in provenance traits according to Suri (1984) and Krishnamoorthy (1989). Kedharanath (1986) conducted a survey and evaluated teak plantations in India and identified 700 plus trees.

Rajaram (1990) reported that, significant variation was found with reference to height, basal diameter, number of branches,

biomass, specific gravity and leaf nutrient content in *Gliricidia sepium* provenances.

Manaturagimath *et al.* (1991) observed significant variation in *Eucalyptus cloeziana* provenances for survival percentage, height, diameter at breast height (dbh) volume and mean annual increment.The variability study in *Eucalyptus camaldulensis* provenances showed significant variation for eight morphological characters of leaves (Burley *et al.* 1977). A one percent progeny test in *E. grandis* was assessed for plant height at four different ages (Kedharnath, 1982). It was noticed that there was a large magnitude of genetic variation in mean plant height between the families. Kapur and Dogra (1987) showed that provenances of *E. Camaldulensis* and *E. Tereticornis* varied significantly for the growth parameters viz., height, diameter and volume.

Clonal variation for growth and morphological traits was observed in *Populus deltoides* (Ying and Bagley, 1976). Jha *et al.* (1991) reported that when data on popular clones (*Populus deltoides* and *P.* xeuramericana) both exotic and indigenous were subjected to statistical analysis at half the rotation age the variation in height was insignificant, while in diameter and survival it was significant.

Half – sib seedlings belonging to eight Santalum album trees located at different places were quantitatively measured for nursery characters to assess variability and superiority in seedlings by Bagchi *et al.* (1987). The results revealed significant inter tree variability in another study (Bagchi and Veerendra, 1991). The variability of growth performances was observed after pruning two years old even-aged *Santalum album* plants. It was found that without host treatment expressed lower mean and variabilituy, whereas the with host conditions showed higher mean and variability. There was maximum variation in sandal (*Santalam album*) leaves within (intra) and between (inter) trees. Rao and Badami (1930) reported the foliar variations as

the important taxonomic character indicators that were discernible even at the seedling stage. Further, the variation in leaf length and area were subjected to biometrical analysis by Kulkarni and Srimathi (1982). Second degree quadratic equations were computed and based on these equations, the occurrence of six biotypes in sandal was confirmed. It was suggested that results of biometric analysis of leaf morphology would be helpful for delimiting different types of sandal. The length and width of leaf were significant between the trees studied except in a few plus trees, indicating that the trees would be genetically alike (Bagchi and Veerendra, 1985). It was concluded that they were governed by genetic factors based on the variation in the magnitude of standard error from tree to tree.

2.6.2 Molecular markers

The discovery of molecular markers in recent years has greatly enhanced the scope for detailed genetic analysis and approaches to improvement of crop plants (Tanksely, 1983). The DNA-based markers viz., Restriction Fragment Length Polymorphism (RFLP) and Randomly Amplified Polymorphic DNA (RAPD) provides excellent tools to study the genetic diversity, allows the elimination of duplicates in germplasm, study genetic relationships, gene tagging, genome mapping, PVR (Plant Varietal Rights) purposes etc. These markers measure diversity at DNA level and are seldom influenced by environmental conditions as is in case of morphological markers.

Markers based on differences in DNA sequence between individuals generally detect more polymorphism than morphological and protein based markers (Tanksley *et al.,* 1988). DNA markers because of their heritable nature were found to act versatile tools in the fields like taxonomy, physiology, embryology, genetic engineering etc. Recent advances in the field of molecular biology has provided tools such as DNA markers, which can detect differences in genetic information carried by two or more individuals. Such information is of

tremendous importance in forensic studies, paternity testing identifying genes responsible for disease, evolutionary linkage mapping, map based cloning, genetic diversity studies and so on. DNA based markers are superior to other markers since they are more in number, highly polymorphic and seldom influenced by the environment.

There are different types of DNA markers and many more are being discovered and find application in various aspects of crop improvement. Broadly they can be classified as a) hybridization based markers and b) PCR based markers. The former type of DNA markers involves the use of radioactive isotopes and is tedious viz., RFLP and hybridized SSR markers. PCR based markers are relatively easier, cheaper and more widely used viz., RAPD, AFLP, SSR, etc. In this review, an attempt has been made to explain some of the widely used DNA markers to estimate genetic diversity.

2.6.2.1 Randomly Amplified Polymorphic DNA (RAPD)

Among the different types of molecular markers used for genetic diversity analysis, Random Amplified Polymorphic DNA (RAPD) analysis is fast and these markers are easily generated by PCR (Williams *et al.,* 1990). RAPD was the first marker used for the analysis of coconut ecotypes in Philippines and Tanzania (Rodriguez *et al.,* 1997; Duran *et al.,* 1997) Single strands DNA of known sequences (primers) generally 10 base pair long are used to prime the polymerase reaction with *Taq* Polymerase, deoxy nucleotide triphosphates (dNTPs) to generate RAPDs. In case of RAPDs, each amplified product is derived from a region of the genome that contains two short segments which share sequence similarty to the single primer and which are on opposite strands and sufficiently close together for amplification to occur (100bp to 3000bp). These amplified products are resolved on agarose gel and visualized under ultra violet light after staining with ethidium bromide.

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., 1993). Because the RAPD technique is amplification-based assay, only nanogram quantities of DNA are required and automation is feasible.

2.6.2.2 RAPD Marker Studies in Tamarind (*Tamarindus indica*)

Boukary Ousmane Diallo *et al.* (2007) observed high value of intra population genetic variability from the studies of 10 population of *Tamarindus indica* using RAPD markers with the seeds collected from Asia (India and Thailand), Africa (Burkina Faso, Senegal, Kenya and Tanzania) and from three islands (Madagascar, Reunion and Guadeloupe).

Yang-Shi Yu (2008) employed RAPD to study the genetic variation and genetic relationship among 10 sweet tamarind (*Tamarindus indica*) cultivars introduced from Thailand. The RAPD analysis showed that 34 of 52 amplified bands (65.38%) were polymorphic bands within the species, while the mean percentage of polymorphic bands within cultivars was 25.96%. The largest proportion of the total RAPD diversity was found within, rather than among the cultivars. The genetic distance between cultivars ranged from 0.0131 to 0.1244, and on average was 0.047.

Anil kumar *et al.* (2006) determined the genetic diversity of tamarind from India and Thailand by morphological and RAPD analysis. A considerable genetic variability between the indigenous tamarind and that introduced from Thailand were observed at DNA level as well as in terms of fruit and seed shape and size. This variability may be used to properly assign the status as of a plant material as a sub-species/variety or cultivar.

2.6.2.3 Other tree species

Gemmas *et al.* (2000) performed RAPD analysis to characterize and to estimate genetic diversity among the three commercial varieties of *Olea europaea* L. viz., 'Galega Vulgar' (GV), 'Cordovil de Serpa' (CS) and 'Verdeal Alentejana' (VA). One hundred and fifty six amplified products generated using 20-decamer primers, were used to assess diversity among 28 trees of CS, 28 trees of GV and 22 VA trees. The dendrogram constructed clearly formed three distinct clusters for CS,GV and VA t among 28 trees of CS, 28 trees of GV and 22 VA trees. The dendrogram constructed clearly formed three distinct clusters for CS,GV and VA types.

Sheidai *et al.* (2007), studied RAPD marker variations in 11 olive cultivars collected from different parts of Iran. Seventeen RAPD primers out of 27 produced 610 bands in total. Some 444 bands were polymorphic bands (72.70%) and 166 bands were monomorphic (27.20%). Primer C05 produced the highest number of bands (56), while primer C03 produced the lowest number of bands (7). Specific bands were observed in some of the cultivars, which may be used in the cultivar discrimination. The highest value of similarity was observed between cultivars Kroneiki and Manzanila. Different clustering methods showed distinctness of the olive cultivars studied. The grouping of the cultivars did not correlate completely with their place of origin or fruit characteristics.

Seventeen olive cultivars, including oil and table olive cultivars originating from throughout the Mediterranean area, were screened using RAPD markers (Fabbri and Bergmanm., 1978). The results indicated a high degree of polymorphism and the analyses revealed two main groups in olive germplasm: the one comprising primarily small-fruited cultivars grown mainly for oil production and the other, characterized by having large fruit. There was no apparent clustering of olive cultivars according to their geographic origins.

Molecular characterization of common olive varieties in Israel and West Bank using RAPD markers revealed significant biodiversity was demonstrated among 'Nabali' olive trees growing along the Central mountain ridge of West Bank, suggesting that the grouping known as 'Nabali' is actually a mixture of genetically distinct variants (Weisman *et al.,* 1998). On the other hand, RAPD profiles of selected variants of 'Souri', cultivated mainly in the northern mountains of Israel, revealed a high degree of similarity, indicating that these variants represent environmental phenotypes of the same genome. Molecular differences were demonstrated between the 'Nabali' group variants and 'Souri

Russel *et al.* (2000) estimated the genetic differences in twenty-five Cocao accessions using RAPD. IMCs and PAs collected from Peru and LCTEENs collected from Ecuador were difficult to distinguish using morphological or biochemical descriptors however they could be fingerprinted using a minimum of three oligonucleotide primers. Analysis of the variability detected using RAPDs clearly discriminated the Cocao accessions between the geographical origins. Partitioning of variability within and between population components revealed that most variation was detected within a population.

A study of the genetic diversity in the coconut populations was made by Ashburner *et al.* (1997) by means of RAPD analysis on a representative sample from 17 distinct south pacific coconut populations to characterize the germplasm presenting the region. A moderate level of genetic diversity occurred within populations, but this level varied between the various populations. By this result they concluded that generally low but variable influence of gene migration between populations. Based on the cluster analysis they grouped the coconut populations in to Southern populations, a northern-eastern group, and single population from the Marquesas and Hawaii groups. Because of isolation and artificial selection Rennell island populations were grouped separately.

Anuradha *et al.* (2004) used RAPD markers to analyze genetic diversity and genetic relationship among coconut accessions. DNA from 81 palms representing 20 accessions, 15 Indian and 5 exotic, were amplified with 8 highly polymorphic primers. The 8 primers yielded 77 markers, with an average of 9.6 markers per primer. The within-accession genetic diversity ranged from 0.057 to 0.196. In general, tall accessions were more heterozygous as they had higher proportions of polymorphic bands and genetic diversity. The proportion of variation explained within accession and between accession diversity was 0.58 and 0.42, respectively. Similarly exotic accessions exhibited more variation. Dwarfs from geographically distant regions did not cluster separately. Based on the similarity matrix, cluster and principal coordinate analysis was performed. A dendrogram of genetic relationship was obtained.

Manimekalai *et al.,* (2007) evaluated the effectiveness of 456 RAPD primers based on polymorphism information content (PIC) and marker index (MI) in coconut. Among the RAPD primers, marker index ranged from 6.280 to 0.031. the primer OPBE 06 had the higher MI and accordingly other primers were ranked. Five , ten and fifteen primers were identified as informative primers. The dendrogram were constructed separately for informative primers and 45 primers using software NTSYS pc. The dendrogram obtained using 15 informative primers was comparable to that of 45 primers.

Yae *et al.* (1995) classified forty apple cultivars by RAPD analysis using 16 random primers. Of the 139 clear and reproducible bands, 106 were polymorphic. Form these polymorphisms the cultivars were divided into 6 groups by cluster analysis. Group I contained 'Rall's Janet', 'Fuji'; group II contained 'Sekaiichi', 'EarliBlaze', 'Delicious' and its bud mutations; group III contained only 'Indo; Gala', 'Mutsu', 'Jonagold' and their strains formed group IV; 'Jonathan', 'Jonared', 'Kogetsu' and 'Mollies Delicious' formed groupV and group VI contained only 'Spur Golden Delicious'.

Identification of 11 apple cultivars was done using RAPD markers by Koller *et al.* (1993). The variability of the technique and the origin of the DNA extract were analyzed. A set of bands consistent in their presence or absence was chosen to create a difference in banding pattern. A key is proposed by which one can differentiate apple cultivars using commercial available primers.

Cheng *et al.* (1996) identified co-dominant RAPD markers that are tightly linked to skin color of fruit in apple using bulked segregant analysis. Identified one 10-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 color. The three allelic DNA fragments (1160, 1180 and 1230 bp) that were associated with skin colour were sequenced and found to share high sequence homology, suggesting that these were generated from the same locus. 'Rome Beauty' X 'White Angel' populations, two fragments were associated red skin colour: one fragment designated as A1 (1160 bp) was from 'Rome Beauty' and another fragment A2 (1180 bp) was from 'White Angel'. Progeny possessing both fragments, and either of them had red fruits. Both parents displayed an alternate fragment, a1 (1230 bp), associated with yellow-skinned fruit. In three other crosses tested, only fragment A1 co-segregated with red skin colour and fragments, a1 and a2 (1230 and 1320 bp), were associated with yellow skin colour.

Karihaloo *et al.,* (2003) carried out random amplified polymorphic DNA analysis in 29 Indian mango cultivars comprising popular landraces and some advanced cultivars. PCR amplification with 24 primers generated 314 bands, 91.4% of which were polymorphic. Jaccard's similarity between pairs of cultivars ranged between 0.318 and 0.75 with a mean of 0.565. A UPGMA dendrogram showed the majority of the cultivars from northern and eastern regions of India clustering together and separate from southern and

western cultivars. Analysis of molecular variance revealed that 94.7% of the genetic diversity in mango existed within regions. However, differences among regions were significant; northern and eastern regions formed one zone and western and southern regions formed another zone of mango diversity in India.

Identification of cultivars and validation of genetic relationships in *Mangifera* L. was carried out using RAPD markers (Schnell *et al.,* 1995). Twenty-five accessions of mango were examined with 80 decamer random primers. Of the 80 primers screened, 33 did not amplify, 19 were monomorphic and 28 polymorphic for the study. The number of bands generated was primer and genotype dependent and ranged from one to ten. No primer gave unique banding pattern for each of the 25 accessions; however, ten different combinations of two primer banding patterns produced unique fingerprints for each accession. A maternal half-sib (MHS) family was included among the 25 accessions to see if genetic relationships could be detected. RAPD data were used to generate simple matching coefficients, which were analyzed phenotypically and by means of principal co-ordinate analysis (PCA). The MHS clustered together in both the phenotic and the PCA while the randomly selected accessions were scattered with no apparent pattern.

Hemanthkumar *et al.* (2001) screened fifty mango cultivars, chosen from different parts of South India to represent a wide range of geographical sources, by using RAPD markers with decamer primers of arbitrary sequence. In the cluster, alternate bearers and regular bearers formed separate groups and the members in each group were very closely linked. Another analysis based on Pearson's co-efficient of similarity revealed a high degree of genetic diversity. This study showed clearly that cultivars from South India possess a high degree of genetic diversity.

Pillay *et al.* (2001) evaluated genetic and phylogenetic relationship of 29 East African highland banana (*Musa ssp*.) cultivars and 2 among M. *acuminata* Calcutta-4 and Agbagba using RAPD markers. Analysis revealed narrow banana genetic base in East African highland germplasm. The major cluster in the dendrogram consisted of all AAA types with the two beer varieties (Isha and Ikigeregere) and two cooking varieties (Igisahira and Kibungo). The cultivars 'Calcutta 4' (AA), 'Kamaramas' (AB) and 'Kisubi' (AB) clustered separately from AAA types. Similar work was also initiated by Antoine and Johannes (2007) in 49 accessions of bananas from The National Banana Collection at Rubona using Random Amplified Polymorphic DNA markers. Fifteen random primers were selected for more detailed analysis. The genetic similarity was estimated using a simple matching coefficient which showed the lowest value of 0.46 between 'Ingumba' and 'Ishika' and the highest value of 0.85 between 'Kirayenda' and 'Inyabukuwe'. The data of matrix of coefficient of similarity was subjected to cluster analysis using the unweighted pair group method with arithmetic average (UPGMA). Each accession was clearly separated. The results of this study are important for the curation of the banana germplasm collection in Eastern Central Africa and for future breeding of this crop.

Damasco *et al.* (1996) detected dwarf off-types in micropropagated Cavendish (*Musa* spp. AAA) bananas using RAPD markers. A marker specific to the dwarf off-type from micropropagated Cavendish group cultivars 'New Guinea Cavendish' and ' Williams' was identified following an analysis of 57 normal and 59 dwarf plants generated from several micropropagated plants. Of 66 random decamer primers used in the initial screen, 28.8 per cent revealed polymorphism between normal and dwarf plants primer OPJ-4 (5'CCGAACACGG3') amplified a 1.5 kb band which was consistently present in normal but 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. Use of this marker could facilitate early detection and elimination of dwarfs from batches nof micropopagated bananas.

MirAli and Nabulsi (2003) used RAPD technology to study the genetic relationships between 19 almond cultivars grown at two gene banks in southern Syria (Izraa and Jillin). The 39 primers used in the study ranged in their amplification fragments between one (OP-I19 and OP-N20) and eight (OP-A20, OP-N14, OP-N16, OP-R16, and OP-Z17). The generated similarity matrix showed that the genetic diversity within the tested genotypes was limited (average similarity index=0.78). Similarity values among the studied genotypes ranged between 0.70 and 0.96. The resulting dendogram divided the cultivars into two clusters (at 0.77 similarity value) with two cultivars (ACSAD12 and Ferralise) as the most distant from the group (similarity value 0.75). The obtained clustering based on RAPD markers agreed to some extent with the geographical origin of the studied set of almond cultivars.

Ali Sarkhosh *et al.* (2009) studied soft-seeded pomegranate accessions using fruit morphological traits and DNA markers to reveal their relatedness. Thirty-six fruit characteristics were measured in these accessions together with applying 29 random decamer primers already reported to be polymorphic on pomegranate. Factor analysis on mean values of fruit characteristics determined 10 main factors and applied for grouping of the accessions using Ward's method. Also 14 of the random primers showed good amplification and polymorphism. Estimates of genetic similarity, using Jaccard's similarity coefficient, ranged from 0.13 to 1.0 using the RAPD data. Grouping based on the fruit traits compared with that based on RAPD data did not produce a significant correlation ($r = -0.36$). This study showed that information based on fruit characteristics and RAPD

markers are complementary for genetic discrimination in soft-seed pomegranate accessions.

The genetic diversity among Tunisian pomegranate cultivars has been investigated by Nejib *et. al.,* (2010). RAPD method was used to generate banding profiles from a set of twelve cultivars. 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. In spite of the relatively low number of primers and cultivars, RAPD constitutes an appropriate procedure to assess the genetic diversity and to survey the phylogenetic relationships in this crop.

Sarkhosh *et al.* (2006) used RAPD markers to determine the diversity level among 24 Iranian pomegranate genotypes. One hundred decamer random primers were used for PCR reactions, among which 16 showed reliable polymorphic patterns. These primers produced 178 bands, of which 102 were polymorphic. Cluster analysis of the genotypes revealed the highest and lowest similarities between genotypes as 0.89 and 0.29, respectively. At a similarity of 60%, the genotypes were divided into four sub-clusters. Cophenetic correlation coefficient between similarity matrix and cophenetic matrix of dendrogram was relatively high (r = 0.9) showing the goodness of fit of the dendrogram. RAPD markers showed to be a useful tool for studying the genetic diversity of pomegranate.

Zhongping Cheng (2007) amplified DNAs of 180 accessions in 10 demes in *Prunus persica* with twenty-two, 10-base primers selected from 200 arbitrary primers using Randomly Amplified Polymorphic DNA (RAPD) technology. One hundred and eighty loci were observed and recorded. With statistical analyses of the data from the study, genetic diversity of the demes was expressed as follow: yellow peach group > honey peach group > flat peach group > red leaf peach

group > crisp peach group > bitao group and juicy peach group > nectarine group > shouxingtao group > weeping peach group. Genetic variations among and within groups by AMOVA analyses were 11.9, 88.1%, respectively. Demes clustered by UPGMA modified from NEIGHBOR procedure of PHYLIP Version 3.5, the edible peaches of which were combined as a section, while the ornamental species were classified into separate sections. Through analyses of genetic diversity and genetic structure, the results could provide molecular biological evidence for conservation and utilization of *P. persica* germplasm.

Casas *et al.* (1999) used RAPD markers to characterize *Prunus* rootstocks from different species, both commercial, and selected clones from the breeding program at Aula Dei Experimental Station (Zaragoza, Spain). Molecular markers were used to study the genetic variation among different species, and within species. Forty one genotypes were used in this study. They included *P. amygdalo-persica*, and *P. persica* × *P. davidiana* hybrids; *P. cerasifera, P. domestica*, and *P. insititia* clones, and other diverse interspecific hybrids, which were divided in three groups according to postulated taxonomic classification. Diversity patterns obtained from 80 RAPD primers were evaluated in a representative subset of genotypes. This screening helped to identify 7 RAPD primers that were selected to produce a combined classification of the whole set of rootstock clones. This analysis successfully clustered rootstocks according to the classification scheme widely used to characterize *Prunus* clones, mainly based on morphological descriptors. Further than that, it supported the alleged origin of some interspecific materials, and confirmed a case of possible misclassification ('Myrobalan 29 C'). A more thorough diversity analysis was conducted within each group of materials, using larger sets of primers (12–14). After this analysis, disjointed clusters were formed for *P. amygdalo-persica* and *P. persica* × *P. davidiana* hybrids in one group, and for Myrobalan (*P. cerasifera*) and Marianna (*P. cerasifera* × *P. munsoniana*) plums in another group.

P. insititia and *P. domestica* clones, however, formed a jumbled cluster, possibly due to genetic interchange among them during their domestication and breeding history.

Eighteen peach (*Prunus persica*) rootstock cultivars were screened for RAPD markers using 80 decamer oligonucleotide primers (Xiang et al., 1996). Based on combined banding patterns, all the18-rootstock cultivars were identified with only six of the 80 primers. Cluster analysis of the 18 rootstocks using 40 RAPD markers produced a dendrogram of genetic relatedness and is in agreement with their putative pedigrees and the data also revealed bifurcation of rootstock into two groups that correlated with their resistance or susceptibility to root knot nematodes (*Meloidogyne incognita* and *Mjavanica javanica*).

Machado *et al.,* (1996) carried out RAPD analysis to evaluate polymorphism and genetic similarity between 39 Mediterranean mandarin genotypes. One hundred and eleven amplification products were identified using 21 random primers. An average of 2.2 RAPD markers was obtained for each primer, corresponding to 42 percent of the amplification products. UPGMA cluster analysis revealed the low level of genetic variation between accessions of Mediterranean mandarins, whereas their hybrids with other Citrus species showed greater genetic dissimilarity.

Amel *et al.,* (2006) studied the genetic diversity in Tunisian fig (Ficus carica L.) using RAPD markers. Thirtyfive fig cultivars originating from diverse geographical areas and belonging to three collections were analysed. Random decamer primers were screened to assess their ability to detect polymorphisms in this crop. Fortyfour RAPD markers were revealed and used to survey the genetic diversity and to detect cases of mislabelling. As a result, considerable genetic diversity was detected among the studied F. carica accessions. The relationships among the 35 varieties were studied by cluster analysis.

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. Our data proved that RAPD markers are useful for germplasm discrimination as well as for investigation of patterns of variation in fig. Since this designed procedure has permitted to establish a molecular database of the reference collections, the opportunity of this study is discussed in relation to the improvement and rational management of fig germplasm.

Hassan *et al.,* (1998) studied genetic variation among 43 date palm (*Phoenix dactylifera* L.) accessions, including 37 accessions from Morocco and 6 cultivars from Iraq and Tunisia, using Random Amplified Polymorphic DNA (RAPD) markers. The pre-screening of 123 primers on four genotypes allowed selection of 19 primers which revealed polymorphism and gave reproducible results. All 43 analysed genotypes were distinguishable by their band patterns. RAPD technology therefore appears very effective for identifying accessions of date palm. RAPD-based genetic distance was used to determine the relationships between the accessions.

Feng *et al.* (1997) studied relationships among species in *Morus* L. using RAPD markers. Relationships among operational taxonomic units (12 species and 2 varieties) of *Morus* were examined with 20 random 10-mer primer, generating 238 polymorphic RAPD bands. According to dendrogram constructed using Nei's genetic similarity values and an UPGMA programme, *M. notabilis* was far removed from the other species, indicating its unique differentiation. All groups were identical with morphological classifications, indicating the usefulness of RAPD for systematic studies.

Cai *et al.* (2007) analyzed RAPD variation among eight cherry species and two interspecific progenies. Fourty eight arbitrary

oligonucleotide primers were used for amplifiction. The phylogenetic analysis was carried out using two distance-matrix methods and a dendrogram was generated to show the relationships among species and cultivars. The results showed that there were 840 amplified loci in total; 23 sweet cherry and four sour cherry cultivars were clustered together with 569 and 247 polymorphic loci respectively which accounted for 67.74% and 29.40% of the total variation. Using these specific markers, cherry species and varieties could be identified and there is therefore the potential to select for good characteristics of hybrids at an early stage.

Samal *et al.,* (2003) described genetic relationships of twenty varieties of cashew on the basis of morphological characters and RAPD markers. Results obtained for the phenotypic characters based on similarity coefficient were divided into four clusters with 70% similarity. By means of similarity coefficients (SG), cluster I was found to consist of twelve varieties. Cluster II consisted of a single variety, NRCC-1, cluster III consisted of six varieties and cluster IV had only one variety, Vridhachalam-2. Cluster analysis clearly showed that 20 varieties of cashew grouped into two major clusters based on similarity indices. The first major cluster comprised one minor cluster. The other major cluster was divided into two sub-minor clusters, one sub-minor cluster having three varieties and the other sub-minor cluster was represented by 15 varieties. The analysis of genetic relationships in cashew using morphological traits and RAPD banding data can be useful for plant improvement, descriptions of new varieties and also for assessment of variety purity in plant certification programmes.

Shen-YongBao *et al.,* (2004) studied the genetic diversity of, Castanea heyyi and Castanea sequinii using RAPD DNA marker, and made a comparison with varieties of Chinese chestnut from all over the country. The results showed the percent polymorphic loci of C. heyyi (44.9%) was lower remarkably than that of C. sequinii (53.7%)

and C. mollissima (52.7%). The further analysis on their effective allele amounts ranged from 1.608 8-1.621 6, Nei's gene diversity ranged from 0.348 0-0.352 9 and Shannon information index ranged from 0.517 9-0.535 7, indicated higher genetic diversity in these three species.

Oliveira *et al.* (1999) investigated molecular characterisation and phenetic similarities between several cultivars of P. *communis* and P. *pyrifolia*, and genotypes of P. *cordata*, P. *bourgaeana* and P. *pyraster* through RAPD markers. Sixty decamer primers were screened, generating polymorphic patterns of Occidental and Oriental pear genotypes. Twenty-two selected primers originated clear and reproducible patterns, produced a total of 358 bands, 327 of them polymorphic. For 10 of the 12 genotypes analysed it was possible to find genotype-specific RAPDs and fragment patterns which could be used for cultivar identification. The patterns distinguished between genotypes and their analysis established a first approach to phenetic classification within the Pyrus genus based on DNA markers, clustering the genotypes according to their geographic origin. RAPD analysis of in vitro and in vivo material of seven cultivars was also performed, resulting in identical patterns for each genotype.

Kim *et al.,* (2005) evaluated the phylogenetic relationships among *Pyrus pyrifolia* and *Pyrus communis* were evaluated using RAPD and the conserved rDNA sequences. The patterns observed distinguished between genotypes and their analysis established the approach to phenetic classification within the *Pyrus* genus based on DNA markers, clustering the genotypes according to their geographic origin. In RAPD analysis, UPGMA separated the cultivars into two main groups; 19 *P. pyrifolia* cultivars and 6 *P. communis* cultivars. On the other hand, the conserved 18S rDNA nucleotide sequences of the amplified fragment were completely determined. In this study, the phenogram resulting from the Neighbor-Joining analysis separated the cultivars into four main groups. The first group included 18 *P.*

pyrifolia cultivars and the second cluster comprises 5 *P. communis* cultivars. 'Sunwhang', which belonged to *P. pyrifolia*, was excluded from the first group and giving rise to the third group. 'Conference', *P. communis* cultivar, made up a forth group.

Jia-YanLi *et al.* (2007) used random amplified polymorphic DNA (RAPD) polymorphism to evaluate DNA polymorphism among 21 pear (*Pyrus pyrifolia*, P. *bretschneideri,* P. *communis* and P. *ussuriensis*) cultivars. Thirty-three of the 40 primers were highly polymorphic. Some 339 DNA alleles were amplified, among which 292 were polymorphic (86.1% polymorphism). The genetic similarity coefficient and cluster analysis by unweighted pair group method with arithmetic averages revealed significant diversity among the cultivars.

The effectiveness of RAPD analysis for cultivar identification of persimmons (*Diospyors kaaki*) was evaluated by using 10 base primers. Among 20 primers, two (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', 'Tonewawe' and 'Sugitawase', showed different DNA patterns with a few additional minor bands using OPA-6 primer.

Levi and Rowland (1997) identified blueberry cultivars and evaluated their genetic relationships using RAPD and simple sequence repeat (SSR)- anchored primers. Fifteen high bush (or high bush hybrid) blueberry cultivars (*Vaccinium corymbosum*)., two rabbit eye blueberry cultivars (*V.ashei*) and one southern low bush (*V. darrowi*) selection from the wild were examined using seventeen 10-base RAPD and seven 15 to 18-base SSR-anchored primers. Fifteen RAPD and three SSR markers resulting from these reactions were chosen to construct a DNA fingerprinting table to distinguish among genotypes including in this study. Similarly values were calculated based on 132 RAPD and 51 SSR bands and a dendrogram was constructed based on

the similarity matrix. The *V. ashei* cultivars and the *V. darrowi* selection grouped out separately from the *V. corymbosum* cultivars as expected. However, estimates of relative genetic similarity between genotypes within the *V. corymbosum* group did not agree well with known pedigree data and thus, indicated that RAPD and SSR data did not accurately assess the genetic relationships of cultivars within the species.

Identification of avocado cultivars with RAPD markers using 'Hass', 'Fuerte' and 'Edranol' was carried out by Lewis, (1992) and results suggested that 'Fuerte' and 'Edranol' were found to be closely related.

Ten arbitrary selected primers were adopted for RAPD assay of peach (*Prunus persica*), plum (*P. glandulosa*), *P. mume* and apricot (*P. armeniaca*). The data obtained indicated that the relationship between *P. mume* and apricot is closer than between plum and peach (Gao-Zhang *et al.,* 2001).

RAPD-PCR was used by Pan-XinFa (2002) to determine the DNA polymorphism of 16 loquat cultivars. Two of the used primers could amplify DNA fragments in 16 cultivars. Nineteen DNA fragments were amplified in 16 cultivars by the two primers. Three fragments were common and 16 were polymorphic or unique, indicating rich genetic diversity in the loquat cultivars. The results of DNA fingerprinting showed that 16 cultivars could be distinguished from each other.

Prakash *et al.* (2002) used RAPD markers to estimate molecular diversity of 41 genotypes of guava consisting of five *Psidium* species, 23 varieties, 12 selections and a hybrid. The genetic dissimilarity matrix calculated based on Squared Euclidean Distance revealed maximum genetic distance of 54 per cent between the variety Mirjapur Seedling (*P. guajava*) and *P. quadrangularis*, while the

minimum distance was only 11 per cent between SWY-1 and GR-1 Navalur selections. All the individuals on the dendrogram were grouped into two major clusters according to their geographical locations and species. The studies showed that the genetic base of Indian guava can be rated as low to moderate diversity and also indicated that various triploid seedless cultivars of guava are not genetically identical and have independent origins.

Devanshi *et al.,* (2007) studied the genetic relationship with RAPD markers among 50 ber genotypes representing *Z. mauritiana, Z. nummularia* and *Z. spina-christi.* Fourty six highly reproducible primers generated 368 RAPD markers with 86.2% polymorphism (316 polymorphic bands). The number of amplification product per primer ranged from 2 (OPF-9) to 17 (OPD-3) with an average of 8 bands per primer. Nineteen primers distinguished atleast one genotype that would prove to be highly useful for identification of genotype and designing future breeding strategy. Genetic relationships between the accessions were established based on Jacquard's similarity coefficient and it ranged from 26.3% to 78.9% suggesting that *Ziziphus* germplasm is genetically diverse. UPGMA cluster analysis generated dendrogram with six clusters seperatin two wild genotypes, *Z. nummularia* (collection 1) and *Z. spina-christi* from rest of the genotypes. Cluster-I and II comprised of two genotypes each, whereas, the biggest cluster, cluster VI comprised of 20 out of remaing 44 genotypes. In cluster II to VI, genotypes were separated from each other at different similarity levels in successive branching. The degree of genetic variation detected in *Ziziphus* species with RAPD analysis in the present study suggests that it is an efficient marker technology for delineating genetic relationships among genotypes and estimating genetic diversity, thereby enabling the formulation of appropriate strategy for conservation and improvement programmes.

2.7 Identification of elite trees

Prabhushankar *et al.* (2004) evaluated fifteen different tamarind clones based on the parameters pertaining to yield components and quality parameters and the study revealed wide variation for fruit yield, pulp weight, shell weight, fiber weight, seed weight, titratable acidity and total soluble solids studied among the clones under studied. The study find out the promising genotypes from the population with respect to excellent pulp recovery, higher acidity content and fruit yield per tree.

Fartais *et al.,* (2009) studied the percentage of the elite (*plus*) trees in Norway Spruce and Silver fir seed reservations. Such samples are usually found in the dominant forestry floor, exceeding the neighbouring trees by rapidity of growth, trunk straightness and increased resistance against pests and diseases. The total number of elite (*plus*) trees in the three testing areas was of 19 samples, 16 of them belonging to Norway Spruce and three to Silver fir trees. The total number of inventoried trees was 66 (57 Norway Spruce and nine Silver fir trees). All the elite trees belonged to the first quality class. The results of this study have shown that the analysed arboretum could be considered as an important seeds and cuttings purveyor in the afforestation activity, as well as for the conservation of the most valuable forest genetic resources.

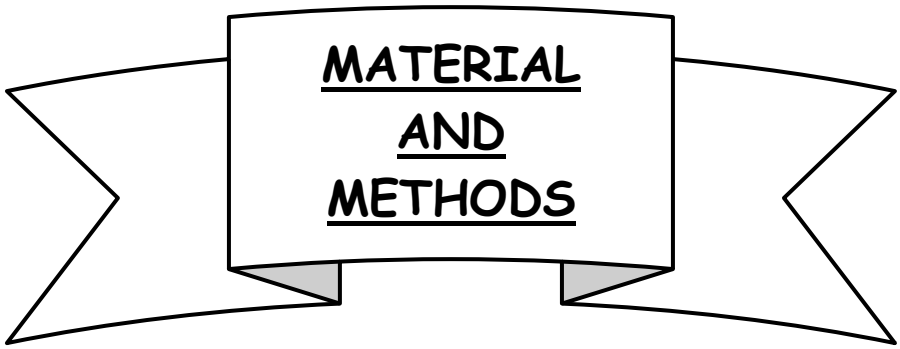
Yang Kai *et al.,* (2008) investigated and measured the fruit-bearing quantities of nut Korean pines (*Pinus Koraiensis*) of natural stands in Changbai Mountain, Xiaoxing'an Mountain, and Wanda Mountain and of artificial forest in Hegang area of Heilongjiang Province by seed collection of singletree during 1988–1998. In order to evaluate the elite nut tree of fructification, the characteristics of fructification of Korean pine, including, the fruit-bearing quantity, output of seed, quantity of cone, cone size, seed size, the ratio of null seed by solid seed, seed percentage of cone, rate of the cones infested

with pest, and fruit-bearing index, etc., were analyzed with the variance analysis, multiple comparison and stepwise regression to obtain the contribution ratio of each fruit-bearing factor to fruit-bearing quantity. The multiple correlation factors and the partial correlation factors for fruit-bearing quantities of Korean pine were determined for different geographical areas, and the cone length, thousand-grain-weight, and the seed percentage of cone were considered as important indices for selection of elite trees.

Harold (1955) used the term high quality in the sense usually employed by both foresters and laymen. That is, fast-growing, vigorous, pest-resistant trees with long, straight, clear boles, free of all visible defects. In the interest of breeding truly elite trees, however, it should be expanded to include the basic anatomical and physical properties need to be included to determine the suitability of wood for a given use. Properties like the density of the wood, the proportion of summerwood to springwood in the annual rings, the number of rings per inch, the length and thickness of the wood fibers, the orientation of the fibrils with respect to the axis of the fibers, and the occurrence of compression wood or other abnormalities that affect strength, shrinkage, warpage, or pulp quality and yields are of importance.

Carolyn *et al.,* (2004) focused on early growth and survival of *E. globulus and E. nitens* as these directly influenced the success of the plantation. As the trees grew, other traits relating to tree shape and size, such as stem straightness and branching quality, were included. Following this, more attention was paid to what may be called risk traits, those relating to the ability of genotypes to survive environmental threats such as winter frosts, browsing animals or insects, salinity or drought. Risk traits have attached both a probability of occurrence and a probability of severity. Later still, attention turned to the quality traits and assessed large numbers of individual trees and families for traits of economic importance.

Saadat *et al.,* (2001) studied to identify elite trees of Persian walnut (*Juglans regi*a L) in Fars province. The Persian walnut populations were evaluated during 1993 and 1994 and 101 elite trees selected in different areas. We found that considerable generic variability existed within the populations of Persian walnut grown from seeds. The most important characteristics of trees which were evaluated were lateral bud flowering, vigor, tree shape and nut qualities: including the morphology of nut, the average weight of nut, the average weight of shell, the average of kernel percentage, kernel quality and the average length and diameter of nut. Some elite trees with high quality of nuts were identified that could be recommended for mass propagation.


MATERIAL
AND
METHODS

III. MATERIAL AND METHODS

The present study was carried out at the Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru on the Nallur tamarind biodiversity heritage site, which is a few kilometres from Bengaluru International Airport occupying an area of 53 acres.

3.1 Plant Materials

The plant materials for both the phenotypic as well as the molecular studies were collected from the tamarind grove at Nallur; Devanahalli taluk. The study was carried out on a 100 randomly selected trees consisting of very old trees, middle and young aged trees which were as follows:

1. Tree 1
2. Tree 5
3. Tree 6
4. Tree 9
5. Tree 12
6. Tree 14
7. Tree 15
8. Tree 16
9. Tree 18
10. Tree 20
11. Tree 21
12. Tree 23
13. Tree 26
14. Tree 30
15. Tree 31
16. Tree 33
17. Tree 39
18. Tree 41
19. Tree 44
20. Tree 47
21. Tree 49
22. Tree 50
23. Tree 52
24. Tree 55
25. Tree 56
26. Tree 58
27. Tree 60
28. Tree 61
29. Tree 64
30. Tree 65
31. Tree 69
32. Tree 71
33. Tree 74
34. Tree 75
35. Tree 78
36. Tree 80
37. Tree 81
38. Tree 82
39. Tree 84
40. Tree 85
41. Tree 92
42. Tree 94
43. Tree 95
44. Tree 98
45. Tree 100
46. Tree 102
47. Tree 104
48. Tree 106
49. Tree 109
50. Tree 110
51. Tree 112
52. Tree 114
53. Tree 117
54. Tree 119
55. Tree 120
56. Tree 121
57. Tree 124
58. Tree 125
59. Tree 130
60. Tree 131

61. Tree 134
62. Tree 137
63. Tree 140
64. Tree 143
65. Tree 149
66. Tree 153
67. Tree 155
68. Tree 157
69. Tree 159
70. Tree 161
71. Tree 162
72. Tree 163
73. Tree 167
74. Tree 169
75. Tree 172
76. Tree 173
77. Tree 174
78. Tree 176
79. Tree 178
80. Tree 181
81. Tree 183
82. Tree 186
83. Tree 192
84. Tree 194
85. Tree 197
86. Tree 201
87. Tree 204
88. Tree 206
89. Tree 208
90. Tree 210
91. Tree 212
92. Tree 217
93. Tree 221
94. Tree 230
95. Tree 238
96. Tree 240
97. Tree 243
98. Tree 244
99. Tree 244
100. Tree 246

3.2 Phenotypic Studies

3.2.1 Phenological studies of qualitative parameters:

Phenological studies of the trees studied at vegetative and reproductive phase of June-July 2009 and the scoring given for analysis are represented in Table: 1.

3.2.2 Studies on quantitative parameters:

The observations taken for quantitative parameters during October - November 2009 from twenty pods per branch per tree were as follows :

1. Pod length - Measured in cm from tip of the fruit to the point of attachment of the fruit to the stalk.

2. Pod width - Width of the pod measured with the help of slide callipers and expressed in cm.

3. Beak length - Length of the tip portion of the fruit measured in cm.

4. Number of ridges - Number of raised regions present per fruit.

5. Number of furrows - Number of grooved regions present per fruit.

6. Fruit weight - Weight of whole fruit measured in gm.

7. Epicarp weight - Weight of outer cover of the fruit measured in gm.

8. Fibre number - Total number of fibres present per fruit.

9. Fibre weight - Weight of the fibres present per fruit measured in gm.

10. Pulp weight - Weight of pulp per fruit measured in gm.

11. Seed weight - Weight of the seeds present per fruit measured in gm.

12. Seed number - Total number of seeds present per fruit.

3.2.3 Statistical analysis

One way analysis of variance was carried out along with a tree joining dendrogram to cluster the genotypes based on their metric traits.

Cluster Analysis: A tree joining dendrogram was constructed to indicate the relative clustering of genotypes based on their qualitative traits. The analysis was done following the STATISTICA software package programme.

Table 1: Diagnostic qualitative characters, their classes and scores used for classifying the genotypes.

Sl. No.	Characteristics	Classes	Scores
1.	Crown shape	Cone	1
		Dome	2
		Semicircle	3
		Oval	4
		Circle	5
		Irregular	6
2.	Foliage characteristics	Dense & dark green	1
		Dense & light green	2
		Sparse & light green	3
		Sparse & dark green	4
3.	Trunk characters	Monotrunk	1
		Double trunk	2
		Multiple trunk	3
4.	Bearing habit	Sparse	1
		Medium	2
		Dense	3
5.	Bract colour	Pale green	1
		Pale yellow	2
		Yellow	3
		Pale pink	4
		Pink	5
		Deep pink	6
6.	Bud colour	Pale green	1
		Yellow	2
		Pink	3

Sl. No.	Characteristics	Classes	Scores
7.	Petal colour	Pale yellow with red streak	1
		Yellow with red streak	2
		Orange yellow with red streak	3
		Orange red with red streak	4
8.	Sepal colour	Lemon chiffon	1
		Pale yellow	2
		Yellow	3
		Gold	4
9.	Anther colour	Dark orange	1
		Deep pink	2
		Reddish brown	3
		Deep red	4
10.	Pod colour	Light Brown	1
		Dark Brown	2
11.	Fruit shape	Straight	1
		Curve	2
12.	Pulp colour	Red brown	1
		Light brown	2
13.	Seed shape	Flat	1
14.	Seed colour	Glassy black	1
		Light brown	2

3.3 RAPD Experiment:

3.3.1 Leaf materials

The leaf samples of all the 100 genotypes studied were collected from the Nallur tamarind grove. The nearly matured and disease free healthy leaves were selected for isolation of DNA. The collected leaves from each trees were sealed in a plastic cover and put in thermo cool bag containing ice cubes. Later in the laboratory the leaves were stored at -20ºC for further use.

3.3.2 Genomic DNA Isolation

3.3.2.1. Sample Preparation

The leaflets were separated carefully from the pinnately compound leaves and were washed thoroughly with distilled water to remove any dirt present on the leaves. The leaves were then air dried properly at normal room temperature.

3.3.2.2. Extraction Protocol

DNA of all the 100 samples were extracted using modified Della Porta *et. al.,*(1983) method. The protocol followed were as follows:

1. Two grams of the leaf samples were ground with liquid nitrogen and add 0.6gm Polyvinyl pyrollidone (PVP). To this ground tissue, 12 ml of extraction buffer (containing 6 % CTAB, 1M Tris, 0.5M EDTA and 5M NaCl) which was pre- heated at 65ºC was added.

2. The tube containing the buffer and powdered leaf tissue were added with 120µl β- mercaptoethanol. The contents were mixed slowly and incubated for 1 hr in water bath at 65ºC with intermittent shaking.

3. It was brought to room temperature and then centrifuged for 10 minutes at 8000 rpm at 4ºC.

4. The supernatant was transferred to a fresh tube slowly and the same volume of chloroform isoamyl alcohol (24:1) was added and again centrifuged for 10 minutes at 8000 rpm at 4°C.

5. The clear supernatant was again transferred to another fresh tube and 1ml NaCl and 7ml ice cold isopropanol were added. In case a clear supernatant is not obtained step 4 is repeated by taking half volume of chloroform isoamyl (24:1).

6. The tube containing the supernatant was shaken slowly and kept at -20°C for 1 hr or at 4°C overnight.

7. The tube is then kept at room temperature for 10 minutes and centrifuged at 10,000 rpm for 20 minutes.

8. The supernatant is discarded slowly except the pellet found in the lower part of the tube. In the tube containing the pellet 1000µl of 70 percent ethanol was added and centrifuge at 2000 rpm for 5 minutes.

9. Pipette out the ethanol from the tube and air dry the pellet for 10 minutes.

10. The pellet is then dissolved in 500µl TE buffer and kept at 4°C for 3-4 hrs.

3.3.2.3. Quantification of DNA

Spectrophotometer was first turned for 20 minutes to stabilize the equipment. 3 ml of TE buffer was taken in quartz cuvette and placed in the reference sample and the equipment was autozeroed. 10 µl of DNA sample in 990 µl of TE buffer (1X) was taken in another cuvette and mixed well. This was placed in the main reading slot and the absorbance at 260 nm and 280 nm was measured. Using the relationship, 1 OD at A_{260} = 50 µg/ml, the DNA concentration/µl and concentration/ml was calculated. The ratio of A_{260}/A_{280} was also calculated to check the purity of the sample. With a pure sample the

ratio of absorbance at 260 nm and 280 nm i.e., OD_{260}/OD_{280} will be 1.8.

3.3.2.3. Quality Checking of Genomic DNA

The quality of the genomic DNA was verified by electrophoresis on 0.8 percent agarose gel.1X TE buffer was used as a running buffer for electrophoresis and for preparing gels. Wells were loaded with 7 µl DNA and 3 µl of loading dye (Bromophenol blue) together. Electrophoresis was conducted at 45-50 V for 1- 2 hours and the DNA band was checked using UV transilluminator.

3.3.3. Random Amplified Polymorphic DNA (RAPD) Analysis

RAPD analysis was performed following the method recommended by Williams *et al.* (1990) with required modification. A total of eight random primers with their sequences selected from peer reviewed literature were shown in Table: 2.

Table 2: Eight random primers used for screening.

Name of primer	Sequence of primer (5' to 3')
OPA-A09	GGGTAACGCC
OPA-B06	TGCTCTGCCC
OPA-K06	CACCTTTCCC
OPA-R15	GGACAACGAG
OPA-X01	CTGGGCACGA
OPA-Y01	GTGGCATCTC
OPA-K17	CCAGCTGTG
OPA-WO1	CTCAGTGTCC

Genomic DNA (30 ng) was amplified in vitro in a 20 µl reaction mixture containing 3mM Magnesium Chloride, 0.2 mM dNTPs, 10pm random primer, 1 unit of *Taq* polymerase with 1X *Taq* assay buffer. Reaction mix was overlaid with mineral oil and placed in thermo cycler programmed for the following set of of conditions.

Profile 1 : Initial Denaturation - 94ºC for 4 minutes

Profile 2 : Denaturation - 94ºC for 1 minutes

Profile 3 : Annealing - 34ºC for 1 minutes

Profile 4 : Extension - 72ºC for 3 minutes

Profile 5 : Final elongation - 72ºC for 5 minutes

Profile 2, 3 and 4 were programmed to run for fourty cycles and a final hold at 4ºC.

3.3.3.1 Agarose Gel Electrophoresis

Amplification products were resolved by electrophoresing on a 1.5 % agarose gel containing ethidium bromide (0.5µg/ml) using 1X TBE buffer. 20µl of PCR products were mixed with 4µl of loading buffer and applied on the agarose gel. Electrophoresis was carried out at a constant voltage of 50-80 V for 1-2 hr. The gels were visualized under UV light and documented using gel documentation unit.

3.4 Data analysis

3.4.1 Scoring and analysis

Amplification profiles of the primers were compared with each other and bands of DNA fragments were scored as present (1) or absent (0). Only clear and unambiguous bands were taken into account and the bands were not scored if they were faint or diffuse. The band were determined by comparing with the 100 bp DNA ladder marker, which was run along with the amplified products.

3.4.2 Statistical analysis and estimating genetic distances

The scored band data was subjected to statistical analysis using the computer programme 'STATISTICA'. The cluster analysis on genotypes was based on dissimilarity measure Squared Euclidean Distances (SED). This gives the geometric distances in the multidimensional space. The Squared Euclidean Distances place the cultivars progressively by greater weight on objects that are further apart. The distance is computed as:

$$SED(x,y)= S2\ I\ (Xi-yi)$$

3.4.3 Cluster analysiss

The agglomerative method of clustering using Ward's method (Ward, 1963) for developing dendrogram was adopted. In this method, the incremental sum of squares (ISS) then fuses the two groups, which increases the within-group sum of squares, the least. It is monotonic, strongly clustering the SED measure can be used, the advantages of this strategy is that it is a generalised form of the analysis of variances and enables the properties of the classification to be integrated into standard theory (DeLaecy and Cooper, 1990).

3.5 Criteria for selection of elite trees based on morphometric traits

Different species have by nature different architecture. Selection traits may vary between different species and improvement programmes. Therefore, in the present investigation after the morphological data were analysed the elite trees from among the population will be identified based on the following criteria selected from peer reviewed literature:

a. Crown shape - Oval or dome
b. Foliage characteristics - Dense and dark

c.	Trunk characteristics	- Monotrunk
d.	Bearing habit	- Dense and regular bearing
e.	Fruit weight	- High
f.	Pulp weight	- High
g.	Pulp weight to seed weight ratio	- High
h.	Pulp colour	- Red brown
i.	Seed shape and colour	- Flat and glassy black


RESULTS

IV. RESULTS

The results of the current investigation are presented under the following heads:

1. Morphological characterization of the 100 tamarind (*Tamarindus indica L.*) genotypes.
2. Evaluation of the genotypes for quantitative traits.
3. PCR-based RAPD technique to detect polymorphism among the genotypes.
4. Identification of the elite genotypes from the population based on morphometric traits.

4.1 Morphological characterization of the 100 tamarind (*Tamarindus indica L.*) genotypes.

Observations were recorded on 14 qualitative traits (Table 3) from each of the 100 genotypes. They are explained below individually followed by clustering of individuals based on Squared Euclidean Distances.

4.1.1 Crown shape

Six different shapes of the crown were recorded in the tamarind population *viz.,* cone, dome, circle, semicircle, oval and irregular (Plate 1). Among them, irregular shape crown were recorded in maximum numbers of forty six trees followed by dome shape crown in twenty three trees, cone shape crown in fifteen trees, Oval shape crown in eight trees and semicircle shape crown in seven trees. Circle shape crown was recorded in minimum number of only one in tree number 49 (Table 3).

4.1.2 Foliage characters

The different foliage characteristics found among the genotypes were dense and dark green, dense and light green, sparse and dark green and sparse and light green (Plate 2). Among them, dense and

a) Dome Shape

b) Circle Shape

c) Oval Shape

d) Cone Shape

e) Semi circle Shape

f) Irregular Shape

Plate 1: Different shape of crown

a) Dense and dark green

b) Dense and light green

c) Sparse and dark green

d) Sparse and light green

Plate 2: Different foliage characteristics

TABLE 3: Diagnostic qualitative parameters of 100 tamarind genotypes

Sl.no.	Tree no.	Bud colour	Bract colour	Colour of petal	Sepal colour	Anther Colour	Pod colour	Fruit shape	Pulp colour	Seed shape	Seed colour	Crown shape	Foliage characters	Trunk characters	Bearing habit
1.	1	pale green to pale yellow and turns green	pale green to pale yellow	gold colour with red streak	lemon chiffon	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Multiple	Sparse
2.	5	yellow at early stage and turns green	pale yellow	yellow with red streak	lemon chiffon	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Semicircle	Dense & dark green	Mono	Sparse
3.	6	yellow at early stage and turns green	pale yellow	yellow with red streak	lemon chiffon	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Oval	Dense & dark green	Mono	Sparse
4.	9	yellow at early stage and turns green	pale yellow	yellow with red streak	lemon chiffon	deep pink	Dark brown	Straight	Red brown	Flat	Glassy black	Semicircle	Sparse & Light green	Mono	Medium
5.	12	yellow at early stage and turns green	pale yellow	pale yellow with red streaks	lemon chiffon	dark orange	Dark brown	Straight	Red brown	Flat	Glassy black	Cone	Sparse & Light green	Multiple	Sparse
6.	14	pink at early stage and turns green	pink	pale yellow with red streaks	yellow	deep pink	Dark brown	Straight	Red brown	Flat	Glassy black	Semicircle	Dense & dark green	Multiple	Medium
7.	15	pink at early stage and turns green	pink	yellow with red streak	yellow	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Sparse & Light green	Mono	Sparse
8.	16	pink at early stage and turns green	pink	orange red with red streak	lemon chiffon	dark orange	Dark brown	Curve	Red brown	Flat	Glassy black	Dome	Dense & dark green	Mono	Sparse
9.	18	yellow at early stage and turns green	yellow	pale yellow with red streaks	pale yellow	dark orange	Light brown	Curve	Red brown	Flat	Glassy black	Cone	Sparse & Light green	Mono	Medium
10.	20	pink and turns green	pale pink	yellow with red streak	gold	deep pink	Dark brown	Straight	Red brown	Flat	Glassy black	Dome	Sparse & Light green	Mono	Sparse
11.	21	pink at early stage and turns green	pink	yellow with red streak	gold	deep pink	Light brown	Curve	Light brown	Flat	Glassy black	Semicircle	Dense & dark green	Mono	Sparse
12.	23	pink at early stage and turns green	pink	orange red with red streak	pale yellow	deep red	Light brown	Curve	Red brown	Flat	Glassy black	Cone	Dense & dark green	Mono	Medium

TABLE 3: Diagnostic qualitative parameters of 100 tamarind genotypes

Sl.no.	Tree no.	Bud colour	Bract colour	Colour of petal	Sepal colour	Anther Colour	Pod colour	Fruit shape	Pulp colour	Seed shape	Seed colour	Crown shape	Foliage characters	Trunk characters	Bearing habit
1.	1	pale green to pale yellow and turns green	pale green to pale yellow	gold colour with red streak	lemon chiffon	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Multiple	Sparse
2.	5	yellow at early stage and turns green	pale yellow	yellow with red streak	lemon chiffon	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Semicircle	Dense & dark green	Mono	Sparse
3.	6	yellow at early stage and turns green	pale yellow	yellow with red streak	lemon chiffon	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Oval	Dense & dark green	Mono	Sparse
4.	9	yellow at early stage and turns green	pale yellow	yellow with red streak	lemon chiffon	deep pink	Dark brown	Straight	Red brown	Flat	Glassy black	Semicircle	Sparse & Light green	Mono	Medium
5.	12	yellow at early stage and turns green	pale yellow	pale yellow with red streaks	lemon chiffon	dark orange	Dark brown	Straight	Red brown	Flat	Glassy black	Cone	Sparse & Light green	Multiple	Sparse
6.	14	pink at early stage and turns green	pink	pale yellow with red streaks	yellow	deep pink	Dark brown	Straight	Red brown	Flat	Glassy black	Semicircle	Dense & dark green	Multiple	Medium
7.	15	pink at early stage and turns green	pink	yellow with red streak	yellow	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Sparse & Light green	Mono	Sparse
8.	16	pink at early stage and turns green	pink	orange red with red streak	lemon chiffon	dark orange	Dark brown	Curve	Red brown	Flat	Glassy black	Dome	Dense & dark green	Mono	Sparse
9.	18	yellow at early stage and turns green	yellow	pale yellow with red streaks	pale yellow	dark orange	Light brown	Curve	Red brown	Flat	Glassy black	Cone	Sparse & Light green	Mono	Medium
10.	20	pink and turns green	pale pink	yellow with red streak	gold	deep pink	Dark brown	Straight	Red brown	Flat	Glassy black	Dome	Sparse & Light green	Mono	Sparse
11.	21	pink at early stage and turns green	pink	yellow with red streak	gold	deep pink	Light brown	Curve	Light brown	Flat	Glassy black	Semicircle	Dense & dark green	Mono	Sparse
12.	23	pink at early stage and turns green	pink	orange red with red streak	pale yellow	deep red	Light brown	Curve	Red brown	Flat	Glassy black	Cone	Dense & dark green	Mono	Medium

Sl.no.	Tree no.	Bud colour	Bract colour	Colour of petal	Sepal colour	Anther Colour	Pod colour	Fruit shape	Pulp colour	Seed shape	Seed colour	Crown shape	Foliage characters	Trunk characters	Bearing habit
13.	26	pink at early stage and turns green	pink	orange red with red streak	lemon chiffon	deep red	Dark brown	Straight	Light brown	Flat	Light brown	Dome	Sparse & dark green	Mono	Sparse
14.	30	pink at early stage turns green	pink	orange yellow with pink streak	lemon chiffon	deep pink	Light brown	Curve	Light brown	Flat	Glassy black	Semicircle	Dense & dark green	Multiple	Dense
15.	31	pale green and turns green	pale green to pale yellow	pale yellow with red streaks	pale yellow	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Semicircle	Dense & dark green	Double	Sparse
16.	33	yellow at early stage and turns green	pale yellow	lemon chiffon	yellow	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Cone	Dense & light green	Double	Dense
17.	39	pink at early stage turns green	pink	orange red with red streak	lemon chiffon	deep red	Light brown	Straight	Red brown	Flat	Glassy black	Irregular	Dense & light green	Mono	Sparse
18.	41	pink at early stage & turns green	deep pink	yellow with red streak	pale yellow	dark pink	Light brown	Curve	Red brown	Flat	Light brown	Cone	Dense & dark green	Mono	Sparse
19.	44	yellow at early stage and turns green.	pale yellow	lemon chiffon	yellow	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Dome	Sparse & Light green	Mono	Sparse
20.	47	yellow at early stage and turns green.	pale yellow	lemon chiffon	yellow	deep pink	Dark brown	Straight	Red brown	Flat	Glassy black	Oval	Dense & dark green	Mono	Dense
21.	49	pale green to pale yellow & turns green	pale green to pale yellow	pale yellow with red streaks	pale yellow	dark orange	Dark brown	Curve	Red brown	Flat	Light brown	Circle	Dense & dark green	Mono	Sparse
22.	50	pale green and turns green	pale green to pale yellow	pale yellow with red streaks	pale yellow	dark orange	Light brown	Curve	Red brown	Flat	Glassy black	Dome	Dense & dark green	Mono	Dense
23.	52	pink and turns green	pale pink	pale yellow with red streaks	lemon chiffon	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Dome	Dense & dark green	Mono	Medium
24.	55	pale green and turns green	pale green to pale yellow	pale yellow with red streaks	lemon chiffon	dark red	Light brown	Straight	Light brown	Flat	Glassy black	Oval	Dense & dark green	Mono	Sparse

Sl.no.	Tree no.	Bud colour	Bract colour	Colour of petal	Sepal colour	Anther Colour	Pod colour	Fruit shape	Pulp colour	Seed shape	Seed colour	Crown shape	Foliage characters	Trunk characters	Bearing habit
25.	56	yellow at early stage and turns green.	pale yellow	lemon chiffon	yellow	deep pink	Light brown	Curve	Light brown	Flat	Glassy black	Oval	Sparse & Light green	Multiple	Medium
26.	58	yellow at early stage and turns green	pale yellow	yellow with red streak	yellow	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Cone	Sparse & Light green	Multiple	Sparse
27.	60	pink at early stage and turns green	pink	yellow with red streak	pale yellow	deep pink	Dark brown	Straight	Red brown	Flat	Light brown	Cone	Dense & dark green	Multiple	Dense
28.	61	pink at early stage & turns green	deep pink	orange yellow with pink streak	lemon chiffon	deep pink	Dark brown	Curve	Red brown	Flat	Light brown	Irregular	Sparse & dark green	Multiple	Medium
29.	64	pink and turns green	pale pink	yellow with red streak	yellow	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Multiple	Medium
30.	65	yellow at early stage and turns green	yellow	yellow with red streak	gold	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Oval	Dense & dark green	Mono	Dense
31.	69	pale green and turns green	pale green to pale yellow	yellow with red streak	lemon chiffon	dark pink	Dark brown	Curve	Red brown	Flat	Glassy black	Oval	Dense & dark green	Mono	Medium
32.	71	pink at early stage & turns green	pink	orange yellow with red streak	yellow	dark pink	Light brown	Curve	Red brown	Flat	Glassy black	Cone	Sparse & Light green	Mono	Medium
33.	74	yellow at early stage and turns green.	pale yellow	lemon chiffon	yellow	deep pink	Dark brown	Straight	Light brown	Flat	Glassy black	Irregular	Dense & dark green	Mono	Sparse
34.	75	yellow and turns green	pale yellow	yellow with red streak	gold	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Dome	Dense & dark green	Mono	Medium
35.	78	yellow & turns green	pink	yellow with red streak	lemon chiffon	dark pink	Light brown	Straight	Light brown	Flat	Light brown	Irregular	Dense & dark green	Mono	Dense
36.	80	yellow colour & turns green	yellow	orange yellow with pink streak	pale yellow	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Mono	Dense
37.	81	pink and turns green	pink	yellow with red streak	Yellow	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Double	Sparse

Sl.no.	Tree no.	Bud colour	Bract colour	Colour of petal	Sepal colour	Anther Colour	Pod colour	Fruit shape	Pulp colour	Seed shape	Seed colour	Crown shape	Foliage characters	Trunk characters	Bearing habit
38.	82	pink and turns green	pale pink	yellow with red streak	yellow	deep pink	Light brown	Curve	Light brown	Flat	Light brown	Oval	Dense & dark green	Double	Medium
39.	84	pink and turns green	pale pink	yellow with red streak	lemon chiffon	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Dome	Sparse & Light green	Mono	Medium
40.	85	pink at early stage and turns green	pink	yellow with red streak	gold	deep pink	Dark brown	Curve	Light brown	Flat	Glassy black	Dome	Sparse & Light green	Double	Sparse
41.	92	pink and turns green	pale pink	yellow with red streak	gold	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Irregular	Sparse & Light green	Mono	Sparse
42.	94	yellow and turns green	pale yellow to pale green	yellow with red streak	lemon chiffon	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Dome	Dense & dark green	Double	Sparse
43.	95	yellow at early stage and turns green	pale yellow	yellow with red streak	lemon chiffon	deep pink	Dark brown	Straight	Red brown	Flat	Glassy black	Cone	Sparse & Light green	Double	Sparse
44.	98	yellow at early stage and turns green	pale yellow	yellow with red streak	lemon chiffon	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Mono	Sparse
45.	100	pink and turns green	pink	yellow with red streak	gold	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Dome	Sparse & Light green	Double	Medium
46.	102	pink and turns green	pink	yellow with red streak	gold	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Mono	Sparse
47.	104	yellow at early stage and turns green	pale yellow	yellow with red streak	lemon chiffon	deep pink	Dark brown	Straight	Red brown	Flat	Glassy black	Dome	Sparse & Light green	Mono	Dense
48.	106	yellow at early stage and turns green	pale yellow	yellow with red streak	yellow	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Mono	Sparse
49.	109	pink at early stage and turns green	deep pink	yellow with red streak	lemon chiffon	deep pink	Light brown	Curve	Red brown	Flat	Light brown	Irregular	Dense & dark green	Mono	Sparse
50.	110	yellow at early stage and turns green	yellow	pale yellow with red streaks	lemon chiffon	deep pink	Dark brown	Straight	Red brown	Flat	Glassy black	Irregular	Dense & light green	Mono	Sparse
51.	112	pink and turns green	pink	yellow with red streak	gold	deep pink	Dark brown	Straight	Red brown	Flat	Light brown	Irregular	Dense & light green	Mono	Sparse
52.	114	pink at early stage and turns green	pink	yellow with red streak	lemon chiffon	deep pink	Light brown	Light Curve	Red brown	Flat	Glassy black	Dome	Dense & light green	Mono	Dense

Sl.no.	Tree no.	Bud colour	Bract colour	Colour of petal	Sepal colour	Anther Colour	Pod colour	Fruit shape	Pulp colour	Seed shape	Seed colour	Crown shape	Foliage characters	Trunk characters	Bearing habit
53.	117	yellow at early stage and turns green.	pale yellow	lemon chiffon	yellow	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Sparse & dark green	Mono	Sparse
54.	119	pale green and turns green	pale green to pale yellow	yellow with red streak	gold	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Dome	Sparse & Light green	Mono	Sparse
55.	120	pink at early stage and turns green	deep pink	yellow with red streak	yellow	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Multiple	Dense
56.	121	pink and turns green	deep pink	yellow with red streak	lemon chiffon	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Sparse & Light green	Multiple	Dense
57.	124	pink and turns green	pink	yellow with red streak	lemon chiffon	deep pink	Dark brown	Curve	Light brown	Flat	Glassy black	Irregular	Dense & dark green	Multiple	Dense
58.	125	pink at early stage & turns green	deep pink	pale yellow with red streaks	lemon chiffon	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Dome	Sparse & dark green	Multiple	Medium
59.	130	pink and turns green	deep pink	yellow with red streak	gold	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Dome	Sparse & Light green	Mono	Sparse
60.	131	yellow at early stage and turns green	pale yellow	yellow with red streak	yellow	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Oval	Dense & dark green	Mono	Medium
61.	134	pink and turns green	pink	yellow with red streak	lemon chiffon	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Cone	Sparse & Light green	Mono	Sparse
62.	137	pink at early stage and turns green	pink	yellow with red streak	gold	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Sparse & Light green	Mono	Sparse
63.	140	pink at early stage and turns green	deep pink	orange red with red streak	lemon chiffon	deep pink	Dark brown	Curve	Red brown	Flat	Light brown	Irregular	Dense & dark green	Double	Sparse
64.	143	pink & turns green	deep pink	orange yellow with pink streak	pale yellow	reddish brown	Dark brown	Light Curve	Red brown	Flat	Glassy black	Irregular	Sparse & Light green	Double	Sparse
65.	149	yellow at early stage and turns green	pale yellow	pale yellow with red streaks	lemon chiffon	deep pink	Light brown	Straight	Red brown	Flat	Light brown	Irregular	Sparse & Light green	Double	Sparse

Sl.no.	Tree no.	Bud colour	Bract colour	Colour of petal	Sepal colour	Anther Colour	Pod colour	Fruit shape	Pulp colour	Seed shape	Seed colour	Crown shape	Foliage characters	Trunk characters	Bearing habit
66.	153	pink at early stage and turns green	pink	yellow with red streak	gold	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Irregular	Dense & light green	Multiple	Sparse
67.	155	yellow turns green	pale yellow	orange yellow with pink streak	pale yellow	reddish brown	Dark brown	Curve	Red brown	Flat	Glassy black	Irregular	Sparse & dark green	Multiple	Sparse
68.	157	yellow at early stage and turns green	pale yellow	yellow with red streak	lemon chiffon	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Sparse & dark green	Multiple	Sparse
69.	159	pink at early stage and turns green	deep pink	orange yellow with red streak	pale yellow	reddish brown	Light brown	Straight	Red brown	Flat	Glassy black	Irregular	Dense & light green	Mono	Dense
70.	161	pink, turns green	dark pink	orange yellow with pink streak	lemon chiffon	reddish brown	Dark brown	Curve	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Mono	Sparse
71.	162	yellow turns green	yellow	orange yellow with pink streak	lemon chiffon	deep red	Light brown	Straight	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Mono	Medium
72.	163	pink turns green	pink	yellow with red streak	light yellow	dark pink	Light brown	Curve	Red brown	Flat	Glassy black	Dome	Dense & dark green	Mono	Medium
73.	167	yellow turns green	pale yellow to pale green	yellow with red streak	lemon chiffon	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Dome	Sparse & Light green	Mono	Medium
74.	169	pink at early stage and turns green	pink	pale yellow with red streaks	yellow	dark pink	Dark brown	Straight	Red brown	Flat	Glassy black	Irregular	Sparse & Light green	Mono	Medium
75.	172	yellow at early stage and turns green	pale yellow	yellow with red streak	lemon chiffon	deep pink	Dark brown	Straight	Red brown	Flat	Light brown	Irregular	Sparse & Light green	Mono	Dense
76.	173	yellow at early stage and turns green	pale yellow	yellow with red streak	lemon chiffon	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Dome	Sparse & Light green	Multiple	Sparse
77.	174	pink at early stage and turns green	pink	yellow with red streak	lemon chiffon	deep pink	Light brown	Straight	Red brown	Flat	Light brown	Irregular	Sparse & Light green	Multiple	Medium
78.	176	yellow at early stage and turns green	yellow	yellow with red streak	yellow	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Cone	Dense & light green	Multiple	Sparse
79.	178	pink and turns green	deep pink	orange yellow with pink streak	gold	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Irregular	Dense & light green	Multiple	Sparse

Sl.no.	Tree no.	Bud colour	Bract colour	Colour of petal	Sepal colour	Anther Colour	Pod colour	Fruit shape	Pulp colour	Seed shape	Seed colour	Crown shape	Foliage characters	Trunk characters	Bearing habit
80.	181	pink at early stage turns green	deep pink	orange red with red streak	gold	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Cone	Sparse & Light green	Multiple	Sparse
81.	183	pink at early stage and turns green	deep pink	orange red with red streak	yellow	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Semicircle	Sparse & dark green	Double	Dense
82.	186	yellow and turns green	pale yellow to pale green	yellow with red streak	gold	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Sparse & Light green	Multiple	Sparse
83.	192	pink at early stage and turns green	pink	orange red with red streak	gold	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Cone	Dense & dark green	Multiple	Dense
84.	194	yellow at early stage and turns green.	pale yellow	lemon chiffon	yellow	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Mono	Medium
85.	197	yellow at early stage and turns green	pale yellow to pale green	yellow with red streak	lemon chiffon	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Oval	Dense & light green	Mono	Dense
86.	201	yellow at early stage and turns green.	pale yellow	lemon chiffon	yellow	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Mono	Sparse
87.	204	pink at early stage and turns green	pale pink	yellow with red streak	gold	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Irregular	Sparse & Light green	Mono	Sparse
88.	206	yellow at early stage and turns green.	pale yellow	lemon chiffon	yellow	deep pink	Light brown	Light Curve	Red brown	Flat	Glassy black	Irregular	Sparse & Light green	Multiple	Sparse
89.	208	pink at early stage and turns green	pink	yellow with red streak	yellow	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Cone	Dense & light green	Multiple	Sparse
90.	210	yellow at early stage and turns green	pale yellow to pale green	yellow with red streak	lemon chiffon	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Oval	Dense & dark green	Multiple	Dense
91.	212	pink and turns green	pale pink	yellow with red streak	gold	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Mono	Medium

Sl.no.	Tree no.	Bud colour	Bract colour	Colour of petal	Sepal colour	Anther Colour	Pod colour	Fruit shape	Pulp colour	Seed shape	Seed colour	Crown shape	Foliage characte-rs	Trunk characte-rs	Bearing habit
92.	217	pink at early stage and turns green	pale pink	yellow with red streak	pale yellow	deep pink	Light brown	Straight	Light brown	Flat	Glassy black	Irregular	Sparse & Light green	Mono	Sparse
93.	221	green at early stage and turns green	pale green to pale yellow	yellow with red streak	gold	deep pink	Dark brown	Curve	Red brown	Flat	Glassy black	Dome	Sparse & Light green	Mono	Sparse
94.	230	pink at early stage and turns green	pale pink	yellow with red streak	gold	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Dome	Sparse & Light green	Double	Sparse
95.	238	pink at early stage and turns green	deep pink	orange red with red streak	yellow	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Irregular	Dense & light green	Mono	Medium
96.	240	yellow at early stage and turns pale pink	pale yellow	yellow with red streak	gold	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Mono	Sparse
97.	243	yellow at early stage and turns green	pale yellow to pale green	yellow with red streak	lemon chiffon	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Dome	Dense & dark green	Mono	Dense
98.	244	pink at early stage and turns green	deep pink	yellow with red streak	gold	deep pink	Light brown	Straight	Red brown	Flat	Glassy black	Dome	Dense & dark green	Mono	Dense
99.	245	yellow colour & turns green	yellow	yellow with red streak	gold	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Dome	Dense & dark green	Mono	Dense
100	246	pink at early stage and turns green	deep pink	orange red with red streak	gold	deep pink	Light brown	Curve	Red brown	Flat	Glassy black	Irregular	Dense & dark green	Mono	Dense

dark green foliage were recorded in maximum numbers of forty seven trees followed by sparse and light green foliage in thirty four trees and dense and light green foliage in twelve trees. Sparse and dark green colour foliage were recorded in minimum numbers of seven trees (Table 3).

4.1.3 Trunk characters

Three different features of the trunk were observed among the population *viz.,* monotrunk, double trunk and multiple trunk (Plate 3). Monotrunk trees were recorded in maximum numbers of sixty one trees followed by multiple trunk trees in twenty six trees and double trunk trees recorded in minimum numbers of thirteen trees (Table 3).

4.1.4 Bearing habit

The different bearing habits among the genotypes recorded were sparse, medium, and dense bearing trees. Among them, sparse bearing trees were recorded in maximum numbers of fifty four trees followed by medium bearing trees in twenty six trees and dense bearing trees with a minimum in twenty three trees (Table 3).

4.1.5 Bract colour

Six different bract colours were observed in the tamarind population *viz.,* pale green, pale yellow, yellow, pale pink, pink and deep pink (Plate 4). Among them, pale yellow colour bract were recorded in maximum number of trees (thirty two trees) and yellow coloured bract were recorded only in seven trees (Table 3).

4.1.6 Bud colour

The different types of colour of the bud found among the population are pale green, yellow and pink (Plate 5). Among them, pink colour bud were recorded in maximum number of fifty two trees followed by yellow colour bud in 40 trees and pale green colour bud were recorded in only eight trees (Table 3).

b) Monotrunk

a) Double trunk

c) Multiple trunk

Plate 3: Different trunk characteristics

a) Pale Green

b) Pale yellow

c) Yellow

d) Pale pink

e) Pink

f) Deep pink

Plate 4: Variation in bract colour

a) Pale green

b) Yellow

c) Pink

Plate 5: Three different bud colours

4.1.7 Petal colour

The colour of the petal varied from pale yellow with red streak, yellow with red streak, orange yellow with red streak, orange red with red streak and lemon chiffon (Plate 6). Yellow colour petal with red streak were recorded in maximum number of fifty nine trees followed by pale yellow with red streak in twelve trees, both orange yellow with red streak and orange red with red streak in ten trees and lemon chiffon colour petal were recorded in minimum number of nine trees (Table 3).

4.1.8 Sepal colour

Four different colour of the sepal were recorded in the population *viz.,* pale yellow, yellow, gold and lemon chiffon. Lemon chiffon colour sepal were recorded in maximum number of thirty nine trees followed by gold colour sepal in twenty six trees and yellow colour sepal in twenty three trees. Pale yellow colour sepal were recorded in minimum number of twelve trees (Table 3).

4.1.9 Anther colour

The colour of the anther varied from dark orange, deep pink, reddish brown and deep red (Plate 7). Among them, deep pink colour anther were recorded in maximum numbers of eighty six trees followed by both dark orange and deep red colour anther in five trees and reddish brown colour anther recorded in minimum numbers of four trees (Table 3).

4.1.10 Pod colour

Two different pod colours were observed among the genotypes *viz.,* light brown and dark brown. Among them, light brown colour pods were recorded in maximum numbers of sixty three trees and dark brown colour pods recorded in minimum numbers of thirty seven trees (Table 3).

a) Pale yellow with red streaks

b) Yellow with red streaks

c) Lemon chiffon

d) Orange yellow with streaks

d) Orange red with red streaks

Plate 6: Variation in petal colour

a) Dark orange

b) Deep pink anthers

c) Reddish brown

d) Deep red

Plate 7: Variation in anther colour

Plate 8: Variation in pod size

a) Curve pod

b) Straight pod

Plate 9: Variation in pod shape

4.1.11 Fruit shape

The shape of the fruit or pod varied from straight to curve (Plate 9). Curved shape fruit were recorded in maximum number of sixty five trees and straight fruits recorded in minimum numbers of thirty five trees (Table 3).

4.1.12 Pulp colour

Two types of pulp colour were observed *viz.,* red brown to light brown. Among them, pulp having red brown colour recorded in maximum numbers of eighty nine trees and light brown colour pulp recorded in minimum numbers of eleven trees. (Table 3).

4.1.13 Seed shape

The shape of the seed recorded among the population were found to be flat in all the genotypes (Table 3).

4.1.14 Seed colour

The colour of the seed varied from glassy black to light brown. Among them, glassy black colour seed were recorded in maximum numbers of eighty three trees and light brown pulp were recorded in minimum numbers of thirteen trees (Table 1).

4.1.15 Diversity analysis among the genotypes of tamarind

All the 100 genotypes of tamarind were analysed to study their genetic diversity based on 14 qualitative traits. Based on Squared Euclidean Distance using Ward's method (Ward, 1963), a dendrogram of clustering among the genotypes was constructed. In the dendrogram (Fig. 1), the genotypes clearly divides into 2 major clusters A and B. These two major clusters were separated at a linkage of 108 units. The major cluster A was subdivided into A_1 and

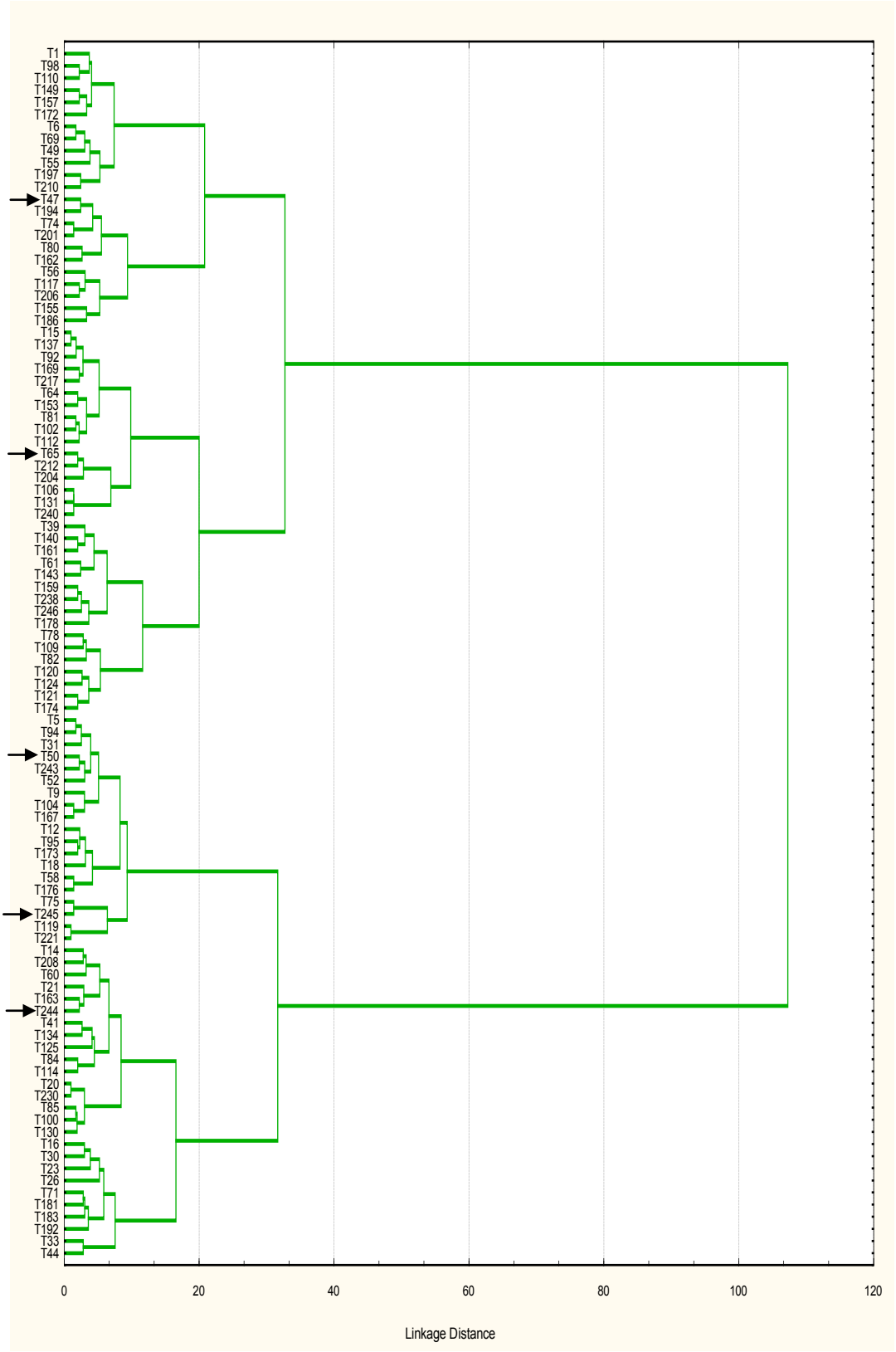


Fig 1: Dendrogram showing clustering of 100 genotypes based on 14 qualitative parameters (Arrow indicates the location of the elite trees)

A_2 at a linkage distance of 34 units. The subcluster A_1 was further divided into $A_{1.1}$ and $A_{1.2}$ at 21 units, while A_2 was further divided into $A_{2.1}$ and $A_{2.2}$ at a linkage distance of 20 units.

The cluster A consisted of 55 genotypes distributed between tree no. 1 and 174 and the remaining 45 genotypes were clustered in cluster B. The subcluster A_1 consisted of 23 genotypes where the subgroup $A_{1.1}$ includes genotypes like tree 1, 98, 110, 149, 157, 172, 6, 69, 49, 55, 197 and 210 and subgroup $A_{1.2}$ includes tree no. 47, 194, 74, 201, 80, 162, 56, 117, 206, 155 and 186. The subcluster A_2 consists of 32 genotypes where subgroup $A_{2.1}$ includes genotypes like tree 15, 137, 92, 169, 217, 64, 153, 81, 102, 112, 65, 212, 204, 106, 131 and 240 and subgroup $A_{2.2}$ includes tree 139, 140, 161, 61, 143, 159, 238, 246, 178, 78, 109, 82, 120, 124, 121 and 174.

The cluster B was divided into 2 subclusters *viz.,* B_1 and B_2 at 32 units of linkage distance. B_1 comprised of 19 genotypes while B_2 had 26 individuals. The subcluster B_1 consisted of 2 groups $B_{1.1}$ and $B_{1.2}$ with a linkage distance of 10 units. In $B_{1.1}$ genotypes like tree 5, 94,31, 50, 243, 52, 9, 104, 167, 12, 95, 173, 18, 58 and 176 were clustered together. The subgroup B_2 was further divided into $B_{2.1}$ and $B_{2.2}$ at 9units of linkage distance. In $B_{2.1}$ genotypes like tree 14, 208, 60, 21, 163, 244, 41, 134, 125, 84, 114, 20, 230, 85, 100 and 130 clustered togetherand in $B_{2.2}$ genotypes like tree 16, 30, 23, 26, 71, 181, 183, 192, 33 and 44 clustered together. Thus, the genotypes falls at different clusters indicates that they are diverse genotypes.

4.2 Evaluation of the genotypes for quantitative traits.

The data on mean performance of each of 100 genotypes for the 12 metric traits, pod length, pod width, beak length, number of ridges and furrows, fruit weight, epicarp weight, fibre number, fibre weight, pulp weight, seed weight and seed number are given in Table 4. Significance differences were noticed for all the metric traits under study (Table 5).

4.2.1 Pod length (cm)

Pod length ranged from 6.65 to 20.04 cm (Tree 110 and 167) with a mean pod length being 10.43 cm.

4.2.2 Pod width (cm)

Pod width ranged from 2.30 to 4.84 cm (Tree 69 and 47) with a mean width of 3.21 cm.

4.2.3 Beak length (cm)

A mean of 0.13 cm and a range from 0.04 to 0.45 cm (Tree 47 and 155) beak length was observed among the genotypes under study.

4.2.4 Number of ridges (per fruit)

The number of ridges ranged from 2.7 to 9.3 (Tree 92 and 82) with a mean number of 5.36 among the genotypes under study.

4.2.5 Number of furrows (per fruit)

The number of furrows ranged from 1.75 to 8.3 (Tree 92 and 82) with a mean number of 4.37 among the genotypes under study.

4.2.6 Fruit weight (gm/fruit)

Fruit weight ranged from 2.34 to 16.41 gm (Tree 217 and 197) with a mean fruit weight of 7.69 gm among the hundred genotypes.

4.2.7 Epicarp weight (gm/fruit)

A range from 1.03 to 4.22 gm (Tree 169 and 130) with a mean epicarp weight of 2.13 gm was observed in the population of hundred genotypes.

4.2.8 Fibre number (per fruit)

The maximum number of fibres were observed in Tree 181 (5.6) and minimum in Tree 119 and 172 (2.6) with a mean number of 3.72 in the population.

4.2.9 Fibre weight (gm/fruit)

The weight of the fibre ranged from 0.12 to 0.89 gm (Tree 26 and 161) with a mean fibre weight of 0.33 gm was observed among the hundred genotypes.

4.2.10 Pulp weight (gm/fruit)

Pulp weight ranged from 0.93 to 6.99 gm (Tree 217 and 131) with a mean pulp weight being 3.28 gm in the population of hundred genotypes.

4.2.11 Seed weight (gm/fruit)

A range from 0.41 to 5.31 gm (Tree 26 and 6) with a mean seed weight of 1.97 gm was observed among the genotypes under study.

4.2.12 Seed number (per fruit)

The maximum number of seeds were observed in Tree 16 (7.7) and minimum in Tree 162 and 212 (1.7) with a mean number of 4.36 among the hundred genotypes.

Table 4: Mean performance of 100 tamarind genotypes for 12quantitative parameters

Sl.no	Tree.no.	Pod Length (cm)	Pod width (cm)	Beak length (cm)	No. of ridges	No. of furrows	Fruit wt. (gm)	Epicarp wt. (gm)	Fiber no.	Fiber wt. (gm)	Pulp wt. (gm)	Seed wt. (gm)	Seed No
1.	1	7.60	3.10	0.13	2.90	1.90	5.30	1.69	3.00	0.16	2.20	1.13	2.40
2.	5	8.60	3.10	0.18	4.00	3.00	5.69	1.88	3.60	0.17	2.43	1.16	4.70
3.	6	9.20	3.00	0.09	5.10	4.10	12.77	3.27	3.90	0.53	5.93	5.31	7.50
4.	9	9.69	3.16	0.09	5.70	4.70	7.32	1.91	2.90	0.31	2.79	2.29	5.40
5.	12	8.65	3.29	0.14	4.20	3.20	5.61	1.70	2.80	0.29	2.45	1.17	2.80
6.	14	8.15	3.05	0.10	4.80	3.80	6.46	2.13	3.80	0.44	2.63	1.38	4.20
7.	15	7.71	2.85	0.12	3.80	2.80	10.90	3.01	3.90	0.66	4.51	2.73	4.80
8.	16	7.76	2.69	0.16	4.50	3.50	10.88	3.32	4.30	0.31	4.52	2.75	7.70
9.	18	7.26	2.53	0.13	3.80	2.80	6.21	1.87	4.00	0.21	2.09	2.02	5.70
10.	20	8.48	3.32	0.12	3.20	2.20	4.95	2.50	3.60	0.17	1.97	0.47	4.30
11.	21	8.47	3.31	0.09	4.95	3.95	5.42	1.65	3.30	0.27	2.34	1.30	3.30
12.	23	10.70	3.30	0.11	5.65	4.65	6.29	2.02	3.20	0.23	2.22	1.77	4.90
13.	26	8.07	3.23	0.15	3.55	2.55	2.94	1.14	2.80	0.12	1.24	0.41	2.00
14.	30	12.02	3.49	0.14	6.35	5.35	6.22	2.69	3.00	0.13	2.31	0.87	7.00
15.	31	9.46	2.99	0.13	5.05	4.05	7.33	2.14	3.60	0.23	2.79	2.21	4.30
16.	33	12.26	3.66	0.10	8.10	7.10	12.10	3.59	4.50	0.52	4.92	2.75	6.00
17.	39	7.40	3.04	0.06	3.40	2.60	6.52	1.55	3.60	0.24	3.55	1.12	3.40
18.	41	9.40	3.40	0.10	5.20	4.10	4.25	1.44	4.50	0.23	1.55	0.96	1.80
19.	44	8.48	3.21	0.14	4.00	3.00	5.28	1.52	4.30	0.34	1.91	1.37	3.30
20.	47	16.69	4.84	0.04	7.15	6.15	11.41	1.74	3.30	0.25	6.73	2.82	3.50
21.	49	8.38	3.07	0.13	5.60	4.60	7.21	2.17	3.60	0.26	2.93	1.51	4.20
22.	50	9.89	3.73	0.21	5.50	4.50	11.78	2.18	4.00	0.26	6.66	2.73	3.80
23.	52	12.03	3.32	0.18	6.65	5.65	13.36	3.59	4.50	0.57	6.29	2.76	8.00
24.	55	8.47	3.37	0.14	3.85	2.85	7.36	2.27	3.90	0.41	2.89	1.96	4.20

Sl.no	Tree.no.	Pod Length (cm)	Pod width (cm)	Beak length (cm)	No. of ridges	No. of furrows	Fruit wt. (gm)	Epicarp wt. (gm)	Fiber no.	Fiber wt. (gm)	Pulp wt. (gm)	Seed wt. (gm)	Seed No
25.	56	8.11	3.39	0.23	3.25	2.25	7.28	2.26	3.50	0.36	2.88	1.67	2.80
26.	58	9.44	3.34	0.10	3.80	2.80	6.88	1.85	3.20	0.16	3.06	1.52	4.00
27.	60	13.10	3.50	0.10	5.80	4.85	4.27	1.34	3.10	0.30	1.76	0.85	1.90
28.	61	12.30	3.50	0.15	6.10	5.10	5.17	1.24	3.00	0.57	2.11	1.23	3.60
29.	64	13.50	3.20	0.18	8.10	7.10	7.77	1.92	4.40	0.56	3.19	2.00	4.80
30.	65	14.70	3.90	0.12	6.20	5.25	10.86	2.49	3.20	0.65	6.18	1.54	3.90
31.	69	9.13	2.30	0.11	7.70	6.75	8.73	2.36	4.40	0.27	3.56	2.43	4.80
32.	71	7.71	3.68	0.13	4.00	3.00	6.39	1.57	3.40	0.27	2.58	1.98	3.60
33.	74	9.68	2.98	0.12	4.60	3.60	7.14	1.81	3.40	0.25	2.77	2.43	5.00
34.	75	9.94	3.14	0.12	4.75	3.75	5.45	1.67	2.70	0.19	2.45	1.09	3.50
35.	78	11.56	2.68	0.10	7.45	6.45	6.03	1.74	3.00	0.15	3.18	1.04	3.00
36.	80	10.97	2.95	0.11	5.40	4.40	6.68	1.82	3.70	0.26	2.62	1.82	4.70
37.	81	8.02	3.05	0.14	4.55	3.55	8.64	2.36	4.00	0.43	4.08	1.71	5.20
38.	82	13.11	2.67	0.18	9.30	8.30	7.91	3.22	3.90	0.23	2.86	1.56	8.40
39.	84	13.37	3.40	0.09	7.20	6.20	4.94	1.37	3.40	0.20	2.18	0.92	4.40
40.	85	11.64	3.39	0.15	4.85	3.85	9.25	2.05	3.40	0.46	4.19	2.47	4.80
41.	92	6.70	3.04	0.09	2.70	1.75	7.37	2.22	3.50	0.29	2.91	1.97	4.40
42.	94	8.99	3.49	0.15	4.50	3.55	3.74	1.24	3.90	0.12	1.50	0.84	2.40
43.	95	7.93	2.62	0.08	3.60	2.60	8.39	2.58	3.30	0.33	3.52	1.87	3.40
44.	98	9.86	3.08	0.11	5.45	4.45	9.43	2.75	3.10	0.42	4.52	1.63	4.10
45.	100	9.06	3.53	0.07	4.30	3.30	10.20	2.62	3.00	0.18	3.77	3.37	7.50
46.	102	10.84	2.80	0.19	7.05	6.05	7.59	2.07	3.20	0.28	3.56	1.93	3.60
47.	104	11.19	3.43	0.09	5.20	4.20	12.37	2.59	3.80	0.35	4.14	5.38	5.20
48.	106	12.90	3.80	0.15	5.70	4.75	5.69	1.74	5.40	0.36	3.04	0.73	3.20
49.	109	12.86	3.30	0.12	5.60	4.60	6.11	2.20	3.80	0.24	2.59	0.91	3.30
50.	110	6.65	2.71	0.21	3.10	2.10	7.28	2.12	4.00	0.43	2.75	1.88	4.40
51.	112	9.80	3.32	0.12	5.00	4.00	3.73	1.19	3.40	0.19	1.67	0.59	2.00

Sl.no	Tree.no.	Pod Length (cm)	Pod width (cm)	Beak length (cm)	No. of ridges	No. of furrows	Fruit wt. (gm)	Epicarp wt. (gm)	Fiber no.	Fiber wt. (gm)	Pulp wt. (gm)	Seed wt. (gm)	Seed No
52.	114	11.81	3.50	0.13	5.90	4.90	6.75	1.95	3.90	0.27	2.28	2.14	4.80
53.	117	8.30	2.72	0.16	4.35	3.35	7.17	2.26	3.80	0.22	2.97	1.60	4.40
54.	119	10.55	2.83	0.10	4.05	3.05	9.73	2.94	2.60	0.84	3.93	2.69	4.30
55.	120	13.40	2.84	0.20	6.90	5.90	9.59	2.63	4.10	0.20	4.14	2.57	7.10
56.	121	13.72	3.08	0.09	8.40	7.40	10.39	2.99	3.50	0.28	3.86	4.41	5.90
57.	124	8.86	2.95	0.09	5.45	4.45	6.87	2.20	3.10	0.23	2.56	1.69	5.90
58.	125	9.64	3.40	0.16	5.35	4.35	6.28	2.06	3.70	0.31	2.98	1.41	3.90
59.	130	12.57	3.35	0.15	8.05	7.05	12.25	4.22	3.60	0.58	4.64	3.32	6.90
60.	131	14.68	3.78	0.15	5.20	4.20	14.39	3.06	3.90	0.45	6.99	4.03	5.50
61.	134	11.86	3.61	0.09	6.25	5.25	9.17	2.54	3.70	0.55	3.83	2.51	5.90
62.	137	8.30	3.18	0.13	3.50	2.50	10.99	3.11	4.70	0.27	4.57	3.33	7.10
63.	140	7.75	2.85	0.16	4.80	3.85	7.45	1.75	3.80	0.26	3.33	2.05	3.80
64.	143	11.60	2.99	0.18	5.55	4.55	9.76	2.37	3.60	0.26	3.93	3.07	7.40
65.	149	9.40	2.98	0.15	5.30	4.30	5.16	1.55	4.10	0.29	1.80	1.11	3.10
66.	153	10.06	3.58	0.14	6.45	5.45	5.15	1.57	4.30	0.26	1.81	1.37	3.90
67.	155	8.57	2.92	0.45	5.45	4.40	8.26	1.69	4.00	0.39	3.53	2.21	4.70
68.	157	17.20	3.75	0.07	6.90	5.90	10.07	2.21	4.80	0.51	5.24	2.24	2.30
69.	159	9.84	2.86	0.16	4.95	3.95	6.15	1.65	4.00	0.24	2.28	1.91	3.90
70.	161	12.01	3.12	0.06	6.20	5.15	9.07	2.18	5.50	0.89	3.76	2.07	4.80
71.	162	10.20	3.32	0.13	4.70	3.70	4.09	1.33	3.50	0.21	1.94	0.59	1.70
72.	163	10.13	3.24	0.15	5.10	4.05	5.39	1.53	4.00	0.26	2.05	1.49	4.30
73.	167	20.04	3.51	0.12	8.20	7.20	9.38	2.28	4.50	0.47	4.60	1.98	3.60
74.	169	10.74	3.45	0.14	6.05	5.05	3.75	1.03	3.30	0.16	1.65	0.89	3.00
75.	172	9.22	3.16	0.10	3.85	2.85	5.77	1.52	2.60	0.19	2.40	1.58	3.40
76.	173	6.95	3.49	0.09	3.90	2.90	6.51	2.02	3.90	0.33	2.73	1.40	3.50
77.	174	11.70	3.70	0.16	5.60	4.60	9.23	2.16	3.70	0.33	4.06	2.61	3.70
78.	176	10.85	3.54	0.18	5.60	4.60	4.78	1.45	3.30	0.16	1.77	1.33	2.70
79.	178	10.56	3.59	0.14	5.35	4.35	5.96	1.31	3.50	0.17	1.66	3.36	2.70

Sl.no	Tree.no.	Pod Length (cm)	Pod width (cm)	Beak length (cm)	No. of ridges	No. of furrows	Fruit wt. (gm)	Epicarp wt. (gm)	Fiber no.	Fiber wt. (gm)	Pulp wt. (gm)	Seed wt. (gm)	Seed No
80.	181	10.94	4.03	0.07	6.00	5.00	13.85	3.15	5.60	0.78	6.07	3.93	6.30
81.	183	10.85	3.23	0.11	5.50	4.47	8.73	2.34	4.40	0.26	3.63	2.46	5.10
82.	186	9.18	2.80	0.09	4.90	3.90	5.89	2.14	3.00	0.23	2.47	1.05	2.80
83.	192	12.70	2.69	0.08	8.60	7.60	11.41	2.86	4.90	0.31	4.50	3.70	7.40
84.	194	12.60	3.37	0.16	7.16	6.16	7.36	1.89	3.60	0.30	2.76	2.31	5.50
85.	197	13.92	3.11	0.07	7.20	6.20	16.41	3.89	4.10	0.57	6.30	5.65	9.00
86.	201	10.15	2.51	0.19	4.45	3.45	6.02	1.85	4.20	0.49	3.27	0.29	2.10
87.	204	9.67	3.38	0.20	4.15	3.15	8.32	2.58	3.30	0.34	3.52	1.99	3.50
88.	206	10.67	3.04	0.12	5.15	4.15	6.21	1.88	4.40	0.29	2.94	0.89	3.40
89.	208	10.35	2.79	0.10	5.45	4.45	4.74	1.83	3.90	0.24	2.11	0.42	3.10
90.	210	11.27	2.91	0.15	5.45	4.45	9.44	2.88	4.50	0.46	3.09	2.88	4.80
91.	212	8.23	2.98	0.17	6.65	5.65	4.15	1.29	3.20	0.19	1.87	0.75	1.70
92.	217	7.17	2.76	0.16	3.60	2.60	2.34	0.86	2.80	0.13	0.93	0.41	2.00
93.	221	11.06	3.39	0.18	5.55	4.55	8.86	2.44	3.50	0.34	3.93	2.10	4.50
94.	230	7.90	3.38	0.20	3.35	2.35	5.14	1.25	2.70	0.75	2.06	0.98	4.00
95.	238	12.60	3.54	0.13	6.70	5.70	7.48	1.91	4.10	0.33	2.88	2.30	5.00
96.	240	12.28	3.22	0.12	5.60	4.60	6.95	2.34	4.40	0.29	2.53	1.97	4.30
97.	243	13.07	3.25	0.11	5.95	4.95	7.11	1.82	3.80	0.36	3.12	1.72	2.80
98.	244	11.02	3.36	0.11	5.20	4.20	11.92	1.73	3.60	0.28	6.51	3.54	4.00
99.	245	8.76	3.06	0.21	4.60	3.60	10.38	2.70	3.90	0.39	5.97	1.43	4.20
100.	246	10.66	3.06	0.10	5.55	4.55	7.77	2.24	3.30	0.21	2.84	2.57	4.70
101.	**Mean**	**10.43**	**3.21**	**0.13**	**5.36**	**4.37**	**7.69**	**2.13**	**3.72**	**0.33**	**3.28**	**1.97**	**4.36**
102.	**Range**	**6.65-20.04**	**2.30-4.84**	**0.04-0.45**	**2.7-9.3**	**1.75-8.3**	**2.34-16.41**	**1.03-4.22**	**2.6-5.6**	**0.12-0.89**	**0.93-6.99**	**0.41-5.31**	**1.7-7.7**

☐ indicate the highest and lowest value of each trait

Table 5: Analysis of variance table for metric traits in tamarind genotypes.

Sl. No.	Metric traits	Treatment MSS	Error MSS
1.	Pod length	19.03394**	5.619834
2.	Pod width	0.631732**	0.048882
3.	Beak length	0.007438**	0.002059
4.	Number of ridges	6.112954**	2.725732
5.	Number of furrows	6.191266**	2.661742
6.	Fruit weight	19.98482**	9.012739
7.	Epicarp weight	1.282729**	0.623087
8.	Fibre number	1.475511**	0.431655
9.	Fibre weight	0.074764**	0.041738
10.	Pulp weight	5.727607**	1.980766
11.	Seed weight	3.126903**	1.315643
12.	Seed number	7.826312**	2.86746

***Significant at 1 per cent level*

4.2.13 Cluster Analysis

Dendrogram showing clustering of the 100 tamarind genotypes based on the 12 quantitative parameters is shown in Fig. 2. From the figure the genotypes divided into 2 major clusters A and B separated at a linkage distance of 112 units. The major cluster A consisted of totally 49 genotypes which further divided into two sub clusters, A_1 and A_2 at a linkage distance of 32 units. A_1 comprised of 27 genotypes while A_2 had 22 individuals.

The subcluster A_1 consisted of two subgroups $A_{1.1}$ and $A_{1.2}$ with a linkage distance of 19 units. In $A_{1.1}$ genotypes like tree 1, 20, 230, 44, 5, 55, 58, 71, 173, 56, 95, 18, 92, 110, 9, 246, 124, 31, 74, 125, 14, 49, 117, 140, 81 and 155 were clustered together while $A_{1.2}$ consists of only one genotypes i.e., tree no. 78. The subgroup A_2 was further divided into $A_{2.1}$ and $A_{2.2}$ at 11 units of linkage distance. In $A_{2.1}$ tree 12, 172, 21, 186, 75, 159, 163, 206 and 201 clustered together while in $A_{2.2}$ genotypes included are tree 26, 217, 41, 94, 112, 162, 149, 208, 153, 169, 176, 178 and 212 clustered together.

The cluster B consisted of 51 genotypes which further divided into two sub clusters *viz.,* B_1 and B_2 at 49 units of linkage distance. B_1 comprised of 29 genotypes and B_2 had 22 genotypes. The subcluster B_1 consisted of two subgroups $B_{1.1}$ and $B_{1.2}$ with a linkage distance of 34 units. In $B_{1.1}$ tree 6, 52, 181, 131, 197, 33, 192, 121 and 130 clustered together and in $B_{1.2}$ tree 15, 16, 137, 100, 39, 50, 245, 244, 85, 183, 221, 210, 98, 161, 119, 204, 104, 120, 134 and 143 clustered together. The subcluster B_2 was futher divided into $B_{2.1}$ and $B_{2.2}$ at 26 units of linkage distance. In $B_{2.1}$ genotypes like tree 23, 80, 114, 240, 102, 174, 60, 61, 109, 243, 106 and 84 clustered together and in $B_{2.2}$ tree 30, 64, 194, 238, 69, 82,47, 65, 157 and 167 clustered together.

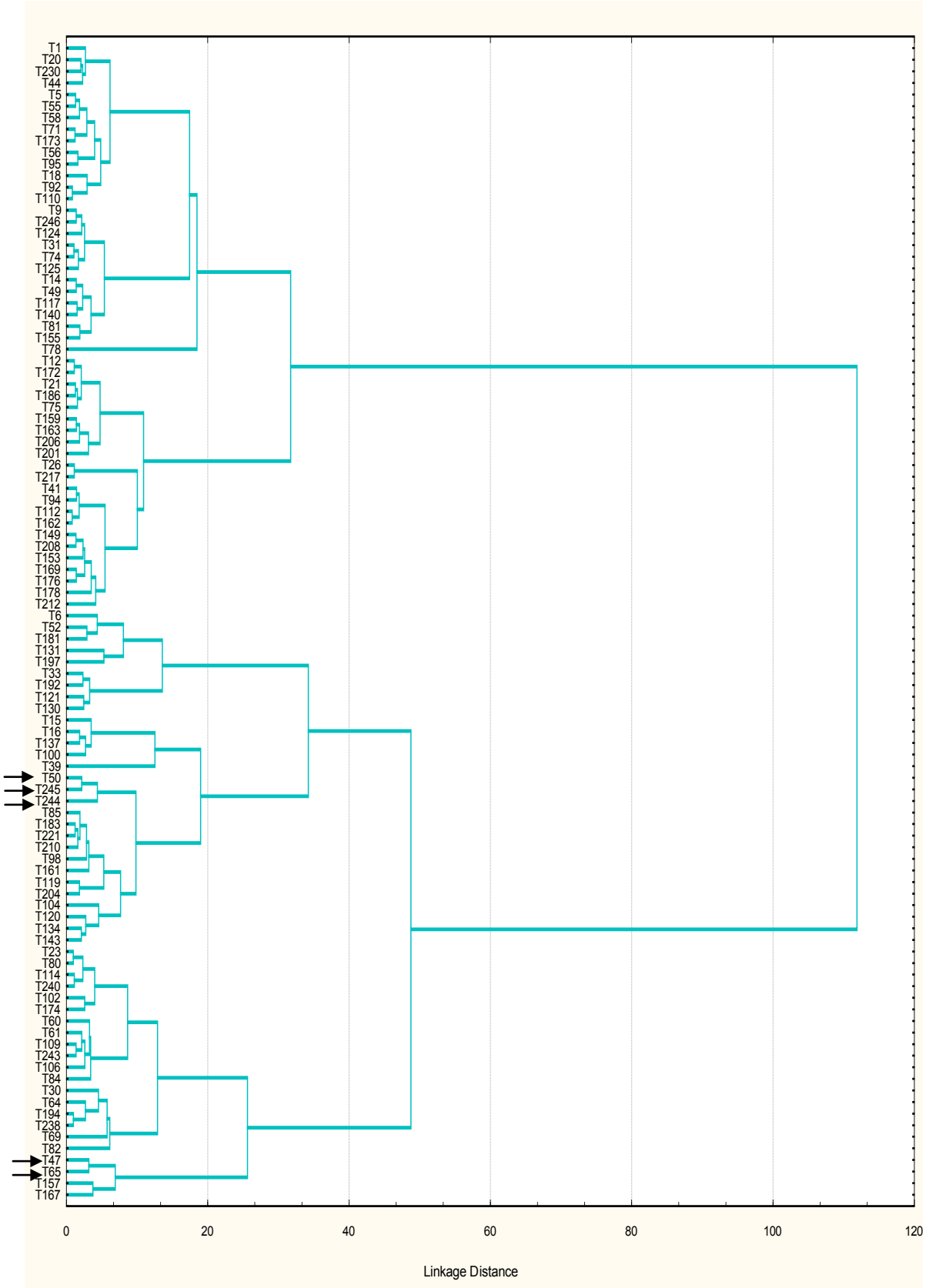


Fig 2: Dendrogram showing clustering of 100 genotypes based on 12 quantitative parameters (Arrow indicates the location of the elite trees)

Table 6: Pulp weight to seed weight ratio

Sl. No.	Tree no.	Pulp wt. per fruit (gm)	Seed wt. per fruit (gm)	Pulp wt. to seed wt. ratio
1.	1	2.2	1.13	1.94:1
2.	5	2.43	1.16	2.1:1
3.	6	5.93	5.31	1.11:1
4.	9	2.79	2.29	1.21:1
5.	12	2.45	1.17	2.1:1
6.	14	2.63	1.38	1.9:1
7.	15	4.51	2.73	1.65:1
8.	16	4.52	2.75	1.64:1
9.	18	2.09	2.02	1.03:1
10.	20	1.97	0.47	4.19:1
11.	21	2.34	1.3	1.8:1
12.	23	2.22	1.77	1.25:1
13.	26	1.24	0.41	3.02:1
14.	30	2.31	0.87	2.65:1
15.	31	2.79	2.21	1.26:1
16.	33	4.92	2.75	1.78:1
17.	39	3.55	1.12	3.17:1
18.	41	1.55	0.96	1.61:1
19.	44	1.91	1.37	1.39:1
20.	47	6.73	2.82	2.38:1
21.	49	2.93	1.51	1.94:1
22.	50	6.66	2.73	2.43:1
23.	52	6.29	2.76	2.27:1
24.	55	2.89	1.96	1.47:1
25.	56	2.88	1.67	1.72:1
26.	58	3.06	1.52	2.01:1
27.	60	1.76	0.85	2.07:1
28.	61	2.11	1.23	0.27:1
29.	64	3.19	2	1.59:1
30.	65	6.18	1.54	4:1
31.	69	3.56	2.43	1.46:1
32.	71	2.58	1.98	1.3:1
33.	74	2.77	2.43	2.54:1
34.	75	2.45	1.09	2.24:1
35.	78	3.18	1.04	3.05:1
36.	80	2.62	1.82	1.43:1
37.	81	4.08	1.71	2.38:1
38.	82	2.86	1.56	1.83:1
39.	84	2.18	0.92	2.36:1
40.	85	4.19	2.47	1.69:1
41.	92	2.91	1.97	1.47:1
42.	94	1.5	0.84	1.78:1
43.	95	3.52	1.87	1.88:1
44.	98	4.52	1.63	2.77:1
45.	100	3.77	3.37	1.11:1
46.	102	3.56	1.93	1.84:1
47.	104	4.14	5.38	0.76:1
48.	106	3.04	0.73	4.16:1
49.	109	2.59	0.91	2.84:1
50.	110	2.75	1.88	1.46:1
51.	112	1.67	0.59	2.83:1
52.	114	2.28	2.14	1.06:1
53.	117	2.97	1.6	1.85:1
54.	119	3.93	2.69	1.46:1

Table 6: cont.....

Sl. No.	Tree no.	Pulp wt. per fruit (gm)	Seed wt. per fruit (gm)	Pulp wt. to seed wt. ratio
55.	120	4.14	2.57	1.61:1
56.	121	3.86	4.41	0.87:1
57.	124	2.56	1.69	1.51:1
58.	125	2.98	1.41	2.07:1
59.	130	4.64	3.32	1.39:1
60.	131	6.99	4.03	1.73:1
61.	134	3.83	2.51	1.52:1
62.	137	4.57	3.33	1.37:1
63.	140	3.33	2.05	1.62:1
64.	143	3.93	3.07	1.28:1
65.	149	1.8	1.11	1.62:1
66.	153	1.81	1.37	1.32:1
67.	155	3.53	2.21	1.59:1
68.	157	5.24	2.24	2.33:1
69.	159	2.28	1.91	1.19:1
70.	161	3.76	2.07	1.81:1
71.	162	1.94	0.59	3.28:1
72.	163	2.05	1.49	1.37:1
73.	167	4.6	1.98	2.32:1
74.	169	1.65	0.89	1.85:1
75.	172	2.4	1.58	1.51:1
76.	173	2.73	1.4	1.95:1
77.	174	4.06	2.61	1.55:1
78.	176	1.77	1.33	1.33:1
79.	178	1.66	3.36	0.49:1
80.	181	6.07	3.93	1.54:1
81.	183	3.63	2.46	1.47:1
82.	186	2.47	1.05	2.35:1
83.	192	4.5	3.7	1.21:1
84.	194	2.76	2.31	1.19:1
85.	197	6.3	5.65	1.11:1
86.	201	3.27	0.29	11.2:1
87.	204	3.52	1.99	1.76:1
88.	206	2.94	0.89	3.3:1
89.	208	2.11	0.42	5.02:1
90.	210	3.09	2.88	1.07:1
91.	212	1.87	0.75	2.49:1
92.	217	0.93	0.41	2.26:1
93.	221	3.93	2.1	1.87:1
94.	230	2.06	0.98	2.10:1
95.	238	2.88	2.3	1.25:1
96.	240	2.53	1.97	1.28:1
97.	243	3.12	1.72	1.81:1
98.	244	6.51	3.54	1.83:1
99.	245	5.97	1.43	4.17:1
100	246	2.84	2.57	1.1:1

4.3 PCR-based RAPD technique to detect polymorphism among the genotypes

4.3.1 Genomic DNA isolation and quantification

The DNA isolated was intact as checked on 0.8 per cent agarose gel (Plate 1) and ratio of absorption was 1.8 to 1.9 conforming good quality of the isolated DNA. The quantity of DNA isolated ranged from 5.75-5760 ng/µl of tissue, DNA was diluted accordingly to obtain a uniform concentration of 30 ng/µl and used for PCR amplification.

4.3.2 RAPD profiles

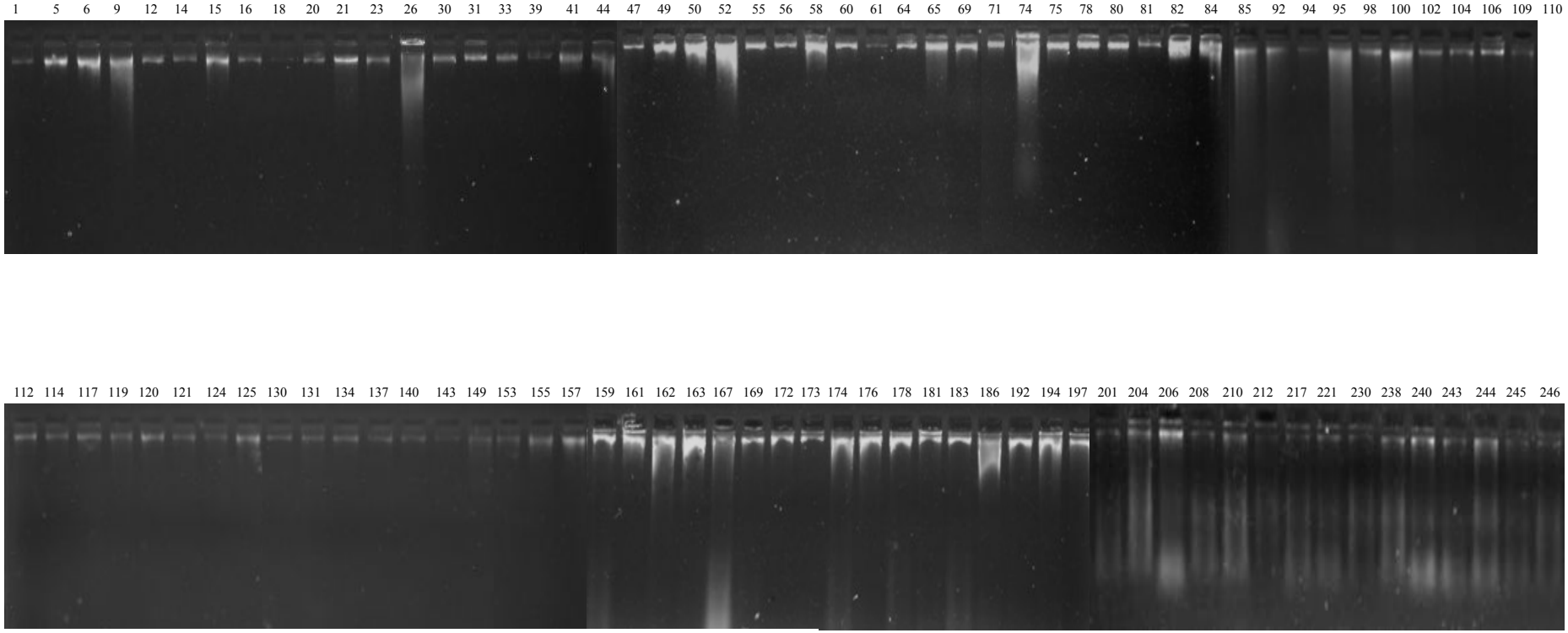
All the 100 genotypes were initially screened with 8 random primers to detect primers showing amplification. Of the 8 primers used, DNA was amplified with six primers. The photographs showing RAPD products for all the 100 genomic DNA with 6 primers are presented in Plates 11, 12, 13, 14, 15 and 16.

In total, 61 scorable bands were produced in 100 genotypes with 6 primers (Table 7). The number of bands produced per primer varied from 8-13 with an average of 10.16 bands per primer. Out of 61 bands recorded, all the 61 bands (i.e. 100 %) were polymorphic. Among the selected primers OPA-Y01 produced maximum number of bands (13 bands) and minimum of 8 bands produced by the primer OPA-R15.

4.3.3 Detection of polymorphism among the genotypes

Primer OPA-A09

The amplification profile of OPA-A09 with the 100 genotypes detected 9 polymorphic fragments. The DNA of tree 245 amplified a unique eighth fragment of 210 bp. The primer failed to amplified the DNA from tree 21, 30, 39, 58, 65, 69, 82, 84, 94, 100, 106, 130, 134, 143, 155, 162, 172, 181, 186, 201, 208, 230, 238 and 246.

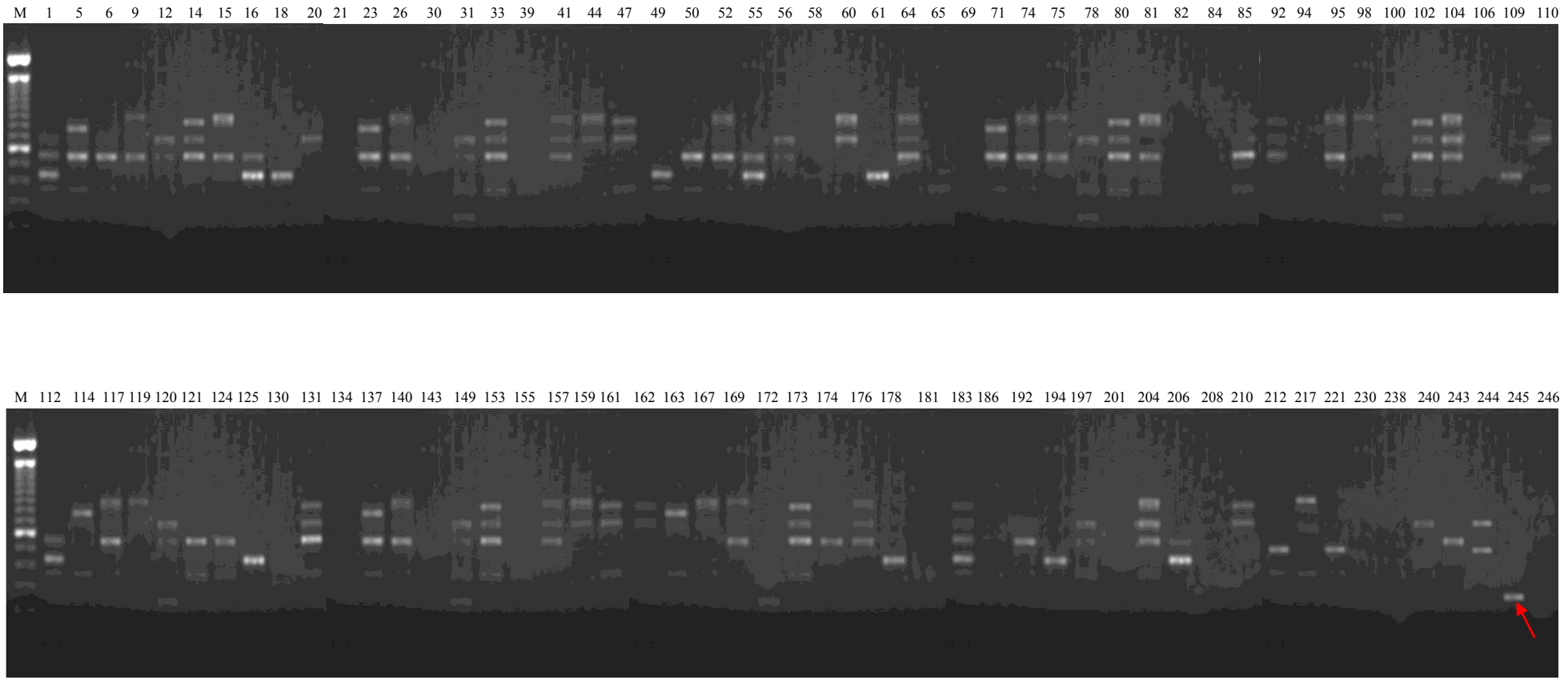


Numericals indicates the corresponding tree numbers

Plate 10: Genomic DNA isolated from the 100 tamarind genotypes

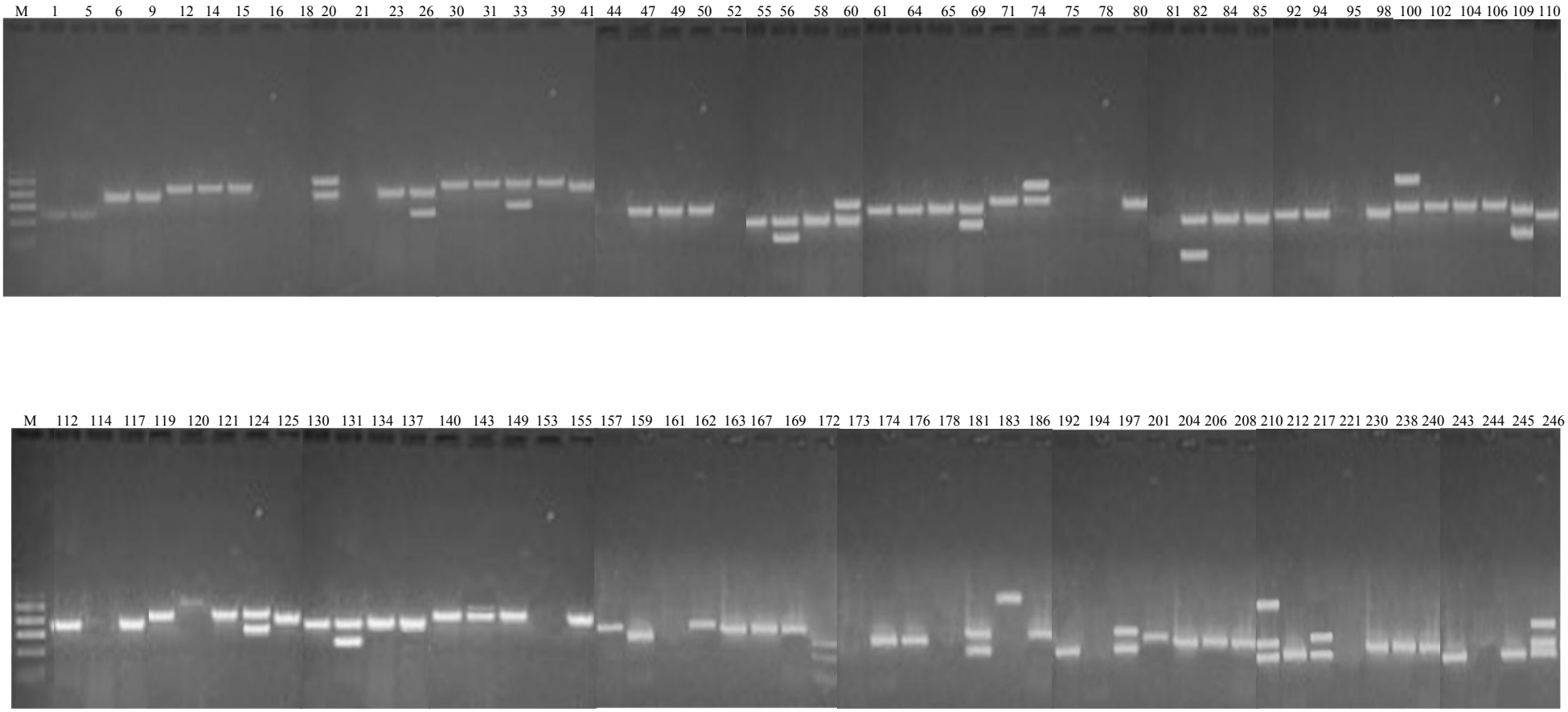
Table 7: Scorable DNA bands generated by different random decamer primers through PCR

Primer	Sequence of primer (5' to 3')	Total Amplified Product	Polymorphic bands
OPA-A09	GGGTAACGCC	9	9
OPA-B06	TGCTCTGCCC	10	10
OPA-K06	CACCTTTCCC	11	11
OPA-R15	GGACAACGAG	8	8
OPA-X01	CTGGGCACGA	10	10
OPA-Y01	GTGGCATCTC	13	13
Pooled		61	61
Average		10.16	10.16



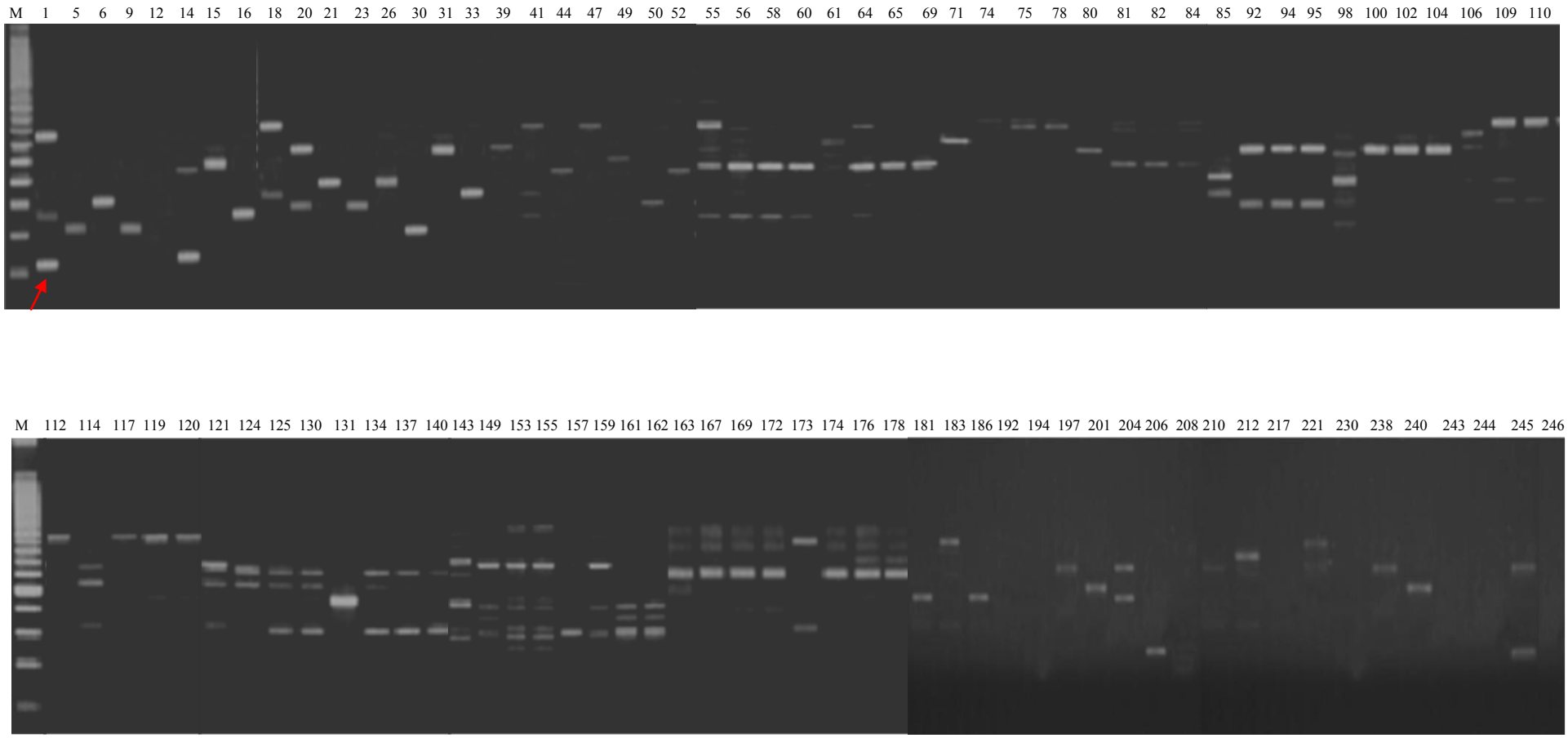
M= 100bp marker DNA ; Numericals indicates the corresponding tree numbers ; Red arrow indicates the unique fragment

Plate 11: RAPD profiles of 100 tamarind genotypes using the primer OPA-A09



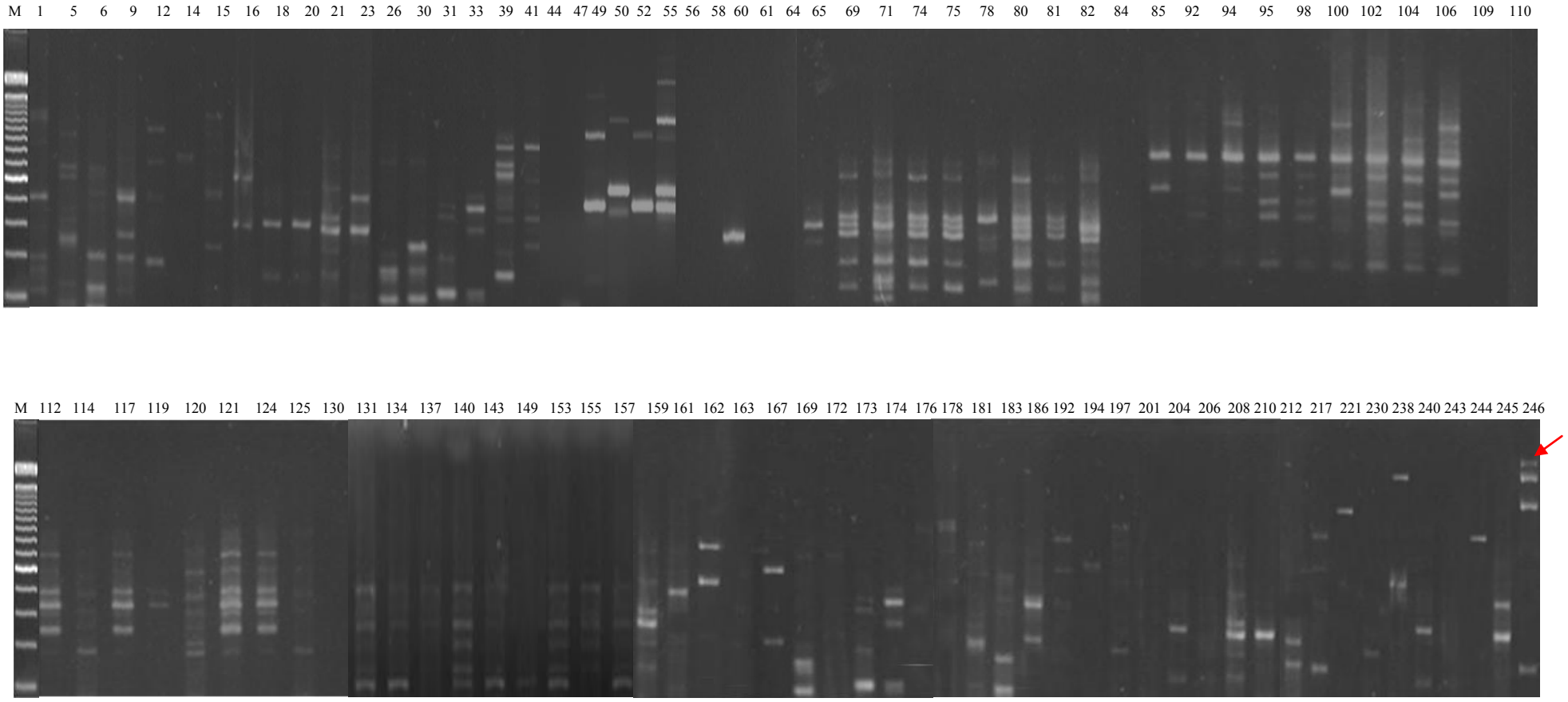
M= 100bp marker DNA ; Numericals indicates the corresponding tree numbers ; Red arrow indicates the unique fragment

Plate 12: RAPD profiles of 100 tamarind genotypes using the primer OPA-X01



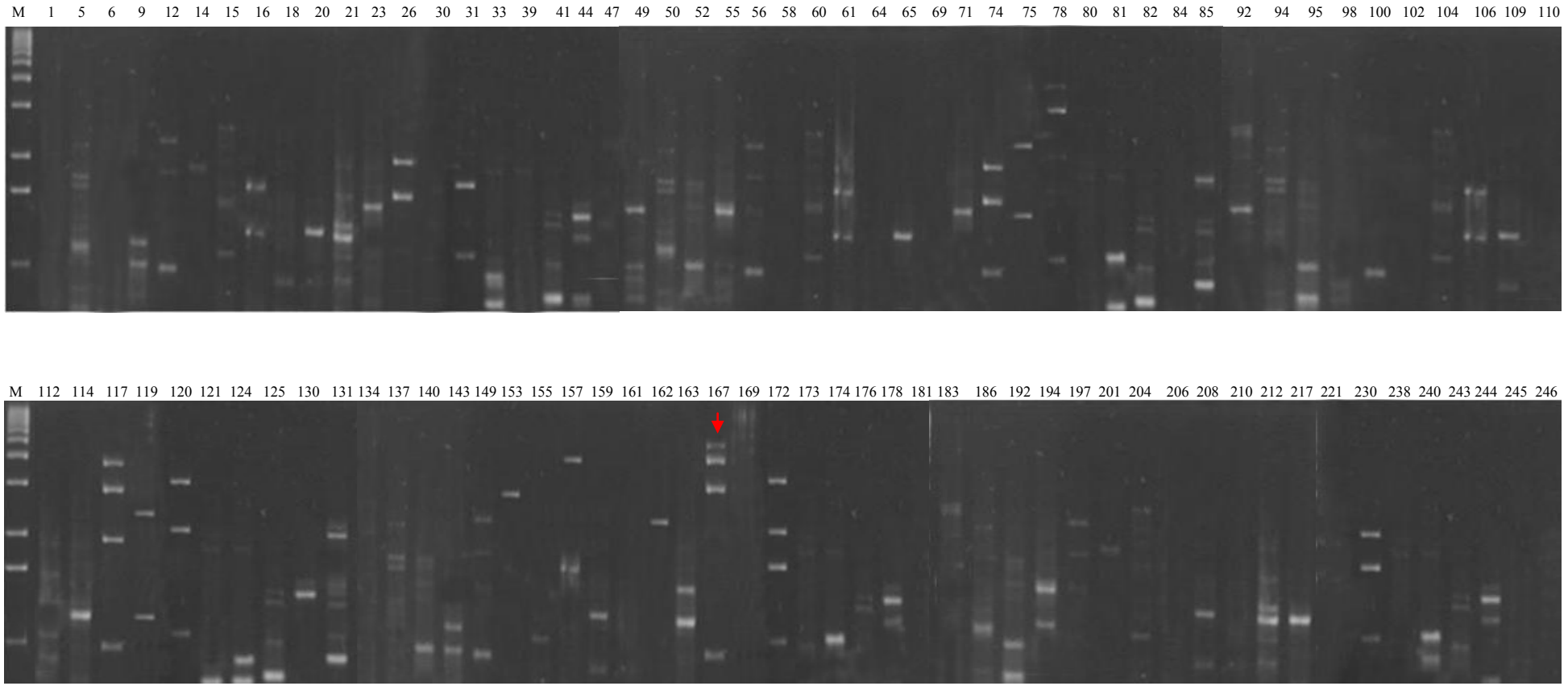
M= 100bp marker DNA ; Numericals indicates the corresponding tree numbers ; Red arrow indicates the unique fragment

Plate 13: RAPD profiles of 100 tamarind genotypes using the primer OPA-K06



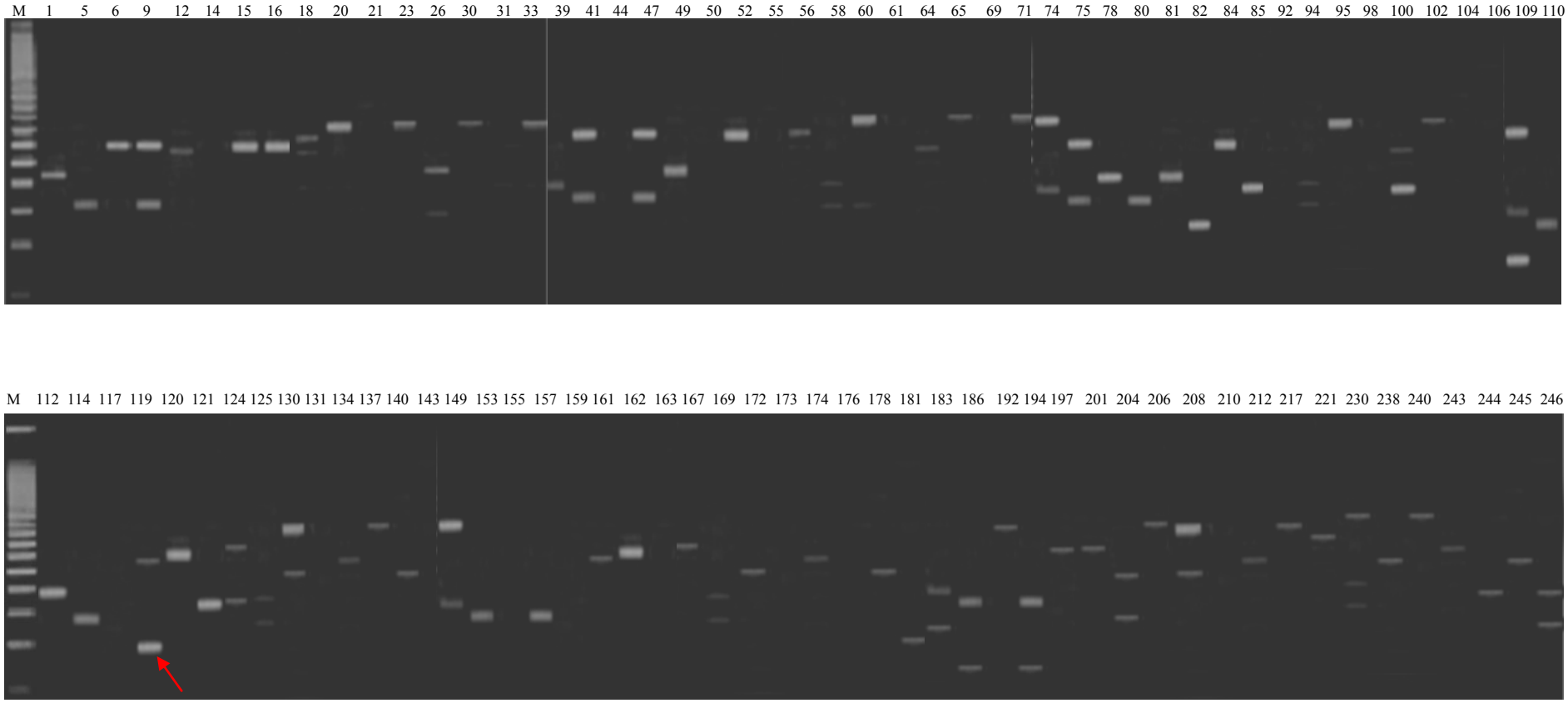
M= 100bp marker DNA ; Numericals indicates the corresponding tree numbers ; Red arrow indicates the unique fragment

Plate 14: RAPD profiles of 100 tamarind genotypes using the primer OPA-Y01



M= 100bp marker DNA ; Numericals indicates the corresponding tree numbers ; Red arrow indicates the unique fragment

Plate 15: RAPD profiles of 100 tamarind genotypes using the primer OPA-B06



M= 100bp marker DNA ; Numericals indicates the corresponding tree numbers ; Red arrow indicates the unique fragment

Plate 16: RAPD profiles of 100 tamarind genotypes using the primer OPA-R15

Primer OPA-B06

This primer failed to amplified DNA from tree 1, 6, 14, 30, 39, 47, 58, 64, 69, 80, 84, 98, 102, 110, 134, 161, 169, 173, 181, 183, 206, 210, 221, 238, 243, 245 and 246. It amplifies a unique fragment of 740 bp from DNA of tree no. 167 which was absent in the other genotypes.

Primer OPA-K06

This primer amplified a total of 11 fragments and the eleventh fragment of 120 bp was amplified only by DNA of tree 1 which was absent in other genotypes.

Primer OPA-R15

This primer totally amplified 8 fragments where the seventh fragment of 200 bp was amplified only by DNA of tree 119. The primer failed to amplified DNA from tree 14, 21, 31, 44, 50, 55, 61, 69, 92, 98, 104, 106, 117, 131, 143, 155, 159, 163, 173, 176 and 210.

Primer OPA-X01

This primer amplified a total of 10 fragments but it fails to amplified DNA from tree 16, 18, 21, 44, 52, 75, 78, 81, 95, 114, 153, 161, 173, 178, 194, 221 and 244.

Primer OPA-Y01

This primer amplified a maximum of 13 fragments. The first fragment of 600 bp was amplified only by DNA of tree 246 which was absent in other genotypes.

4.3.4 Genetic diversity analysis for the 100 genotypes

The dendrogram of 100 tamarind genotypes drawn based on RAPD markers is represented in Fig. 3. From the dendrogram, the genotypes clearly divides into two major clusters A and B separated at a linkage distance of 62 units. The major cluster A was subdivided into A_1 and A_2 at a linkage distance of 57 units. The subcluster A_1 further divided into subcluster $A_{1.1}$ and $A_{1.2}$ at 42 units, while A_2 into $A_{2.1}$ and $A_{2.2}$ at a linkage distance of 31 units.

The cluster A consisted of 64 genotypes distributed between tree 1 and 124. The subcluster A_1 consisted of 50 genotypes in which the subgroup $A_{1.1}$ includes genotypes like tree 1, 12, 149, 197, 161, 194, 6, 9, 20, 23, 26, 143, 30, 31, 174, 5, 153, 173, 183, 14, 44, 204, 15, 52, 117, 95, 125, 140, 39, 85, 98, 100, 120, 167 and 169 and the subgroup $A_{1.2}$ had genotypes like tree 55, 157, 110, 172, 163, 130, 134, 58, 60, 137, 92, 94, 114, 176 and 162.

The subcluster A_2 consisted of 14 genotypes where in the subgroup $A_{2.1}$ genotypes like tree 33, 71, 74, 78, 65, 69, 81, 75, 159 and 80 clustered together and in the subgroup $A_{2.2}$ tree 102, 104, 121 and 124 clustered together.

The cluster B consisted of totally 36 genotypes which further divided into two sub clusters *viz.,* B_1 and B_2 at 32 units of linkage distance. B_1 consisted of 26 genotypes while B_2 had 10 individuals.

The subcluster B_1 consisted of two subgroups $B_{1.1}$ and $B_{1.2}$ with a linkage distance of 23 units. In $B_{1.1}$ 14 genotypes like tree 16, 18, 21, 84, 178, 206, 47, 61, 64, 109, 49, 119, 50 and 106 clustered together and in $B_{1.2}$ genotypes like tree 41, 112, 155, 221, 181, 230, 240, 186, 245, 201, 208 and 238 clustered together. The subcluster B_2 was further divided into $B_{2.1}$ and $B_{2.2}$ at 24 units of linkage distance. In $B_{2.1}$ genotypes like tree no. 56, 82 and 131 clustered together and in $B_{2.2}$ tree 192, 246, 217, 210, 244, 212 and 243 clustered together.

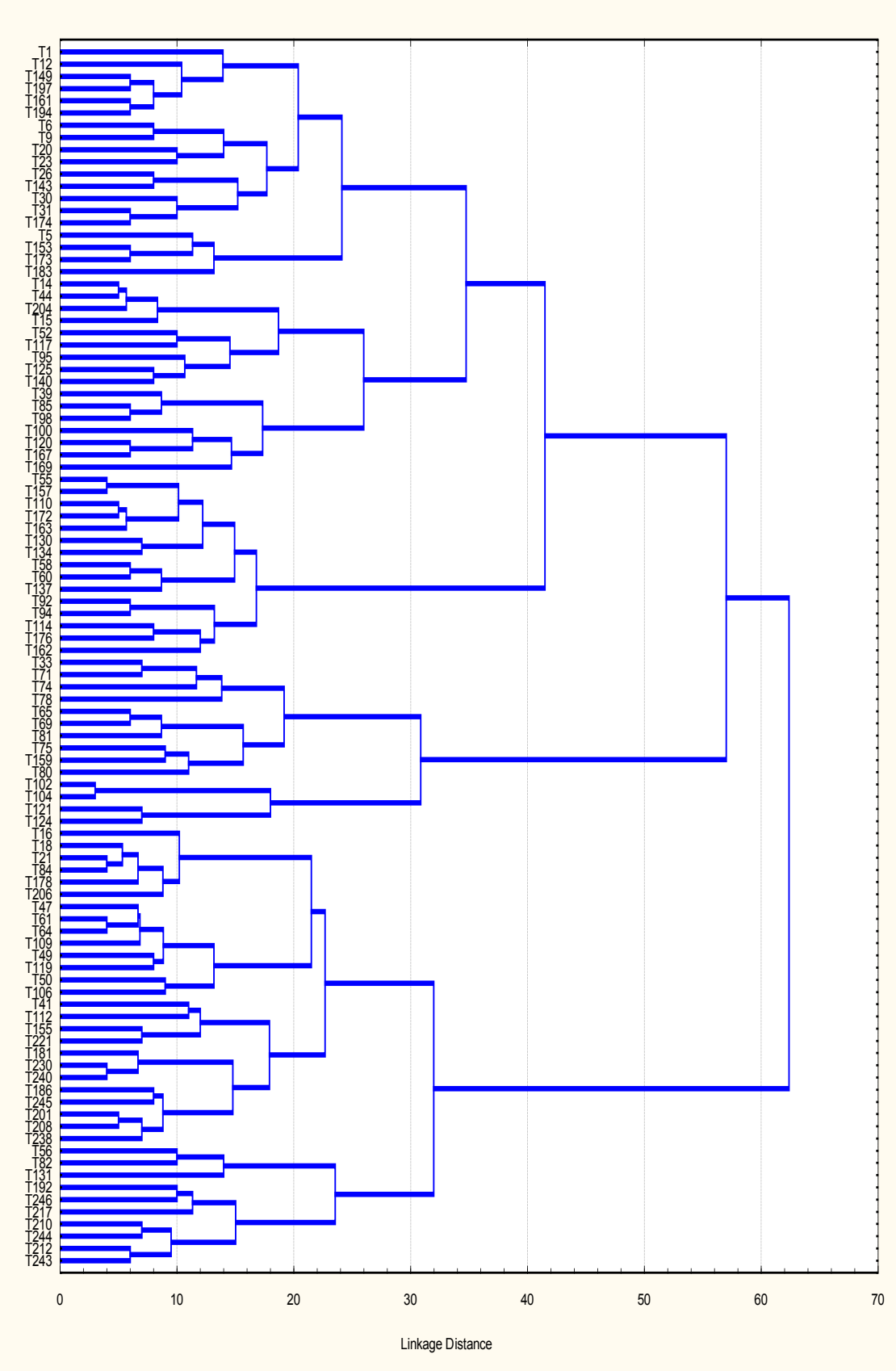


Fig 3: Dendrogram showing clustering of 100 genotypes based on RAPD studies

Fig 4: Dissimilarity matrix of 100 tamarind genotypes based on Squared Euclidean

4.3.5 Genetic Distance

The genetic dissimilarity matrix based on Squared Euclidean Distance is given in fig. 4. The genetic distance in the distance matrix ranged from 3-22 percent. The minimum genetic distance of 3 percent was found in between the genotype tree 104 and 102. The maximum genetic distance of 22 percent was observed in between the genotypes tree 169 and 23, 246 and 5, 246 and 95 and also between 246 and 121.

4.4 Identification of the elite genotypes from the population based on morphometric traits.

After analysing the morphometric parameters which served the criteria for selection of the elite or plus trees described in the Materials and Methods (3.5), five genotypes (Tree 47, 50, 65, 244 and 245) were selected as elite trees from among the population as they fulfilled the criteria to be considered as an elite tree (Table 8).

Table 8: Morphometric parameters of five plus trees

Sl. no	Genotypes	Selection Criteria								
		Crown shape	**Foliage characteris-tics**	**Trunk characterist-ics**	**Bearing habit**	**Seed shape and colour**	**Pulp colour**	**Fruit weight (gm/fruit)**	**Pulp weight (gm/fruit)**	**Pulp wt. to seed wt. ratio**
1	Tree 47	Oval	Dense & dark green	Monotrunk	Dense & regular	Flat & glassy black	Red brown	11.41	6.73	2.38:1
2	Tree 50	Dome	Dense & dark green	Monotrunk	Dense & regular	Flat & glassy black	Red brown	11.78	6.66	2.43:1
3	Tree 65	Oval	Dense & dark green	Monotrunk	Dense & regular	Flat & glassy black	Red brown	10.86	6.18	4:1
4	Tree 244	Dome	Dense & dark green	Monotrunk	Dense & regular	Flat & glassy black	Red brown	11.92	6.51	1.83:1
5	Tree 245	Dome	Dense & dark green	Monotrunk	Dense & regular	Flat & glassy black	Red brown	10.38	5.97	4.17:1

Tree 47

Tree 50

Tree 65

Tree 244

Tree 245

Plate 17: Dense bearing elite trees.


DISCUSSION

V DISCUSSION

Tamarind (*Tamarindus indica* L.) is a domesticated species of high economic value and has been recorded as a variable species for many of its morphological characters like pulp colour, fruit size and shape (Von Mueller, 1881). Genetic improvement activities in tamarind were initiated in India a decade ago and its domestication was mainly aimed at improving the pulp yield. At present in India a few good germplasm bank and single progeny trials are available (Nagarajan *et al.,* 1998). Since there is an extensive variation in characters like foliage, flower and pod production, considerable scope to improve the species and improvement holds the key for boosting productivity and yield of the orchards leading to the development and propagation of genotypes with desirable characters.

Keeping this in view, the present investigation was undertaken to study the phenology and the extent of genetic diversity of the tamarind grove at Nallur to identify candidate plus trees based on pod size and fruit pulp to seed ratio etc. As tamarind can also be easily propagated by grafts, the selected plus trees can be utilized for clonal propagation. Very recently tamarind clonal populations are becoming popular because of their uniform growth and performance and therefore such of the selected plus trees from among the genotypes in Nallur tamarind grove can also be utilized for the same purpose.

Keeping these factors, efforts were made to study the phenological variations and analyze genetic diversity of the Nallur tamarind grove using RAPD markers. The results of the present investigaton are discussed as under.

Very low fruit setting noticed in tamarind under open pollination is not a rarity, such a condition is quite common in many tropical trees (Bawa, 1974). A low fruit set mean value is normally an indicator of pollinator limitation (Calvo, 1990). This is severe especially when monoculture is practiced but however it can be

overcome by introducing captive bee hives. Specialized anther arrangement makes pollinator interaction effective (Harder and Barrett, 1993; Nilsson, 1988) while herkogamy promotes a strongly outcrossing breeding system under natural conditions. Colouration in reproductive organs, a qualitative trait has been earlier recorded only in temperate trees and in comparison no such inheritance has so far been reported in tropical trees (Sanjay *et al.,* 2008). However, a range of colouration of floral parts have been recorded in the present investigation and can be of immense breeding value and can be effectively used as a morphological marker in progeny testing programmes.

Considering the phenological traits at reproductive phase five categories of petal colouration were observed and the most common was yellow coloured petal with red coloured streaks. Most of the trees having this colour also exhibited a fruit weight ranging from 2 to 16 gm/fruit. Similarly, the fruit colour dimorphism in the population in our investigation can also be used as a morphometric marker. Fruit pulp colour was either reddish brown or light brown. Among the population of 100 trees most of them were mono trunked while double trunked and even multiple trunked trees were also observed.

A large amount of variation was noticed in quality traits like crown shape, varied from irregular to dome shaped, foliage were either dense and dark green to sparse and light green. While some were heavy bearing trees and some were either medium or sparse bearers. It was evident from the present studies that all the hundred genotypes bear fruit in 2009 fruiting season however, during the previous year nine trees did not fruit at all. So, they were irregular bearers, whether they exhibit alternate bearing habits like mango is a matter of future consideration to be investigated.

The observations for 14 qualitative traits were then used to cluster the genotypes which grouped the genotypes into two main clusters. From the dendrogram (Fig. 1), tree number 1 and tree number 44 were found to be placed at a larger genetic distance of 108 units and can be utilized for crossing programme.

Though tamarind is an economically important crop in India, so far not much work has been done to understand and improve tamarind as a cash crop. Presently, tamarind is grown throughout India and majority of them are of seedling origin. The population under study has shown a high degree of variation and a wide range of heterozygosity with respect to size and pulp quality of fruit. This heterozygous nature of plant gives scope for further selection and establishing desirable plus trees. Therefore, twelve quantitative traits like pod length, pod width, beak length, number of ridges, number of furrows, fruit weight, epicarp weight, fibre number, fibre weight, pulp weight, seed weight and seed number have been analysed to establish plus trees and identify their yield levels. Similar work has been done for evaluating the elite clones in tamarind by Prabhusankar *et al.,* in 2004.

The data revealed wide range of variation in the fruit characteristics. The longest length of fruits were recorded in genotype 167 with 20.04 cm to 6.65 in the genotype 110. Beak length varied from 0.04 to 0.45 cm, fibre number ranged from 2.6 to 5.6 fibres/fruit, pulp weight was the highest in tree 131 (6.99 gm) and lowest (0.93gm) in genotype 217. Similar trend of variation in fruit characteristics was observed in tamarind by Divakara in 2008.

The pulp to seed ratio was found to be the highest in genotype 201 (11.2:1). Although, the pulp to seed ratio was highest in this genotype, the tree was associated with certain undesirable characters like irregular crown shape and sparse bearing which did not fulfilled the genotype to termed as one among the elite or plus trees. But

however, most of the trees selected as plus trees had a higher pulp to seed ratio. They are genotypes 245 (4.17:1), 65 (4:1), 50 (2.43:1), 47 (2.38:1) and 244 (1.83:1). The selected elite trees were also found to be resistance against pod borer, which is considered as one of the desirable characteristic of an elite tree (Yang Kai *et al.,* 2008).

Intrapopulation clustering of the genotypes was developed using all the twelve quantitative traits and compared with the clustering of the population using fourteen qualitative parameters. On comparison it was found that there was lack of consistency in obtaining similar results. Difficulties in classifying these individuals based on morphogeny could be as a result of the confounding influences of environmental effects on the whole plant phenotype. These influences may result in an over or under estimation of genetic diversity. The limitations of this classification suggest that a classification scheme based on molecular marker could thus provide an accurate structure of the diversity and this diversity may be used to properly assign the status of a plant material and their genetic relationship (Anil *et al.,* 2006). Hence, the samples were further subjected to molecular analysis using RAPD markers.

Of the six primers used in the study all of them produced polymorphic bands. In total 61 scorable bands were produced in the 100 genotypes. The number of bands produced per primer varied from 8 to 13. Primer OPA-A09 amplified a unique fragment of 100 bp specific to genotype tree 245 which incidently had higher pulp weight to seed weight ratio of about 4.17:1. Fifteen samples were monomorphic for the primer OPA-A09 while twenty five samples did not amplified at all. Similar such unique fragments of 740, 600, 120 and 200 bp have amplified exclusively from DNA of genotypes tree 167, 246, 01 and 119 with primers OPA-B06, OPA-Y01, OPA-K06 and OPA-R15 respectively. The 120 bp fragment amplified by DNA of genotype 01 has eleven natural clones arising from the mother tree. The uniqueness of the tree is that the massive trunk is completely

deteriorated without any cambial connections, completely split into two pieces but is still surviving under all natural calamities exposed over the years deriving its nutrients through the supporting clones. The 740 bp fragment amplified exclusively by the primer OPA-B06 from the DNA of genotype 167 may be consider as an associated marker for having the highest pod length among the genotypes.

The cluster analysis based on Squared Euclidean distance using Ward's method with 6 random primers allowed discriminating tamarind genotypes. The distinctive discriminative profiles from each cluster are very useful in identifying the genotypes and it is useful to document each genotypes. Similar discriminative ability of RAPD markers in identifying the genotypes has been documented in several other fruit crops like avocado (Lewis, 1992), apple (Koller *et al.,* 1993), mango (Schnell *et al.,* 1995; Hemanthkumar *et al.,* 2000), guava (Prakash *et al.,* 2002).

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 traits by correlating it with the RAPD profile. In the following paragraphs an attempt has been made to describe the relationship between genotypes based on RAPD analysis to the morphological characters.

The dendrogram along with Squared Euclidean distances drawn on 100 tamarind genotypes clearly indicated a low to moderate genetic diversity among the genotypes. The measure of dissimilarity namely Squared Euclidean distance showed different levels of diversity. The dissimilarity for the 100 tamarind genotypes ranged from 3 to 22 percent.

It is clear from the dendrogram (Fig. 3) that the three genotypes *viz.,* Tree 56, 82 and 131 belonging to one cluster ($B_{2.1}$). This is the shortest cluster from among the 100 genotypes studied. These three genotypes have many characters that are in common. For example, all

Plate 18 : Tree 1 with its clones (Arrow indicates the clones)

the three genotypes developed the same yellow sepal colour. Further, the pod colour of both the genotypes is light brown. Similarly, even the shape of the fruit is curved. The seed shape and colour of the three genotypes are found to be flat with an attractive glassy black colour. The shape of the crown of these three genotypes was also found to be similar with an oval shape structure. Bearing habit which is one of the most important characteristic noticed in fruit crops was medium which can be attributed to genetic make up of the plant.

Comparing the dendrogram constructed based on the quantitative parameters and with the one constructed from RAPD studies there was certain fluctuation in the clustering of genotypes. In the dendrogram using quantitative traits (Fig. 2) the genotypes having highest pod length and highest pod width were clustered at one cluster ($B_{2.2}$). Similarly, genotypes having highest fruit weight and high pulp weight also clustered at the same cluster ($B_{1.1}$). But, when they were compared with the dendrogram based on molecular studies (Fig. 3) they clustered differently i.e. the genotypes having highest pod length clustered at $A_{1.1}$, highest pod width clustered at $B_{1.1}$, highest fruit weight clustered again at $A_{1.1}$ and highest pulp weight clustered at $B_{2.2}$. Hence, morphologically similar genotypes were now found to be placed apart at greater genetic distance and such genotypes can be selected as diverse parents for future breeding programmes. Similar studies have been reported in tamarind by Boukary *et al.,* in 2007 and in coconut by Rodrigruez *et al.,* in 1997.

Genetic dissimilarity within the population was 3-22 percent. A maximum distance of 22 percent was between genotypes 169 and 23, 246 and 5, 246 and 95 and also between 246 and 121 while a minimum dissimilarity of 3 percent was between 104 and 102.

From the dendrogram constructed based on molecular markers (Fig. 3) it is clear that subcluster $A_{1.1}$ consists of 35 genotypes and a maximum dissimilarity of 22 percent between genotypes 16 and23

was noticed. Similarly, subcluster $A_{1.2}$ consists of 15 genotypes with a maximum dissimilarity of 13 percent between genotypes 60 and 163.

The subcluster $A_{2.1}$ have 10 genotypes with a maximum dissimilarity of 15 percent between genotypes 33 and 159 as well as between 33 and 69 while subcluster $A_{2.2}$ consists of 4 genotypes with a maximum dissimilarity of 12 percent between 102 and 121.

The subcluster $B_{1.1}$ consists 14 genotypes with a maximum dissimilarity of 14 percent between genotypes 16 and 47 and subcluster $B_{1.2}$ consists of 12 genotypes with a maximum dissimilarity of 14 percent between genotypes 16 and 47 was observed.

The subcluster $B_{2.1}$ consists of only 3 genotypes having the maximum dissimilarity of 14 percent between genotypes 82 and 131 while the subcluster $B_{2.2}$ consists of 7 genotypes with a maximum dissimilarity of 17 percent between 246 and 131.

Therefore, the genotypes showing higher percentage of dissimilarity are highly diverse genotypes and hence can be utilized as parents for future breeding programmes including clonal propagation. For example, genotype 23 has higher pod length of 10.70 cm than the genotype 16 (7.76). On the other hand, genotype 16 has higher fruit weight (10.78gm) and pulp weight (4.52gm) compare to genotype 23. Therefore, these two diverse genotypes can be used as parents in future crossing programme.

Considering the clustering of the plus trees in all the three dendrograms constructed based on qualitative parameters, quantitative parameters and RAPD markers, the genotype 65 was present in cluster A. Moreover, it was found to have the higher pulp to seed ratio among the plus trees which is on par with genotype 245. Although, genotype 47 was clustered at A in the morphological

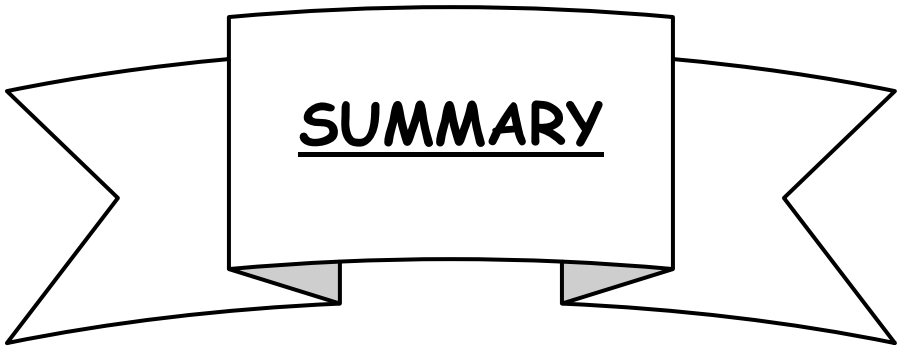
dendrogram, it was represented in the B cluster in the dendrogram elucidated out of RAPD analysis and it was found to have the highest pulp weight of 6.73 gm/fruit among the plus trees. Similarly, genotype 244 was also found to have the highest fruit weight 11.92 gm/fruit . In addition to this, among the plus trees, the maximum genetic distance of 14 % was found between genotypes 65 and 244 followed by genotypes 47 and 65 with a genetic distance of 13 %. Therefore, the three genotypes i.e. genotype 65, 47 and 244 can be used as parentage in future tree improvement programmes to introgress desirable trait of interest.

Our study thus indicated that clustering of genotypes using phenotypic traits are confounded by environmental interaction. While DNA method of analysing genetic diversity presented in the study shall provide a useful supplement to traditional morphological and agronomic data for estimating genetic diversity within the population. This will also help in choosing the core collection which represents the genetic spectrum of the entire collection. The identified plus trees having a higher pulp weight to seed weight ratio can thus be suggested for clonal propagation.

Thus, from the studies it can be concluded that tamarind is an economically important multipurpose tree species. With the increasing population pressure, the demand for tamarind pulp has increased considerably. This has necessitated identifying superior elite trees like genotypes 47, 50, 65, 244 and 245 for monoculture plantations, without causing genetic erosion. Thus tree improvement through the application of genetic principles is basically directed towards modifying the heredity of tree populations to meet the needs of the farmers.

Future line of work

The genotypes used for the present study to study the intrapopulation diversity can further utilized to analyze their biochemical and nutritional values like vitamin C, iron, calcium phosphorous, carbohydrates, proteins, titrable acidity, total soluble solids etc. to identify the super elite trees for further multiplication and propagation. The selected five plus trees can further be used for clonal propagation. The principles of genetics can also be applied to the remaining trees in the tamarind grove by using those markers or primers that have not been tried on a larger scale in the present study.


SUMMARY

VI SUMMARY

A comprehensive study on the morphological and molecular diversity studies in the tamarind grove at Nallur revealed the following salient features.

❖ A range of colouration in the floral parts like the sepals and petals have been reported. Such a colouration is restricted to only temperate regions and not in tropical regions. But our studies have shown that such an intrapopulation variation for floral colours existing in the Tamarind Hereitage Site can be used to enhance pollinators for efficient fruit setting. Similarly, the fruit colour dimorphism observed can also be used as a morphological markers in progeny testing programme.

❖ The genotype 201 has the highest pulp to seed ratio, can be utilized as one of the parent for breeding programmes to introgress the trait of high pulp to seed ratio.

❖ At the molecular level, RAPD profiles of all the genotypes were compared. In all, sixty one scorable bands were produced with six primers, with the number of bands ranging from eight for OPA-R15 to thirteen for OPA-Y01. All the six primers used in the present study produced polymorphic bands.

❖ A 120 bp fragment has been amplified exclusively by primer OPA-K06 in the genotype 01 and not in any other samples. This genotype is considered to be one of the oldest trees having eleven clones which are not self sown but arising out of the mother tree whose cambial connections have been completely deteriorated but with a regular and sparse fruit set.

❖ The 740 bp fragment amplified exclusively by primer OPA-B06 from the DNA of the genotype 167 has the highest pod length from among the genotypes of the population.

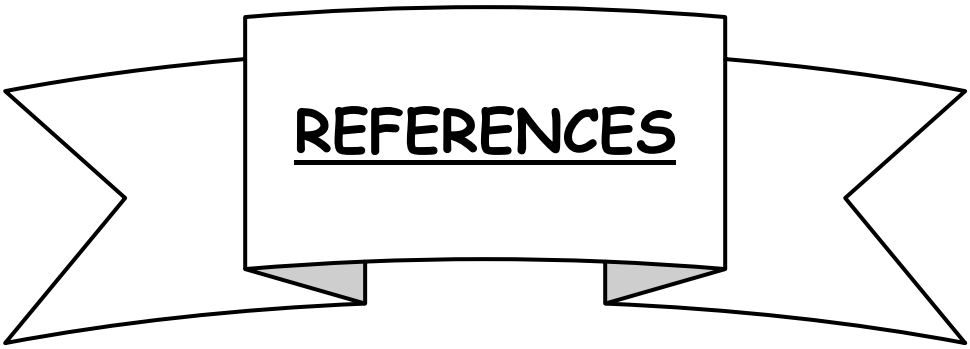
❖ The dendrogram showing clustering of tamarind genotypes based on morphological studies were not in accordance with molecular markers. Genotypes which were closely related were found to be separated with a greater distance at the molecular level.

❖ A considerable amount of intrapopulation diversity observed can be used to identify diverse parents like genotypes 16 and 23, 163 and 60, 33 and 159, 33 and 69, 102 and 121, 16 and 47, 82 and 131 and 246 and 131 which can be utilize in many hybridization programmes to efficiently transfer the desirable trait of interest.

❖ All the hundred genotypes had borne fruits during the fruiting season of 2009. However, previous year's data indicates that nine of them *viz.,* 75, 84, 163, 174, 184, 187, 204, 210 and 212 did not fruit at all. Whether they are alternate bearers needs to be further investigated.

❖ Of the hundred genotypes, five trees fulfilled the basic criteria to be termed as elite have been identified that can served as the base material to develop a clonal orchard or single progeny trials. These elite or plus trees are genotypes 47, 50 65, 244 and 245.

❖ Among the plus trees identified, the three genotypes 65, 47 and 244 were found to be very diverse in terms of fruit weight, pulp weight and pulp to seed ratio. Therefore, they can be used as parents in future tree breeding programmes.


REFERENCES

VII REFERENCES

ABRAHAM, Z., MALIK, S. K., GANGADHAR EASHWAR RAO., LAKSHMI NARAYANAN, S. AND BIJU, S., 2006, Collection and characterisation of Malabar tamarind [*Garcinia cambogia* (Gaertn.) Desr.]. *Genet. Resour. Crop Evol.,* **53**: 401-406.

ALI SARKHOSH., ZABIHOLLAH ZAMANI., REZA FATAHI AND HAMID RANJBAR., 2009, Evaluation of genetic diversity among Iranian soft-seed pomegranate accessions by fruit characteristics and RAPD markers. *Scientia Hort.,* **121**(3): 313-319.

*AMEL SALHI-HANNACHI1., KHALED CHATTI., OLFA SADDOUD., MESSAOUD MARS., ABDELMAJID RHOUMA., MOHAMED MARRAKCHI AND MOKHTAR TRIFI., 2006, Genetic diversity of different Tunisian fig (Ficus carica L.) collections revealed by RAPD fingerprints. *Hereditas,* **143**: 15-22

ANTOINE NSABIMANA AND JOHANNES VAN STADEN.,2007, Assessment of genetic diversity of Highland bananas from the National Banana Germplasm Collection at Rubona, Rwanda using RAPD markers. *Scientia Hort.,* **113**(3): 293-299.

*ANON., 1976, *Tamarindus indica* L. In The Wealth of India (Raw Materials Series) Council of Scientific and Industrial Research, New Delhi, **10:** 144-122.

ANIL KUMAR., SHARGA, A. N. AND PUSHPAGADAN, P., 2006, Genetic diversity of tamarind (*Tamarindus indica* Linn.). *Physio. and Mol. Biol. of Plt.,* **12**(2): 185-187.

ANURADHA UPADHYAY., JAYADEV, K., MANIMEKALAI, R. AND. PARTHASARATHY, V. A., 2004, Genetic relationship and diversity in Indian coconut accessions based on RAPD markers, *Scientia Hort.,* **99**(3): 353-362.

ASHBURNER, G. R., THOMPSON, W. K. AND HALLORAN, G. M., 1997, RAPD analysis of south pacific coconut palm population. *Crop Sci.*, **37**: 992-997.

BAGCHI, S. K., CHOUBEY, A. K. AND KULKARNI, H. D. 1987, Variability analysis among half-sib seedlings of *Santalum album* L. *Indian Forester*, **113** (5): 370-374.

BAGCHI, S. K., AND VEERENDRA, H. C. S., 1985, Study on intra-tree and inter-tree variations in leaves of *Santalum album*. *My Forest*, **21**: 33-39.

BAGCHI, S. K., AND VEERENDRA, H. C. S., 1991, Variation and relationship in developmental growth phases of *Santalum album* after pruning. *Indian Forester*, **117** (12): 1053-1058.

*BARAGANO DE MOSQUEDA, M., 1980, Technology of clarified tamarind juice. In Tropical and Subtropical Fruits. (Eds) Nagy, S. and Shaw, P.E. AVI Publishing, Westport, Conn, pp: 375.

*BAWA, K. S., 1974, Breeding systems of tree species of a lowland tropical community. *Evol.,* **28**: 85-92.

*BENERO, J.R., COLLAZO DE RIVERA, A.L. AND GEORAGE, L.M.I., 1974, Studies on the preparation and shelf life of soursoup, tamarind and blended soursop-tamarind soft drinks. *J. of the Agric. Univ, Puerto Rico,* pp: 58-99.

BOUKARY OUSMANE DIALLO., HELENE, I., JOLY., DOYLE MCKEY., MARTINE HOSSAERTMCKEY. AND MARIE HELENE CHEVALLIER., 2007, Genetic diversity of *Tamarindus indica* populations: Any clues on the origin from its current distribution? *Afr. J. Biotechnol.,* **6** (7): 853-860.

BRANDIS, D., 1921, Indian Trees. *Tamarindu*s Linn. Constable and Co. Ltd. London. **13**: 52-253.

*BURLEY, J., WOOD, P.J. AND HANS, A. S., 1977, Variation in leaf characteristics among provenances of *Eucalyptus camaldulensis* Dehn. Growth in Zambia. *Australian J. Bot.,* **21**: 69-77.

*CALVO, R. N., 1990, Inflorescence size and fruit distribution among individuals in three orchid species. *Amer. J. Bot.,* **77**: 1378-1381.

CASAS, A. M., IGARTUA, E., BALAGUER, G. AND MORENO, M.A., 1999, Genetic diversity of Prunus rootstocks analyzed by RAPD markers. *Euphytica.* **110**(2): 139-149.

CAI, Y. L., CAO, D.W. AND ZHAO G.F., 2007, Studies on genetic variation in cherry germplasm using RAPD analysis. *Scientia Hort.,* **111**(3): 248-254.

*CAROLYN RAYMOND, A AND LUIS APIOLAZA, A., 2004, Incorporating wood quality and deployment traits in *Eucalyptus globulus* and *Eucalyptus niten. Plantation For. Biotechnol., for the 21st Century,* pp: 87-99

*CHATURVEDI, A.N., 1985, Firewood farming on the degraded lands of the Gangetic plain.U. P. Lucknow, India Government of India Press. *For. Bull.,* **50**(1): 286.

CHAPMAN, K.R., 1984, Tamarind. In Tropical Tree Fruits for Australia. Compiled by P.E. Page. Queensland Department of Primary Industries. Information series Q183018, Brisbane, pp: 83-86.

CHENG, F. S., WEEDEN, F. AND BROWN, S. K., 1996, Identification of codominant RAPD markers tightly linked to fruit skin colour in apple. *Theor. Appl. Genet.,* **93**(1-2): 222-227.

*COATES-PALGRAVE, K., 1988, Trees of Southern Africa. *Tamarindus indica* L. C. S.Striuk Publishers, Cape Town. **10**: 278-279.

*CORONEL, R.E., 1991, *Tamarindus indica* L. In Plant Resources of South East Asia, Wageningen, Pudoc. No.2. Edible fruits and nuts. (Eds.) Verheij, E.W.M. and Coronel, R.E., PROSEA Foundation, Bogor, Indonesia. pp: 298-301.

*DALZIEL, J.M., 1937, The Useful Plants of West Tropical Africa. London. Crown Agents for Overseas Governments and Administrations. pp: 612.

DALE, I.R AND GREENWAY, P.J., 1961, Kenya Trees and Shrubs. Buchanan's Estate Ltd. pp: 654.

DAMASCO, O.P., GRAHAM, G.C, HENRY, R.J., ADKINS, S.W., SMITH, M.K. AND GODWIN, I.D., 1996, Random Amplified Polymorphic DNA (RAPD) detection of dwarf off-types in micropropagated Cavendish (*Musa* spp. AAA) bananas. *Plant Cell Reports*. **16**(1-2): 118-123.

*DE VRIES, H., 1912, Species and varieties, their origin by mutation. Open Court pupl. Co., Chicago.pp: 124-136.

DELLAPORTA, S. L., WOODS, J. AND HOCKS, J. B., 1983, A plant DNA mini preparation. *Plt. Mol. Biol. Rep.,* **1**: 19-21.

DELAECY, I. H. AND COOPER, M., 1990. Pattern analysis of regional variety traits. **In**: Kang, M. S. (ed) Genotype by environmental interaction and plant breeding. Baton, Range, Louisiana, U.K. State University Agricultural Center

DEVANSHI., SINGH, A. K., PANKAJ SHARMA., BHANUMATI SINGH., RAKESH SINGH AND SINGH, N. K., 2007, Molecular profiling and genetic relationship among ber (*Ziziphus* sp.) genotypes using RAPD markers. *Indian J. Genet,* **67**(2): 121-127.

DIVAKARA, B. N., 2008, Variation and character association for various pod traits in *Tamarindus indica* L. *Indian Forester,* **134**(5): 687-696.

DURAN, Y., ROHDE, W., KULLAYA, A., GOIKOETXEA, P. AND RITTER, E., 1997, Molecular analysis of East African tall coconut genotypes by DNA marker technology. *J. Genet. Breed.,* **51**: 279-288.

*EGGELING, W.J. AND DALE, I.R., 1951, The Indigenous Trees of the Uganda Protectorate Entebbe, Uganda. The Government Printer. pp: 491.

EL-SIDDIG, K., EBERT, G., LUDDERS, P., 1999, Tamarind (*Tamarindus indica* L.): a review on a multipurpose tree with promising future in the Sudan. *J. Appl. Botany* **73**: 202-205.

*FARTAIS, L., RISCA, I.M AND TEODOROVICI, D., 2009, Percentage of elite (plus) trees in norway spruce and silver fir seed reservation. *Cercetari Agronomice in Moldova,* **141**(1): 43-47.

*FABBRI, P. P. AND BERGMANM, F., 1978, The collection of tuba and lambanog Toddy, *Phillip. J. of Coconut Stud.,* pp: 73-80.

*FALKENHAGES, E. R., 1991, Provenance variation in Pinus radius at six sites in South Africa. *Silvae Genetica.,* **40**(2): 41-56.

*FEUNGCHAN, S., YIMSAWAT, T., CHINDAPRASERT, S. AND KITPOWSONG, P. (1996) Tamarind (*Tamarindus indica* L.). Plant genetic resources in Thailand. *Thai J. of Agric. Sci.*, **1**: 1-11.

*FENG, L., YANG,G., YU, M., ZHANG, X, Y., XIANG, Z, A, I., FENG, L, C., YANG, G. W., YU,M, D., ZHANG, X,Y. AND XIANG, Z, H., 1997, Study of relationships among species in *Morus* L. using random amplified polymorphic DNA (RAPD). *Scientia Agricultura Sinica.,* **30**(1): 52-56.

GAMBLE, J.S., 1922, A Manual of Indian Timbers. London Sampson Low, Marston & Co. pp: 866.

GAO, Z., ZHANG, Z., SHENG, B.C., YAO, Q., GAO, Z.H., ZHANG,Z., SHENG, B.C., YAO, Q.H, 2001, Preliminary study on RAPD fringerprinting of some stone fruits. *J. of fruit Sci.,* **18**(2): 120-121.

GEMMAS, V. J., RIJO- JOHANNSEN, M. J., TENREIRO, R. AND FEVEREIRO, P., 2000, Inter and intra – varietal analysis of three *Olea europa* L. cultivars using RAPD technique. *J. Hort. Sci. Biotech.,* **75**: 312-319.

*GROLLIER, C., DEBIEN, C., DORNIER, M., AND REYNES, M., 1998, Principales caracteristiques et voies de valorisation du tamarin. *Fruits,* **53**: 271-280.

*GUNASENA, H.P.M., 1999, Tamarind, an unexploited species in Sri Lanka. Paper presented at the National Workshop on Fruits for the Future. Gannoruwa, Sri Lanka. 4 November. Gannoruwa, Peradeniya, Sri Lanka.

HARDER, L. D. AND BARRETT, S. C. H., 1993, Pollen removal from tristylous *Ponderia cordata*: effects of anther position and pollinator specialization. *Ecology,* **74**: 1059-1072.

HANAMASHETTI, S.I., 1996, Studies on genetic diversity and evaluation of promising genotypes in tamarind (*Tamarindus indica* L.), PhD thesis, Univ. of Agric. Sci, Dharwad.

HASSAN SEDRA, M.Y., PHILIPPE LASHERMES., PIERRE TROUSLOT. AND MARIE-CHRISTINE COMBES., 1998, Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers. *Euphytica*, **103**(1): 75-82.

*HAROLD MITCHELL, L., 1955, Breeding for high- quality wood, Paper presented at the Second Lake States Forest Tree Improvement Conference held at Wisconsin Rapids, Wis., August. pp: 29-31.

HEMANTHKUMAR, N.V., NARAYANASWAMY, P., PRASAD, T. G., MUKUNDA, G. K. AND SONDUR, S. N., 2001, Estimation of genetic diversity of commercial mango (Mangifera indica L.) cultivars using RAPD markers. *J. Hort. Sci. And Biotech.,* **76**(5): 529-533.

HERNANDEZ-UNZON, N.Y. AND LAKSHIMINARAYANA, S.L., 1982, A development physiology of the tamarind fruit (*Tamarindus indica* L.). *Hort. Sci.*, **6** : 938-940.

*HONG, T.D., LINNINGTON, S. AND ELLIS R.H., 1996, Seed Storage Behaviour: A Compendium. Handbook for Genebanks No.4 International Plant Genetic Resources Institute, Rome.

*IRVINE, F.R., 1961, Woody Plants of Ghana. Oxford University Press, London. pp: 868.

*JAYAWEERA, D.M.A., 1981, Medicinal Plants (Indigenous and Exotic) Used in Ceylon. Part 111. *Flacourtiaceae-Lytharaceae*. A publication of the National Science Council of Sri Lanka. pp: 244-246.

JHA, K. K., GUPTA, C. AND VERMA, R. S., 1991, Field testing of few promising clones of poplar in Terai. *Indian J. For.,* **14**(2): 83-89

JIA-YANLI., TIAN-YIKE., WANG-CAIHONG AND WANG-LIANG., 2007, Analysis of the genetic diversity among pear cultivars by RAPD. *J. of Fruit Sci.*, **24**(4): 525-528

KAPUR, S. K. AND DOGRA, A. S. 1987, Provenance trial of *Eucalyptus camaldulensis* in Punjab. *Indian Forester,* **113**(2): 471-475.

KARIHALOO, J. L., DWIVEDI, Y. K., SUNIL ARCHAK and AMBIKA BALDEV GAIKWAD., 2003., Analysis of genetic diversity of Indian mango cultivars using RAPD Markers. *J. Hort. Sci. and Biotech.,* **78** (3): 285-289.

*KEDHARNATH, S., 1982, Genetic variability and heritability of juvenile height growth in *Eucalyptus grandis*. *J. of Tree Sci.*, **1**(1-2): 46-49.

KEDHARNATH, S., 1986, Genetics and improvement of forest trees. *Indian J. of Genet.*, **46**: 172-180.

KIM, C.S., LEE, K.W., PARK, C.H., KANG, S.J., SHIN, I.S. AND LEE, G.P., 2005, Phylogenetic relationships among *Pyrus pyrifolia* and *P. communis* detected by randomly amplified polymorphic DNA (RAPD) and conserved rDNA sequences. *Scientia Hort.,* **106**(4): 491-501.

*KOLLER, B.,LEHMANN, A., MC DERMOTT, T.M. AND GESSLER, C.,1993, Identification of apple cultivars using RAPDs and RLFPs. *Agronomie,* **17**(6-7): 323-333.

*KRISNAMOORTHY, A. V. R. G., 1989, Provenance trials of Teak in Andhra Pradesh- A status paper in : Seminar on Provenance Research , Jan 18th and 19th , Institute of Forest Genetics and Tree breeding, Coimbatore.

KULKARNI, H. D. AND SRIMATHI, R. A., 1982, Variation in foliar characteristics in sandal. Biometric Analysis in Improvement of Forest Biomass (Ed. Khosla, P.K.). International Book Distributors, Dehradun. pp: 63-69.

KUMARAN, K., 1991, Genetic analysis of seed and juvenile seedling attributes in neem (*Azadirachta indic*a A. Juss) and Pungam (*Pongamia pinnata* (Linn) Pierre). MSc. Thesis, Tamil Nadu Agric. Univ, Coimbatore.

LAKMINARAYANA, S., 1980, Tropical and subtropical fruits; composition, properties and uses in mango, (Eds. Nagy, S. & Shaw, P.E.) AVI publishing, Westport, Connecticut, pp: 184-257.

*LEFEVRE, J. C., 1971, Revue de la littérature sur tamarinier. *Fruits,* **26**: 687-695.

*LEONARD, J., 1957, Genera des Cynometreae et des Amherstieae africaines (Leguminosae-Caesalpinioideae). *Mémoire Acad. Royale Belgique*, **30**(2): 1-314.

*LEVI, A. AND ROWLAND, L, J., 1997, Identifying blueberry cultivars and evaluating their genetic relationships using randomly amplified polymorphic DNA (RAPD) and simple sequence repeat-(SSR-) anchored primers. *J. Amer. Soc. Hort. Sci.*, **122**(1): 74-78.

LEWIS, G. SCHRIRE, B. MACKINDER, B. AND LOCK, M. (eds.), 2005, Legumes of the World. Royal Botanic Gardens, Kew. pp: 14-20.

LEWIS, Y.S. AND NEELAKANTAN, S., 1964, The chemistry, biochemistry and technology of tamarind. *J. of Sci. and Industrial Res.*, **23**: 204.

*LEWIS, E., 1992, Identification of avocado cultivars with RAPD markers. *Inligtinsbullten-Instituut- vir-Tropies-en-Subtropiese-Gewasse.* **241**:7-9.

*LITTLE, E.L. AND WADSWORTH, F.W., 1964, Common Trees of Puerto Rico and the Virgin Islands, Agriculture. Handbook 249, Washington DC, US Department of Agriculture, pp: 548.

LIU, S., CANTRELL, R. G., McCARTY, J. C. AND STEWART, J. McD., 2000, SSR based assessement of genetic diversity in cotton erace accessions. *Crop Sci.,* **40**: 1459-1469.

*LYNCH, M., 1998, Estimation of relatedness by DNA fingerprinting. *Mol. Biol. Evol.,* **5**: 584-599.

MANATURAGIMATH, B. B., BULGANNAVAR, G. N., PARAMESWARAPPA, S. AND BURLEY, J.,1991,Provenance trial on *Eucalyptus cloeziana* in Western Ghats of Karnataka, India. *Indian Forester,* **117**(12): 1013-1020.

MANIMEKALAI, R., NAGARAJAN, P., BHARATHI, M., JAYAPRAGASAM, M., ANITHA KARUN, KUMARAN, P. M. AND PARTHASARATHY, V. A., 2007, RAPD as useful marker for coconut (*Cocos nucifera* L.) DNA analysis. *Indian J. Hort.,* **64**(1): 1-4.

MACHADO, M, A., COLETTA, F. H. D., TARGON, M. L. P. N. AND POMPEW, J. Jr., 1996. Genetic relationships of Mediterranean mandarins (*Citrus deliciosa Tenore)* using RAPD markers. *Euphytica*, **92**(3): 321-326.

MIRALI, N. AND NABULSI, I., 2003, Genetic diversity of almonds (*Prunus dulcis*) using RAPD technique. *Scientia Hort.,* **98**(4): 461-471.

NAGARAJAN, B., NICODEMUS, A., MANDAL, A. K., VERMA, R. K., GIREESAN, K., AND MAHADEVAN, N. P., 1998, Phenology and controlled pollination studies in tamarind. *Silvae Genetica,* **47**: 5-6.

*NAS., 1979, Tropical Legumes: Resources for the Future. Washington DC. pp: 117-121.

NARIAN, P., 2000, Genetic diversity- conservation and assessement. *Curr. Sci.,* **75**: 170-175

NEJIB HASNAOUI ., MESSAOUD MARS ., JEMNI CHIBANI AND MOKHTAR TRIFI ., 2010, Molecular Polymorphisms in Tunisian Pomegranate (*Punica granatum* L.) as Revealed by RAPD Fingerprints. *Diversity*, **2**: 107-114.

*NILSSON, L. A., 1998, The evolution of flowers with deep corolla tube. *Nature,* **334**: 147-149.

OLIVEIRA, C. M., MOTA, M., MONTE-CORVO, L., GOULAAO, L. AND SILVA, D. M., 1999, Molecular typing of Pyrus based on RAPD markers. *Scientia Hort.,* **79**: 163-174.

*PARROTTA, J.A., 1990, *Tamarindus indica* L. Tamarind. SO-ITF-SM-30 June, USDA Forestry Service. Rio Piedras, Puerto Rico. pp: 1-5.

*PATINO, V.M., 1969, Plantes cultivadas y animales domesticos en America equinoccial. Tomo IV. Plantes introducidas Imprenta Departamental, Cali, Colombia, pp: 233-235.

PAN-XINFA, MENG-XIANXUN, CAO-GUANGLI, XU-CHUNMING, PAN, X.F., MENG,X.X. AND CAO, G.L. AND XU, C.M, 2002, Application of RAPD-PCR technique for identification of varieties in loquat. *J. of Fruit Sci.,* **19**(2) :136-138.

*PETTIGREW, C.J. AND WATSON, L., 1977, On the classification of Caesalpinioideae.
Taxon, **26**(1): 57-64.

PILLAY, M., OGUNDIWIN, E., NWAKANMA, D. D., UDE, G. AND TENKOUANO, A., 2001, Analysis of genetic diversity and relations in East African banana germplasm. *Theor. Appl. Genet.,* **102**: 965-970.

*POUPON, J., CHAUVIN, G., 1983, Les arbres de la Martinique, , *In* ONF, ed. Direction régionale pour la Martinique. pp: 256.

*POLHILL, R.M. AND RAVEN, P.H (Eds.)., 1981, Advances in legume systematics. Kew, 24-29 July, held under the auspices of the Royal Botanic Gardens, Kew, the Missouri Botanical Garden, and the University of Reading. *Proc. of the International Legume Conf.,* **2**: 2-8.

PRAKASH, D.P., NARAYANASWAMY, P. AND SONDUR, S.N., 2002, Analysis of molecular diversity in guava using RAPD markers. *J. Hort. Sci. and Biotech.,* **77**(3):287-293.

PRABHUSANKAR, D. S., MELANTA, K. R. AND CHANDREGOWDA, M., 2004, Evaluation of elite clones of tamarind. *Karnataka J. Agri. Sci.,* **17**(3): 512-514.

PURSEGLOVE, J.W., 1987, Tropical Crops. Dicotyledons. *Longman Science and Technology*. pp: 204-206.

.

RADHAMANI, A., NICODEMUS, A., NAGARAJAN, B. AND MANDAL, A.K., 1998, Reproductive biology of tropical trees. In: Madal, A.K. and Gibson, G.I. (Eds.) *For. Genet. and Tree Breed.*, CBS Publishers, New Delhi, India.

RAJARAM, S., 1990, Provenances studies in Gliricidia sepium (Jacq) Walp. MSc. (Agri) Thesis, Tamil Nadu Agric. Univ., Coimbatore, pp: 118.

RAO, M. G. V. AND BADAMI, K., 1930, A preliminary note on varieties of *Santalum album* L. Mysore Sandal Spike Investigation Committee Bulletin. **3**: 5.

RAO, S.Y., 1995, Tamarind economics. *Spice in India*, pp: 10-11.

*RELWANI, L. L., 1993, Tamarind: A multipurpose tree for diverse environments. *The Bharathia Agric. and Industrial Foundation J.*, **13**(2): January: 10.

*RIDLEY, H.N., 1922, The Flora of the Malay Peninsula. *Tamarindu*s Linn. Polypetalae L. Reeve & Co, Ltd. London. **1** : 636.

RODRIGUEZ, M. J. B., ESTIOKO, L. P., NAMIA, T.I. AND SONIEGA, J. A., 1997. Analysis of genetic diversity in coconut by RAPD. *Phillip. J. Coconut Stud.,* **22**: 1-7.

RONG, L.Z., YONEMORRI, K., SUGIURA, A. AND LUO, Z.R., 1995, Evaluation of RAPD analysis for cultival identification of persimmons. *J. Japanese Soc. Hort. Sci.,* **64**(3): 535-541.

RUSSELL, J. R., HOSEIN, F., JOHNSON, E., WAUGH, R. AND POWELL, W., 2000, Genetic differentiation of cocao (*Theobroma cacao* L.) populations revealed by RAPD analysis. *Mol. Ecol.,* **2**(2): 89-97.

SAADAT, Y.A. AND ZANDI, P., 2001, Identification and evaluation of Persian walnut elite trees in Fars province. *Natural Resources.,***14** (3): 14-18

SAMIULLAH, R., 1984, Biometric studies in tamarind (Tamarindus indica L.). Ph.D. Thesis, Univ. of Mysore, **pp** :1-154.

SAMIULLAH, R., 1993, Genetic diversity in tamarind, Abstracts of Golden jublee Symposium on Horticulture Research- Changing Scenario. Publication of Horticulture Society of India, New Delhi. pp: 473.

SAMAL, S., ROUT, G. R. AND LENKA, P. C., 2003, Analysis of genetic relationships between populations of cashew (*Anarcardium occidentale* L.) by using morphological characterisation and RAPD markers. *Plt. Soil Environ.,* **49**(4): 176–182.

SANJAY SINGH., SINGH, A. K., AND JOSHI, H. K., 2008, Genetic variability for floral traits and yield attributes in tamarind. *Indian J. Hort.,* **65**(3): 328-331.

SARKHOSH, A., ZAMANI, Z., FATAHI, R. AND EBADI , V., 2006, RAPD markers reveal polymorphism among some Iranian pomegranate (*Punica granatum* L.) genotypes. *Scientia Hort.,* **111**(1): 24-29.

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

SHANKARACHARYA, N.B., 1998, Tamarind - Chemistry, Technology and Uses - a critical appraisal. *J. of Food Tech.,* **35**(3): 193-208

*SHEN-YONGBAO., SHI-JISEN AND LIN-TONGLONG., 2004, The genetic diversity of *Castanea heyyi* and C. *sequinii* in Jianou by RAPD markers. *J. Nanjing Fores. Univ. Nat. Sci.,* **28**(6): 15-17.

SHEIDAI, M., VAEZI-JOZE, S., SHAHRIARI, Z.H., NOORMOHAMMADI, Z., PARSIAN, H AND FARAHANEI, F., 2007, Study of genetic diversity in some olive (Olea europaea L.) cultivars by using RAPD markers. *Pakistan J. Biol. Sci.,* **10**(17): 2972-2975.

SHIVANANDAM, V. N., 1980, Studies on systematic selection of productive types of tamarind (*Tamarindus indica* L.). MSc. (Agri) thesis, Univ. of Agric. Sci., Bangalore.

SHIVANANDAM, V.N. AND RAJU, K.R.T., 1988, Correlation between some fruit characters of four tamarind (*Tamarindus indica* L.) types. *Mysore J. of Agric. Sci.*, **22**(2): 229-231.

SINGH, N. B. AND CHAUDHARY, V. K., 1992, Multivariate analysis of genetic divergence in wild apricot (Prunus armeniaca Linn.). *Indian J. For.,* **15**(3):211-216.

SMITH,J.C.S. AND SMITH,O.S., 1991, Fingerprinting crop varieties. *Adv. Agron.,* **47** : 85-140.

SOZOLNOKI, T.W., 1985, Food and Fruit Trees of Gambia. Hamburg, Federal Republic of Germany: 132 Tiftung Walderhatung in Africa.

SRIVASTAVA, D. P., SRIVASTAVA, P. K., GOEL, A. K. AND THANGAVELU, K., 1993, Genetic variability in *Terminalia arjuna* Beld. *Indian J. For.,* **16**(3): 223-225.

*STORRS, A.E.G., 1995, Know Your Trees. Some common trees found in Zambia. Regional Soil Conservation Unit (RSCU), Zambia.

SURI, S. K., 1984, Analytical study of teak provenance tests in North Raipur division of Madhya Pradesh. *Indian Forester,* **110**: 345-363.

TAI, T. H. AND TANKSLEY, S. D., 1990, A Rapid and inexpensive method for isolation of total dehydrated plant tissue. *Plt. Mol. Bio. Rep.,* **8**(4): 297-303.

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

TANKSELY, S. D., MILLER, J., PATERSON, A. AND BERNATZKY, R., 1988, Molecular mapping of plant chromosomes. In: Chromosome structure and function- impact new concepts (Eds. GUSTAFSON, J.P. AND APPLELS, R., 18th Stadler genetics symposium). Plenum press, New York.

THIMMARAJU, K. R., NARAYAN REDDY, M. A., SWAMYRAO, N. AND SULLADMATH, U. V., 1978, Studies on floral biology of tamarind (Tamarindus indica L.). *Mysore J. of Agric. Sci.*, **11**: 293-298.

TROUP, R.S., 1921, The Silviculture of Indian Trees. Leguminosae (Caesalpinieae) to Verbenaceae. *Tamarindus indica* L.: Oxford, Clarendon Press., **11**(7): 263-363.

ULRICH, R.,1970, Organic acids. In The Biochemistry of Fruits and Their Products, Hulme, A.C., (Ed.), Academic Press, New York., Vol **1**.

VIRK, P., NEWBURY, H.J., JACKSON, M. T., AND FORD-LLOYD, B. V., 1995, The identification of duplicate accessions within a rice germplasm collection using RAPD analysis. *Theor. Appl. Genet.,* **90**: 1049-1055.

VON MUELLER, B.F., 1881, Select Extra Tropical Plants Readily Available for Industrial Culture or Naturalisation. *Tamarindus indica* L. Thomas Richards, Government Printer, Sydney. pp: 328.

*WARD, J. H., 1963, Hierarchic grouping to optimize an objective function. *J. Amer. Stat. Assoc.,* **58**: 236-239.

WATANABE, S. AND DISSANAYAKE, M.D., 1999, Tamarind. *Tamarindus Indica* L. Major Plant Genetic Resources of Sri Lanka: An Illustrated Guide: **73.**

WATT, G., 1893, *Tamarindus.* Linn. Dictionary of Economic Products of India. Part III. (Silk to Tea) **6**: 404-409.

WIESMAN, Z., AVIDAN, N., LAVEE, S. AND QUEBEDEAUX, B., 1998, Molecular characterization of common olive varieties in Tsrael and the West Bank using Randomly Amplified Polymorphic DNA (RAPD) markers. *J. Amer. Soc. Hort. Sci.,* **123**(5): 837-841.

WILLIAMS, J. G. K., HANAFEY, M. K., RAFALSKI, J, A. AND TINGEY, S, V., 1993, Genetic analysis using randomly amplified polymorphic DNA markers. *Methods in Enzymology.,* **218**: 704-741.

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.

*WUNDERLIN, R. P., 1998, Guide to the vascular plants of Florida Gainesville : University Press of Florida, pp: 806.

XIANG, L, Z., REIGHARD, G. L., BAIRD, W. V., ABBOTT, A, G., RAJAPAKSE. S. AND LU, Z. X., 1996, Identification of peach rootstock cultivars by RAPD markers. *Hort. Sci.,* **31**(1): 127-129.

*YAE, B., KO, K.,YAE, B,W. AND KO,K,C., 1995, Classification of *Malus domestica* cultivars by random amplified polymorphic DNA markers. *J. Korean. Soc. Hort. sci.,* **36**(6): 824-828.

YANG – SHIYU., HE- CHENZHONG., ZHAO- YIHE., LUI JUAN., WANG-BINGYI., 2008, Analysis of the genetic diversity of sweet Thai tamarind introduced from Thailand based on RAPD markers. *For. Res.,* **21**(1): 60-63.

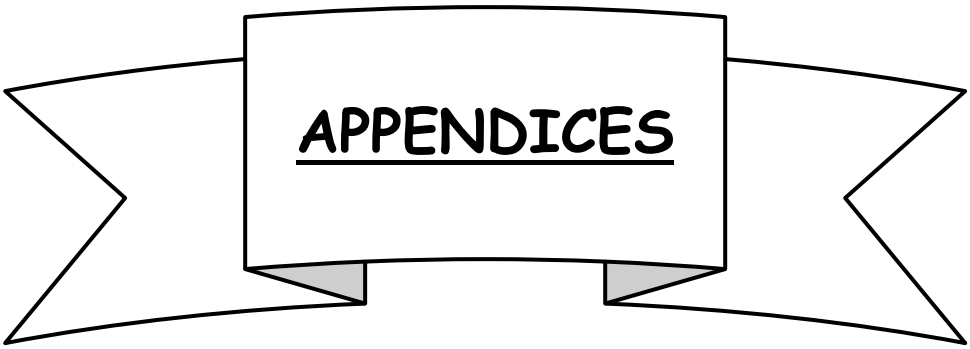
YANG KAI., GU HUI-YAN., LUAN HAI-YAN AND YANG FENG-JIAN., 2008, Method and standard for selecting elite nut trees of Korean pine. *J. For. Res.,* **12**(2): 84-88.

YING, C, C. AND BAGLEY, W.T., 1976, Genetic variation of Eastern Cotton Wood in an eastern Nebraska Provenance Study. *Silvae Genetica,* **25**: 65-73.

ZHONGPING CHENG., 2007, Genetic characterization of different demes in *Prunus persica* revealed by RAPD markers. *Scientia Hort.,* **111**(3): 242-247.

---

* *Original not seen*


APPENDICES

APPENDIX I

COMPOSITION OF BUFFERS AND SOLUTION USED IN THE STUDY

EXTRACTION BUFFER

Chemicals	Concentration	Amount (For 100 ml)
Tris base	1M (pH=8)	18.612g
NaCl	5M	29.22g
EDTA	0.5M (pH=8)	15.76g
CTAB	6%	6g

OTHER BUFFERS AND SOLUTION

Buffers	Composition	Concentration
1X TE buffer	1. Tris base 2. EDTA	1M 0.2M
10 X TBE buffer (1 litre)	1. Tris base 2. Boric acid 3. EDTA	121 g 51.3 g 3.7 g
Loading buffer (per litre)	1. Bromophenol blue	0.25 %
Solutions		
70% Ethanol	1. Ethanol 2. Distilled water	70 ml 30ml
Chloroform : Isoamyl alcohol (24:1) (For 100ml)	1. Chloroform 2. Isoamyl alcohol	96 ml 4 ml

APPENDIX II

COMPOSITION OF PCR MIXTURES

Ingredients	**Initial concentration**	**Final concentration**	**Amount**
DNA Assay buffer	10X	1X	2µl
dNTPs	2mM	0.2mM	2µl
MgCl2	100mM	3mm	0.7 µl
Primer	100pm	10pm	2 µl
Taq polymerase	3U	0.7U	0.2 µl
Genomic DNA	30ng/µl		2 µl
Distilled water			11.1
Total			20 µl