MORPHOLOGICAL AND MOLECULAR ANALYSES OF COCONUT (*Cocos nucifera* L.)

S.SELVARAJU

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Department of Plant Breeding and Genetics
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695 522

DECLARATION

I hereby declare that this thesis entitled '**Morphological and molecular analyses of coconut (*Cocos nucifera* L.)**' is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellayani,
-2008.

S.SELVARAJU
(2006-11-127)

CERTIFICATE

Certified that this thesis entitled '**Morphological and molecular analyses of coconut (*Cocos nucifera* L.)**' is a record of research work done independently by Mr. S. Selvaraju (2006-11-127) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Vellayani,
-2008.

Dr. JAYALEKHSMY V. G
(Chairperson, Advisory Committee)
Associate Professor,
Department of Plant Breeding and Genetics,
College of Agriculture, Vellayani,
Thiruvananthapuram.

Approved by

Chairperson:

Dr. JAYALEKHSMY V. G
Associate Professor,
Department of Plant Breeding and Genetics,
College of Agriculture, Vellayani,
Thiruvananthapuram – 695 522.

Members:

Dr.P.MANJU
Professor and Head,
Department of Plant Breeding and Genetics,
College of Agricuture, Vellayani,
Thiruvannthapuram – 695 522.

Dr. K.RAJMOHAN
Professor and Head,
Department of Plant Biotechnology,
College of Agriculture, Vellayani,
Thiruvannthapuram – 695 522.

Dr. D. GEETHA
Professor,
Instuctional Farm, Vellayani,
Thiruvannthapuram – 695 522.

External Examiner:

DEDICATED
TO
MY BELOVED PARENTS,
BROTHER, SISTER
AND
FRIENDS

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LIST OF ABBREVATION

CGD	Chowghat Green Dwarf
COD	Chowghat Orange Dwarf
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
EAT	East African Tall
g	Gram (s)
GCV	Genotypic coefficients of variations
h^2	Heritability
ISSR	Inter Simple Sequence Repeat
ISTR	Inverse Sequence Tagged Repeat
Kb	Kilo base
LO	Laccadive ordinary
m	Meter
M	Molar
mm	Millimeter
NCD	Natural Cross Dwarf
ng	Nanogram
Nm	Nanometer
OPB	Operon Primer B series
OPC	Operon Primer C series
OPD	Operon Primer D series
OPE	Operon Primer E series
OPH	Operon Primer H series
OPK	Operon Primer K series
OPP	Operon Primer P series
PCR	Polymerase Chain Reaction
PCV	Phenotypic coefficients of variations

Pmoles	Pico moles
QTL	Quantitative Trait Loci
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
Rpm	Revolution per minute
SSR	Simple Sequence Repeats
TAE	Tris acetate EDTA buffer
Taq	*Thermus aquaticus*
TE	Tris EDTA buffer
Tris HCl	Tris (hydroxy methyl) aminomethane Hydrochloride
UPGMA	Unweighted Pair Group Method for Arithmetic average
V	Volts
WCT	West Coast Tall
µg	Micro gram
µl	Microliter

Introduction

1. INTRODUCTION

Coconut (*Cocos nucifera* L.) is a perennial oil yielding crop of tropics, belonging to the family Arecaceae. This versatile palm has its importance in subsistence agriculture and also as a major trading commodity. The tree is rightly called the tree of heaven "Kalpa Vriksha" as very few other cultivated plants have such highly diversified utility as the coconut.

Coconut is grown in the tropical belt lying between 23.5° N and 23.5° S of the equator. It is cultivated in about 90 countries of which about 90 per cent of the world's total area and production of coconut comes from the five major coconut producing countries viz., India, Philippines, Indonesia, Srilanka and Malaysia.

In India the four southern states viz Kerala, Tamilnadu, Karnataka and Andhra Pradesh accounts for 92.43 per cent area and 91.31 per cent production in this crop. Among these states, Kerala accounts for largest area and production with 50.76 per cent of total area and 43.66 per cent of total production

Even though coconut is regarded by most taxonomists as monotypic, there is considerable variation in coconut. The high genetic variation observed in this palm may be due to high outcrossing nature, spontaneous mutation and selection in the only species (Harland, 1957).

Assessment of the nature and extent of variability will be of immense value in identifying superior genotypes and in formulating breeding programmes. Coconut has not received the attention that it deserved mainly due to some unique problems encountered with the crop. The perennial habit, long juvenile period, high outcrossing and heterozygous nature, difficulties in clonal propagation and requirement of large area for systematic experimentation are some of the barriers to achieve rapid progress in coconut breeding.

Coconut palm has a wide range of genetic divergence mainly in colour, shape and size of the fruit. The current knowledge of such variability is insufficient for proper characterization of this species. Over the years, different attempts have been made to use morphological and physiological traits as markers for the study of genetic diversity in coconut. The traits studied comprises of the morphology of fruit and vegetative organs, earliness of seed nut germination, floral characteristics and physicochemical properties of the seed. However, these studies have shown that the use of such markers is limited by their susceptibility to environmental changes.

The detection and exploitation of naturally occurring DNA sequence polymorphisms represent one of the most significant recent developments in molecular biology. These DNA based markers are in abundance and allow clear comparison of the genotypes avoiding any environmental influence on gene expression. RAPD (Randomly Amplified Polymorphic DNA) is a specific class of marker which provides a useful system to monitor levels of diversity detected between and within populations. This procedure has the advantages of being technically simple, quick to perform, requires only small amount of DNA and involves no radioactivity.

The present study was undertaken to genetically analyze the prominent six genotypes of this locality viz. West Coast Tall and Laccadive Ordinary (two popular Tall types), CGD and COD (two prominent Dwarf types) and Komadan a coconut cultivar of high demand among the cultivators and NCD a natural cross hybrid obtained from Chowghat Orange Dwarf by open pollination,with morphological and molecular markers.

The major objectives of this study include

- Genetic analysis of six coconut cultivars with respect to fifteen morphological characters (vegetative and fruit characters)
- RAPD analysis of the coconut cultivars with 14 oligonucleotide primers.
- Comparison of genetic divergence of the six coconut cultivars with respect to morphological and molecular markers

KALPA VRIKSHA - the coconut palm is the traditional source of edible oil. Gainful achievements have been obtained through plant breeding and genetic strategies MOLECULAR MARKERS can further enhance the improvement in the TREE OF HEAVEN

Review of literature

2. REVIEW OF LITERATURE

Coconut (*Cocos nucifera* L.) (2n=2x=32) is a member of the monocotyledonous family Arecaceae. Coconut palm was the first grown plantation crop in the 1840s (Child, 1974). The family Arecaceae is widely distributed throughout the tropical and temperate regions of the world.

Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Arecales

Family: Arecaceae

Subfamily: Arecoideae

Tribe: Cocoeae

Genus: **Cocos**

Species: **nucifera**

Binomial name: *Cocos nucifera*

Grimwood (1975) while reviewing the literature on origin suggested that there are two schools of thought on the origin and subsequent dispersal of coconut. The first expounds a New World origin with subsequent dispersal to Polynesia and Asia. Debate on the origin and dispersal of *Cocos nucifera* has generally concluded that the palm has a South East Asian Polynesian origin. The means of dispersal to the margin of its pre-industrial range is in more disputes.

A stochastic simulation model of the wind currents of islands of the Pacific Ocean was developed by Ward and Brookfield (1992) and they tested the hypothesis of trans-pacific drift dispersal. The model suggested that the probability of coconuts drifting to the west coast of the Panama while remaining viable is extremely low and dispersal to Panama by man seems to be more likely.

Loy et al. (1992) reported Coconut , as a monospecific palm, which evolved in the Indo-Pacific region, where fossils from the Tertiary and Quaternary have been found and where archeological antecedents indicate its presence as far back as 28 000 years ago.

2.1 VARIETAL DIVERSITY

Taxonomic studies and investigations of monocotyledon anatomy reports *Cocos nucifera* to be monotypic. However, different varieties and cultivars are recognized.

John and Narayana (1949) revealed five different varieties viz., Typica –tall palms with both male and female flowers, Nana-dwarf delicate palms bearing in three years, Javanica- dwarf palms bearing in four years, Spicata-tall palms with unbranched inflorescence having one or two small spikes only and Androgena with male flowers only.

Nair and Ratnambal (1994) assessed the genetic resources of coconut and reported Tall and Dwarf are the two distinct varieties of coconut universally found. They described tall and dwarf palms as follows.

Tall palms

The Tall palms, sometimes referred as var. typica, are mostly cultivated in all the coconut growing areas of the world. Tall palms generally grow to a height of 15 to 18m or more and have a comparatively long pre-bearing age of 6-10 years. They are normally

cross-pollinated as there is usually no overlapping of male and female phases. Fruit is generally medium to large in size and nuts mature within a period of 12 months. The copra content is usually over 150g/nut and oil percentage varies from 66 to 70.

West Coast Tall, Laccadive Ordinary, East Coast Tall and Andaman Ordinary are some of the distinct Tall types present in India.

West Coast Tall (WCT) coconut is grown in large numbers on the west coast of India. It is a hardy palm yielding copra, oil and fiber of good quality. But its population is highly variable. Nuts vary in size, shape and colour and it has generally medium yielding capacity. Since the last half century, more than 95 per cent of the coconut area in Kerala continues to be cultivated under this local variety. WCT is a locally adapted plant type with considerable genetic variation for yield of nut and copra. During the survey conducted to study the performance of coconut hybrids in the cultivator's fields in Kerala, it was found that under identical condition of poor management, WCT performed far better than the hybrids (Kannan 1982). WCT has been reported to be a stable cultivar (Balakrishnan et al. 1991).

Laccadive Tall or Laccadive Ordinary commonly cultivated in Islands of India is more or less similar to WCT, except for its high oil content (72 per cent), more number of medium size nuts and high out turn of copra. The female flower production and the setting percentage are also high (Ohler, 1984).

Kappadam is another tall variety from the west coast of India with large nuts (Gangolly et al., 1957). The fruit is ellipsoid and it has a rather high content of thick and hard copra. The nut yield is rather low. It is not stable in annual yield due to its biennial (alternate) bearing tendency (Balakrishnan et al. 1991).

Komadan is a local coconut off type, popular in the erstwhile Central Travancore area of Kerala associated with the family history of an old Tharavadu called Komattu house. Gopimony (1982) reported that Komadan was superior to WCT in morphological characters of the palms including nut and copra characters in a preliminary observation done on the available Komadan palms in the Vellayani campus of Kerala Agricultural University. The study also indicated the overlapping nature of male and female phases suggesting a self pollinated breeding system in this local type. Local enquiries made at the centre of origin of this coconut type revealed that a curious process of shrewd selection for yield linked phenotypic characters like bronze colour and oblong shape of nuts was conducted for many generations (Shylaraj et al. 1991). They also reported that Komadan type further exhibited superior seedling vigour in terms of germination percentage, height, collar girth, mean number of total leaves and mean number of split leaves when they compared Komadan mother palms and seedlings with those of WCT.

Manju (1992) reported that Komadan types showed significant superiority for majority of mother palm characters especially number of bunches and spadices and number of nuts per palm per year. Number of nuts per palm per year and number of female flower per bunch had high heritability combined with moderate to high genetic advance indicating the predominance of additive genes. Among the Komadan palms, 33 per cent were of self pollinating nature thereby occupying a position in between WCT and NCD regarding pollination system. All the Komadan palms had nuts of different shades of brown while 70 per cent of WCT palms had nuts of green shade and NCD palms had varying shades of green, olive and brown nuts indicating the distinction of Komadan as a separate group.

Dwarf palms

Dwarf palms also referred to as var. nana are characterized by their short stature. They are early bearing (3-4 years), easier to harvest and short lived. They have thin trunks without a swollen base or `bole' and fully developed fronds rarely exceeds 4m. Though the dwarf palms yield heavily, they have tendency for irregular bearing. Dwarfs

are identified mainly by the colour of their nuts. They are presumed to have originated from tall palms either through mutation (Menon and Pandalai, 1958) or by inbreeding in talls (Swaminathan and Nambiar, 1961). Chowghat Green and Orange are a few Dwarf cultivars found in the central region of Kerala. Chowghat Green cultivar with dark green nuts has a very high female flower production but with a low setting percentage. Its copra is of very low quality with low oil content.

The Chowghat Orange cultivar produces medium sized orange coloured nuts having a copra content of about 135 g and the oil content as low as 55 per cent or less (Nelliat, 1978). Unfertilized female flowers are retained on the spikes, being characteristic of these varieties (Ohler, 1984).

Intermediate types

In India, in addition to these two groups, there are distinct tall types such as Laccadive Micro, Andaman Giant, Catangute, Nadora and Benaulim. Ramachandran et al., (1977) reported Ayiramkachi, an intermediate type between tall and dwarf in Tamil Nadu.

Satyabalan (1982) considered Ayiramkachi palm as intermediate between Tall and Dwarf. The nuts are green and copra is good quality. It has high female flower production which may be exploited for breeding purposes. However, it is an alternate bearer (Ohler, 1984).

Natural Cross Dwarf

Pillai (1991) reported that the natural hybrids obtained from Chowghat Orange Dwarf were till recently known as Natural Cross Dwarf and that in Central Kerala, these hybrids are known by the name Komadan. He opined that the percentage recovery of hybrids (DxT) greatly depends on the level of the homozygosity of Dwarf and Tall parents involved in crossing.

2.2 GENETIC ANALYSIS IN COCONUT WITH MORPHOLOGICAL MARKERS

2.2.1 Assessment of genetic variability

The study of variability in genetic stocks of coconut palms with regard to phenotypic and genotypic variability and genetic advance is a pre-requisite for any breeding programme.

Nambiar and Ravindran (1974) compared open pollinated and inbred progenies of twelve varieties of coconut to study the pattern of genetic variation for reproductive characters and its impact on yield potential. There were substantial differences between inbreds as well as between varieties and open pollinated progenies of same variety for number of female flowers per spike and number of sterile spike.

Louis (1981) studied the phenotypic and genotypic variability in 25 varieties and hybrids and reported that number of leaves per year, number of spathes per year, number of female flowers per palm, setting percentage and number of nuts had high genetic advance and recommended consideration of these characters for exercising selection. Moderately high genetic advances were combined with moderately high heritability for the length of the leaf and number of leaves in the crown indicating the predominance of additive genes, which was considered a desirable feature for selection.

Shylaraj et al., (1991) reported that seednut characters of the two coconut types viz., Komadan and WCT did not show any significant difference. Nut production and number of flowers per spadix were reported to be more variable than other characters by Ovasuru et al., (1991). But Pillai et al. (1991) reported that there was not much variation in the number of female flowers produced in an inflorescence between cultivars. Absence of significant variation for kernel thickness in the 13 genotypes studied was reported by Patil et al. (1993).

Sindhumole and Ibrahim (2000) reported that economic characters showed higher genotypic coefficient of variation compared to vegetative and reproductive characters. Jayalekshmy and Sree Rangasamy (2002(a)) studied variability for twenty morphological traits in 30 genotypes of coconut and observed significant differences for both vegetative and reproductive characters. They also reported that the range of mean values for all the characters studied were much wider in the tall varieties than in the dwarf ones.

Medium and high phenotypic and genotypic coefficients of variation was observed for number of nut per palm per year, number of female flowers per bunch and number of nuts per bunch by Manju and Gopimony (2001) while studying three generations of Komadan along with WCT and NCD. They also reported high heritability combined with moderate to high genetic advance for nut yield per palm per year and number of female flowers per bunch.Ganesamurthy et al. (2002) analysed genetic variability of nut and copra yield along with six other nut characters in 14 genotypes of coconut and reported high degree of variability for copra yield, dehusked nut weight, nut yield and whole nut weight. All these characters showed high heritability and genetic advance.

Vanaja and Sreekumariamma (2002) reported that the number of female flowers varied with the season and the season had no effects on the average nut production per bunch, however significant interaction effects were recorded between cultivars and seasons.

2.2.2 Correlation Studies

Yield is the most important criterion for selection. Complexity of this character made up of other component characters makes it the subject of distinct study. Correlation studies would facilitate effective selection for improvement of one or many yield contributing components. An estimate of inter-relationship of yield with other characters is of immense help in crop improvement programme.

Harland (1957) observed that veritieswith heavy husked nuts had a higher weight of copra per nut and considered the correlation between the weight of copra per nut as the most important.

Positive significant correlation of number of female flowers with yield was observed by Nampoothiri et al. (1975).Ramanathan (1984) studied the correlation of yield per plant with eight of its components in four dwarf and 26 tall cultivars and observed that the characters were positively correlated with yield.

Balakrishnan et al., (1991) in an experiment with 15 different hybrid combinations of coconut involving T x D and D x T, reported high correlation between number of nuts produced per year and total number of leaves produced.

Kalathiya and Sen (1991) worked out correlation coefficient between floral and yield traits in coconut variety Dwarf green. They found nut yield to be significantly and positively correlated with number of spadices. It is suggested that number of spadices can be considered as selection criterion for nut yield improvement in Dwarf green. Liyanage (1991) reported high and psitive genetic correlation between yield of nuts and copra per palm. Mathew and Gopimony (1991) in their studies conducted with super mother palms and WCT found that the number of bunches per tree failed to show significant correlation with number of spadices per year and number of nuts per bunch.

Narayanankutty and Gopalakrishnan (1991) reported that there was significant positive correlation for total number of leaves retained by the palm and length of leaves with yield. Manju (1992) reported that the number of bunches and spadices was significantly and positively correlated with number of female flowers per bunch, number of nuts/bunch and number of nuts per palm per year and indicated the scope for selection based on these characters.

N'cho et al. (1993) reported that the inflorescence characters are positively correlated with yield and they can be effectively used as selection indices in coconut.

Jayalekshmy and Sree Rangasamy (2001) studied association among twenty morphological traits in coconut which included seven vegetative characters, seven reproductive characters and six yield contributing characters. The point of insertion of first female flower, number of female flowers and the number of nuts per bunch had significant positive correlation with yield. Plant height had no significant correlation with any of the characters, but had significant negative correlation with number of leaf scars.

Sindhumole and Ibrahim (2001) reported that the genotypic correlation was mostly negative with respect to vegetative characters but positive for other pairs. Only nut yield among the four economic characters was correlated with both vegetative and reproductive characters. Other economic characters viz. copra yield, oil content (per cent) and oil yield were dependent only on vegetative characters. Correlation and regression analysis suggested that reproductive characters had less effect on economic characters.

2.2.3 Path Analysis

Path coefficient analysis was done by Sukumaran et al. (1981) for yield of nuts during stabilized period of yield and reported that the major contributing characters which influenced yield directly or indirectly are the average number of female flowers, the number of functioning leaves at 19 years and internodal distance at a fixed mark.

Louis (1981) stated that the selection strategy for yield of nuts may be indirectly based on component characters such as number of leaves, leaves on the crown, number of spathes per year and number of female flowers.

Louis and Chopra (1991) reported that among the five characters significantly correlating with copra weight, kernel weight, length of the petiole and thickness of shell had positive direct effects. Negative direct effects on copra weight were observed with the pre-flowering period and thickness of the kernel. Highest direct and positive effects

on copra were observed by the kernel weight per unit, followed by leaflets and number of leaves produced in a year. The thickness of the shell had the least direct positive effect on copra.

Ganesamurthy et al. (2002) reported that direct effects of dehusked nut weight, percentage of husk to whole nut weight, percentage of kernel to whole nut weight and nut yield on copra yield were positive and high.

2.2.4 Genetic Divergence

Murthy and Arunachalam (1966) while studying accessions collected from different Islands reported that no correlation exists between genetic diversity and geographical diversity. Absence of correlation between genetic diversity and geographic diversity was also reported by Louis and Chopra (1989). Ovasuru et, al (1991) analyzed coconut germplasm of Papua New Guinea and reported the same.

Rao et al. (1983) studied divergence in 10 coconut cultivars based on fruit components and reported that the Andaman Tall and Benaulim were falling in two separate clusters while all the rest of the Tall populations formed into a single cluster in the D^2 analysis based on fruit components.

According to Balakrishnan and Namboodiri (1987), in general there was no correlation between the genetic diversity and geographical diversity in coconut varieties collected from different geographical locations. The 24 cultivars studied fell into six different clusters based on the genetic distances among them. Cultivars of the same planes of origin fell into different clusters while those of diverse origin fell into the same cluster. The clusters showing maximum diversity (clusters IV and VI) come under the Niu Vai and Nio Kafa types.

Harries (1991) stated the concept of introgressive hybridization and explained how the variability observed in the plant populations, such as coconut in India or elsewhere, has risen and how it affects choice and selection of hybrid parents. The effects of introgressive hybridization can be observed in many aspects of the palms performance. The introgressed populations can be classified as either predominantly wild type or predominantly domestic type.

Ashburner et al. (1997) conducted a study on diversity in the genetic resources of South Pacific region using fruit component analysis. A large diversity in fruit morphology was found that ranged from population.

Manju and Gopimony (1998) reported that fifty mother palms belonging to the five coconut types can be grouped into three clusters based on the seedling characters when subjected to D^2 analysis. All the WCT and NCD palms were found constellated in cluster I which may be due to the common heritage. The Komadan palms belonging to the three generations were seen distributed in all the three clusters indicating the comparative unstable genetic identity of Komadan as against WCT and NCD.

Zizumbo and Pinerio (1998) studied 41 populations using 17 morphological fruit characters and indicated four major groups of coconut populations. They also reported that Dwarf being autogamous was found to be less diverse and different from three groups of Tall based on analysis of pattern of phenotypic diversity in Mexico.

Kumaran et al. (2000) clustered coconut population of Indian Ocean islands of Madagascar, Mauritius and Seychelles using principal component analysis. A total of 28 vegetative, reproductive and fruit characters were used for analysis and obtained five clusters.Jayalekshmy and Sree Rangasamy (2002(b)) clustered 30 genotypes in coconut based on twenty morphological traits into six clusters. They found nut characters to be more efficient in assessing genetic divergence in coconut. Meerow et al. (2003) reported that allogamous Nio Leka dwarf had shown the highest genetic diversity among dwarf samples analyzed.

Arunachalam et al. (2005) studied seven Tall groups and four Dwarf groups representing seven Island territories using 206 individuals. Diversity estimate was the highest in Nicobar Tall group whereas it was low in Tall genotypes of Fiji and Tonga.

Ratnambal et al. (2005) reported that important characters that cause divergence as obtained from the canonical analysis were weight of fruit, length of fruit, volume of cavity, weight of shell and per cent husk to fruit weight.

2.3 RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) AS MOLECULAR MARKERS FOR GENETIC ANALYSIS IN COCONUT

Comparative anatomy, physiology and embryology were employed for variability analysis at intra and inter specific levels. With the advent of molecular markers, these were replaced and are now widely applied in genetical analysis, taxonomy and in plant breeding. Biochemical constituents such as secondary metabolites in plants or macromolecules viz. proteins and deoxyribonucleic acids (DNA) are used as molecular markers. Among these DNA markers are more suitable and ubiquitous to most of the living organisms and ideally neutral to environmental effects or management practices (Joshi et al. 1999).

Molecular markers have revolutionized the plant genomics as it paves way for complete genome analysis. During the last few decades, a broad range of molecular markers have become available, which are being used in a variety of ways not only to supplement and expedite the conventional methods of crop improvement, but also for characterization and maintenance of plant genetic resources that are so vital for crop improvement programmes (Gupta et al. 2002).

RAPD is a molecular marker based on the differential PCR amplification of a sample of DNA using short oligonucleotide sequences as primers. The procedure detects nucleotide sequence polymorphisms in DNA. On an average each primer directs amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence polymorphism between individuals (Williams et al. 1990). The advantage of RAPD is that it is less labour intensive, requires smaller quantities of genomic DNA and is less costly and quicker. It can be used to detect even

single gene mutations. RAPD markers are well suited for genome mapping, for plant and animal breeding application and for DNA fingerprinting for analyzing genetic structure of populations.

According to Tingey and Tufo (1993) the RAPD technique is an efficient tool for identifying variation and estimating diversity in different biological system. RAPDs had been successfully utilized for varietal characterization in many perennial crops as well (Shah et al., 1994,Koller et al. 1993).

Jayalekshmy (1996) reported RAPD analysis to be an effective method for developing molecular markers to differentiate the coconut genotypes. Ashburner et al. (1997) analysed population variation among the south pacific accessions using RAPD markers and reported high amount of variation between populations. Even though very few unique fixed markers were found, 47 per cent of polymorphic bands between one Tall and one Dwarf coconut accession indicated a moderate level of polymorphism.

Duran et al. (1997) analyzed a total number of 48 coconut types belonging to the East African Tall (EAT) by different DNA marker techniques including RAPDs, microsatellite-primer PCR and ISTR analysis. All the three approaches detected large polymorphism among the set of genotypes and allowed the identification of single genotype by individual-specific fingerprinting.

Duran et al. (1999) and Rodriguez et al. (1997) conducted bulk analysis with combined DNAs from 20 palm trees per population by using only 8 RAPD primers and 15 scorable polymorphic markers which were sufficient to distinguish all the 10 Philippine ecotypes they studied.

The biodiversity of coconut was analysed using RAPD, SSR and ISTR markers by Rhode et, al., (1999). They found that populations of Tall varieties show a higher degree of heterogenity than Dwarf types. Herran et, al. (2000) observed that RAPD markers were less efficient in QTL mapping of coconut populations.

RAPD markers were used by Ratnambal et al. (2001) to characterize the coconut germplasm. Hundreds of primers were screened for their ability to detect polymorphism in coconut. Among these, only 34 per cent primers were polymorphic. The number of polymorphic bands/primer ranged from 1-16. Daher et, al. (2002) assessed the genetic divergence among 19 coconut tree populations by Random Amplified Polymorphic DNA. The markers used permitted the identification of each of the populations showing that they were genetically different (absence of duplicity). The use of compound samples was effective to investigate the interpopulational genetic diversity.

Upadhyay et al. (2002) analysed fourteen coconut accessions (9 tall, 4 dwarf, and 1 intermediate type) using RAPD markers to establish genetic similarity among some indigenous and exotic coconut accessions maintained in the coconut germplasm Centre in Kerala, India.

Jayalekshmy and SreeRangasamy (2003) reported that intervarietal variation could be detected by RAPD markers. Out of the 10 primers which gave amplification products, seven gave amplification products showing polymorphism between tall and dwarf varieties. Genetic relationship between the two varieties could be deduced from the degree of similarity in the amplified product profiles. RAPD markers appeared to be of high value for characterization of genetic resources.

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According to Upadhyay et al. (2004), the data on genetic distance and heterozygosity indicated that more variance exists among Tall accessions than among Dwarf accessions and the RAPD markers were able to distinguish coconut accessions with high efficiency. He also reported that the number of polymorphic bands detected by each primer depends on the primer sequence.

Parthasarathy et al. (2005) reported that DNA fingerprinting of coconut using molecular markers detect variation at the DNA level overcoming most of the limitations of morphological and biochemical markers. Manimekalai et al. (2006) investigated the effectiveness of Randomly Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR) and Simple Sequence markers to identify polymorphism among

worldwide collection of coconut (*Cocos nucifera* L.) germplasm accessions. Ten RAPD markers produced 97 polymorphic markers in 33 coconut germplasm accessions which accounted for 76.7 per cent of polymorphic markers. Moreover, mean polymorphism information content value for RAPD markers was 0.23. There was good correspondence between RAPD and ISSR similarity matrices.

Material and methods

3. MATERIALS AND METHODS

The present study was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during the period 2006-2008. The experimental material consisted of thirty palms belonging to six coconut cultivars or varieties viz., Komadan, Laccadive ordinary, WCT, Chowghat Orange Dwarf, Chowghat Green Dwarf and Natural Cross Dwarf .

Five palms each of Laccadive Ordinary (LO), Komadan and West Coast Tall (WCT), palms of the similar age group and yield group were selected from C, D and E Block respectively of Instructional Farm, College of Agriculture, Vellayani.

Five palms each of Natural Cross Dwarf (NCD), Chowghat Green Dwarf (CGD) and Chowghat Orange Dwarf of the same age were selected from N8 and J Block of Regional Agricultural Research Station, Pilicode.

3.1 Morphological characters

The following observations were recorded on all the selected five palms in each of the six cultivars during the period 2006-2008.

(1)Plant height (m)

Height of the palm was measured from the base of the stem to the crown region using a graduated meter tape.

(2) Number of leaves per palm

Fully opened leaves on the crown were counted.

(3) Number of spikelets per inflorescence

The number of spikelets per inflorescence in each accession was counted and the average worked out.

(4) Number of female flowers/ inflorescence

The number of female flower sockets in each harvested bunch was counted with number of nuts in that particular bunch which gives the number of female flowers in that inflorescence.

(5) Length of bunch stalk (cm)

The bunch stalk length of each accession was measured and expressed in centimeters.

(6) Girth of bunch stalk (cm)

The bunch stalk girth of each accession was measured and expressed in centimeters.

(7) Number of bunches harvested/year

Number of bunches harvested in each harvest was added together to obtain number of bunches per year. Average of four harvests was recorded.

(8) Number of nuts per bunch

Numbers of nuts per bunch in each harvest added together and mean number of nuts per bunch was obtained as follows.

$$x = a/b$$

where

x – mean number of nuts per bunch

a = total number of nuts produced in an year

b = total number of bunches produced during the year

(9) Number of nuts per palm per year

This was obtained by adding the total number of nuts harvested in each harvest for one year.

(10) Weight of unhusked nut (kg)

Unhusked nuts were weighed in a pan balance and mean weight expressed in kilograms.

(11) Weight of husked nut (g)

Each husked nut was cleaned and weighed and weight measured in grams.

(12) Husk/nut ratio

The difference in weight of unhusked nut and husked nut divided by weight of unhusked nut gave the husk/ nut ratio.

(13) Fruit polar perimeter (cm)

The length of the nut from one pole to other was measured by setsquare blocking of the nut and measuring the distance using a meter scale gave the polar diameter of the fruit in centimeter.

(14) Fruit equatorial perimeter (cm)

The breadth of the nut at the middle portion measured by setsquare blocking of the nut and measuring the distance using a meter scale gave the equatorial diameter of the nut in centimeter.

3.2.3 Statistical Analysis

The data collected were subjected to the following statistical analyses.

3.2.3.1 Analysis of Variance

The analysis of variance was carried out for various characters (Panse and Sukhatme, 1957).

- To test the significance of differences among the genotypes with respect to various characters and
- To estimate the variance components and other genetic parameters like coefficients of variation, heritability and genetic advance.

Estimation of components of variance

1. Variance (for a trait X)

Environmental variance, $\sigma^2 e_X = E_{XX}$

Genotypic variance, $\sigma^2 g_X = \frac{G_{XX} - E_{XX}}{r}$

Phenotypic variance, $\sigma^2 p_X = \sigma^2 g_X + \sigma^2 e_X$

Where

E_{XX} = observed mean square for error

G_{XX} = observed mean square for genotype

2. Coefficient of variation

Phenotypic and genotypic coefficients of variation (PCV and GCV) for a trait X were estimated as suggested by Singh and Chaudhary (1979).

3.2.3.3 Correlation Analysis

The correlation coefficients (phenotypic, genotypic and environmental) were worked out based on the formulae given by Singh and Chaudhary (1979).

3.2.3.4 Path Analysis

The path analysis was done by the method developed by Wright (1954) to study the cause and effect relationship among a system of variables which helps to measure the direct influence along each separate path in such a system and to find the degree to which the variation of a given effect is determined by each particular cause.

3.2.3.5 D^2 Analysis

Genetic divergence was studied using Mahalanobis D^2 Statistic as described by Rao (1952). The genotypes were clustered by Tocher's method.

3.3 MOLECULAR CHARACTERIZATION OF COCONUT

3.3.1 Isolation of Genomic DNA

Genomic DNA was isolated from fresh leaves using Modified CTAB method. (Guillemaut and Marechat-Drouard,1992 and Porebski et al,1997).

- About 20 mg of leaves powdered using liquid nitrogen were transferred to 2 ml eppendorf tube containing pre-warmed (60˚C,15 min)extraction buffer (3% CTAB, EDTA, NaCl, 2%PVP and 1% β-mercaptoethanol).
- Incubated at 65˚C for1 hour.
- The homogenate extracted once with Chloroform: Isoamylalcohol (24:1) then treated with RNase and twice with equal volume Chloroform.
- To this 100µl of 3M Sodium acetate and equal volume of absolute ethanol was added and kept overnight at -20 ˚C.
- The DNA was pelleted, washed with 70% ethanol and dissolved in100µl TE.
- The quality of the isolated DNA was checked in 1% agarose gel and the quantity of DNA determined spectrophotometrically.

3.3.2 Quantification of DNA

The quantification of DNA is necessary before it is subjected to amplification. The quantification of DNA was carried out with the help of UV-vis spectro photometer (Spetronic Genesys 5).

The buffer in which the DNA was already dissolved was taken in a cuvette to calibrate the spectrophotometer at 260 nm and 280 nm wavelength. The optical density (OD) of the DNA samples dissolved in the buffer was recorded at both 260 and 280 nm. The concentration of the DNA was found out using the formula

$$\text{Amount of DNA } (\mu g/\mu l) = \frac{A_{260} \times 50 \times \text{dilution factor}}{1000}$$

where

A_{260} is the absorbance at 260nm

A_{280} is the absorbance at 280nm

The quantity of the DNA could be judged from the ratio of the OD values recorded at 260 nm and 280 nm. The A_{260}/A_{280} ratio between 1.8 and 2 indicates best quality of DNA.

3.3.3 Agarose Gel Electrophoresis

- Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit.
- Required amount of agarose was weighed out (0.8% for genomic DNA and 1.4% for visualizing the amplified products) and melted in 1 x TAE buffer (0.004 M Tris acetate, 0.001 M EDTA, pH 8.0) by boiling.
- After cooling to about 50°C ethidium bromide was added to a final concentration of 0.5 μg ml^{-1}.
- The mixture was then poured to a preset template with appropriate comb.
- After solidification of the agar, the comb and the sealing tapes were removed and the gel was mounted in an electrophoresis tank.

- The tank was loaded with 1 x TAE buffer, so that it just covered the entire gel. Required volume of DNA sample and gel loading buffer (6 x loading dye with 40% sucrose, 0.25 % bromophenol blue) were mixed.

- Each well was loaded with 15 μl of sample. One of the wells was loaded with 5.0 μl of PCR molecular weight marker along with required volume of the gel loading buffer.

- Electrophoresis was performed at 75 volts until the loading dye reached $3/4^{th}$ length of the gel. The gel was documented using gel document system (BIORAD).

3.3.4 Standardization of RAPD Analysis

The procedure of Williams et al. (1990) was used for the amplification of DNA. The amplification was done in 20 µl reaction mixture containing,

Template DNA	30 ng,
10 x Assay buffer (A)	2.5 µl (With 15mM MgCl2)
Taq DNA polymerase	0.5 µl
Primer	1 µl (10 pmoles)
DNTP (0.5µl) each	2.0µl.
Sterile water	13µl
TOTAL	20.0µl

Amplification was done in a programmable thermocycler (MJ Research Inc., USA) that was programmed.

	Temperature	time (min)
Step-1	94°C	5
Step-2	94°C	1
Step-3	37°C	1
Step-4	72°C	2
Step-5	72°C	7
Step-6	Hold at 15°C	1.5
Step-7	Storage 4°C	over night

3.3.5 Number of Monomorphic and Polymorphic Band

From the amplified products separated by agarose gel electrophoresis using 1.4% gel as described earlier and photographed using gel documentation system the number of monomorphic bands and number of polymorphic bands were recorded.

3.3.6 Data Analysis

The reproductive bands for all the primers were scored for their presence (1) or absence (0) for all the coconut cultivar studied. A genetic similarity matrix was constructed using Jaccard's similarity coefficient method (Jaccard, 1908).

$Sj = a/(a+b+c)$

where

a: number of bands present in both the varieties

b: number of bands present in the first variety but not in the second one

c: number of bands present in the second variety but not in the first

Based on the similarity coefficient, the distance between the genotypes was computed with the help of the software package NTSYS (Version 2.02). Using these values of distance between cultivars, a dendrogram was constructed by following UPGMA (Unweighted Pair Group Method for Arithmetic average) methods.

Results

4. RESULTS

In the present investigation, the selected six cultivars were subjected to genetic analysis based on 14 morphological characters and RAPD markers generated using 14 oligonucleotide primers. The data were analyzed statistically and the results are presented in this chapter.

4.1 MORPHOLOGICAL MARKERS

4.1.1 Variability Studies

Data on 14 morphological traits were recorded on thirty palms belonging to six genotypes. Analysis of variance, mean, standard error, critical difference and coefficient of variation for the fourteen variables are given in Table 1. Analysis of variance showed significant F values for all the variables studied. F value ranged from 372.41 to 7.68.

Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance for different characters are given Table 2. Coefficients of variation for the fourteen variables are compared in the histogram (Fig.1).Heritability and genetic advance for the variables were also compared in a histogram (Fig.2).

1. Plant height (m)

The plant height ranged from 4.10 m (COD) to17.72 m (WCT). Mean plant height was 9.86m. The genotypic coefficient of variation (53.82 per cent) and the phenotypic coefficient of variation (54.18 per cent) were found to be the maximum for plant height. Heritability was highest (98 per cent) for this character indicating more genetic influence for this character. A genetic advance of 11.13 was indicated for selection.

Table 1. Mean values of different characters for the genotypes studied

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
WCT	**17.72**	27.80	28.60	28.20	33.80	11.40	11.20	10.60	1090	464	**0.625**	52.00	47.30	89.4
LO	7.44	**42.20**	**53.20**	**28.80**	55.40	19.30	9.00	**16.80**	860	474	0.446	53.5.	42.40	**119.6**
Komadan	12.17	32.00	36.00	28.40	**580**	**22.00**	**11.20**	11.60	**1251**	**541**	0.588	**58.10**	**47.80**	118.6
NCD	12.94	28.40	35.40	23.20	29.80	10.00	9.00	10.20	933	485	0.507	56.70	45.40	95.6
CGD	4.80	26.80	**26.20**	27.20	35.48	**8.20**	**6.20**	9.20	**552**	**248**	0.461	51.10	**39.70**	59.6
COD	**4.10**	**26.80**	32.40	**17.60**	**25.94**	9.17	6.80	**7.80**	730	506	**0.300**	**45.90**	43.10	**54.8**
Mean	**9.86**	**30.67**	**35.30**	**25.57**	**36.71**	**13.35**	**8.90**	**11.03**	**902.77**	**453.00**	**0.49**	**52.88**	**44.28**	**89.60**
SE	0.389	1.93	1.63	2.32	4.12	1.46	1.05	2.23	3943.0	567.08	0.01	6.85	6.33	86.78
CD	0.812	1.82	1.67	1.99	2.65	1.58	1.34	1.95	81.97	31.09	0.09	3.42	3.29	12.16
F value	372.42	92.10	279.81	41.94	327.81	116.24	21.24	21.62	79.17	95.51	14.33	13.85	7.68	44.80

X1 Plant height (m)
X2 Number of leaves/palm
X3 Number of spikelets/inflorescence
X4 Number of female flowers/inflorescence
X5 Length of bunch stalk
X6 Girth of bunch stalk
X7 Number of bunches harvested/year
X8 Number of nuts per bunch
X9 Weight of unhusked nut (kg)
X10 Weight of husked nut (g)
X11 Husk/nut ratio
X12 Fruit polar perimeter (cm)
X13 Fruit equatorial perimeter (cm)
X14 Number of nuts per palm per year

WCT : West Coast Tall
NCD : Natural Cross Dwarf
CGD : Chowghat Green Dwarf
COD : Chowghat Orange Dwarf
LO : Laccadive Ordinary
SE: Standard error
CD: Critical Difference

Table 2. Heritability, genetic advance, genotypic and phenotypic variances

Sl.No	Characters	GCV	PCV	Heritability %	Genetic advance at 5%
1	Plant height (m)	53.82	54.18	98.00	11.01
2	Number of leaves/palm	19.35	19.88	95.00	11.90
3	Number of spikelets/inflorescence	27.04	27.28	98.00	19.49
4	Number of female flowers/inflorescence	17.03	18.04	89.00	8.47
5	Length of bunch stalk(cm)	44.72	45.06	98.00	33.56
6	Girth of bunch stalk(cm)	43.41	44.34	96.00	11.68
7	Number of bunches harvested/year	23.16	25.87	80.00	3.80
8	Number of nuts per bunch	27.50	30.66	80.00	5.61
9	Weight of unhusked nut (kg)	27.50	28.37	94.00	495.86
10	Weight of husked nut (g)	22.86	23.45	95.00	207.85
11	Husk/nut ratio	22.83	26.77	73.00	0.20
12	Fruit polar perimeter (cm)	7.93	9.35	72.00	7.33
13	Fruit equatorial perimeter (cm)	6.57	8.69	57.00	4.53
14	Number of nuts per palm per year	30.77	32.48	90.00	53.81

GCV Genotypic Coefficient of Variation

PCV Phenotypic Coefficient of Variation

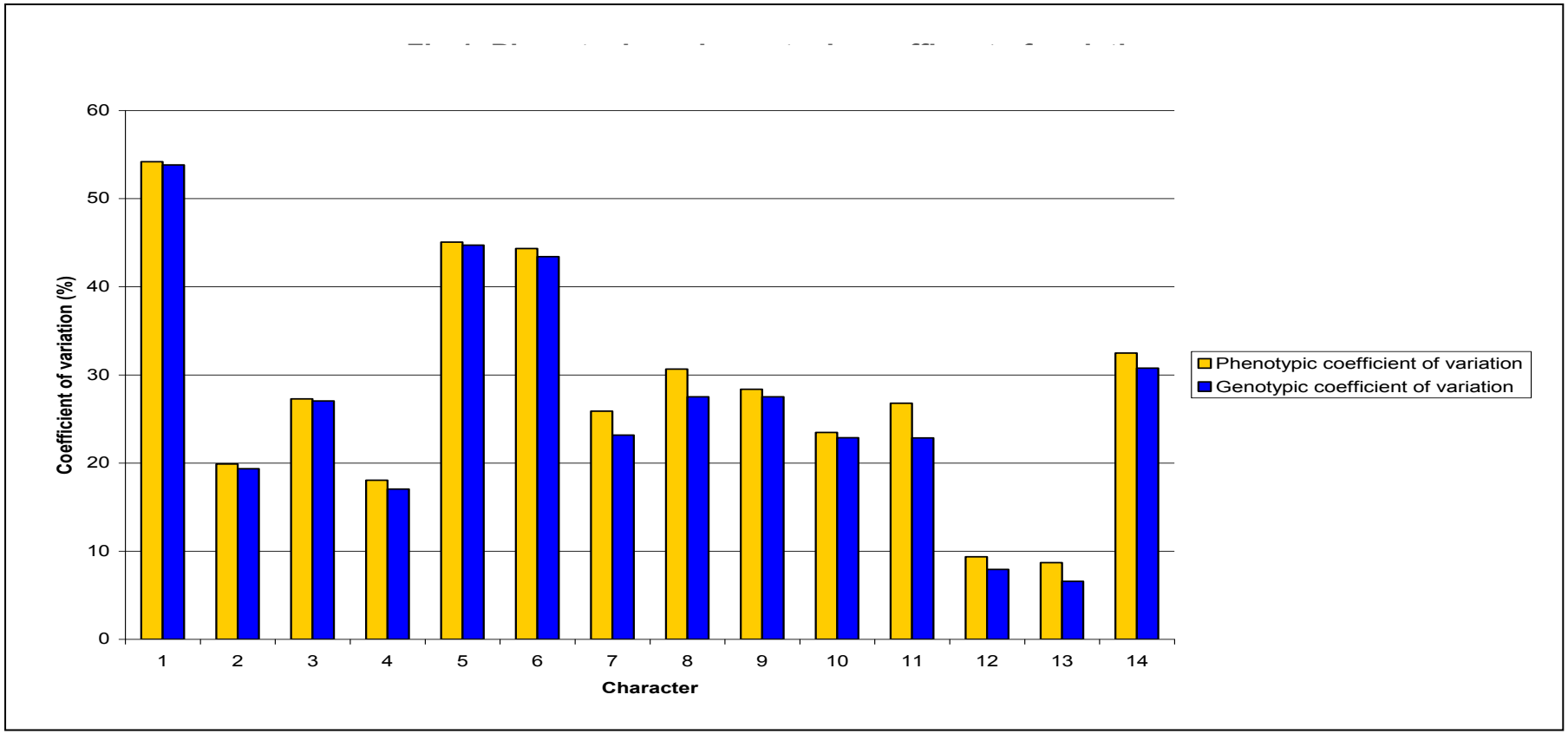

Fig 1.Phenotypic and genotypic coefficient of variation

X1 Plant height (m)
X2 Number of leaves/palm
X3 Number of spikes/inflorescence
X4 Number of female flowers/inflorescence
X5 Length of bunch stalk (cm)
X6 Girth of bunch stalk (cm)
X7 Number of bunches harvested/year
X8 Number of nuts per bunch
X9 Weight of unhusked nut (kg)
X10 Weight of husked nut (g)
X11 Husk/nut ratio
X12 Fruit polar perimeter (cm)
X13 Fruit equatorial perimeter (cm)
X14 Number of nuts/palm/year

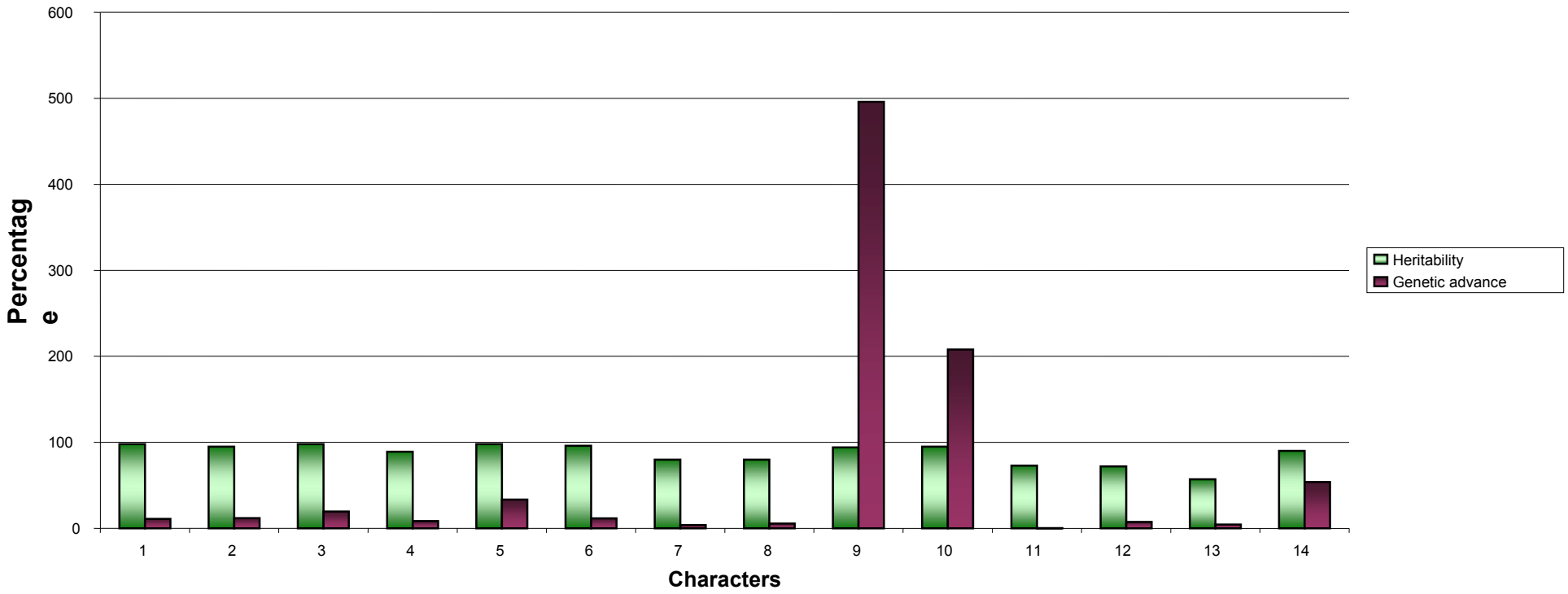

Fig 2. Heritability and genetic advance for characters studied

X1 Plant height (m)
X2 Number of leaves/palm
X3 Number of spikes/inflorescence
X4 Number of female flowers/inflorescence
X5 Length of bunch stalk (cm)
X6 Girth of bunch stalk (cm)
X7 Number of bunches harvested/year
X8 Number of nuts per bunch
X9 Weight of unhusked nut (kg)
X10 Weight of husked nut (g)
X11 Husk/nut ratio
X12 Fruit polar perimeter (cm)
X13 Fruit equatorial perimeter (cm)
X14 Number of nuts/palm/year

Fig 4. Diagramatic representation of Clustering diagram of six coconut genotypes

 - Intra cluster distance

——— - Inter cluster distance

Cluster I West Coast Tall and Natural Cross Dwarf

Cluster II Chawghat Green Dwarf and Chawghat Orange Dwarf

Cluster III Komadan and Laccadive Ordinary

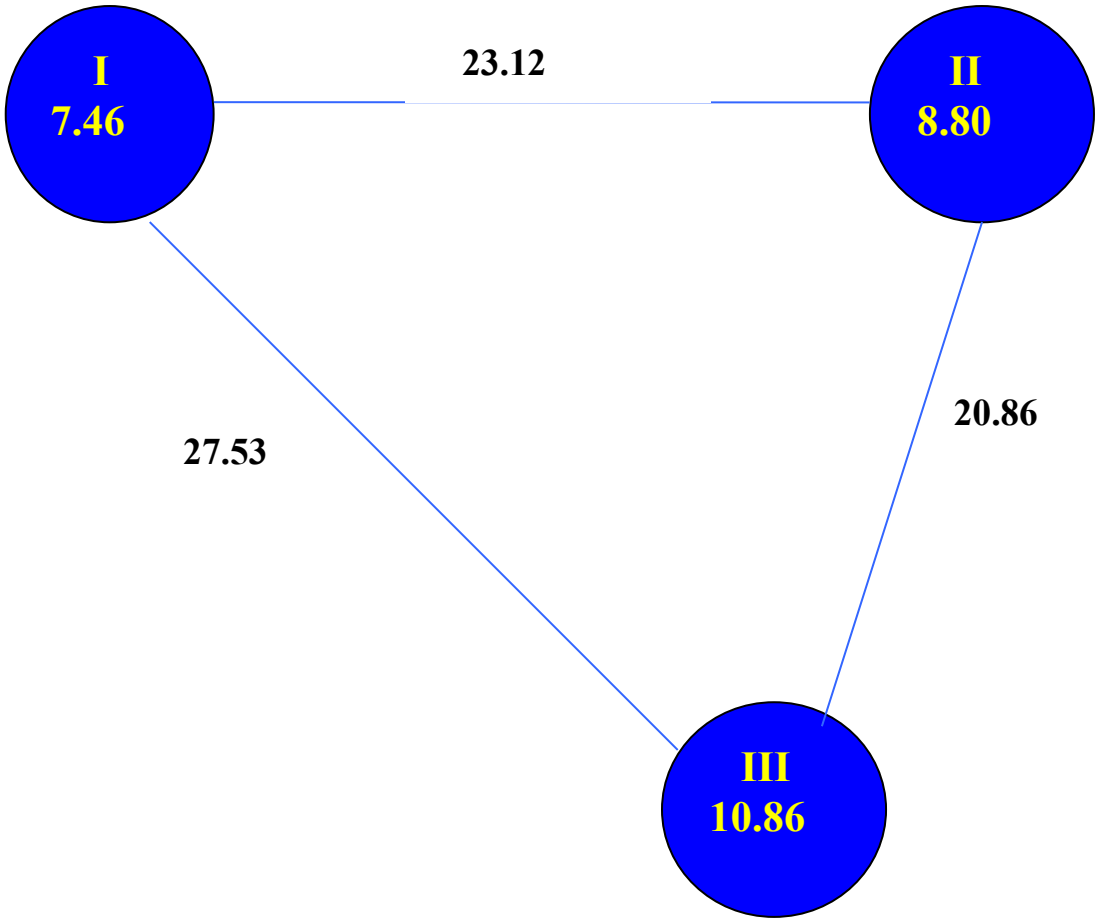

Fig 4. Diagramatic representation of Clustering diagram of six coconut genotypes

2. Number of leaves per palm

The mean number of leaves per palm was 30.67. Minimum number of leaves was recorded by both COD and CGD (26.8) and LO recorded the maximum number of leaves (42.2). This character showed medium GCV of 19.35per cent and PCV of 19.88 per cent. High heritability of 95 per cent was noted indicating high genotypic influence for this character with a low genetic advance of 11.90.

3. Number of spikelets per inflorescence

The number of spikelets ranged from 26.20 (CGD) to 53.2 (LO). The mean number of spikelet was 35.30. High genotypic and phenotypic coefficient of variation was recorded (27.04 per cent and 27.28 per cent). Highest heritability (98 per cent) was recorded indicating maximum genetic influence on the character. Genetic advance 19.49 was indicated for selection. For this character NCD and Komadan were on par.

4. Number of female flowers per inflorescence

The mean number of female flowers per inflorescence ranged from 17.60 (COD). to 28.8 (LO). The mean value of this character was 25.57. The GCV and PCV were 17.03 and 18.04 percent respectively. High heritability of 89 per cent and a low genetic advance of 8.47 were recorded.

5. Length of bunch stalk (cm)

The mean length of bunch stalk ranged from 25.94 cm (COD) to 58.00 cm (Komadan). The mean was 36.71 cm. High GCV (44.72 per cent) and PCV (45.06 per cent) were recorded. Maximum heritability value of 98 per cent and a medium genetic advance of 33.56 was noted.

6. Girth of bunch stalk (cm)

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The maximum girth of bunch stalk (22.00cm) was noted for Komadan and the mimimum (8.20cm) for CGD with a mean of (13.35cm). High GCV (43.41 per cent), PCV (44.34 per cent) and high heritability (96 per cent) were noted for the character. Genetic advance of 11.68 was recorded.

7. Number of bunches harvested per year

The highest number of bunches harvested per year (11.20) was recorded by Komadan and WCT and lowest value of 6.20 recorded for CGD. This character recorded high GCV and PCV values (23.16 and 25.87 per cent respectively). High heritability (80 per cent) denoting high genetic influence was also noted. Very low genetic advance of 3.80 was noted.

8. Number of nuts per bunch

The number of nuts per bunch ranged from 7.80 (COD) to 16.80 (LO) with a mean of 11.30. The GCV (27.50 per cent) and PCV (30.66 per cent) were recorded along with a heritability of 80 per cent and a genetic advance of 5.61.

9. Weight of unhusked nut (kg)

The mean for this variable was 0.90 kg with a range of 0.55 kg for CGD to 1.25kg for Komadan. High GCV and PCV (27.50 and 28.37 per cent respectively) and a high heritability of 94 per cent was also noted. This character showed the highest value of 495.86 for genetic advance.

10. Weight of husked nut (g)

The mean weight of husked nut was 453.00g. Komadan recorded the highest value of 541g and the lowest value of 248.00g by CGD. GCV and PCV were 22.86 per cent and 23.45 percent respectively. High heritability of 95 per cent along with a very high genetic advance of 207.85 was recorded.

11. Husk/ nut ratio

The husk/ nut ratio ranged from 0.300 (CGD) and 0.625 (WCT) with a mean of (0.490). High GCV (22.83 per cent) and PCV (26.77 per cent) and a high heritability (73 per cent) were noted for this character. A very low genetic advance of 0.20 was recorded for the character.

12. Fruit polar perimeter (cm)

The mean fruit polar perimeter ranged from 45.90cm (COD) to 58.10 cm (Komadan). The mean fruit polar perimeter of this character was 52.88cm. A low GCVand PCV (7.93 per cent and 9.35 per cent respectively) and a heritability of 72 per cent was noted for this character along with a low genetic advance of 7.33 for selection.

13. Fruit equatorial perimeter (cm)

The mean of fruit equatorial perimeter ranged from 47.86cm (Komadan) to 39.70 cm (CGD). The mean fruit equatorial perimeter recorded 44.28cm. A low GCV and PCV (6.57per cent and 8.69 per cent respectively) along with a medium heritability of 57 per cent was noted indicating moderate genotypic influence for this character. A low genetic advance of (4.57) was indicated for this character.

14. Number of nuts per palm per year

The mean number of nuts per palm per year ranged from 54.80 (COD) to119.60 (LO). The mean of this character recorded was 89.60. A high GCV and PCV (30.77 per cent and 32.48 per cent respectively) and a high heritability 90 per cent were noted. A moderate genetic advance (53.81) was recorded.

4.1.2 Correlation Studies

Genotypic correlation between the 14 variables were estimated and presented in Table 3. Tree height showed significant positive correlation at 1% level with number of bunches harvested per year (0.896), weight of unhusked nut (0.769), husk/ nut ratio (0.899), fruit polar perimeter (0.583) and fruit equatorial perimeter (0.900). This character showed significant positive correlation at 5% level with number of nuts per palm per year (0.498).

Number of leaves per palm showed high significant positive correlation with number of spikelets per inflorescence (0.961), length of bunch stalk (0.789), girth of bunch stalk (0.754), number of nuts per bunch (0.996) and number of nuts per palm per year (0.764). Correlation of this character with number of female flowers per inflorescence (0.491) was significant only at 5% level.

High significant positive correlation of number of spikelets per inflorescence was noted with length of bunch stalk (0.735), girth of bunch (0.667), number of nuts per bunch (0.918) and number of nuts per palm per year (0.712).

Number of female flowers per inflorescence had significantly high positive correlation with length of bunch stalk (0.509), girth of bunch stalk (0.561), number of bunches harvested per year (0.553), number of nuts per bunch (0.670), husk/nut ratio (0.783), fruit polar perimeter (0.646) and number of nuts per palm per year (0.650).

Table 3. Genotypic correlation between different characters

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
X1	1.000	-0.079	-0.124	0.425	0.276	0.195	0.896**	0.115	0.796**	0.388	0.899**	0.583 **	0.900**	0.498*
X2		1.0000	0.961**	0.491*	0.789**	0.754**	0.260	0.996**	0.191	0.271	0.012	0.327	-0.078	0.764**
X3			1.0000	0.238	0.735**	0.667**	0.187	0.918**	0.161	0.419	-0.154	0.282	-0.051	0.712**
X4				1.0000	0.509**	0.561**	0.553**	0.670**	0.381	-0. 216	0.783**	0.646 **	0.168	0.650**
X5					1.0000	0.984**	0.699**	0.793**	0.715**	0.639**	0.360	0.609**	0.523*	0.924**
X6						1.0000	0.635**	0.748**	0.672**	0.531*	0.369	0.627**	0.450*	0.887**
X7							1.0000	0.385	0.981**	0.630 **	0.867**	0.690**	0.994**	0.796**
X8								1.0000	0.282	0.226	0.217	0.447*	0.007	0.841**
X9									1.0000	0.744**	0.749**	0.683**	1.017	0.750**
X10										1.0000	0.092	0.258	0.792**	0.540**
X11											1.0000	0.765**	0.750**	0.583**
X12												1.0000	0.512*	0.835**
X13													1.0000	0.575**
X14														1.0000

*Significant at 5 per cent level **Significant at 1 per cent level

X1 Plant height (m)
X2 Number of leaves/palm
X3 Number of spikelets/inflorescence
X4 Number of female flowers/inflorescence
X5 Length of bunch stalk (cm)
X6 Girth of bunch stalk (cm)
X7 Number of bunches harvested/year
X8 Number of nuts per bunch
X9 Weight of unhusked nut (kg)
X10 Weight of husked nut (g)
X11 Husk/nut ratio
X12 Fruit polar perimeter (cm)
X13 Fruit equatorial perimeter (cm)
X14 Number of nuts per palm per year

High significant positive correlation for length of bunch stalk was noted with girth of bunch stalk (0.984), number of bunches harvested per year (0.699), number of nuts per bunch (0.793), weight of unhusked nut (0.715), weight of husked nut (0.639), fruit polar perimeter (0.609), and number of nuts per palm per year (0.924). It showed significant positive correlation at 5% level with fruit equatorial perimeter (0.521).

Girth of bunch stalk showed high significant positive correlation with number of bunches harvested per year (0.635), number of nuts per bunch (0.748), weight of unhusked nut (0.672), fruit polar perimeter (0.629) and number of nuts per palm per year (0.887) and it showed positive significant correlation with fruit equatorial perimeter (0.450) and weight of husked nut (0.530).

Number of bunches harvested per year had high positive significant correlation with weight of unhusked nut (0.981), weight of husked nut (0.630), husk/nut ratio (0.867), fruit polar perimeter (0.690), fruit equatorial perimeter (0.994) and number of nuts per palm per year (0.796).

Number of nuts per bunch showed high significant positive correlation with number of nuts per palm per year (0.841) and significant positive correlation with fruit polar perimeter (0.447).

Weight of unhusked nut registered high positive significant correlation with weight of husked nut (0.744), husk/nut ratio (0.749), fruit polar perimeter (0.683) and number of nuts per palm per year (0.750).

Weight of husked nut showed high significant positive correlation with fruit equatorial perimeter (0.792) and number of nuts per palm per year (0.540).

High significant positive correlation for husk/nut ratio was observed with fruit polar perimeter (0.765), fruit equatorial perimeter (0.750) and number of nuts per palm per year (0.583).

High significant positive correlation for fruit polar perimeter was seen with number of nuts per palm per year (0.8348). It showed significant positive correlation at 5% level with fruit equatorial perimeter (0.512).

High significant positive correlation was observed between fruit equatorial perimeter and number of nuts per palm per year (0.575).

None of the characters had significant negative correlation with yield.

4.1.3 Path analysis

To study the direct and indirect effects of 14 characters considered for the estimation of the genotypic correlation coefficient, path coefficient analysis was done and presented in Table 4. A path diagram was constructed with characters showing significant correlation with yield (Fig. 3).

Eight characters viz., plant height, number of spikelets/inflorescence, number of female flowers/inflorescence, length of bunch stalk, number of nuts per bunch , weight of unhusked nut, weight of husked nut and fruit polar perimeter showed positive correlation and positive direct effect. Five characters viz., number of leaves per palm, girth of bunch stalk, number of bunches harvested per year, husk/nut ratio and fruit equatorial perimeter showed negative direct effect.

Plant height had positive direct effect (0.538) and positive correlation (0.498).

Table 4. Path coefficient analysis

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	Correlation with yield
X1	**0.538**	0.025	-0.023	0.060	0.187	-0.003	-0.322	0.031	0.088	0.051	-0.118	0.178	-0.195	0.498
X2	-0.042	**-0.317**	0.181	0.069	0.536	-0.013	-0.093	0.271	0.021	0.036	0.001	0.100	-0.017	0.764
X3	-0.066	-0.305	**0.188**	0.033	0.499	-0.011	-0.067	0.249	0.017	0.055	0.020	0.086	0.011	0.712
X4	0.228	-0.156	0.044	**0.142**	0.345	-0.009	-0.199	0.182	0.042	-0.028	-0.103	0.197	-0.036	0.650
X5	0.148	-0.250	0.138	0.072	**0.679**	-0.017	-0.251	0.215	0.079	0.084	-0.047	0.186	-0.113	0.924
X6	0.104	-0.239	0.125	0.079	0.668	**-0.017**	-0.228	0.203	0.074	0.070	-0.048	0.192	-0.097	0.887
X7	0.482	-0.082	0.035	0.078	0.474	-0.011	**-0.360**	0.104	0.108	0.083	-0.114	0.211	-0.216	0.796
X8	-0.062	-0.316	0.173	0.095	0.538	-0.013	-0.138	**0.272**	0.031	0.029	-0.028	0.137	-0.001	0.841
X9	0.428	-0.060	0.030	0.054	0.485	-0.011	-0.353	0.076	**0.110**	0.098	-0.098	0.209	-0.221	0.750
X10	0.208	-0.086	0.079	-0.030	0.433	-0.009	-0.227	0.061	0.082	**0.132**	-0.012	0.078	-0.172	0.540
X11	0.484	-0.003	-0.029	0.111	0.244	-0.006	-0.312	0.059	0.083	0.012	**-0.131**	0.234	-0.163	0.583
X12	0.313	-0.103	0.053	0.092	0.413	-0.010	-0.248	0.121	0.075	0.034	-0.100	**0.306**	-0.111	0.835
X13	0.4849	0.024	-0.009	0.023	0.355	-0.007	-0.357	0.002	0.112	0.105	-0.098	0.156	**-0.217**	0.575

RESIDUAL EFFECT= .1242985

Bold letters are the direct effects

Off diagonal values are indirect effects

X1 Plant height (m)
X2 Number of leaves/palm
X3 Number of spikelets/inflorescence
X4 Number of female flowers/inflorescence
X5 Length of bunch stalk (cm)
X6 Girth of bunch stalk (cm)
X7 Number of bunches harvested/year
X8 Number of nuts per bunch
X9 Weight of unhusked nut (kg)
X10 Weight of husked nut (g)
X11 Husk/nut ratios
X12 Fruit polar perimeter (cm)
X13 Fruit equatorial perimeter (cm

Number of leaves per palm showed negative direct effect (-0.317) but positive correlation (0.764) which is accounted by indirect effects via length of bunch per stalk (0.536).

Highest positive direct effect (0.679) as well as high positive correlation (0.924) was observed for length of bunch stalk.

Fruit polar perimeter recorded a positive direct effect of (0.306) and a high positive correlation of (0.835). The high correlation was due to the indirect effect via length of bunch stalk (0.413) and plant height (0.314).

Positive direct effect of number of spikelets per inflorescence (0.188), number of female flowers per inflorescence (0.142), number of nuts per bunch (0.272) and weight of husked nut (0.110)) were less but had a high positive correlation of 0.712, 0.841, and 0.540 respectively. The high indirect effect, through length of bunch of stalk, for all these characters clearly accounts for high correlation.

Weight of unhusked nut has negligible positive direct effect (0.110) but had high positive correlation (0.750) which is accounted by the indirect effects of length of bunch of stalk (0.485) and (0.428).

Negative direct effect was showed by girth of bunch stalk (-0.017), but had a high positive correlation of 0.887. This character showed high positive indirect effect via length of bunch of stalk (0.668) which accounts for high positive correlation.

Negative direct effect was by husk/ nut ratio (-0.131) but it had a positive correlation (0.583) which was accounted by indirect effect through plant height (0.484).

Fruit equatorial perimeter had a negative indirect effect of (-0.217) but a high positive correlation (0.575) accounted by the indirect effect through plant height (0.485) and length of bunch stalk (0.355).

Number of bunches harvested per year had a negative direct effect of (-0.017) but a positive correlation of (0.796) accounted by indirect effects through length of bunch stalk (0.668).

The residual effect (0.124) indicates that the selected characters explain the total correlation well and the remaining characters have only minor contribution towards the variability of yield.

4.1.4. Genetic Divergence

Genetic divergence of the six coconut genotypes based on eight morphological traits was worked out using D^2 analysis. The D^2 values for the six genotypes given in Table 5. The most divergent pair was Komadan and CGD with D^2 value 1325.870. The least value was between the pair WCT and NCD (83.280).

4.1.5. Group constellation: Intra and inter cluster D^2

The clustering of the cultivars was done by Tocher's method based on the D^2 totals. The six cultivars were grouped in to 3 clusters (Fig.4).Cluster II with dwarf palms COD and CGD, cluster I with WCT and NCD and cluster III with Komadan and Laccadive Ordinary.

The means of clusters for eight characters chosen for the D^2 analysis is given in Table 6. Cluster I with the dwarf cultivars had the lowest mean for plant height and highest value for this character was for Cluster II. Cluster mean for length and girth of bunch stalk was highest for cluster III. Mean for number of nuts/bunch and weight of unhusked nut weight was also high for the Cluster III. Fruit polar perimeter was highest for cluster III but equatorial perimeter was highest for Cluster II. The highest mean for nuts per palm/year was for the Cluster III.

Table 5. D2 values for the six coconut genotypes

	1	2	3	4	5	6
1	0.000	678.965	414.763	**83.280**	1111.296	796.684
2		0.000	183.415	464.478	752.583	490.262
3			0.000	391.704	**1325.870**	861.637
4				0.000	663.825	339.776
5					0.000	193.177
6						0.000

Bold figures indicate the lowest D^2 total

1. West Coast Tall (WCT)
2. Laccadive Ordinary (LO)
3. Komadan
4. Natural Cross Dwarf (NCD)
5. Chawghat Green Dwarf (CGD)
6. Chawghat Orange Dwarf (COD

Table 6. Average inter and intra cluster distance

Cluster	I	II	III
I	**7.456**	23.115	27.534
II		**8.800**	20.856
III			**10.858**

Bold figure indicate intra cluster distance

Table 7. Cluster means for different traits

Sl. No	Characters	Cluster I	Cluster II	Cluster III
1	Plant height (m)	4.45	**15.33**	9.80
2	Length of bunch stalk	21.57	31.80	**56.70**
3	Girth of bunch stalk	8.68	10.70	**20.65**
4	Number of nuts per bunch	8.50	10.40	**14.20**
5	Weight of unhusked nut (kg)	641.00	1011.80	**1055.50**
6	Fruit polar perimeter (cm)	48.50	54.35	**55.80**
7	Fruit equatorial perimeter (cm)	41.40	**46.35**	45.35
8	Number of nuts per palm per year	57.20	92.20	**119.10**

Bold figure indicate highest means among the clusters

The inter and intra cluster distances of the three clusters are presented in Table 7. The average intra cluster distance for the three clusters ranged from 7.456 (Cluster I) to 10.858 (ClusterIII). The second cluster had an intra cluster distance of 8.8. The inter cluster distance showed that the Cluster I and Cluster III were the farthest (27.537) and Cluster II and III were closest (20.856).

The relationship between the six genotypes based on the D^2 analysis is shown in a dendrogram Fig.5.

4.2 RAPD markers

Good quality DNA was isolated from 30 palms and DNA from five palms of each accession pooled for analysis. The quantity and quality of DNA used for analysis is given in Table 8. Plate 1 shows the quality of DNA used in agarose gel. Fifty random primers from Operon series –OPB, OPC, OPD, OPE, OPH, OPK and OPP series were screened for producing amplification products in coconut varieties. Out of these, fourteen primers which gave good amplification were selected for the study. Primer number, it's sequence ,the number of amplification products produced and the number of unique products produced are given in Table 9.

A total number of 107 amplified products were produced of which 29 were polymorphic. Amplified fragments were produced between 2kb to 0.5kb.The number of amplicons produced by each primer is compared in a histogram (Fig.6).The percentage of polymorphism for each primer ranged from 10 to 43 per cent with an overall average of 27 per cent. Primers OPE 7 and OPB 05 showed the highest percentage of polymorphism. The percentage of polymorphism for fourteen primers compared in Fig.7.

Table 8. Quality and Quantity of DNA of the 6 coconut accessions used in the study

Sl. No	Accession name	A260 nm	A280 nm	A260/A280	DNA yield ng/µl
1	West Coast Tall(WCT)	0.070	0.004	1.75	210
2	Laccadive Ordinary(LO)	0.003	0.006	2.00	180
3	Komadan	0.007	0.004	1.75	210
4	Natural Cross Dwarf(NCD)	0.012	0.008	1.50	360
5	Chawghat Green Dwarf(COD)	0.004	0.008	2.00	240
6	Chawghat Orange Dwarf (CGD)	0.011	0.007	1.57	330

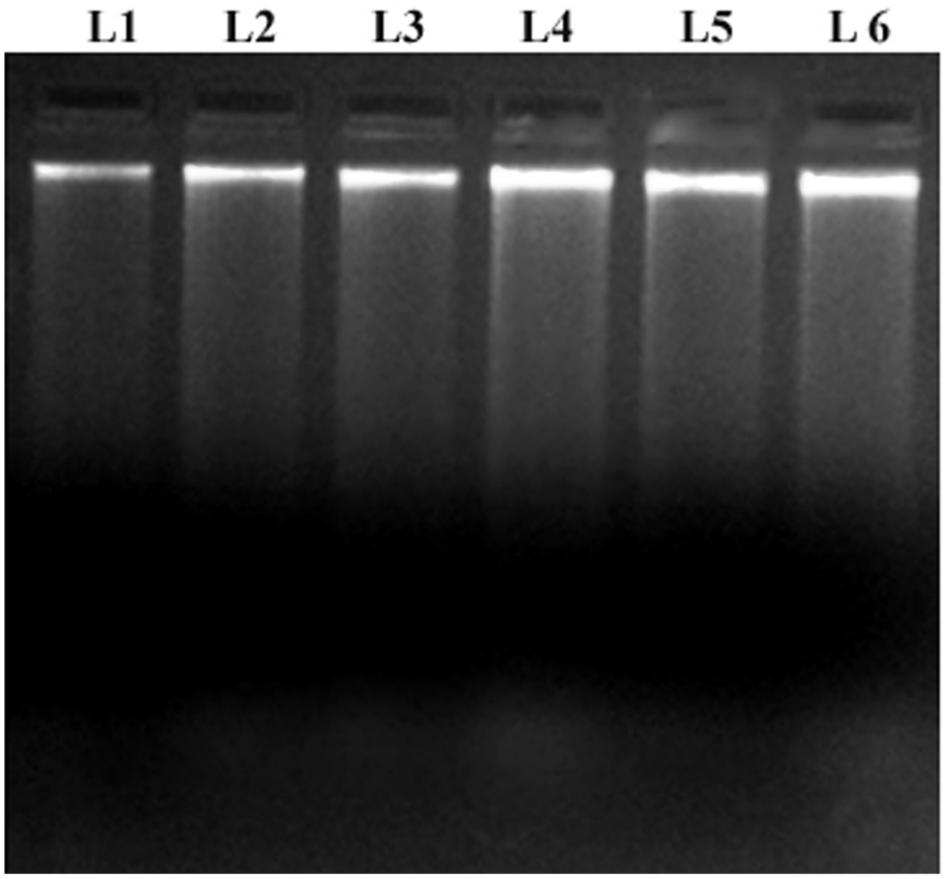


Plate 1 - Genomic DNA of the 6 Coconut accessions

L 1 (WCT) West Coast Tall

L 2 (LO) Laccadive Ordinary

L 3 Komadan

L 4 (NCD) Natural Cross Dwarf

L 5 (CGD) Chowghat Green Dwarf

L 6 (COD) Chowghat Orange Dwarf

Table 9. Base sequence of RAPD primers, number of anplicons and percentage of polymorphsm in coconut genomic DNA

Primer name	Sequence	Number of Amplicons	Number of polymorphic amplicons	Number of monomorphic amplicons	Polymorphism (%)
OPB-05	**TGCGCCCTTC**	7	3	4	43
OPB-08	**GTCCACACGG**	4	1	3	25
OPB-10	**CTGCTGGGAC**	7	2	5	29
OPC-07	**GTCCCGACGA**	6	2	4	33
OPC-15	**GACGGTTCAG**	7	1	6	14
OPC-20	**ACTTCGCCAC**	10	1	9	10
OPD-20	**ACCCGGTCAC**	8	3	5	30
OPE-07	**AGATGCAGCC**	7	3	4	43
OPE-14	**TGCGGCTGAG**	8	2	6	20
OPH-14	**ACCAGGTTGG**	9	2	7	22
OPH-19	**CTGACCAGCC**	6	2	4	33
OPK-14	**CCCGCATCAC**	6	2	4	33
OPP-03	**CTGATACGCC**	11	3	8	27
OPP-05	**CCCCGGTAAC**	7	2	5	29
	Total	**107**	**29**	**78**	**27**
	Average	**7.64**	**2.07**	**5.57**	**1.93**

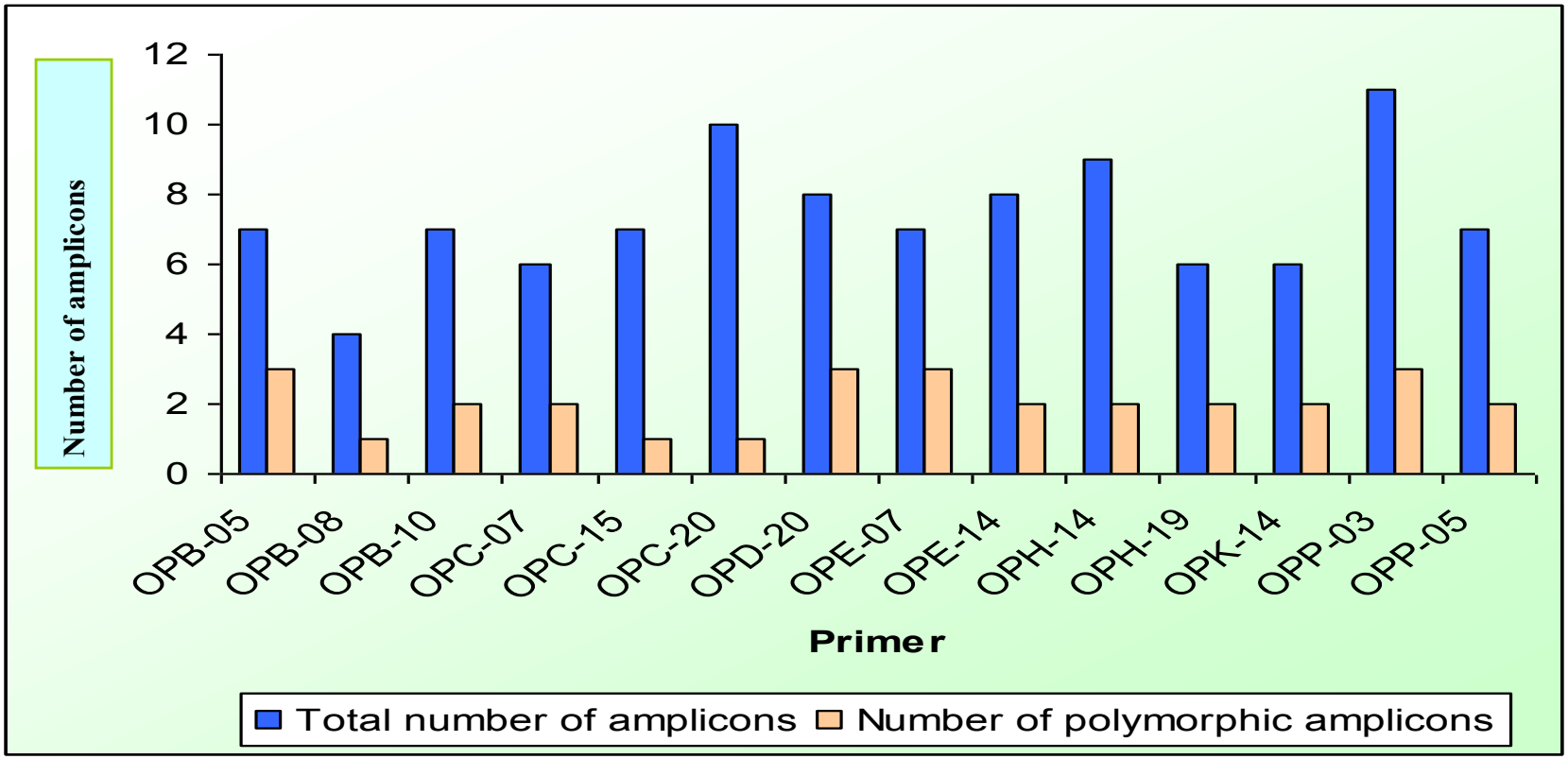


Fig 6. Total number of amplicons and polymorphic amplicons produced by RAPD primers

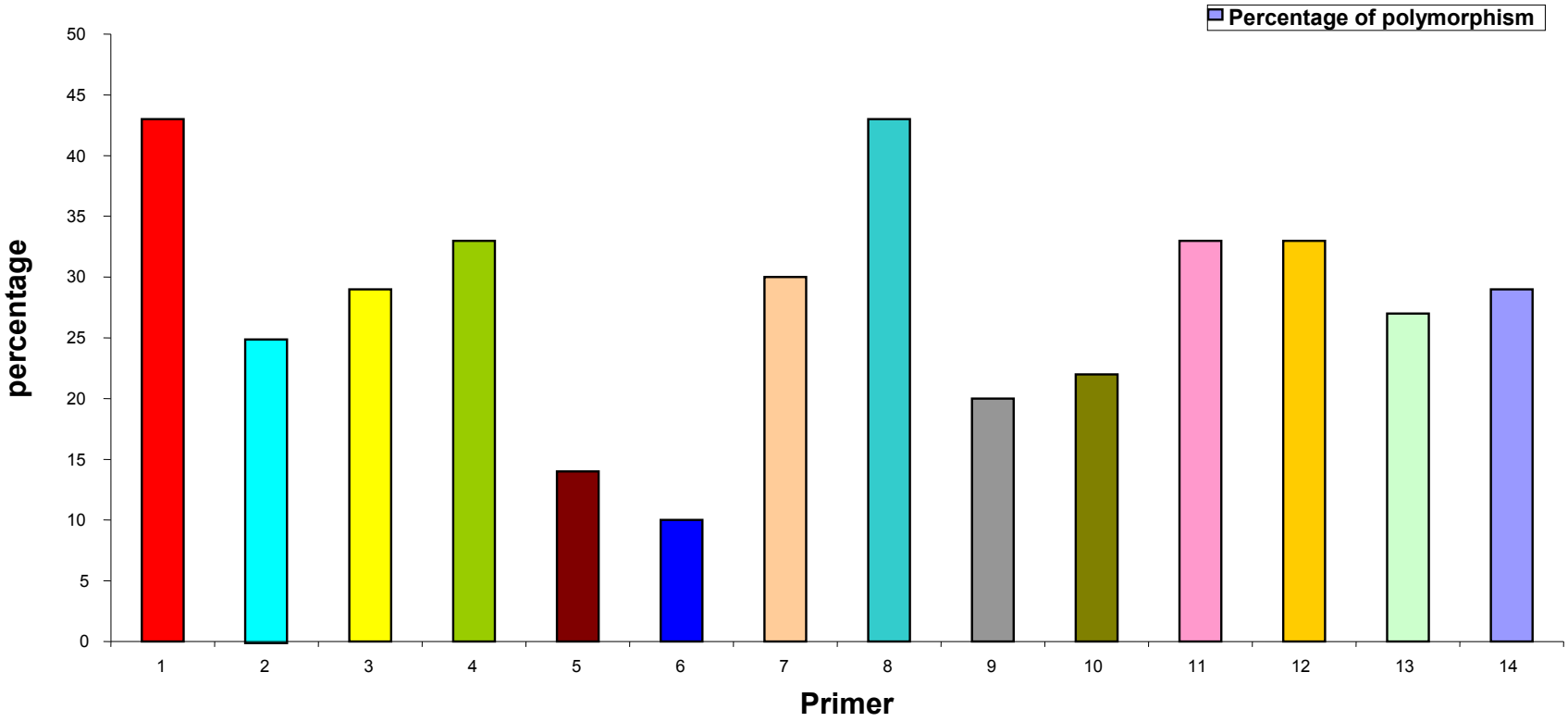
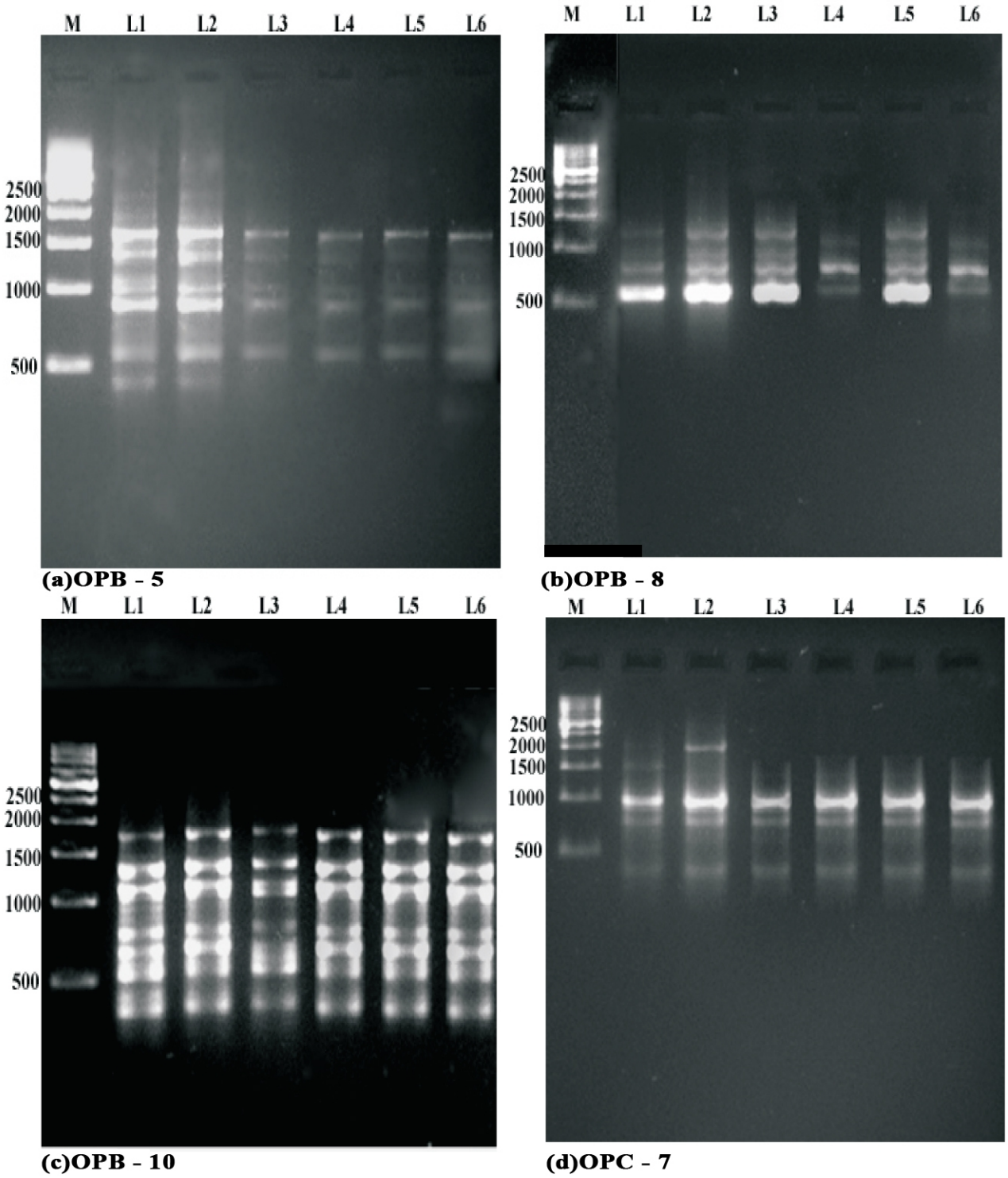


Fig 7. Percentage of polymorphism produced by RAPD primers in coconut

1. OPB-05	**4. OPC-07**	**7. OPD-20**	**10. OPH-14**	**13. OPP-03**
2. OPB-08	**5. OPC-15**	**8. OPE-07**	**11. OPH-19**	**14. OPP-05**
3. OPB-10	**6. OPC-20**	**9. OPE-14**	**12. OPK-14**	



L 1 (WCT) West Coast Tall
L 2 (LO) Laccadive Ordinary
L 3 Komadan
L 4 (NCD) Natural Cross Dwarf
L 5 (CGD) Chowghat Green Dwarf
L 6 (COD) Chowghat Orange Dwarf

Plate 2 - RAPD profile of six coconut genotypes with primers
(a) OPB - 5 (b) OPB-8 (c) OPB - 10 & (d) OPC - 7

Plate 2 shows the amplification profile of six genotypes by primers OPB-5,OPB-8,OPB-10 and OPC-7, Plate 3 shows amplification profile by primers OPC-15,OPC-20,OPD-20 and OPE-7 , Plate 4 shows amplification profile by primers OPE-14,OPH-14,OPH-19 and OPK-14 and Plate 5 shows amplification profile by primers OPP-3 and OPP-5.

The details of amplicons produced by each primer given below.

Primer OPB-5

This primer produced 7 amplicons within the range of 2 kb to 0.5 kb. This primer could produce two polymorphic bands at around 1000 bp. One polymorphic product present in the WCT and LO and one was unique for WCT.

Primer OPB-10

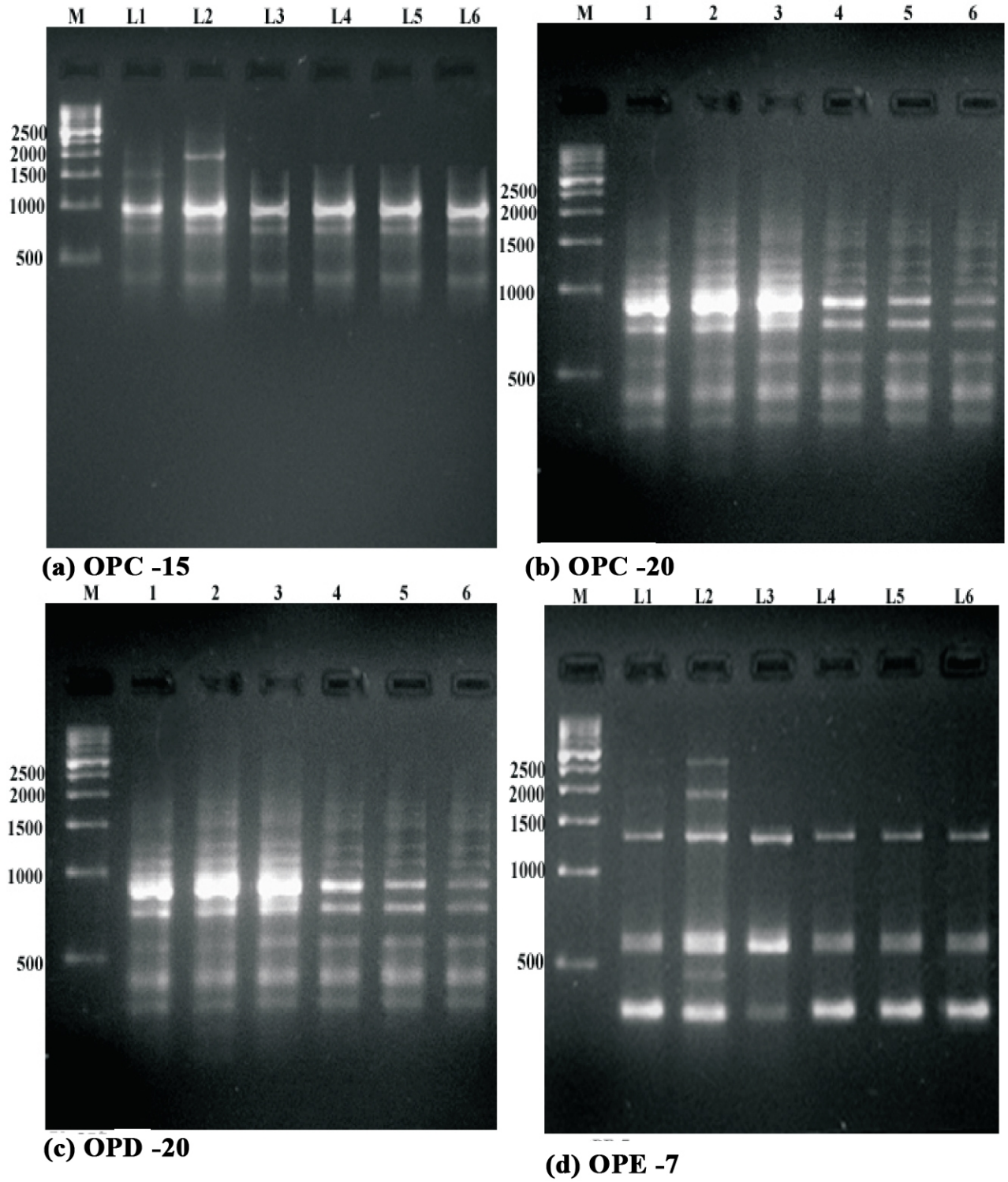
Ten amplification products within the range of 2kb to 0.5 kb were produced by this primer. It could produce two polymorphic products. A product at around 500bp was found only in WCT and one around 1000 bp in WCT and LO.

Primer OPC-7

This primer produced six amplification products within the range of 2.5 kb and less than 500 bp. Out of these, two were polymorphic. The amplicon at 1500 bp was unique for LO.

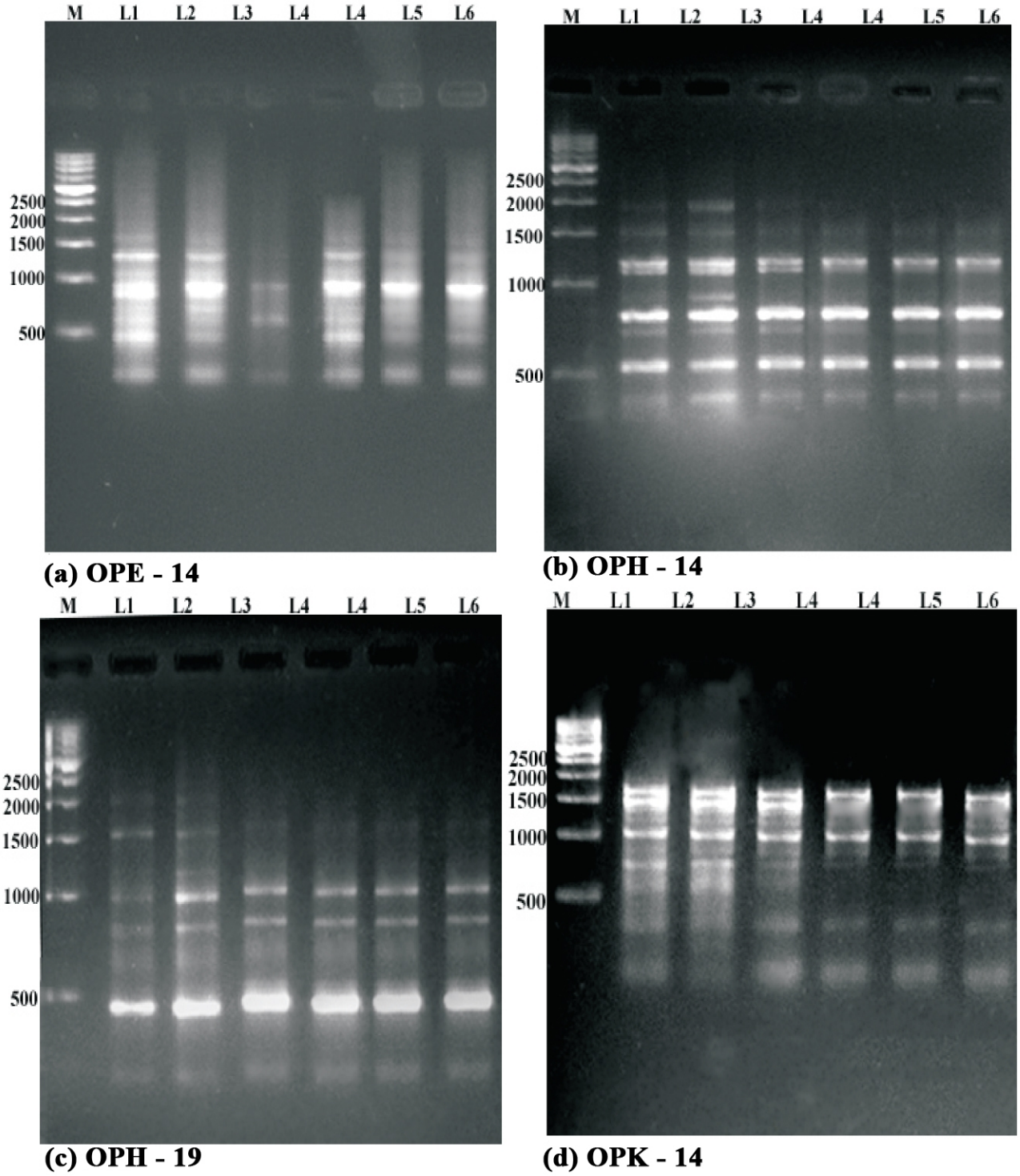
Primer OPC- 15

Seven amplicons within the range of 1500 bp to less than 500 bp were produced. Here also, one polymorphic product was noted at 1500 bp and that was present in WCT and LO.



L 1 (WCT) West Coast Tall
L 2 (LO) Laccadive Ordinary
L 3 Komadan
L 4 (NCD) Natural Cross Dwarf
L 5 (CGD) Chowghat Green Dwarf
L 6 (COD) Chowghat Orange Dwarf

Plate 3 RAPD profile of coconut genotypes with primers
(a) OPC-15, (b) OPC -20, (c) OPD-20 & (d) OPE - 7



L 1 (WCT) West Coast Tall
L 2 (LO) Laccadive Ordinary
L 3 Komadan
L 4 (NCD) Natural Cross Dwarf
L 5 (CGD) Chowghat Green Dwarf
L 6 (COD) Chowghat Orange Dwarf

Plate 4 RAPD profile of coconut genotypes with primers
(a) OPE-14, (b) OPH - 14, (c) OPH-19 & (d) OPK - 14

Primer OPC -20

This primer could produce two bright monomorphic amplicons and eight faint products. There was only one polymorphic product around 1500 bp found in WCT, LO and Komadan. The NCD and the dwarf accessions produced very faint products other than the monomorphic products.

Primer OPD- 20

This primer could produce 8 amplicons, of which five were monomorphic. One amplicon between 500 and 1000 bp was unique and present in the Laccadive ordinary. Two amplicons just above the 500 bp and at 100bp were found only in WCT and LO.

Primer OPE- 7

Seven amplicons within the range of 2.5 kb and less than 500 bp were amplified. This primer produced three unique polymorphic products at around 500bp, 2 kb and 2.5 kb. All these were seen only in LO.

Primer OPE -14

The 8 amplicons produced by this primer were between 2 kb to less than 500 bp. Three monomorphic bright products and five faint products were produced. Of these one product at above 500bp was unique for Komadan.

Primer OPH 14

This primer could produce nine amplicons between 2.5 kb and less than 500 bp. This primer produced two polymorphic products, one between 500 and 1000bp seen only in talls and CGD and one only in WCT and LO.

Primer OPH – 19

All the accessions had a bright monomorphic product at 500bp and three monomorphic products at 1000 and 1500bp. This primer produced polymorphic products one at 2500bp showed by LO and WCT and one between 1000 and 1500bp only for Laccadive Ordinary.

Primer OPP -3

Eleven amplicons between less than 500bp and 1500 bp were produced by this primer. Of these 8 were monomorphic but only 5 bright. This primer could produce a polymorphic product found only in LO,CGD and COD below 500bp and one product for WCT and LO and one for WCT, LO and Komadan.

Primer OPP-5

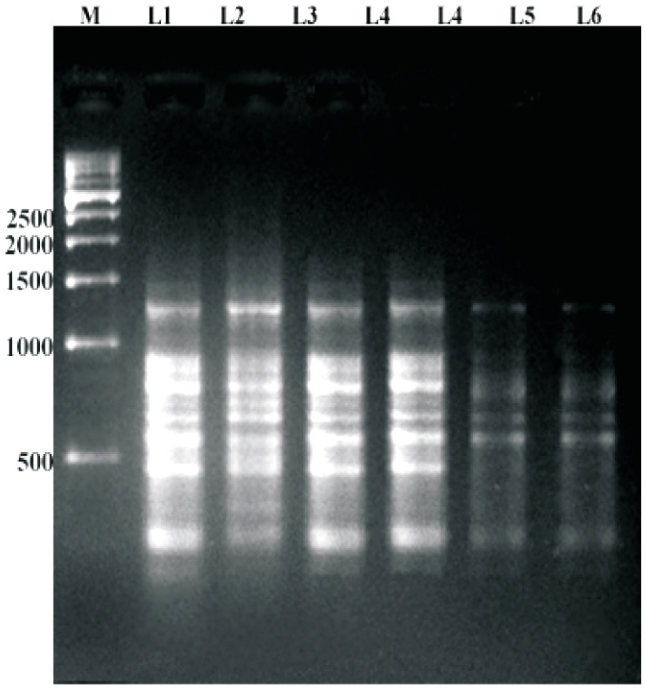
All the 7 amplicons produced were below 1500 bp. This primer also produced two unique products. One for WCT and one for LO and one product for WCT and LO.

Primer OPB – 8

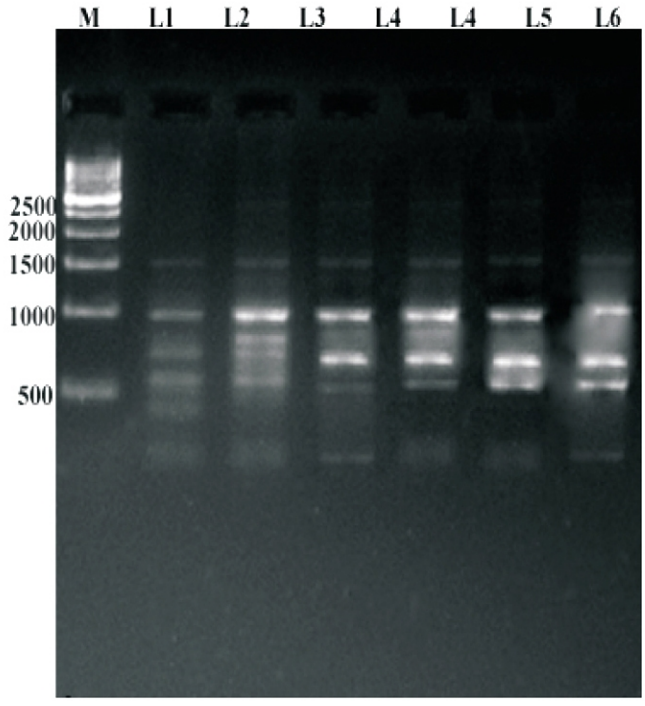
This primer could produce only four amplicons below 1500bp. Of these one faint product was missing in NCD and COD.

4.2.1 RAPD analysis

The RAPD scores were analysed using the software NTSYS (Version 2.02) Jaccard's coefficient of similarity between the accessions ranged from 0.68 to 0.97 (Table 10). Accessions COD and CGD were closest with a similarity percentage of 97, followed by CGD and NCD with a similarity percentage of 92. COD and LO were the farthest with a similarity of only 68 per cent.



(a) OPP - 3



(b) OPP - 5

L 1 (WCT) West Coast Tall
L 2 (LO) Laccadive Ordinary
L 3 Komadan
L 4 (NCD) Natural Cross Dwarf
L 5 (CGD) Chowghat Green Dwarf
L 6 (COD) Chowghat Orange Dwarf

Plate 5 RAPD profile of coconut genotypes with primers (a) OPP- 3 & (b) OPP -

The dendrogram constructed based on the RAPD markers using UPGMA clustering showed two clusters (Fig.8). West Coast Tall and Laccadive Ordinary clustered together at 85 percentage similarity and Komadan, NCD, CGD and COD clustered at 80 per cent similarity. Within the second cluster NCD, CGD and COD clustered at 90 percent similarity. CGD and COD showed 98 percent similarity.

Table 10. Similarity indices for the DNA amplicons in coconut genotypes.

	V1	V2	V3	V4	V5	V6
V1	1.00					
V2	0.83	1.00				
V3	0.79	0.73	1.00			
V4	0.76	0.71	0.89	1.00		
V5	0.71	0.70	0.85	0.90	1.00	
V6	0.69	0.68	0.83	0.92	0.97	1.00

V1 - West Coast Tall WCT
V2 - Laccadive Ordinary (LO)
V3 - Komadan
V4 - Natural Cross Dwarf (NCD)
V5 - Chawghat Green Dwarf (CGD)
V6 - Chawghat Orange Dwarf (COD)

Discussion

5. DISCUSSION

In any crop improvement programme an assessment of the nature and extent of variability will be of immense value in identifying superior genotypes and formulating breeding procedures. The analysis of genetic variation or diversity in coconut has been assessed for many years using morphological traits (Meunier et al. 1992). But this may not be a reliable measure because of the influence of environment on gene expression. The analysis of DNA which is more stable allows a clear assessment of variation in the genotypes. This project was undertaken to assess the genetic diversity in the six popular genotypes of coconut in this locality (WCT, CGD, COD, LO, Komadan and NCD) for the fourteen morphological traits and DNA using Randomly Amplified Polymorphic DNA (RAPD) markers. The results obtained are discussed in this chapter

5.1 GENETIC ANALYSIS BASED ON MORPHOLOGICAL MARKER

Coconut is a robust palm, with tall slender and thick stem and massive crown with large number of leaves bearing bunches of nuts in its axis. Variability exists among different cultivars of coconut on a number of morphological traits. Fourteen biometric traits were used to differentiate the six genotypes under study.

5.1.1 Variability for the morphological traits

The magnitude of variation as represented by range, phenotypic coefficient of variation and genotypic coefficient of variation was moderately high for all the characters studied. Pillai et al. (1991) reported that the coconut cultivars could be characterized and classified successfully based on leaf, stem, inflorescence and nut characters.

Manju (1992) reported that medium to high phenotypic and genotypic coefficient of variation for number of nuts per palm per year, number of female flowers per bunch and number of nuts per bunch indicating scope for selection based on these characters. N'cho et al. (1993) reported that coconut cultivars can be classified based on the inflorescence characters.

Jayalekshmy and Sree Rangasamy (2002b) also reported the role of vegetative and floral traits for distinguishing coconut cultivars.

For all the nut characters except husk/nut ratio Komadan recorded the highest value and for yield it was on par with Laccadive Ordinary which recorded the maximum. Superiority of Komadan for yield and related characters were reported previously by many workers (Gopimony (1982), Shylaraj et al. (1991) and Manju (1992)). In this study Lacadive Ordinary, commonly cultivated in the Islands of India, showed significant superiority over the WCT which is the local cultivar of this area. Lacadive Ordinary has already been released for Kerala in the name of "Chandrakalpa". Ohler (1984) have reported that Lacadive Ordinary is more or less similar to WCT. NCD showed similarity to Komadan only for plant height, number of nuts per bunch and fruit shape characters.For all the rest of the characters Komadan showed significant variation from corresponding traits of NCD. This is contradictory to the report of Pillai (1991) that NCD in Central Travancore is known as Komadan.

The genotypic coefficient of variation is a measure of genetic variability facilitating successful isolation of desirable types. Genotypic coefficient of variation together with heritability estimates can give the best picture of the amount of advance to be expected from selection (Burton, 1952). The heritability estimates were high for most of characters studied. Louis (1981) reported high genetic advance for the vegetative characters in coconut.

High heritability coupled with high genetic advance was observed for weight of unhusked nut, weight of husked nut (g) and number of nuts per palm per year. The result indicates that these characters were highly heritable and hence were less affected by the environment. The coconut breeder therefore may take his selection on the basis of phenotypic expression of these characters in the individual palms. Heritability in conjunction with genetic advance would give a more reliable index of selection value. Hence selection based on phenotypic performance would result in considerable genetic gain of these traits. Ganesamoorthy et al., (2002) had reported high genetic advance for copra yield, dehusked nut weight, nut yield and whole nut weight.

This suggests that selection for all the characters chosen have good role in yield improvement in coconut.

5.1.2 Correlation between variables

The correlation between variables provided an idea of the degree of association existing between the different parameters measured. All the significant correlations existing between the characters studied are positive. All the characters studied, except plant height, had positive correlation with yield at 1 per cent level of significance.

Earlier studies conducted in coconut palms have revealed that the length of the stem, number of leaves, length of leaves and number of flowers per bunch are correlated to yield (Satyabalan et al. 1982, Abeywardana 1976, Ramanathan 1984) but Manju (1992) have reported that number of leaves per palm did not have significant correlation with yield. Sindhumole and Ibrahim (2001) reported that nut yield was significantly correlated with vegetative and reproductive characters. In this study also yield had significant positive correlation with both vegetative and reproductive characters included in the study.

High positive correlation was recorded for number of leaves per palm and number of spikelets per inflorescence, length of bunch stalk and girth of bunch stalk, plant height and number of bunches, number of nuts per bunch and number of leaves per palm and weight of unhusked nut and number of bunches per year.

Path coefficient analysis revealed that eight characters viz., plant height, number of spikelets/inflorescence, number of female flowers/inflorescence, length of bunch stalk, number of nuts per bunch , weight of unhusked nut, weight of husked nut and fruit polar perimeter showed positive correlation and positive direct effect showed positive direct effect on yield. Louis (1981) reported that the selection strategy for yield may be based on number of leaves, number of spathe per year and number of female flowers.Sukumaran et al., (1981) had reported the direct effect of number of leaves and number of female flowers on yield but in this study number of leaves showed only an indirect effect on yield.

5.1.3. Genetic divergence

Based on eight morphological traits (plant height, length and girth of bunch stalk, number of nuts per bunch, weight of unhusked nut, fruit polar and equatorial perimeter and nuts/palm/year), the thirty palms belonging to six genotypes were selected for the study and subjected to D^2 analysis in order to classify them into group constellations.

The two dwarf palms chosen (Chowghat Green Dwarf and Chowghat Orange Dwarf were separately clustered. The uniqueness of dwarf palms were reported by many workers (Nair and Ratnambal 1994, Jayalekshmy and Sree Rangasamy, 2002, Arunachalam 2005). The local cultivar WCT and the Natural Cross Dwarf (NCD) were clustered together and this may be due to the common heritage of NCD and WCT since WCT is the male parent of NCD. The same trend of NCD to cluster with WCT was also reported by (Peter and Rai, 1976 and Manju and Gopimony, 1998).

The well preferred cultivar Komadan got clustered along with Laccadive Ordinary the variety of Lakshadweep released in Kerala as “Chandrakalpa” for its superiority in performance. The yield characters, number of nuts/bunch and number of nuts/palm/year was highest for this group emphasizing the superiority of Komadan as reported by Manju 1992.Maximum divergence was reported between Komadan and NCD and it was contradictory to the observation of to Pillai (1991) who had stated that NCD in central Travancore was called as “ Komadan”

The present study revealed that the importance of nut characters in assessing the genetic divergence in coconut. Similar result were reported by Ovasuru (1993), Rao and Pillai (1983) and Jayalekshmy and Sree Rangasamy (2002).

5.2. GENETIC ANALYSIS BASED ON RAPD MARKERS

Molecular markers have presently become fundamental tools for finger printing varieties, establishing phylogenetics, tagging desirable genes, determining similarities among inbreds and mapping plant genomes (Kang Fu Yu et al. 1993). In perennial plants, the use of these markers may have the most practical value because breeding and genetic studies in these species are difficult using conventional techniques due to the long juvenile period.

The Randomly Amplified Polymorphic DNA (RAPD) reaction performed on genomic DNA with an arbitrary oligonucleotide results in the amplification of several discrete DNA products. These are usually separated on agarose gel and visualized by ethidium bromide staining. The polymorphism between individuals result from sequence difference in one or both of the primer binding sites and are visible as presence or absence of a particular band. Such polymorphisms in general behave as dominant genetic markers. The banding pattern differences, existing between 2 species or varieties can be used for species or varietal identification.

In this study fourteen random primers were used to amplify the genomic DNA of six coconut genotypes. Pooled DNA from five palms of each genotype was used for the analysis. Fifty random primers selected based on the reports of RAPD analysis in coconut (Jayalekshmy and Sree Rangasamy 2003, Parthasarathy et al. 2005, and Manimekhalai et al. 2006) were used for screening and from this fourteen primers were chosen for the RAPD analysis.

The fourteen primers produced a total of 107 amplicons of which only 29 were polymorphic giving 27 percentage polymorphism. The number of markers for each primer ranged from 4 (OPB-8) to 11 (OPP-3) with an average of 7 markers/primer. The amplification product size ranged from 2 Kb to less than 0.5 Kb. Manimekhalai et al. (2006) reported a size range of 0.117 kb to3.103 kb after assessing 45 primers in coconut.

The primers chosen for the study reveal the advantage of GC-rich primers in bringing about amplification. Williams *et al.* (1990) tested a set of primers with GC-content ranging from 0-100 per cent in the amplification of soyabean genomic DNA to find that GC content of 40 per cent or more generated detectable levels of amplification products.

Among the different primers used OPE 7 and OPB 5 produced maximum polymorphism (43 percentage). Primer OPE 7 could produce 3 unique products to distinguished Laccadive Ordinary. Among the fourteen primers studied 9 primers could produce unique products. Three primers produced unique products for Laccadive Ordinary, two primers for WCT and two primers for Komadan. Since the study included only six accessions screening of more accessions is suggested for confirming the uniqueness of these products.

The similarity analysis of the RAPD products showed that the accessions were divergent with respect to RAPD markers. The usefulness of RAPD markers to assess genetic diversity had been emphasized by many workers.

Similarity indices showed that the two accessions COD and CGD are having 98 percentage similarity. The uniformity of dwarf accessions had been reported previously. Jayalekshmy and Sree Rangasamy (2003) had reported 93 percent similarity between Kulasekharam Green Dwarf and Chowghat Orange Dwarf. The least similarity was noted between Laccadive Ordinary and Chowghat Orange Dwarf. These divergent accessions can be used as parents for exploiting maximum heterosis.

Komadan had 89 percentage similarity with NCD and only 77 and 71 percentage similarity with WCT and Laccadive Ordinary. The pedigree of Komadan is to be further investigated for confirming its similarity to NCD. The dendrogram constructed depicts the clustering of Komadan along with NCD and dwarfs. Earlier reports regarding this were controversial (Gopimony, 1982), and Pillai (1991).

5.3 COMPARISON OF MORPHOLOGICAL AND MOLECULAR MARKERS FOR ASSESSMENT OF GENETIC DIVERSITY

This study was conducted with a major objective of comparing the genetic stock of coconut with morphological markers and molecular markers. The fourteen biometrical traits used for the study could give a clear cut idea on the variability existing for these characters, the influence of these characters on yield and the genetic divergence of the six genotypes. The RAPD analysis using fourteen random oligonucleotide primers produced only 27 percentage of polymorphism. These primers could amplify the DNA of the six genotypes and reveal the variability at the molecular level. Since the DNA used for the analysis was collected from five palms of each accessions and pooled to get the working sample, the variability within the accession was also taken care of.

The divergent analysis using these two markers (morphological and molecular) slightly differed. In both analyses the dwarfs were clustered together. In the analysis with biometrical traits the divergence was 56 percent and in the RAPD analysis it had 98 percent similarity. Morphological markers clustered Komadan and Laccadive Ordinary together and West Coast Tall along with NCD. But in the RAPD analysisWest Coast Tall and Laccadive ordinary clustered together and Komadan clustered with dwarfs and NCD at 80 percent similarity. Komadan is an off type selection and the pedigree is yet to be confirmed. The dwarfs and NCD are mostly self pollinated. Komadan also shows higher percentage of self pollination. But the superiority of Komadan is on par with Laccadive ordinary as depicted in the analysis with morphological markers and the superiority is confirmed in many studies (Gopimony 1982, Shylaraj et al. 1991 and Manju 1992).

The molecular analysis with RAPD markers was reported by many workers to be efficient in the genetic divergence analysis. In this study a limited number of primers were only used for the study. The use of more number of primers can give a clearer picture of the molecular divergence of the genotypes studied.

Summary

SUMMARY

The present study Morphological **and molecular analyses of coconut (*Cocos nucifera* L)** was conducted in the Department of Plant Breeding and Genetics College of Agriculture, Vellayani during the year 2006-2008.Morphological observations were recorded in palms at Instructional farm, Vellayani and Regional Agricultural Research Station Pilicode. Molecular studies were done in the department of Biotechnology, College of Agriculture Vellayani.

The major objectives of this study include genetic analysis of six coconut cultivars with respect to fourteen morphological characters (vegetative and fruit characters),RAPD analysis of the coconut cultivars with 14 oligonucleotide primers and comparison of the genetic divergence of the six coconut cultivars with respect to morphological and molecular markers

The study was conducted in six popular coconut genotypes WCT, Komadan, Laccadive Ordinary, Natural Cross Dwarf, Chowghat Green Dwarf and Chowghat Orange Dwarf. Morphological data on fourteen biometrical traits in thirty palms belonging to the six genotypes, with five palms for each genotype were subjected to analysis of variance, correlation analysis, path analysis and divergent analysis. The analyses revealed that magnitude of variation represented by range; phenotypic coefficient of variation and genotypic coefficient of variation were moderately high for all the characters studied. High heritability coupled with high genetic advance was observed for weight of unhusked nut, weight of husked nut (g) and number of nuts per palm per year. The coconut breeder therefore can take his selection on the basis of phenotypic expression of these characters in the individual palms. Correlation studies revealed that all characters except plant height had significant positive correlation with yield. Path coefficient analysis revealed that eight out of thirteen characters showed positive direct effect on yield.

Genetic divergence studies using Mahalanobis D^2 analysis showed that the dwarf accessions are distinctly divergent from the rest of the accessions.The group constellations developed based on D^2 values showed that the local cultivar WCT and NCD were clustered together and the well preferred cultivar Komadan got clustered along with Laccadive Ordinary, the variety of Lakshadweep released in Kerala as "Chandrakalpa" for its superiority in performance. Maximum divergence was reported between Komadan and NCD .With regard to the characters chosen for the divergence analysis, it shows the importance of nut characters in assessing the genetic divergence in coconut.

Good quality DNA was isolated from 30 palms belonging to the five genotypes and the DNA from five palms of a genotype was pooled and used for analysis. The DNA was subjected to PCR using 14 random oligonucleotide primers selected after screening 50 primers reported in coconut. RAPD analysis using the 14 primers produced 107 amplicons within the molecular range of 2 kb to less than 0.5 kb. The primers chosen for the study reveal the advantage of GC-rich primers in bringing about amplification. Among the 14 primers studied, OPE-7 and OPB-05 produced maximum polymorphism of 43 percentages. The percentage of polymorphism produced by the 14 primers ranged from 10-43 with an average of 27 percentage. Among the fourteen primers studied nine primers could produce unique products.The similarity analysis of the RAPD products showed that the accessions are divergent with respect to RAPD markers. Similarity indices showed that the two accessions COD and CGD are having 98 percentage similarities. Komadan had 89 percentage similarity with NCD and only 77 and 71 percentage similarity with WCT and Laccadive Ordinary. The dendrogram constructed also depicts the clustering of Komadan along with NCD and dwarfs. The least similarity was noted between Laccadive Ordinary and Chowghat Orange Dwarf. These divergent accessions can be used as parents for exploiting maximum heterosis.

Divergent analysis with biometrical traits and RAPD markers slightly differed .In both analyses the dwarfs were clustered together. In the analysis with biometrical traits the divergence was 56 per cent and in the RAPD analysis it had 98 per cent similarity. Morphological markers clustered Komadan and Laccadive Ordinary together and West Coast Tall along with NCD. But in the RAPD analysis, West Coast Tall and Laccadive ordinary clustered together and Komadan clustered with dwarfs and NCD at 80 per cent similarity. Eventhough, Komadan is accepted as a superior palm its pedigree is controversial. A detailed molecular study may give a clear picture.

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Abstract

MORPHOLOGICAL AND MOLECULAR ANALYSES OF COCONUT (*Cocos nucifera* L.)

S.SELVARAJU

Abstract of the
thesis submitted in partial fulfilment of the requirement
for the degree of

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Department of Plant Breeding and Genetics
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM-695 522

ABSTRACT

The research project entitled **Morphological and molecular analyses of coconut (*Cocos nucifera* L)** was carried out in the Department of Plant Breeding and Genetics College of Agriculture, Vellayani during the year 2006-2008.

The major objectives of this study include genetic analysis of six coconut cultivars with respect to fourteen morphological characters (vegetative and fruit characters),RAPD analysis of the coconut cultivars with 14 oligonucleotide primers and comparison of the genetic divergence of the six coconut cultivars with respect to morphological and molecular markers

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