MARKER ASSISTED CHARACTERIZATION OF MANGO

(*MANGIFERA INDICA* L.)

by

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(2013 – 12 – 115)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"MARKER ASSISTED CHARACTERIZATION OF MANGO (*MANGIFERA INDICA* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Padannakkad
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Certified that this thesis entitled **"MARKER ASSISTED CHARACTERIZATION OF MANGO (*MANGIFERA INDICA* L.)"** is a record of research work done independently by Ms. Suchithra B. Shenoy under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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10/3/16

Suchithra B. Shenoy

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LIST OF ABBREVIATIONS

%	- Per cent
µm	- Micrometer
^0C	- Degree Celsius
A	- Average leaf area
bp	- Base pair
CD	- Critical difference
cM	- Centi Morgan
cm	- Centimetre
cm^2	- Centimetre square
CoA	- College of Agriculture
CPCRI	- Central Plantation Crops Research Institute
CTAB	- Cetyl Trimethyl Ammonium Bromide
cv.	- Cultivar
DNA	- Deoxyribonucleic acid
dNTPs	- Deoxynucleotides
DW	- Dry weight
EDTA	- Ethylene Diamine Tetra Acetic acid

et al	- and Co-workers
F_1	- First filial generation
g	- Gram
HDP	- High density planting
hrs	- Hours
I	- Internodal length
I.U	- International unit
IARI	- Indian Agricultural Research Institute
IIHR	- Indian Institute of Horticulture Research
L	- Number of leaves
LL	- Leaf Length
Ltd	- Limited
LW	- Leaf width
M	- Molar
m^3	- Meter cube
MAS	- Marker assisted selection
mg	- Milligram
Min	- Minute
ml	- Millilitre

mm	- Millimetre
N	- Normal
nm	- Nanometre
OD	- Optical density
PCR	- Polymerase Chain Reaction
PH	- Plant height
PVP	- Polyvinylpyrrolidone
PXR	- Phloem – xylem ratio
QTL	- Quantitative trait loci
RAPD	- Random Amplified Polymorphic DNA
rpm	- Rotation per minute
RWC	- Relative water content
SD	- Significant difference
Sec	- Second
t/ ha	- tonnes per hectare
TA	- Total leaf area
TCSA	- Trunk cross sectional area
UV	- Ultra violet
v/v	- Volume by volume

Vis	- Visible
viz	- Namely
	- Web based Agricultural Statistics Software Package

INTRODUCTION

1. INTRODUCTION

Mango (*Mangifera indica* L.) is a tropical tree belonging to the family Anacardiaceae and originated in the Indo-Myanmar region (Mukherjee, 1951). Due to its delicious taste, capitative flavour and attractive aroma, it is precisely designated as "King of fruits" (Purseglove, 1972). Among the 41 species described under the genus *Mangifera*, *M. indica* L. is the only species which produces superior quality fruits (Mukherjee, 1949).

Mango is the major fruit crop in India after banana and it shares 20.7% of the total fruit production. It is cultivated world over in about 51 countries and India ranks first in area, production and export (APEDA, 2015a). According to the 2013 – 2014 statistics, mango production in India is about 18000 tonnes. Kerala accounts for 2.4% of the total production with a productivity of 5.9 t/ ha (National Horticultural Board, 2015). Although our country posses topmost position in terms of area, production and export, the productivity is much less than the world's average productivity. Brazil ranks first in productivity (15.83 t/ha), which is much higher than the world's average productivity (7.52 t/ ha), whereas India's productivity is only 6.5 tonnes per hectare (APEDA, 2015b).

High density planting is one of the measures to increase productivity. The standard spacing for mango varies from 8 – 10 meters (Radha and Mathew, 2007), which can accommodate less than 200 plants per hectare. High density planting can be achieved by the use of dwarf statured mango varieties and by using dwarfing rootstocks for grafting elite varieties.

Some of the local mango varieties and few commercial varieties are reported to possess dwarf stature. Incorporation of dwarfing gene (s) from dwarf Indian cultivars could be a solution to induce dwarfness in commercial cultivars (Krishna and Singh, 2007). The local varieties like Olour, Mylepelian and Vellaikolamban induced dwarfness, when they were used as rootstocks (Radha and Mathew, 2007). Identification of dwarfness in the early stage is cumbersome, which could be overcome by marker assisted selection (MAS).

Marker is an indicator of the presence or absence of a character or gene. Marker assisted selection can be defined as an indirect selection process where a trait is selected, not based on the trait itself, but on a marker linked to it. It can be a morphological character or biochemical product of the plant or it can be the DNA sequence linked with the gene.

Marker assisted selection (MAS) is an important tool of modern breeding especially in fruit crops. Since most of the fruit crops are perennial in nature, developing hybrids is a time consuming process. Selection of hybrids for certain characters which are related with the reproductive stage of the crop will make the breeding programme much complicated. Requirement of large area to maintain hybrid population is another constraint. Hence, pre-selection of plants using markers will help to overcome the problems of conventional breeding.

Markers identified from the present study could be used for early screening of dwarf hybrids in the nursery stage. Ascertaining such a dwarf gene through MAS would assist breeding of mango. This could possibly be utilized for developing a dwarf mango variety, which could be effectively incorporated in high density planting (HDP).

The study entitled "Marker assisted characterization of mango (*Mangifera indica* L.)" aims to identify morphological, anatomical, physiological and molecular markers (RAPD) associated with dwarfness in mango [illegible].

1.1. OBJECTIVES OF THE STUDY

1. To study the morphological traits related to dwarfness.

2. To investigate the anatomical and physiological parameters associated with dwarfness in different mango varieties

3. To probe into the molecular markers associated with dwarfness.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Mango is an important fruit crop of India. It is known as king of fruits and recognized as one of the best fruit in the world market. The fruit is rich in vitamins like vitamin A (3000 I.U/ 100 g of pulp) and vitamin C (22-28mg/ 100 g of pulp). It also contains minerals like potassium, calcium, phosphorus and iron.

The present study on "Marker assisted characterization of mango (*Mangifera indica* L.)" is aimed at the early selection of dwarf plants based on morphological, anatomical, physiological and molecular characters associated with it. Development of markers will help to select the desirable plants in the nursery stage and this will reduce the time for the evaluation of hybrids in the field. Dwarfness is the most desirable character for a mango tree.

The productivity of mango is low (4-9 t/ha.) compared to the other fruit crops. Seedling trees and wider spacing are the major reasons for low productivity. These problems could be overcome by using grafts and through the adoption of high density planting (HDP). Dwarf statured/less vigorous varieties are recommended for HDP. The hybrids evolved in India suitable for HDP are Amrapali (Majumder and Sharma, 1989) and Arka Aruna (Reddy *et al.*, 2000). Other low vigorous cultivars reported are Kalapady (Sen, 1939), Malaviya Bhog (Tyagi, 1986), Creeping (Iyer and Subramanyam, 1986), Kerala Dwarf (Iyer, 1991) and Neelam (Ray *et* al., 1995). The hybrids and monoembryonic varieties will not yield true to type plants. Vegetative propagation is the only way to get true to type plants and the success of vegetative propagation depends on the stock scion interaction. Use of dwarfing rootstocks for grafting is an option to achieve dwarf trees, as reported by many workers.

The literature relevant for the present study are reviewed hereunder:

2.1. Classification of rootstocks

2.2. Markers

2.2.1. Morphological characters

2.2.2. Anatomical characters

2.2.3. Physiological characters

2.2.4. Molecular studies

2.1. CLASSIFICATION OF ROOTSTOCKS

The rootstocks were classified by several scientists as dwarf, semi vigorous and vigorous. Kurian *et al.* (1996) reported that the varieties Muvandan, Bappakai and Olour as vigorous rootstocks for Alphonso. For cv. Baneshan, Olour imparted dwarfness (Swamy *et al.*, 1972). Singh *et al.* (1986) classified Olour under dwarfing rootstock based on bark thickness of stem, root and petiole.

Several reports indicate that Vellaikolamban was a dwarfing rootstock based on different characters (Singh and Singh, 1976; Mukherjee and Das, 1980; Srivastava *et al.*, 1980; Kurian *et al.*, 1996). Gawankar *et al.* (2010) reported that Vellaikolamban could impart dwarfness to commercial cultivars like Ratna, Alphonso and Kesar. Vellaikolamban and heterozygous seedlings were used as rootstocks. The percentage reduction in plant volume was 39.1, 24.9 and 26.5 in the case of Alphonso, Ratna and Kesar respectively when Vellaikolamban was used as the rootstock.

Apart from the above, dwarfing rootstocks in mango include Mylepelian (Singh and Singh, 1976; Singh and Singh, 2004), Rumani (Babu *et al.*, 1985; Singh *et al.*, 1986), Creeping (Iyer and Subramanyam, 1986), Kurukkan (Murti and Upreti, 2003) and Chandrakaran (Murti and Upreti, 2003).

Singh and Singh (1976) and Singh and Singh (2004) reported Mylepelian as dwarfing rootstock based on less plant height. High phenol content, a physiological parameter found in dwarf cultivars like Rumani was reported by Babu *et al.*, (1985). Murti and Upreti (2003) also reported cultivars like

Kurukkan and Vellaikolamban as dwarfing rootstocks based on high phenol content.

2.2. MARKERS

First evidence for the linkage between a qualitative character and a quantitative trait was given by Sax (1923). He found the relation between the colour and weight of the seeds in French bean (*Phaseolus vulgaris* L.). The weight of seed is less in white coloured type compared to the pigmented one (both eyed and mottled). Later using QTL (Quantitative Trait Loci) mapping, the result was rediscovered as the genes responsible for seed coat colour (*P*) and weight of seed (*Phs*) are linked at a distance of 10 cM (Gepts *et al.*, 1993).

2.2.1. Morphological characters

2.2.1.1. Plant height

An investigation was carried out by Majumdar *et al.* (1972) using 31 rootstocks including Dashehari, Langra, Chausa, Totapuri Red Small, Neelam, Bombay Green and open pollinated seedlings of these varieties. The results showed that the vigorous varieties Chausa, Goa and Kurukkan showed high rate of stem growth while Totapuri Red Small and Bombay Green showed low rate of stem growth.

Kurian and Iyer (1997) conducted an experiment using nine year old trees of twenty four cultivars. The cultivars were grouped into least vigorous (canopy volume <14 m^3), medium vigorous (14-25 m^3) and most vigorous (>25 m^3). From the analysis of growth parameters the length of new flesh showed high correlation with vigour.

According to Murti and Upreti (2003), the plant height of Muvandan and Bappakai were higher than Vellaikolamban, Chandrakaran and Kurukkan.

2.2.1.2. Number of leaves

At IIHR, Bangalore, Murti and Upreti (2003) conducted an experiment for screening of mango in the nursery stage. The cultivars employed were Bappakai, Muvandan, Olour, Mylepelian, Chandrakaran, Kurukkan and Vellaikolamban. From the data recorded during 30th, 60th and 120th day after planting, the leaf production was more in the cultivars Muvandan, Olour, Bappakai and Mylepelian while it was less in Vellaikolamban, Chandrakaran and Kurukkan. The data analysis showed a positive correlation between number of leaves and plant height.

2.2.1.3. Leaf – length, width and area

The studies for the identification of morphological parameters related to the vigour of mango conducted by Kurian and Iyer (1997) revealed that the leaf area was a good indicator for dwarfness. Dwarf trees recorded less leaf area than tall trees.

A pot study was conducted by Murti and Upreti (2003) in IIHR using the cultivars – Vellaikolamban, Muvandan, Chandrakaran, Olour, Bappakai, Kurukkan, Mylepelian and Alphonso. The observations were recorded during 30th, 60th and 120th days from transplanting. The results showed that, there was a positive correlation between the leaf area and plant height on the 60th day. Kurukkan and Vellaikolamban recorded the lowest leaf area and Muvandan recorded the highest.

As cited by Iyer and Kurian (1992), the work conducted by Agarwal (1986) showed a positive correlation with leaf area and vigour in trifoliate orange.

2.2.1.4. Internodal length

Short internodal length was positively correlated with dwarfness. The mango cv. Creeping showed the shortest internodal length of 2cm (Iyer and Subramanyam, 1986).

Wang and Faust (1987) analysed the relationship between internodal length and plant height in apple, using ten year old hybrid seedlings of Goldspur Delicious X Redspur Delicious. Internodal length had a positive correlation with the plant height.

In papaya, plants with short internodal length had dwarf stature (Lim and Hawa, 2007).

2.2.1.5. Root number and length

In the study conducted in mango by Srivastav *et al.* (2009) using sixteen polyembryonic genotypes including Bappakai, Chandrakaran, Carabao, Cecil, Combodiana, Kerala-1, Kerla-3, Kerala-5, Kurukan, Muvandan, Mylepelian, Olour, Peach, Sabre, Turpentine and Vellaikolamban, root length showed positive correlation with the plant height.

Shaban (2010) screened four polyembryonic cultivars of mango *viz.* Zebda, Sukkary, Sabre and "13-1". There was a positively correlation between root length and plant height.

Mukherjee and Das (1976) reported that the number of secondary roots could be used as an indicator of dwarfness.

2.2.1.6. Dry atter content of root and shoot and total dry matter production

Pal *et al.* (1981) cited by Singh *et al.* (1986) stated that dry matter content could be used for the classification of mango rootstocks, as it had a positive correlation with vigour.

2.2.2. Anatomical characters

2.2.2.1. Stomatal density

Srivastava *et al.* (1980) categorized 24 rootstocks of mango based on stomatal count. Dwarf group comprised of Bappakai, Kurukkan, Goa, Kalapady and Taimuria and Dashehari were included in the vigorous group.

Abirami *et al.* (2011) found that stomatal density had no much correlation with the height in mango. After analyzing ten monoembryonic varieties and twelve polyembryonic varieties for their stomatal distribution, the results revealed that there was no much difference between the dwarf hybrid Amrapali and the vigorous cultivars - Langra and Chausa.

In 2001 Marie and Parameswaran conducted an experiment on dwarfing potential of indigenous mango varieties under Kerala Agricultural University. In the study, the lowest number of stomata was recorded in Muvandan and highest in Vellaikolamban.

Studies on stomatal density in different species of guava revealed that *Psidium chinensis*, a dwarf type recorded less stomatal density (Saroj *et al.*, 1997).

2.2.2.2. Phloem – xylem ratio

An experiment conducted by Iyer and Kurian (1992) for the screening of eight mango cultivars of mango based on its vigour showed that the least vigorous cultivars had phloem to xylem ratio of more than 1, moderately vigorous rootstocks between 0.6 – 1 and the most vigorous had a ratio below 0.6.

From the experiment conducted in apple using ten year old hybrid seedlings of Goldspur Delicious X Redspur Delicious by Wang and Faust (1987), dwarfness was found to be related to the higher phloem to xylem ratio.

Saeed *et al.* (2010) evaluated Troyer citrange (*Citrus sinensis* X *Poncirus trifoliata*), rough lemon (*C. jambhiri*), swingle citrumelo (*C. paradisi* X *P. trifoliata*), sweet lime (*C. limettioides*), carrizo citrange (*C. sinensis* X *Poncirus trifoliata*), sour orange (*C. aurantium*) and flying dragon (*P. trifoliata*) and they found that the high phloem to xylem ratio of root and stem was related to dwarfness.

However, after analysing the anatomical characters of eleven olive cultivars, El Said *et al.* (2013) concluded that there was a negative correlation between phloem to xylem ratio and height.

2.2.2.3. Bark percentage

Majumdar *et al.* (1972) classified Chausa, Goa, Kurukkan and wild mango as vigorous rootstocks based on less bark percentage. While Totapuri Red Small and Olour which recorded high bark percentage were grouped as dwarf rootstocks.

Mukherjee and Das (1980) reported that high bark percentage of mango root was associated with dwarfness.

Bark percentage of stem, root and petiole of ten polyembryonic and 14 monoembryonic varieties were analysed by Singh *et al.* (1986). Lesser bark percentage was observed in the North Indian varieties whereas it was higher in South Indian varieties like Taimuria, Rumani, Olour, Nakkare, Kurukkan, Vellaikolamban, Chandrakaran and Mahmooda-Vikarabad. The bark percentage showed negative correlation with height.

An investigation was conducted by Abirami *et al.* (2011) for identifying the physiological characters related with tree vigour in mango. The study was performed using the twelve polyembryonic genotypes comprising of Bappakai, Chandrakaran, Olour, Vellaikolamban, Kurukkan, Nakkare, Starch, Peach Kensington, Kerala 1, Kerala 2 and Kerala 5, and ten monoembryonic varieties

including Amrapali, Bombay Green, Chausa, Dashehari, Langra, Mallika, Pusa Arunima, Pusa Surya, Sensation and Tommy Atkins. Starch, a dwarf cultivar recorded the highest bark percentage (55.5%) and least was found in Nakkare (22.4%), a tall cultivar. This showed that there was a positive correlation between bark percentage and less plant height.

Beakbane and Thompson (1939) cited by Majumdar *et al.* (1972) stated that the bark to wood ratio in apple was negatively correlated with plant height.

Saroj *et al.* (1997) reported that in guava, higher bark/ wood ratio was observed in dwarf species but it was not suitable for predicting the vigour. They also cited the work by Sharma (1982), which stated that the bark/ wood ratio was positively correlated with dwarfness.

2.2.3. Physiological characters

2.2.3.1. Phenol content

Babu *et al.* (1985) classified mango rootstocks as dwarf based on high phenol content. Rumani contained highest phenol content and Chinnarasam the lowest. This study revealed that there was a negative correlation between the phenol content and tree height.

Kurian *et al.* (1994) assessed the total phenol content of dormant apical buds of twenty four mango varieties and chemically dwarfed Alphonso trees wherein, the dwarfness was induced by drenching paclobutrazol in soil. From the analysis, they found that the height was inversely proportional to the phenol content of the dormant bud.

The studies on endogenous hormones and phenols in rootstock seedlings of mango cultivars and their relationship with seedling vigour by Murti and Upreti (2003) disclosed that the total phenol content of the leaf was negatively correlated

with the vigour. Vellaikolamban recorded 60.87mg/g, while Muvandan recorded 20.69 mg/ g at 30 days after planting.

The total phenol content of dormant apical buds were estimated from the cultivars Bappakai, Chandrakaran, Olour, Vellaikolamban, Kurukkan, Nakkare, Starch, Peach Kensington, Kerala 1, Kerala 2 and Kerala 5, Amrapali, Bombay Green, Chausa, Dashehari, Langra, Mallika, Pusa Arunima, Pusa Surya, Sensation and Tommy Atkins. The experiment proved that the phenol content was negatively correlated with vigour. The phenol content varied from 74.5 mg/ g in the cv. Starch to 18.6mg/ g in the cv. Bombay Green (Abirami *et al.*, 2011).

The study conducted under Kerala Agricultural University by Marie and Parameswaran (2001) revealed that the total phenol content of both leaves and buds were highest in Vellaikolamban and Kalapady while the lowest was recorded in Muvandan and Chandrakaran.

2.2.3.2. Leaf water potential / Relative Water Content (RWC)

Leaf water potential showed negative correlation with plant height. Murti and Upreti (2003) conducted an experiment using the cultivars Vellaikolamban, Muvandan, Chandrakaran, Olour, Bappakai, Kurukkan, Mylepelian and Alphonso as the treatments. The water potential was less in the dwarf cultivars, *viz.* Vellaikolamban (1.24), Kurukkan (1.32) and Chandrakaran (1.44) at 120 days of transplanting.

The results of the experiment conducted by Abirami *et al.* (2011) in IARI, New Delhi, showed that the maximum relative water content was for Bappakai and least for Amrapali.

2.2.4. Molecular studies

2.2.4.1. RAPD marker

Identification of markers associated with dwarfness had not been reported so far in mango. In apple, RAPD marker for dwarfness was identified by Ying *et al.* (2002). They identified a primer (OPE152-1001) with specific bands for dwarf trees. They also found that the distance between the primer binding position and the gene (*Dw*) as 0.69 cM.

Venkatachalam *et al.* (2004) screened F_1 hybrids of rubber trees from the cross between a natural dwarf variant and normal tree using RAPD primers. A specific band was obtained during the amplification of genomic DNA by primer OPB-12, the band was present only in the dwarf parent and the dwarf progenies whereas it was absent in the tall parent.

To identify the marker related to the dwarfing gene (*pcDw*) in pear, Yike *et al.* (2008) conducted an experiment using the F_1 population obtained from the cultivars 'Ahuli' (*Pyrus communis*) and 'Chilli' (*P. bretchneideri)*. From the bulk segregant analysis using SSR marker, KA14210 was identified as marker, which is placed at a distance of 9.3 cM from the *pcDw*.

Fifty mango varieties were screened for identifying RAPD marker related to the polyembryony in mango by bulk segregant analysis. The primer OPM12 amplified a specific band for polyembryonic cultivar with a size of 550 bp (Lopez *et al.*, 1997).

Damodaran *et al.* (2007) carried out an experiment for the identification of molecular markers linked with differential flowering behaviour of mangoes in Andaman and Nicobar Islands using RAPD markers. The primers OPX9, OPX10, OPF4 and OPC2 produced specific bands with a size ranging 200 – 300 bp in multiple flowering clones. The bands were absent in the single flowering clones.

Radha *et al.* (2015) conducted an experiment under Kerala Agricultural University on molecular characterization of popular mango cultivars of Kerala using RAPD markers. The cultivars used were Priyur, Neelum, Bangalora, Kalapady, Banganapally, Muvandan, Chandrakaran, Tholikaipan, Vellaikolamban, Olour, Puliyan and Sindhurum. Cluster analysis was done for the grouping of cultivars based on their genotype. The cultivars Kalapady, Sindhurum, Vellaikolamban, Olour, Priyur, Bangalora and Neelum form a single group.

Simi *et al.* (2013) characterized thirty traditional cultivars of Kerala using RAPD marker.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study on "Marker assisted characterization of mango (*Mangifera indica* L.)" was conducted at the College of Agriculture, Padannakkad during 2013 – 2015. The experimental details are furnished below:

3.1. EXPERIMENTAL SITE

The studies were conducted at College of Agriculture, Padannakkad situated at 12^0 20' 30'' N latitude, 75^0 04' 15'' E longitude and an altitude of 20 m above mean sea level.

3.2. SEASON

The experiment was conducted during May 2014 to July 2015.

3.3. MATERIALS

3.3.1. Crop and varieties

Ten polyembryonic varieties were selected based on the previous reports as vigorous or less vigorous rootstocks in mango. The varieties included in the study are given below (Table 1). Twenty five seeds of each variety, extracted from ripe fruits were sown in pots and the germination percentage and number of plants obtained in each variety were described in Table 1 (Plate 2).

The varieties V_7 and V_8 were excluded from the further studies, because of poor germination and due to the lack of sufficient number of plants required for recording observations.

Table 1. Varieties

Sl. No.	Treatments	Name of variety	Germination percentage (%)	Number of seedlings/ variety
1	V_1	Bappakkai	32.00	8
2	V_2	Chandrakaran	24.00	6
3	V_3	Creeping	28.00	7
4	V_4	Kalapady	36.00	9
5	V_5	Kurukkan	36.00	9
6	V_6	Muvandan	48.00	12
7	V_7	Mylepelian	12.00	3
8	V_8	Olour	16.00	4
9	V_9	Rumani	40.00	10
10	V_{10}	Vellaikolamban	24.00	6

3.3.2. Source of seed material

The seed materials were collected from District Agricultural Farm, Taliparamba, Kannur and from College of Horticulture, Vellanikkara, Thrissur.

3.3.2. Container

Earthen pots with a size of 12 inches were used for raising the seedlings.

3.3.3. Preparation of growing media

The growing media was prepared by mixing sand, soil and vermicompost in the ratio of 1:1:0.5.

3.3.4. Laboratory chemicals and glassware

The chemicals used for the study were purchased from Merck India Ltd. and SRL laboratories. The *Taq* DNA polymerase along with buffer and molecular markers (lambda DNA *Eco RI/ Hind III* double digest, 100 bp ladder, 50 bp

Plate 1. Experimental plot

Plate 2. Varieties

Plate 2. Varieties

ladder) used were from Merck Genei, Banglore. The random primers were selected based on the earlier reports and the oligos were synthesized by Integrated DNA Technologies, Inc., New Delhi.

3.3.5. Equipments and machinery

The available equipments in the departments of College of Agriculture, Padannakkad *viz.* Plant Biotechnology, Plant Physiology and Plant Pathology and CPCRI, Kasaragod were used for the study. For counting stomata, microscope Magnus MLX-DX was used. For reading the absorption, spectrophotometer (Systronics uv-vis spectrophotometer 117) was used.

For histological procedure, the sections were taken using the microtome (Leica RM 2145) and the slides were observed using the light as well as stereo microscope (Leitz Diaplan and Leica respectively). The documentation and observations were done using the software LeicaQWin.

The DNA was quantified using Biophotometer (Eppendorf, Germany) and polymerase chain reaction was done in Mastercycler (Eppendorf, Germany). BioRad imaging system was used for the documentation of the agarose gel.

3.4. METHODS

3.4.1. Design and layout

The experiment was laid out in completely randomized design using eight varieties and three replications (Plate 1). Three plants showing uniform growth characters in each genotype were used for recording observations.

3.5. OBSERVATIONS RECORDED

3.5.1. Morphological characters

Different morphological parameters of the plants were recorded from the first month to six months after germination and at the age of twelve months.

3.5.1.1. Plant height

The height was taken from the base to the tip and expressed in centimeter.

3.5.1.2. Number of leaves

Number of leaves of each plant was counted at the specified intervals.

3.5.1.3. Leaf length, width and area

Leaf length and width of all leaves were recorded for calculating the leaf area constant. The average leaf length and width was calculated and expressed in centimeter. Total leaf area and average leaf area were calculated and expressed in centimeter square. The leaf area was calculated using the leaf area constant derived using leaf area meter and the leaf length and width.

3.5.1.4. Internodal length

The second internodal region from the base was measured in centimeter at the specified intervals.

3.5.1.5. Root number and length

The root number and root length were recorded from the one year old seedlings. The length of longest root was measured in centimeter and the number of primary roots was counted.

3.5.1.6. Dry matter content of root and shoot and total dry matter production

Dry matter content of root and shoot without leaves were found out from one year old plants and expressed in grams. The total dry matter production was worked out by adding the dry weight of leaves along with that of root and stem.

3.5.2. Anatomical characters

3.5.2.1. Stomatal density

Stomatal density of the dorsal side of the leaf was examined using clear nail varnish method (Bajracharya, 1999). The stomatal print was taken from three leaves of same age from each plant and two prints per leaf with three replications per cultivar. From each stomatal print, stomata from three microscopic fields (40X magnification) were counted and average was calculated.

3.5.2.2. Phloem – xylem ratio

For calculating phloem – xylem ratio, the immature shoot of 2 cm length was taken from 3 plants of each variety. Processing of tissue and preparation of the slides were detailed below. The prepared slides were observed under the microscope and the measurements were taken by using the LeicaQWin software.

3.5.2.2.1. Fixation

Immediately after the collection of tissue, it was transferred to the vials containing fixative. The fixative used was Carnoy's B fluid containing chloroform, ethanol and glacial acetic acid in the ratio of 3:6:2 respectively. The tissue was kept in fixative for 48 hours by changing the fixative after 24 hours.

3.5.2.2.2. Dehydration

The fixed tissue was subjected to dehydration using ethanol and butanol as follows.

Table 2. Ethanol and butanol grades for dehydration of tissue

Sl. No.	Ethanol (ml)	Butanol (ml)	Water (ml)	Exposure time
1	70	-	30	24hrs
2	80	-	20	24hrs
3	90	-	10	24hrs
4	100	-	-	24hrs
6	30	10	-	24hrs
7	10	10	-	24hrs
8	10	30	-	24hrs
9	-	100	-	24hrs
10	-	100	-	24hrs

3.5.2.2.3. Infiltration and embedding

A mixture of paraffin wax and bee wax (40:1) of melting point 60^0C was used for infiltration and embedding. Small chips of paraffin wax were added to the vials containing dehydrated tissue and small amounts of fresh butanol. Then the vials with wax and tissue were kept in oven maintained at 60°C, to allow the wax to infiltrate into the tissue. The used molten paraffin wax was replaced with fresh molten wax after 24 hours. This process was continued for twelve days to remove even the last traces of butanol with paraffin. The material was subsequently embedded in paraffin wax.

3.5.2.2.4. Paraffin block preparation

The tissue along with the molten paraffin wax was poured into the block mould. Then the block mould was kept in cold water for cooling. After proper cooling, water was passed over the block mould and the block was removed from the mould.

3.5.2.2.5. Microtome sectioning

The paraffin block was trimmed into a block and fixed on a wooden block. The uniform thin sections of 10µm thickness were cut using Leica RM 2145 rotary microtome.

3.5.2.2.6. Affixing sections on slide

Three percent gelatin containing little amount of potassium dichromate was added on the clean micro slide. The section ribbon of appropriate size was kept on the micro slide and kept on the warmer plate maintained at a temperature of 45^0 C. After the proper stretching of the sections, the slides were kept for drying under dust free condition at room temperature for 48 hours. From each tissue, six slides were prepared.

3.5.2.2.7. Deparaffinization and dehydration of the sections

Deparaffinization and dehydration process involves a series of procedure as described in Table 3. After each treatment, the slides were dried by keeping them in a slanting position.

Table 3. Deparaffinization and dehydration process

Sl. No.	Chemical	Exposure time	Purpose
1	Xylene I	2 minutes	Deparaffinization
2	Xylene II	2 minutes	Deparaffinization
3	Dry	-	-
4	Butanol I	2 minutes	Dehydration
5	Butanol II	2 minutes	Dehydration
6	Dry	-	-

3.5.2.2.8. Staining of sections

Periodic acid, Schiff's reagent (magenta) and toluidine blue (blue) were used for staining. From the prepared slides, three were stained with periodic acid, Schiff's reagent and remaining with toluidine blue. The procedure for staining is given in Table 4.

3.5.2.2.8.1. Preparation of periodic acid Schiff's reagent

Periodic acid (H_5IO_6) was prepared by dissolving 1g periodic acid in 100 ml of distilled water and stored in an amber coloured bottle.

Schiff's reagent was prepared by dissolving 1g of basic fuchsine powder in 90 ml of distilled boiling water. After cooling, 15 ml of 1N HCl was added by shaking the mixture at frequent intervals. Then 1.8 g of potassium metabisulphate was added to the mixture. The solution was transferred to an airtight container and kept in shaker for overnight in dark. Later, one gram activated charcoal was added and the mixture was shaken and filtered immediately. This step was repeated for five times. The pink coloured filtrate was stored in an amber coloured bottle.

3.5.2.2.8.2. Preparation of toluidine blue

For preparing toluidine blue, 1g of Toluidine blue powder was dissolved in 100 ml of distilled water.

Table 4. Procedure for staining of slides

Sl. No.	Chemical		Exposure time
	Toluidine blue	Periodic acid – Schiff's reagent	
1	Distilled water	Distilled water	-
2	-	Periodic acid	30 sec
3	-	Dry	-
4	Toluidine blue	Schiff's reagent	1 min
5	Distilled water	Distilled water	-
6	Dry	Dry	-
7	Butanol I	Butanol I	1 min
8	Butanol II	Butanol II	1 min
9	Dry	Dry	-
10	Xylene I	Xylene I	1 min
11	Xylene II	Xylene II	1 min
12	Dry	Dry	-

3.5.2.3. Bark percentage

The bark percentage was ascertained by taking stem sections as mentioned in the section 3.5.2.2.

3.5.3. Physiological characters

3.5.3.1. Phenol content

Phenol was determined using Folin Ciocalteau reagent. The 4th mature leaf from the top of three plants of each variety was collected for determining the total phenol content. 1g of the leaf was ground with ten times volume of 80 per cent ethanol. The supernatant was obtained by centrifuging the sample at 10,000 rpm for 20 minutes. The residue was re-extracted with five time volume of 80 per cent ethanol. Supernatant was collected and pooled with the first supernatant. Then

the supernatant was evaporated at 50^0 C. The remaining residue was dissolved in 50ml of water for dilution. From the dilution, 0.5ml was pipetted out and made up the volume to 3ml. To this, 0.5ml Folin Ciocalteau reagent was added and 2ml of 20 per cent of Na_2Co_3 added after three minutes. The well mixed tubes were kept in boiling water for one minute. After cooling the absorbance was measured at 650nm. Similarly a blank was also prepared. The standard curve was drawn from the standard catechol. The phenol content of the sample was calculated from the standard curve.

3.5.3.2. Leaf water potential / Relative Water Content (RWC)

Fifth leaf from the top of each plant was taken. After recording its weight, it was kept in distilled water for five hours and recorded the weight for getting the turgid weight. Then it was dried at 60^0 C and the dry weight was recorded. Using the following formula, the relative water content was worked out:-

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

3.5.4. Molecular studies

3.5.4.1. Isolation of total genomic DNA

3.5.4.1.1. Stage of leaf for genomic DNA extraction

Tender and brown, brownish green as well as green immature leaves combined with different protocols were used for the standardization of the DNA extraction.

3.5.4.1.2. Protocol for genomic DNA extraction:

Three standard protocols were tried along with few modifications as detailed below.

Protocol 1: CTAB method (Rogers and Bendich, 1994)

One gram leaf sample (devoid of midribs) was ground in the sterilized mortar and pestle along with 2 – 5µl β-mercaptoethanol and a pinch of sodium metabisulphate in the presence of liquid nitrogen. To the ground powder, 4 ml pre-heated (60 – 65^{0}C) extraction buffer (2% CTAB, 100mm Tris buffer, 20mm EDTA and 1.4M NaCl) was added. The ground material was transferred to the 2ml eppendorf tube. The tubes were incubated in water bath for 30 minutes at 65^{0}C by giving intermittent inversion. After 30 minutes 1/3rd volume of chloroform: isoamyl alcohol (24:1) was added and inverted for several times. Then the tubes were centrifuged for 10 minutes at 10,000rpm. The aqueous phase was transferred to a sterilized fresh tube and added 1/6th volume of chilled isopropanol. The tube was inverted several times for allowing the DNA to precipitate and pellet was obtained by centrifuging at 10,000rpm for 10 minutes. Then decanted the supernatant and the pellet was washed for 2 – 3 times using 70 per cent alcohol. Pellet was dried under room temperature and dissolved using sterile water.

Modified procedure

Instead of 2-5µl β-mercaptoethanol, 20µl was added. Additional to the other chemicals 10µl of 20 per cent PVP was added to the extraction buffer. For 1g sample, 7ml extraction buffer was added. All other procedures were same as the standard CTAB method.

Protocol 2: Porebski *et al.* (1997)

The leaf sample (devoid of midribs) of 0.5g was wrapped in aluminium foil and allowed to freeze in the liquid nitrogen. Then the leaf was transferred to the sterile chilled mortar and pestle and to this 25µl of β-mercaptoethanol and 10µl PVP was added. The leaf sample was ground to a fine powder in the presence of liquid nitrogen and 7 ml of warm CTAB buffer (10% CTAB, 1M Tris buffer, 0.5M EDTA and 4M NaCl) was added. Then the sample was transferred to the

sterile oak ridge tube and mixed thoroughly by inversion. Then the tube was incubated at 65^0C for 30 minutes by occasional inversion. After incubation, chloroform: isoamyl alcohol (24:1) was added in the ratio of 1:1 (v/v). Tube was inverted for several times for proper extraction of chlorophyll and protein from the leaf. Centrifugation was done at 12,000rpm for 15 minutes. The supernatant was transferred to a fresh tube. To the supernatant 0.5 volume of chilled 5M NaCl and 1.5 volume of 95 per cent alcohol was added. For precipitating the DNA, the tube was inverted for several times and kept in freezer for 10 minutes for complete precipitation. For pellet formation the precipitate was centrifuged at 10,000rpm for 5 minutes. Then the supernatant was decanted and the pellet was washed using 70 per cent alcohol. The air dried pellet was dissolved in the sterile water.

Modified procedure

50µl PVP (20%) and 30µl β-mercaptoethanol were added. The remaining procedures were same as mentioned under 3.5.4.1.1.2.

Protocol 3: DNA kit

The DNA extraction kit used was 'Origin' from Origin Diagnostics and Research, Karunagapalli, Kerala.

Materials supplied by the kit manufacturer:

Buffer GP1, Buffer GP2, Buffer GD, Buffer TE, Spin columns CB3, Collection tube (2ml) and Chloroform. The buffer GD and PW was prepared by adding 96 – 100% alcohol as mentioned in the bottle and mixed thoroughly. β-mercaptoethanol was added to the buffer GP1 in a volume that obtain a final concentration of 0.1% β-mercaptoethanol.

Steps involved in the procedure:

1. 300mg plant sample was ground thoroughly using liquid nitrogen

2. 2 ml of pre heated GP1 was added to the ground tissue and vortex for 10 – 20 seconds for proper mixing and to disperse the clumps.

3. The tube was incubated at 65^{0}C for 20 minutes and inverted the tubes for proper mixing.

4. 2.1 ml chloroform was added and mixed by inverting for several times. Then centrifuged at 12,000rpm for 5 minutes.

5. The supernatant was transferred to a fresh tube and 2.1 ml GP2 was added and mixed thoroughly.

6. Pipetted out the whole mixture (700µl at a time) in the step 5 into the spin column CB3 kept in the collection tube and centrifuged at 12,000 rpm for 30 seconds. The filtrate was discarded and 500µl buffer GD was added and centrifuged for 12,000 rpm for 30 seconds. Then the filtrate was discarded.

7. Then 700 µl buffer PW was added to the spin column CB3 and centrifuged at 12,000 rpm for 30 seconds to wash the membrane. The filtrate was discarded and replaced the collection tube. Repeated the washing by adding 500 µl buffer PW to the spin column. After discarding the filtrate the spin column was centrifuged at 12,000 rpm for 2 minutes to remove the residue of buffer PW. Then the collection tube was discarded.

8. The spin column was kept in a sterile 1.5 or 2 ml micro centrifuge tube and incubated at room temperature (15 – 20^{0}C) or at 50^{0}C until drying of the membrane.

9. 50 – 200 µl TE buffer was added to the spin column to elute the DNA and centrifuged at 12,000 rpm for 2 minutes after incubating for 2 – 5 minutes.

3.5.4.2. DNA quantification and quality check:

Agarose gel electrophoresis and spectrophotometer method were used. 0.8 per cent agarose gel (for genomic DNA) and 1.2% agarose gel (for PCR product) were prepared using 1X TBE buffer (89mM Tris, 89mM boric acid 2mMEDTA) with a pH of 8. Gel tray, comb and buffer tank of Genie, Bangalore were used.

For preparing the gel, the tray as well as comb was wiped with absolute alcohol and dried properly. The sides of the tray were sealed with tape and the comb was placed by leaving a space between the tray and comb. Then it was heated until the agarose was fully dissolved. Then it was allowed to cool to 42 – 45^0C. After cooling 10µl ethidium bromide was added and poured into the gel casting tray carefully to avoid the bubbles. The gel was kept at room temperature for 30 – 45 minutes for solidifying. After removing the comb, kept the gel in buffer tank containing 1X TBE, in way that the well side was facing towards the cathode side. The DNA was loaded along with the gel loading buffer 6X (Genei).

The gel was visualized and documented using BIO-RAD gel documentation system and software.

Determination of quantity of DNA

The quantification of DNA was done using biophotometer. The equipment works based on the principle of spectrophotometer. The concentration of nucleic acid measures based on the Beer – Lambert law, the peak absorbance of nucleic acid recorded at 260nm while the protein at 280nm. The ratio between OD_{260} and OD_{280} will be 1.8 – 2 for the pure double stranded DNA. If the value was below 1.8 the sample was contaminated with protein and if it was above, contaminated with RNA. The quantity of double stranded DNA was calculated using the following equation.

1 OD at 260nm = 50µg double stranded DNA/ ml of sample

Therefore OD_{260} X 50 = total quantity of double stranded DNA (µg/ ml)

3.5.4.3. RAPD marker

Thirty RAPD primers were used for screening the mango DNA (Table 5) and10 primers giving best results in terms of number of bands and intensity were selected for the study.

Table 5. RAPD primers used for screening

Sl. No.	Primer	Sequence (5' – 3')
1	OPA 05	AGGGGTCTTG
2	OPA 08	GTGACGTAGG
3	OPA 10*	TGCTCGGGTG
4	OPA 11*	CAATCGCCGT
5	OPA 15	TTCCGAACCC
6	OPA 16	AGCCAGCGAA
7	OPA 17*	GACCGCTTGT
8	OPA 18	AGGTGACCGT
9	OPA 20	GTTGCGATCC
10	OPB 01	GTTTCGCTCC
11	OPB 05	TGCGCCCTTC
12	OPB 10	CTGCTGGGAC
13	OPB 12	CCTTGACGCA
14	OPC 05	GATGACCGCC
15	OPD 07	TTGGCACGGG
16	OPH 20	GGGAGACATC
17	OPM 12	GGGACGTTGG
18	OPM 15	GACCTACCAC
19	OPM 20*	AGGTCTTGGG
20	OPX 01*	CTGGGCACGA
21	OPX 02	TTCCGCCACC
22	OPX 08*	CAGGGGTGGA
23	OPX 15	CAGACAAGCC
24	OPX 17*	GACACGGACC
25	OPX 20	CCCAGCTAGA
26	RPI 01*	AAAGCTGCGG
27	RPI 02	AACGCGTCGG
28	RPI 03*	AAGCGACCTG
29	RPI 04*	AATCGCGCTG
30	RPI 05	AATCGGGCTG

* selected for further studies

3.5.4.3.1. RAPD reaction

RAPD reaction was carried out using thermo cycler (Eppendorf, Germany). Each 20µl of reaction mixture contains 2µl of 1X PCR buffer with $MgCl_2$, 1.6µl dNTPs (contains 200 µl of each dNTP), 0.3µl *Taq* DNA polymerase (0.9 U), 1µl primer (0.5µM), 20ng template DNA and 13.1µl distilled water. The thermal profile comprises of initial denaturation at 94^0C for 5 minutes followed by 39 cycles of denaturation at 94^0C for 1 minute, primer annealing at 37^0C for 1 minute and primer extension at 72^0C for 2 minutes. The profile completes with a final extension at 72^0C for 10 minutes. Then the samples were brought down to a temperature of 4^0C.

3.5.5. Statistical analysis

The analysis of variance was done using the software SAS. The correlation analysis was done using Microsoft excel. The Student's-t test was carried out using the online programme for statistical analysis, WASP.

Classification of mango varieties as dwarf, semi tall and tall was done based on the morpho – anatomical and physiological characters. From the 57 observations of 16 characters, 53 observations of 15 characters were used for the classification. The relative water content and the internodal length from the first to fourth month were excluded as the former one was non significant and the later one gives a negative value for the range. The upper and lower limits were calculated using standard deviation in the consideration with the correlation.

Range = Mean ± Standard deviation

Plant height as an example,

Mean = 20.46cm

Standard deviation = 8.87

≥29 cm : Tall

≤12 cm : Dwarf

12 – 29 cm : Semi tall

In the case of negatively correlated characters the upper limit will be the dwarf and lower will be the tall.

Clustering of the genotypes based on molecular markers was done using the programme DaRwin (version: 5.0.158). The dissimilarity coefficient was calculated based on the presence or absence of bands using the Dice coefficient.

$$\text{Dice coefficient, } dij = \frac{b + c}{2a + (b + c)}$$

Where,

a = no. of bands present in both

b= no. of bands present in i but absent in j

c= no. of bands absent in i but present in j

RESULTS

4. RESULTS

An experiment was conducted to identify morphological, anatomical, physiological and molecular markers (RAPD) associated with dwarfness in mango varieties at the College of Agriculture, Padannakkad during 2013-2015. The data collected were analysed and results are presented in this chapter.

4.1. MORPHOLOGICAL CHARACTERS

The morphological characters of seedlings such as plant height, number of leaves, leaf – length, width and area, internodal length, root number and length, dry matter content of root and shoots and total dry matter production were recorded and the analyzed data are furnished below.

4.1.1. Plant height

The plant height was recorded up to 6 months at monthly interval and 1 year after germination (Table 6). In the first month, all the varieties showed significant difference in height except Chandrakaran, Creeping and Kurukkan. The variety Rumani and Kalapady recorded the maximum height while the variety Vellaikolamban recorded the minimum (4.29 cm).

In the second month, Kalapady and Rumani; Muvandan and Creeping, Kurukkan and Chandrakaran were on par. Vellaikolamban and Bappakai were significantly different form all the other treatments. Vellaikolamban recorded the lowest height (5.77 cm) and the highest values were shown by Kalapady and Rumani.

During the third month, Kalapady and Rumani recorded the maximum height and were on par with Bappakai. Creeping, Muvandan, Chandrakaran and Kurukkan were on par. Vellaikolamban recorded the least height.

Fourth month observation showed that varieties Kalapady, Rumani, Creeping and Bappakai were on par. Kurukkan, Chandrakaran and Muvandan were on par and had medium heights. Vellaikolamban recorded the lowest height and it was significantly different from others.

Data on fifth month revealed that the varieties Rumani, Kalapady, Bappakai and Creeping and Kurukkan, Muvandan and Chandrakaran were on par. Plants of Vellaikolamban recorded the least height of 11.67 cm.

Bappakai, Creeping, Kalapady and Rumani and Kurukkan, Chandrakaran and Muvandan were on par during the sixth month. Vellaikolamban was significantly different from all other 7 varieties and recorded the least height (13.9 cm).

After one year's growth, Rumani recorded the maximum height (76.6 cm) followed by Kalapady and Chandrakaran, they were on par with each other. The varieties Bappakai, Kurukkan, Creeping and Muvandan were on Par. Vellaikolamban was significantly different from others with a least height of 37.13cm.

Table 6. Plant height (cm) in eight mango varieties

Variety	First month (cm)	Second month (cm)	Third month (cm)	Fourth month (cm)	Fifth month (cm)	Sixth month (cm)	Twelve month (cm)
V_1 Bappakai	25.27^{c}	28.93^{b}	34.07^{a}	41.20^{b}	47.83^{a}	57.10^{a}	76.60^{d}
V_2 Chandrakaran	17.67^{e}	19.00^{d}	24.60^{bc}	29.77^{c}	33.50^{b}	39.67^{b}	93.30^{ef}
V_3 Creeping	16.57^{e}	22.53^{c}	28.73^{b}	43.00^{ab}	46.87^{a}	53.3^{a}	71.0^{de}
V_4 Kalapady	29.87^{b}	34.30^{a}	38.83^{a}	47.83^{a}	50.67^{a}	52.9^{a}	94.10^{b}
V_5 Kurukkan	15.90^{e}	19.23^{d}	23.17^{c}	30.23^{c}	34.90^{b}	42.1^{b}	72.83^{c}
V_6 Muvandan	22.20^{d}	23.17^{c}	26.80^{bc}	29.33^{c}	33.70^{b}	35.7^{b}	61.20^{d}
V_9 Rumani	31.90^{a}	33.70^{a}	34.73^{a}	45.07^{ab}	51.50^{a}	52.8^{a}	96.67^{a}
V_{10} Vellaikolamban	4.29^{f}	5.77^{e}	8.20^{d}	10.10^{d}	11.67^{c}	13.9^{c}	37.13^{f}
CD (0.05)	2.01	3.02	5.21	5.89	8.27	10.58	11.53

4.1.2. Number of leaves

Number of leaves was recorded during each month up to 6 months after germination and from the one year old seedlings (Table 7). During the first month, all the varieties were on par with values ranging from 2.67 (Vellaikolamban) to 16 (Kalapady).

In the second, third and fourth months, except Kalapady, all others were on par and it recorded the maximum number of leaves and minimum in Vellaikolamban.

Rumani and Kalapady and Bappakai, Creeping, Kurukkan, Muvandan, Chandrakaran and Vellaikolamban were on par during fifth month. Vellaikolamban had the least number (8) of leaves.

Six months after germination, Rumani and Kalapady accounted for maximum number of leaves and they were on par. The varieties, Bappakai, Creeping, Muvandan and Kurukkan and Chandrakaran and Vellaikolamban were on par.

After one year's growth except Rumani, Kalapady and Kurukkan, all other varieties were on par. Vellaikolamban recorded the lowest number of leaves.

Table 7. Number of leaves observed in eight mango varieties

Variety	First month	Second month	Third month	Fourth month	Fifth month	Sixth month	Twelve month
V_1 Bappakai	8.33^{bc}	14.33^{b}	18.00^{b}	30.33^{bc}	36.33^{b}	38.67^{b}	52.00^{d}
V_2 Chandrakaran	4.67^{cd}	4.33^{c}	10.33^{bc}	13.00^{ef}	15.00^{ef}	15.33^{d}	31.00^{ef}
V_3 Creeping	8.00^{bc}	13.00^{b}	18.33^{b}	26.00^{cd}	30.67^{bc}	34.00^{bc}	41.00^{de}
V_4 Kalapady	16.00^{a}	21.67^{a}	31.33^{a}	47.67^{a}	52.67^{a}	55.33^{a}	98.00^{b}
V_5 Kurukkan	8.00^{bc}	9.33^{bc}	13.33^{bc}	21.33^{de}	24.67^{cd}	27.33^{c}	80.00^{c}
V_6 Muvandan	6.00^{cd}	9.33^{bc}	11.67^{bc}	17.33^{de}	19.67^{de}	28.67^{bc}	46.33^{d}
V_9 Rumani	11.67^{ab}	14.00^{b}	17.67^{b}	35.33^{b}	55.33^{a}	65.67^{a}	113.67^{a}
V_{10} Vellaikolamban	2.67^{d}	3.67^{c}	6.00^{c}	7.00^{f}	8.00^{f}	9.33^{d}	17.00^{f}
CD (0.05)	4.59	6.59	11.40	8.78	8.14	10.76	14.62

4.1.3. Leaf length, width and area

The data on leaf length up to six months and thereafter during 12^{th} month were shown in Table 8. In the first month, Kalapady had significantly longer leaves than others. The varieties Rumani, Muvandan, Bappakai, Creeping and Chandrakaran were on par. Vellaikolamban recorded the lowest leaf length.

From the second month onwards, only Vellaikolamban was significantly different from others. Every month, Kalapady and Rumani had the longest leaves and Vellaikolamban had the shortest leaves.

During twelfth month, Creeping, Kalapady, Muvandan and Rumani and Bappakai, Chandrakaran and Kurukkan were on par. Vellaikolamban was significantly different from others with shortest leaves (13.58 cm).

Table 8. Leaf length (cm) observed in eight mango varieties

Variety	First month (cm)	Second month (cm)	Third month (cm)	Fourth month (cm)	Fifth month (cm)	Sixth month (cm)	Twelve month (cm)
V_1 Bappakai	14.17^{bc}	14.44^{bc}	14.64^{cd}	16.52^{c}	16.77^{d}	17.26^{c}	17.26^{e}
V_2 Chandrakaran	13.34^{c}	13.86^{bc}	15.07^{bcd}	15.94^{c}	16.69^{d}	17.65^{bc}	17.74^{de}
V_3 Creeping	13.72^{bc}	13.25^{c}	15.82^{abc}	17.58^{bc}	18.76^{bc}	19.58^{ab}	19.74^{c}
V_4 Kalapady	17.36^{a}	17.89^{a}	18.88^{a}	20.10^{a}	20.50^{a}	20.72^{a}	21.99^{a}
V_5 Kurukkan	11.03^{d}	11.81^{c}	12.56^{d}	15.73^{c}	17.30^{cd}	17.78^{bc}	18.50^{d}
V_6 Muvandan	14.35^{bc}	16.48^{ab}	17.11^{abc}	17.41^{bc}	18.16^{cd}	19.33^{ab}	21.03^{ab}
V_9 Rumani	15.33^{b}	17.83^{a}	18.20^{ab}	19.16^{ab}	19.87^{ab}	20.19^{a}	20.53^{bc}
V_{10} Vellaikolamban	3.92^{e}	5.45^{d}	7.78^{a}	9.28^{d}	10.99^{e}	12.32^{d}	13.58^{f}
CD (0.05)	1.69	3.09	3.18	2.10	1.69	2.69	1.45

Leaf width was on par with all other varieties except Vellaikolamban up to fifth month (Table 9). Bappakai recorded the highest width and Vellaikolamban was the lowest.

During the fifth and sixth month, Bappakai and Vellaikolamban were significantly different from others and recorded the highest and the lowest widths respectively.

From the twelfth month data, the varieties Bappakai, Creeping and Vellaikolamban were significantly different from all other varieties. Bappakai recorded the highest leaf width (6.56 cm) while Vellaikolamban recorded the lowest (3.7 cm).

Table 9. Leaf width (cm) in eight mango varieties

Variety	First month (cm)	Second month (cm)	Third month (cm)	Fourth month (cm)	Fifth month (cm)	Sixth month (cm)	Twelve month (cm)
V_1 Bappakai	5.01^{a}	5.16^{a}	5.25^{a}	5.51^{a}	6.10^{a}	6.12^{a}	6.56^{a}
V_2 Chandrakaran	3.61^{bc}	4.00^{bc}	4.26^{abc}	4.40^{b}	4.53^{b}	4.67^{b}	5.06^{cd}
V_3 Creeping	3.50^{bc}	3.69^{bc}	4.10^{bc}	4.30^{b}	4.50^{b}	4.71^{b}	5.53^{b}
V_4 Kalapady	4.26^{ab}	4.51^{ab}	4.54^{ab}	4.61^{ab}	4.61^{b}	4.69^{b}	4.81^{d}
V_5 Kurukkan	2.97^{c}	3.16^{c}	3.40^{c}	4.27^{b}	4.47^{b}	4.68^{b}	5.08^{c}
V_6 Muvandan	4.07^{b}	4.32^{ab}	4.46^{abc}	4.69^{ab}	4.87^{b}	4.92^{b}	5.11^{c}
V_9 Rumani	3.66^{bc}	3.86^{bc}	3.97^{bc}	4.31^{b}	4.41^{b}	4.64^{b}	5.11^{c}
V_{10} Vellaikolamban	1.26^{b}	1.82^{d}	2.27^{d}	2.80^{c}	3.41^{c}	3.69^{c}	3.70^{e}
CD (0.05)	0.78	0.92	1.07	1.03	0.96	0.93	0.37

The data presented in Table 10 on average leaf area revealed that during the first month, Bappakai and Kalapady; Rumani, Muvandan, Chandrakaran and Creeping were on par. Kurukkan and Vellaikolamban were significantly different and recorded the lowest average leaf area.

In the second month, all the varieties were on par except Vellaikolamban by recording the lowest average leaf area of 7.34 cm.

Kalapady, Bappakai, Rumani, Muvandan and Chandrakaran and Kurukkan and Creeping were on par during the third month. Vellaikolamban was significantly different from others.

All the varieties were on par during the fourth and fifth month except Creeping and Vellaikolamban. The highest leaf area was recorded by Bappakai while Vellaikolamban recorded the least.

During the sixth month, all the varieties were on par except Vellaikolamban (28.26 cm^2).

In the twelfth month, all the varieties were significantly different except Kurukkan and Muvandan. Lowest average leaf area was recorded by Vellaikolamban (32.77 cm^2).

Table 10. Average leaf area (cm^2) in different mango varieties observed at various intervals

Variety	First month (cm^2)	Second month (cm^2)	Third month (cm^2)	Fourth month (cm^2)	Fifth month (cm^2)	Sixth month (cm^2)	Twelve month (cm^2)
V_1 Bappakai	44.82^{a}	46.95^{ab}	48.53^{ab}	56.99^{a}	64.16^{a}	66.40^{a}	76.27^{a}
V_2 Chandrakaran	28.78^{cd}	33.11^{cd}	38.50^{b}	41.99^{c}	45.27^{d}	49.26^{bc}	52.84^{d}
V_3 Creeping	23.13^{d}	25.22^{de}	23.03^{c}	29.17^{d}	35.31^{e}	44.89^{c}	45.58^{e}
V_4 Kalapady	44.32^{a}	48.36^{a}	51.47^{a}	55.72^{ab}	57.43^{ab}	58.33^{ab}	65.94^{b}
V_5 Kurukkan	17.03^{e}	20.99^{e}	26.33^{c}	41.28^{c}	47.56^{cd}	51.18^{bc}	58.80^{c}
V_6 Muvandan	34.00^{bc}	39.06^{bc}	43.62^{ab}	47.70^{bc}	53.34^{bcd}	57.59^{ab}	59.42^{c}
V_9 Rumani	34.99^{b}	42.72^{ab}	44.72^{ab}	51.38^{ab}	54.64^{bc}	58.46^{ab}	65.87^{b}
V_{10} Vellaikolamban	3.34^{f}	7.34^{f}	12.16^{d}	16.53^{e}	23.33^{f}	28.26^{d}	32.77^{f}
CD (0.05)	5.85	7.91	10.10	8.86	9.16	10.72	3.31

From the statistical analysis done for the total leaf area (Table 11), in the first month, Kalapady, Rumani, Bappakai and Muvandan and Kurukkan, Creeping and Chandrakaran were on par. Vellaikolamban was significantly different from the all other varieties and recorded the lowest total leaf area of 6.83 cm^2.

In the second month, Rumani and Muvandan and Creeping and Kurukkan were on par. Kalapady and Creeping had significantly higher total leaf area. Total leaf area was significantly less in Chandrakaran and Vellaikolamban.

Observations on third month show that Bappakai and Kalapady and Creeping, Rumani, Kurukkan and Muvandan were on par. Chandrakaran and Vellaikolamban were significantly different and recorded the lowest leaf area.

In the fourth month, the total leaf area of the varieties was significantly different except Bappakai and Kalapady.

During the fifth month, Bappakai and Kalapady and Creeping and Kurukkan were on par. Rumani recorded significantly higher leaf area and Vellaikolamban recorded the lowest leaf area.

According to sixth month data, Kalapady and Bappakai and Kurukkan and Muvandan were on par. Rumani recorded higher leaf area of 2057.33 cm^2 and Vellaikolamban recorded the lowest leaf area of 114.9 cm^2.

Bappakai, Creeping and Muvandan and Kalapady and Rumani were on par during twelfth month. Vellaikolamban recorded the minimum total leaf area and Kalapady recorded the maximum.

Table 11. Total leaf area (cm^2) in eight mango varieties observed at various intervals

Variety	First month (cm^2)	Second month (cm^2)	Third month (cm^2)	Fourth month (cm^2)	Fifth month (cm^2)	Sixth month (cm^2)	Twelve month (cm^2)
V_1 Bappakai	212.99^{b}	370.19^{b}	538.11^{a}	1035.66^{a}	1210.71^{b}	1243.78^{b}	1469.51^{c}
V_2 Chandrakaran	81.23^{c}	95.19^{e}	138.52^{d}	260.87^{f}	352.11^{e}	482.16^{e}	824.81^{d}
V_3 Creeping	114.91^{c}	169.11^{d}	336.61^{b}	568.03^{d}	795.06^{c}	969.96^{c}	1378.73^{c}
V_4 Kalapady	291.67^{a}	430.26^{a}	479.44^{a}	992.62^{a}	1111.77^{b}	1245.72^{b}	3051.43^{a}
V_5 Kurukkan	123.55^{c}	163.20^{d}	248.46^{c}	647.90^{c}	700.39^{c}	750.09^{d}	2258.58^{b}
V_6 Muvandan	191.68^{b}	247.21^{c}	242.66^{c}	334.99^{e}	579.53^{d}	652.41^{d}	1395.20^{c}
V_9 Rumani	250.73^{ab}	260.21^{c}	292.66^{bc}	744.82^{b}	1358.81^{a}	2057.33^{a}	2947.44^{a}
V_{10} Vellaikolamban	6.83^{d}	16.19^{f}	40.51^{e}	68.85^{g}	75.29^{f}	114.90^{f}	230.55^{e}
CD (0.05)	60.46	54.21	78.30	57.50	112.21	159.44	377.05

4.1.4. Internodal length

During the first month, Muvandan and Kurukkan, Kalapady, Rumani and Creeping and Vellaikolamban and Chandrakaran were on par. Bappakai recorded the maximum internodal length and Vellaikolamban and Chandrakaran recorded the minimum.

Creeping, Muvandan, Kalapady and Kurukkan were on par during the second month. Bappakai and Chandrakaran had significantly higher internodal length than others. The varieties Rumani and Vellaikolamban recorded the least internodal length and they were significantly different

In the third month, Bappakai was significantly different from others with a higher internodal length of 6.9 cm.

The internodal length was on par in all the varieties during the fourth month. Vellaikolamban recorded the least and Kalapady was the highest.

From the data analyzed for sixth month's observation, Creeping, Bappakai and Rumani and Chandrakaran, Kurukkan and Muvandan were on par. Kalapady and Vellaikolamban were significantly different and recorded for the highest and the lowest internodal length respectively.

During twelfth month, the internodal length of all the varieties was significantly different except Bappakai, Chandrakaran and Kurukkan. Kalapady recorded the maximum internodal length of 10.27 cm and the lowest with 3.2 cm was recorded by Vellaikolamban.

Table 12. Internodal length (cm) of eight mango varieties observed at various intervals

Variety	First month (cm)	Second month (cm)	Third month (cm)	Fourth month (cm)	Fifth month (cm)	Sixth month (cm)	Twelve month (cm)
V_1 Bappakai	5.93^{a}	6.53^{a}	6.90^{a}	7.47^{ab}	7.70^{a}	8.53^{b}	7.33^{d}
V_2 Chandrakaran	0.00^{d}	2.80^{b}	3.07^{cd}	3.37^{de}	6.07^{bc}	7.03^{c}	7.03^{de}
V_3 Creeping	0.65^{c}	2.10^{c}	3.63^{bcd}	5.87^{bc}	7.30^{ab}	8.83^{b}	8.50^{c}
V_4 Kalapady	0.73^{c}	1.83^{c}	4.07^{bc}	8.03^{a}	8.53^{a}	10.17^{a}	10.27^{a}
V_5 Kurukkan	1.62^{b}	1.77^{c}	4.50^{b}	4.67^{cd}	5.10^{c}	6.40^{c}	6.77^{e}
V_6 Muvandan	1.63^{b}	1.92^{c}	2.90^{de}	4.57^{cd}	5.07^{c}	6.37^{c}	5.03^{f}
V_9 Rumani	0.70^{c}	0.87^{d}	1.90^{ef}	7.37^{ab}	7.83^{a}	8.20^{b}	9.30^{b}
V_{10} Vellaikolamban	0.00^{d}	0.00^{e}	1.50^{f}	1.73^{e}	2.87^{d}	3.27^{d}	3.20^{g}
CD (0.05)	0.36	0.67	1.09	1.99	1.63	1.01	0.54

4.1.5. Root number and length

The analysis on root number and root length are given in Table 13. The varieties Kurukkan, Muvandan, Kalapady and Rumani and Bappakai and Vellaikolamban were on par. Chandrakaran recorded significantly lowest (22) number of roots and Creeping recorded the highest (102).

The analysis on root length revealed that Rumani, Bappakai and Kurukkan, Chandrakaran and Creeping and Kalapady and Vellaikolamban were on par. Least root length was recorded by Vellaikolamban and the highest was by Rumani.

Table 13. Number and length of roots (cm) of one year old seedlings of mango varieties

Variety	Number of roots	Length of root (cm)
V_1 Bappakai	35.67^{e}	94.33^{a}
V_2 Chandrakaran	18.00^{f}	64.00^{c}
V_3 Creeping	93.67^{a}	58.17^{c}
V_4 Kalapady	68.33^{cd}	29.67^{d}
V_5 Kurukkan	80.33^{b}	94.00^{a}
V_6 Muvandan	76.67^{bc}	79.83^{b}
V_9 Rumani	61.67^{d}	100.33^{a}
V_{10} Vellaikolamban	33.67^{e}	20.67^{d}
CD (0.05)	10.48	12.81

4.1.6. Dry matter content of root and shoot and total dry matter production

The data on dry matter content was recorded after one year (Table 14). In the case of dry weight of roots, all the varieties were on par except Rumani. Rumani recorded more root weight (46.67g) and Muvandan recorded the least.

Creeping and Chandrakaran were on par and all other varieties were significantly different. Rumani recorded the highest dry matter production (215 g) and Vellaikolamban recorded the lowest.

Table 14. Dry matter content of root and stem and total dry matter production of eight mango varieties after one year

Variety	Dry weight of root (g)	Dry weight of stem (g)	Total dry matter production (g)
V_1 Bappakai	26.67^{cd}	84.33^{b}	149.333^{c}
V_2 Chandrakaran	31.67^{bc}	56.67^{e}	126.667^{e}
V_3 Creeping	28.33^{bcd}	60.00^{e}	143.333^{cd}
V_4 Kalapady	35.00^{b}	72.67^{c}	166.000^{b}
V_5 Kurukkan	21.67^{de}	66.00^{d}	134.333^{de}
V_6 Muvandan	15.00^{e}	46.00^{f}	96.000^{f}
V_9 Rumani	46.67^{a}	108.33^{a}	215.000^{a}
V_{10} Vellaikolamban	16.67^{e}	26.67^{g}	66.667^{g}
CD (0.05)	6.84	5.43	11.80

4.2. ANATOMICAL CHARACTERS

The anatomical parameters consisted of stomatal density, phloem – xylem ratio and bark percentage. The details of statistically analyzed data are presented in Table 15.

4.2.1. Stomatal density

The varieties Kurukkan and Rumani were on par and accounted for maximum stomatal count. Muvandan, Chandrakaran and Kalapady and Vellaikolamban, Creeping and Bappakai were on par with moderate and minimum number of stomata respectively.

4.2.2. Phloem – xylem ratio

The phloem xylem ratio found to be on par except Vellaikolamban and Rumani. The maximum phloem – xylem ratio was recorded by Vellaikolamban, followed by Rumani and the minimum by Creeping (Plate 3).

4.2.3. Bark percentage

Bark percentage of the varieties Kalapady, Kurukkan and Chandrakaran and Bappakai, Rumani and Vellaikolamban were on par. Maximum bark percentage was found in Creeping and the least was in Muvandan.

Table 15. Stomatal density (number/ field), phloem – xylem ratio and bark percentage (%) of eight mango varieties

Variety	Stomatal density (number/ field)	Phloem – xylem ratio	Bark percentage (%)
V_1 Bappakai	74.00^{d}	0.55^{cd}	12.58^{d}
V_2 Chandrakaran	95.00^{bc}	0.58^{c}	13.99^{c}
V_3 Creeping	79.00^{d}	0.51^{e}	16.64^{a}
V_4 Kalapady	88.67^{c}	0.51^{e}	15.28^{b}
V_5 Kurukkan	110.67^{a}	0.53^{de}	14.25^{bc}
V_6 Muvandan	98.67^{b}	0.58^{c}	9.90^{e}
V_9 Rumani	110.00^{a}	0.64^{b}	11.81^{d}
V_{10} Vellaikolamban	80.67^{d}	0.78^{a}	11.47^{d}
CD (0.05)	7.38	0.03	1.28

4.3. PHYSIOLOGICAL CHARACTERS

Phenol content and Relative water content (RWC) were analyzed under the physiological parameters (Table 16).

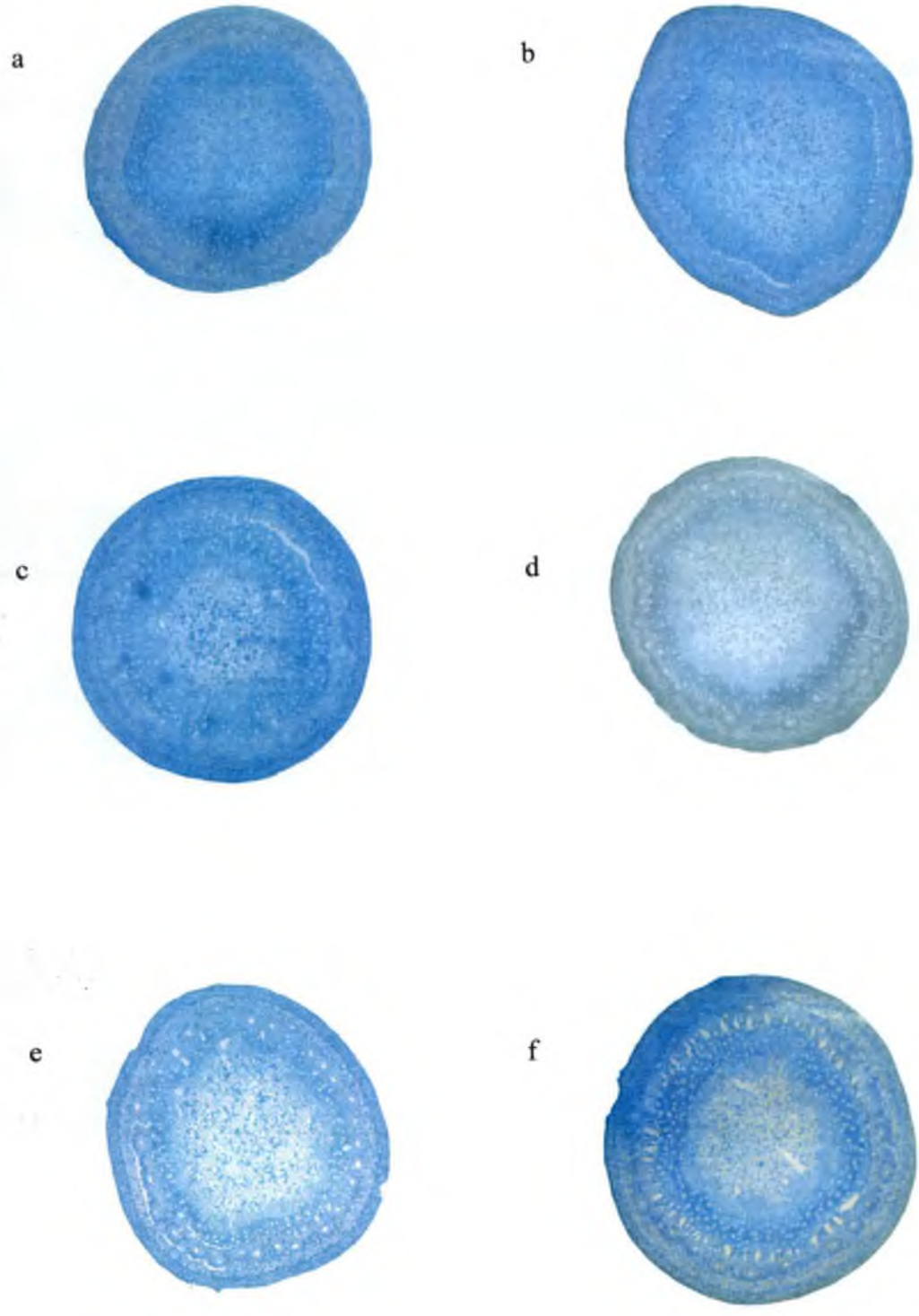
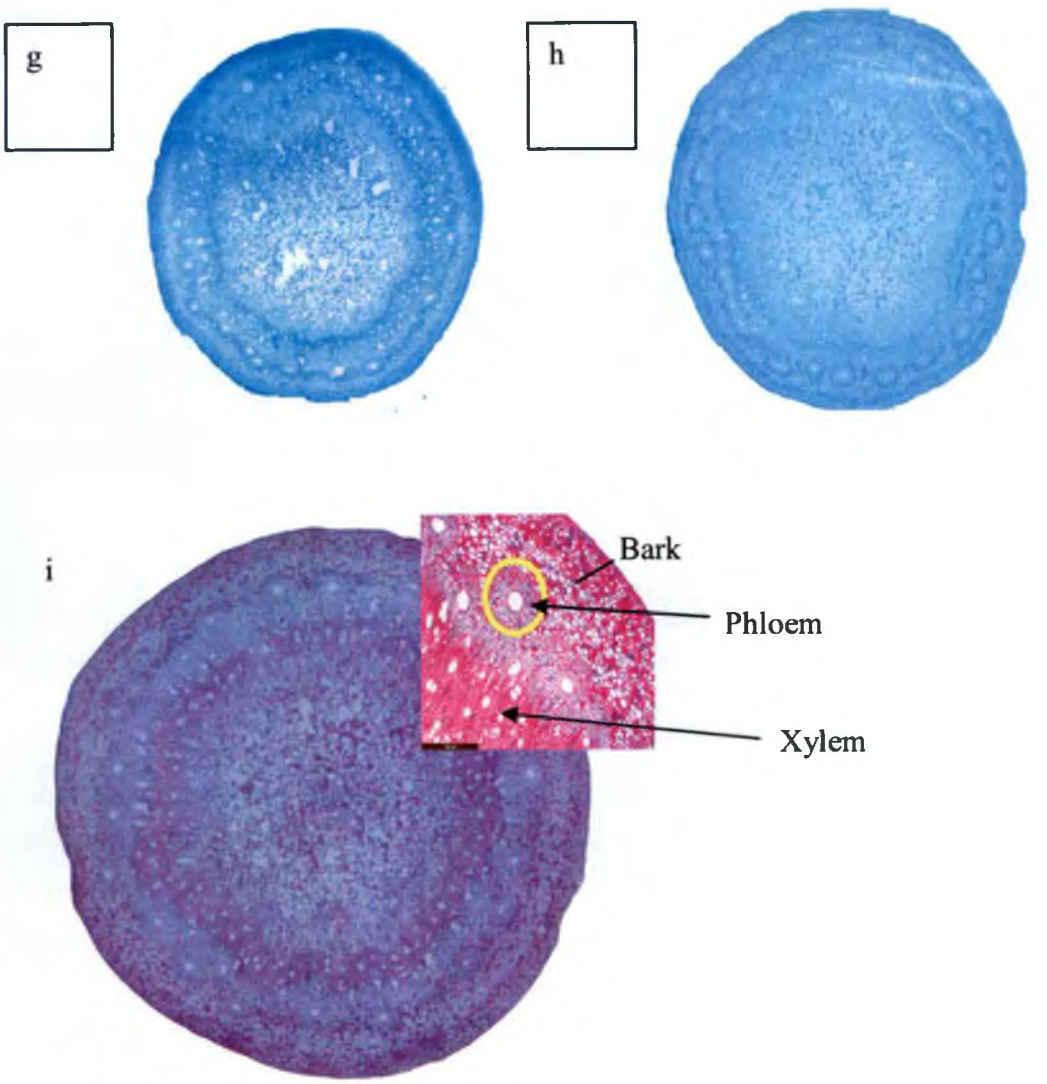


Plate 3. Stem sections of eight varieties



a. Bappakai (V_1) b. Chandrakaran (V_2) c. Creeping (V_3) d. Kalapady (V_4)

e. Kurukkan (V_5) f. Muvandan (V_6) g. Rumani (V_9) h. Vellaikolamban (V_{10})

i. Periodic acid Schiff's reagent staining

Plate 3. Stem sections of eight varieties

4.3.1. Phenol content

The phenol content of all varieties was on par, even though Kalapady and Vellaikolamban recorded maximum phenol content and Bappakai and Muvandan recorded the minimum.

4.3.2. Relative water content (RWC)

The relative water content of the plants was found to be not significant.

Table 16. Phenol content (mg/g catechol equivalent) and relative water content (%) of eight mango varieties

Variety	Phenol content (mg/g catechol equivalent)	Relative water content (%)
V_1 Bappakai	38.67^{c}	96.06
V_2 Chandrakaran	44.53^{bc}	96.42
V_3 Creeping	52.60^{ab}	96.64
V_4 Kalapady	58.20^{a}	97.44
V_5 Kurukkan	52.47^{ab}	96.64
V_6 Muvandan	37.60^{c}	97.50
V_9 Rumani	44.73^{bc}	96.09
V_{10} Vellaikolamban	53.33^{a}	97.62
CD (0.05)	8.33	NS

4.4. CORRELATION STUDIES

The various morphological characters such as number, leaf length, leaf width, average leaf area and total area of leaf, internodal length, number of roots, length of roots, dry weight of roots, dry weight of shoots and bark percentage were correlated with the plant height and the details are presented in the Table 17, 18, 19, 20, 21, 22, 23 and 24 respectively. All the above characters were

positively correlated with the plant height. There was a weak positive correlation between the plant height and stomatal density.

The number of leaves during fifth month was highly correlated with the plant height during the second month.

The leaf length and width of first month showed high positive correlation with third month's height.

Average leaf area during the second month was positively correlated with height at first month.

Total area of first month was highly correlated with the plant height during first and second month.

Internodal length at fifth month was correlated with plant height during fourth month.

Number of roots was highly correlated with plant height during fourth month, whereas the length of roots and height at six month's age was highly correlated.

Dry weight of roots was positively correlated with plant height and the highest correlation was observed during the twelfth month.

Phloem – xylem ratio, phenol content and relative water (Table 24) content was negatively correlated with the plant height. The Phloem – xylem ratio and relative water content showed high negative correlation with the plant height during six months and the phenol content with that of first month.

Table 17. Correlation between plant height (cm) and number of leaves

	PH1	PH2	PH3	PH4	PH5	PH6	PH12	L1	L2	L3	L4	L5	L6	L12
PH1	1.000													
PH2	0.982*	1.000												
PH3	0.945*	0.984*	1.000											
PH4	0.865*	0.938*	0.965*	1.000										
PH5	0.880*	0.945*	0.965*	0.994*	1.000									
PH6	0.799*	0.880*	0.926*	0.965*	0.978*	1.000								
PH12	0.801*	0.800*	0.811*	0.794*	0.795*	0.765*	1.000							
L1	0.817*	0.874*	0.849*	0.845*	0.814*	0.727*	0.687	1.000						
L2	0.781*	0.864*	0.866*	0.865*	0.836*	0.774*	0.543	0.947	1.000					
L3	0.733*	0.822*	0.846*	0.851*	0.805*	0.748*	0.622	0.951	0.969	1.000				
L4	0.841*	0.908*	0.894*	0.893*	0.869*	0.799*	0.676	0.983	0.978	0.963	1.000			
L5	0.882*	0.920*	0.869*	0.882*	0.877*	0.790*	0.710*	0.934	0.894	0.845	0.954	1.000		
L6	0.900*	0.919*	0.850*	0.853*	0.856*	0.751*	0.670	0.894	0.852	0.777	0.909	0.985	1.000	
L12	0.794*	0.793*	0.705	0.693	0.699	0.602	0.672	0.865	0.719	0.672	0.811	0.883	0.895	1.000

* Correlation is significant at 0.05 level

Table 18. Correlation between plant height (cm) and leaf length (cm)

	PH1	PH2	PH3	PH4	PH5	PH6	PH12	LL1	LL2	LL3	LL4	LL5	LL6	LL12
PH1	1.000													
PH2	0.982*	1.000												
PH3	0.945*	0.984*	1.000											
PH4	0.865*	0.938*	0.965*	1.000										
PH5	0.880*	0.945*	0.965*	0.994*	1.000									
PH6	0.799*	0.880*	0.926*	0.965*	0.978*	1.000								
PH12	0.801*	0.800*	0.811*	0.794*	0.795*	0.765*	1.000							
LL1	0.907*	0.927*	0.959*	0.909*	0.905*	0.859*	0.826*	1.000						
LL2	0.949*	0.931*	0.922*	0.842*	0.848*	0.767*	0.798*	0.969	1.000					
LL3	0.903*	0.904*	0.909*	0.862*	0.853*	0.763*	0.781*	0.970	0.982	1.000				
LL4	0.903*	0.933*	0.947*	0.928*	0.921*	0.859*	0.815*	0.979	0.961	0.972	1.000			
LL5	0.860*	0.897*	0.908*	0.911*	0.902*	0.836*	0.792*	0.948	0.930	0.950	0.992	1.000		
LL6	0.831*	0.863*	0.879*	0.880*	0.869*	0.799*	0.757*	0.944	0.931	0.959	0.984	0.994	1.000	
LL12	0.793	0.811	0.811	0.782	0.762	0.664	0.642	0.892	0.908	0.937	0.942	0.956	0.971	1.000

* Correlation is significant at 0.05 level

Table 19. Correlation between plant height (cm) and leaf width (cm)

	PH1	PH2	PH3	PH4	PH5	PH6	PH12	LW1	LW2	LW3	LW4	LW5	LW6	LW12
PH1	1.000													
PH2	0.982*	1.000												
PH3	0.945*	0.984*	1.000											
PH4	0.865*	0.938*	0.965*	1.000										
PH5	0.880*	0.945*	0.965*	0.994*	1.000									
PH6	0.799*	0.880*	0.926*	0.965*	0.978*	1.000								
PH12	0.801*	0.800*	0.811*	0.794*	0.795*	0.765*	1.000							
LW1	0.812*	0.829*	0.885*	0.790*	0.814*	0.835*	0.649	1.000						
LW2	0.799*	0.808*	0.866*	0.759*	0.780*	0.799*	0.643	0.996	1.000					
LW3	0.748*	0.767*	0.839*	0.748*	0.768*	0.799*	0.614	0.990	0.994	1.000				
LW4	0.722*	0.743*	0.808*	0.717*	0.754*	0.812*	0.582	0.977	0.965	0.964	1.000			
LW5	0.567	0.589	0.655	0.560	0.610	0.699	0.377	0.900	0.892	0.901	0.954	1.000		
LW6	0.559	0.586	0.649	0.569	0.623	0.718*	0.380	0.884	0.872	0.882	0.946	0.997	1.000	
LW12	0.522	0.577	0.653	0.645	0.697	0.804	0.428	0.842	0.816	0.845	0.908	0.936	0.955	1.000

* Correlation is significant at 0.05 level

Table 20. Correlation between plant height (cm) and average leaf area (cm^2)

	PH1	PH2	PH3	PH4	PH5	PH6	PH12	A1	A2	A3	A4	A5	A6	A12
PH1	1.000													
PH2	0.982*	1.000												
PH3	0.945*	0.984*	1.000											
PH4	0.865*	0.938*	0.965*	1.000										
PH5	0.880*	0.945*	0.965*	0.994*	1.000									
PH6	0.799*	0.880*	0.926*	0.965*	0.978*	1.000								
PH12	0.801*	0.800*	0.811*	0.794*	0.795*	0.765*	1.000							
A1	0.906*	0.901*	0.923*	0.803*	0.814*	0.785*	0.708*	1.000						
A2	0.940*	0.918*	0.921*	0.794*	0.808*	0.760*	0.736*	0.992	1.000					
A3	0.913*	0.866*	0.859*	0.698	0.714*	0.663	0.724*	0.967	0.983	1.000				
A4	0.910*	0.878*	0.869*	0.724*	0.754*	0.730*	0.733*	0.932	0.945	0.963	1.000			
A5	0.873*	0.845*	0.840*	0.694	0.732*	0.727*	0.660	0.916	0.921	0.935	0.991	1.000		
A6	0.882*	0.867*	0.873*	0.753*	0.794*	0.795*	0.667	0.922	0.922	0.913	0.971	0.986	1.000	
A12	0.862	0.847	0.838	0.716	0.760	0.767	0.656	0.886	0.887	0.889	0.971	0.988	0.978	1.000

* Correlation is significant at 0.05 level

Table 21. Correlation between plant height (cm) and total leaf area (cm^2)

	PH1	PH2	PH3	PH4	PH5	PH6	PH12	TA1	TA2	TA3	TA4	TA5	TA6	TA12
PH1	1.000													
PH2	0.982*	1.000												
PH3	0.945*	0.984*	1.000											
PH4	0.865*	0.938*	0.965*	1.000										
PH5	0.880*	0.945*	0.965*	0.994*	1.000									
PH6	0.799*	0.880*	0.926*	0.965*	0.978*	1.000								
PH12	0.801*	0.800*	0.811*	0.794*	0.795*	0.765*	1.000							
TA1	0.960*	0.960*	0.922*	0.831*	0.835*	0.746*	0.660	1.000						
TA2	0.857*	0.891*	0.893*	0.797*	0.795*	0.753*	0.545	0.943	1.000					
TA3	0.725*	0.812*	0.858*	0.833*	0.837*	0.860*	0.490	0.796	0.923	1.000				
TA4	0.767*	0.844*	0.855*	0.843*	0.852*	0.866*	0.598	0.821	0.892	0.948	1.000			
TA5	0.891*	0.930*	0.896*	0.897*	0.922*	0.885*	0.669	0.882	0.834	0.841	0.912	1.000		
TA6	0.872*	0.883*	0.815*	0.837*	0.864*	0.791*	0.695	0.811	0.672	0.639	0.753	0.950	1.000	
TA12	0.817	0.840	0.780	0.772	0.766	0.675	0.693	0.851	0.719	0.595	0.755	0.817	0.811	1.000

* Correlation is significant at 0.05 level

Table 22. Correlation between plant height (cm) and internodal length (cm)

	PH1	PH2	PH3	PH4	PH5	PH6	PH12	I1	I2	I3	I4	I5	I6	I12
PH1	1.000													
PH2	0.982*	1.000												
PH3	0.945*	0.984*	1.000											
PH4	0.865*	0.938*	0.965*	1.000										
PH5	0.880*	0.945*	0.965*	0.994*	1.000									
PH6	0.799*	0.880*	0.926*	0.965*	0.978*	1.000								
PH12	0.801*	0.800*	0.811*	0.794*	0.795*	0.765*	1.000							
I1	0.304	0.328	0.362	0.278	0.340	0.450	0.022	1.000						
I2	0.316	0.353	0.448	0.379	0.423	0.561	0.260	0.880	1.000					
I3	0.294	0.378	0.477	0.439	0.467	0.608	0.231	0.856	0.897	1.000				
I4	0.892*	0.954*	0.949*	0.944*	0.948*	0.911*	0.675	0.453	0.426	0.514	1.000			
I5	0.858*	0.925*	0.956*	0.978*	0.970*	0.946*	0.826*	0.298	0.444	0.456	0.931	1.000		
I6	0.810*	0.895*	0.951*	0.976*	0.955*	0.940*	0.789*	0.264	0.432	0.499	0.904	0.976	1.000	
I12	0.789	0.865	0.884	0.945	0.921	0.880	0.869	0.044	0.195	0.295	0.857	0.943	0.937	1.000

* Correlation is significant at 0.05 level

Table 23. Correlation between plant height (cm) and number, length (cm) and dry weight of root (g), dry weight of shoot (g) and total dry matter content (g)

	PH12	No. of roots	Length of root	DW of roots	DW of stem	Total dry matter
PH12	1.000					
No. of roots	-0.005	1.000				
Length of root	0.366	0.160	1.000			
DW of roots	0.843*	-0.037	0.228	1.000		
DW of shoot	0.780*	0.123	0.638	0.830	1.000	
Total dry matter	0.846	0.222	0.477	0.917	0.962	1.000

* Correlation is significant at 0.05 level

Table 24. Correlation between plant height (cm) and stomatal density, phloem – xylem ratio, bark percentage (%), phenol content (mg/g of catechol equivalent based on fresh weight) and relative water content (%)

	PH6	Stomata	PXR	Bark (%)	Phenol	RCW
PH6	1.000					
Stomata	0.007	1.000				
PXR	-0.775*	-0.055	1.000			
Bark (%)	0.476	-0.213	-0.600	1.000		
Phenol	-0.116	-0.057	-0.003	0.646	1.000	
RCW	-0.639	-0.133	0.288	-0.201	0.383	1.000

* Correlation is significant at 0.05 level

4.5. CLASSIFICATION OF MANGO VARIETIES

Based on the morpho – anatomical and physiological studies, eight mango varieties were classified as tall, semi tall and dwarf (Table 25). The classification was done by fixing range using mean and standard deviation. Vellaikolamban with a total score of 47 fell under the dwarf category. All other varieties except Kalapady came under the semi tall category. Kalapady scored 27 and 24 for tall and semi tall characters respectively.

Table 25. Classification of mango varieties based on morpho – anatomical and physiological studies

Observations	Category	Range	Bappakai	Chandrakaran	Creeping	Kalapady	Kurukkan	Muvandan	Rumani	Vellaikolamban
PH1	Tall	>29				*			*	
	Semi tall	12 – 29	*	*	*		*	*		
	Dwarf	<12								*
Mean – 20.46 SD – 8.87										
PH2	Tall	>32				*			*	
	Semi tall	14 – 32	*	*	*		*	*		
	Dwarf	<14								*
Mean – 23.33 SD – 9.29										
PH3	Tall	>36				*				
	Semi tall	18 – 36	*	*	*		*	*	*	
	Dwarf	<18								*

Mean – 27.39 SD – 9.45											
PH4	Tall	>47				*					
	Semi tall	21 – 47	*	*	*		*	*	*		
	Dwarf	<21								*	
Mean – 34.57 SD – 12.36											
PH5	Tall	>47									
	Semi tall	26 – 47	*	*	*	*	*	*	*		
	Dwarf	<26								*	
Mean – 38.83 SD – 13.39											
PH6	Tall	>58									
	Semi tall	30 – 58	*	*	*	*	*	*	*		
	Dwarf	<30								*	
Mean – 43.45 SD – 14.17											
PH12	Tall	>96							*		
	Semi tall	54 – 96	*	*	*	*	*	*			
	Dwarf	<54								*	
Mean – 75.36 SD – 20.59											
L1	Tall	>12	*			*			*		
	Semi tall	4 – 12		*	*		*	*			
	Dwarf	<4								*	
Mean – 8.17 SD – 4.16											

L2	Tall	>17				*				
	Semi tall	5 – 17	*		*		*	*	*	
	Dwarf	<5		*						*
Mean – 11.21 SD – 5.87										
L3	Tall	>24				*				
	Semi tall	8 – 24	*	*	*		*	*	*	
	Dwarf	<8								*
Mean – 15.83 SD – 7.61										
L4	Tall	>38				*				
	Semi tall	12 – 38	*	*	*		*	*	*	
	Dwarf	<12								*
Mean – 24.75 SD – 13.04										
L5	Tall	>47				*			*	
	Semi tall	13 – 47	*	*	*		*	*		
	Dwarf	<13								*
Mean – 30.29 SD – 17.07										
L6	Tall	>53				*			*	
	Semi tall	15 – 53	*		*		*	*		
	Dwarf	<15		*						*
Mean – 34.29 SD – 18.93										
L12	Tall	>94				*				

	Semi tall	26 – 94	*	*	*		*	*	*	
	Dwarf	<26								*
Mean – 59.88		SD – 33.86								
LL1	Tall	>17				*				
	Semi tall	11 – 17	*	*	*			*	*	
	Dwarf	<11					*			*
Mean – 12.9		SD – 4.05								
LL2	Tall	>18								
	Semi tall	10 – 18	*	*	*	*	*	*	*	
	Dwarf	<10								*
Mean – 13.88		SD – 4.05								
LL3	Tall	>19								
	Semi tall	11 – 19	*	*	*	*	*	*	*	
	Dwarf	<11								*
Mean – 15.01		SD – 3.56								
LL4	Tall	>19				*			*	
	Semi tall	13 – 19	*	*	*		*	*		
	Dwarf	<13								*
Mean – 16.47		SD – 3.27								
LL5	Tall	>20				*				
	Semi tall	14 – 20	*	*	*		*	*	*	
	Dwarf	<14								*

Mean – 17.38		SD – 2.93								
LL6	Tall	>21								
	Semi tall	15 – 21	*	*	*	*	*	*	*	
	Dwarf	<15								*
Mean – 18.11		SD – 2.66								
LL12	Tall	>19			*	*		*	*	
	Semi tall	18 – 19					*			
	Dwarf	<18	*	*						*
Mean – 18.80		SD – 0.53								
LW1	Tall	>5	*							
	Semi tall	3 – 5		*	*	*	*	*	*	
	Dwarf	<3								*
Mean – 3.54		SD – 1.1								
LW2	Tall	>5	*							
	Semi tall	3 – 5		*	*	*	*	*	*	
	Dwarf	<3								*
Mean – 3.82		SD – 1								
LW3	Tall	>5	*							
	Semi tall	3 – 5		*	*	*	*	*	*	
	Dwarf	<3								*
Mean – 4.03		SD – 0.88								

LW4	Tall	>5	*							
	Semi tall	3 – 5		*	*	*	*	*	*	
	Dwarf	<3								*
Mean – 4.36		SD – 0.75								
LW5	Tall	>5	*							
	Semi tall	3 – 5		*	*	*	*	*	*	*
	Dwarf	<3								
Mean – 4.61		SD – 0.74								
LW6	Tall	>5	*							
	Semi tall	3 – 5		*	*	*	*	*	*	*
	Dwarf	<3								
Mean – 4.76		SD – 0.66								
LW12	Tall	>6	*							
	Semi tall	5 – 6		*	*	*	*	*	*	
	Dwarf	<5								*
Mean – 5.12		SD – 0.16								
A1	Tall	>43								
	Semi tall	15 – 43	*	*	*	*	*	*	*	
	Dwarf	<15								*
Mean – 28.8		SD – 14.05								
A2	Tall	>47				*				

	Semi tall	19 – 47	*	*	*		*	*	*	
	Dwarf	<19								*
Mean – 32.97		SD – 14.27								
A3	Tall	>50				*				
	Semi tall	22 – 50	*	*	*		*	*	*	
	Dwarf	<22								*
Mean – 36.05		SD – 13.97								
A4	Tall	>57								
	Semi tall	29 – 57	*	*	*	*	*	*	*	
	Dwarf	<29								*
Mean – 42.6		SD – 13.86								
A5	Tall	>61	*							
	Semi tall	35 – 61		*	*	*	*	*	*	
	Dwarf	<35								*
Mean – 47.63		SD – 13.09								
A6	Tall	>64	*							
	Semi tall	40 – 64		*	*	*	*	*	*	
	Dwarf	<40								*
Mean – 51.8		SD – 11.6								
A12	Tall	>60	*			*			*	
	Semi tall	54 – 60		*			*	*		
	Dwarf	<54			*					*

Mean – 57.19 SD – 2.64										
TA1	Tall	>253				*				
	Semi tall	65 – 253	*	*	*		*	*	*	
	Dwarf	<65								*
Mean – 159.2 SD – 94.42										
TA2	Tall	>356	*			*				
	Semi tall	82 – 356		*	*		*	*	*	
	Dwarf	<82								*
Mean – 218.94 SD – 137.35										
TA3	Tall	>451	*			*				
	Semi tall	123 – 451		*	*		*	*	*	
	Dwarf	<123								*
Mean – 286.62 SD – 164.22										
TA4	Tall	>927	*			*				
	Semi tall	237 – 927		*	*		*	*	*	
	Dwarf	<237								*
Mean – 581.72 SD – 344.95										
TA5	Tall	>1213							*	
	Semi tall	333 – 1213	*	*	*	*	*	*		
	Dwarf	<333								*
Mean – 772.96 SD – 440.24										

TA6	Tall	>1531							*	
	Semi tall	349 – 1531	*	*	*	*	*	*		
	Dwarf	<349								*
Mean – 939.54 SD – 590.91										
TA12	Tall	>1890				*	*		*	
	Semi tall	1500 – 1890								
	Dwarf	<1500	*	*	*			*		*
Mean – 1694.53 SD – 195.08										
I5	Tall	>8				*				
	Semi tall	4 – 8	*	*	*		*	*	*	
	Dwarf	<4								*
Mean – 6.31 SD – 1.89										
I6	Tall	>9				*				
	Semi tall	5 – 9	*	*	*		*	*	*	
	Dwarf	<5								*
Mean – 7.35 SD – 2.10										
I12	Tall	>8	*	*	*	*			*	
	Semi tall	7 – 8								
	Dwarf	<7					*	*		*
Mean – 7.18 SD – 0.45										
No: of roots	Tall	>86			*					

	Semi tall	32 – 86	*	*		*	*	*	*	*
	Dwarf	<32								
Mean – 58.5		SD – 26.53								
Length of roots	Tall	>98							*	
	Semi tall	38 – 98	*	*	*		*	*		
	Dwarf	<38				*				*
Mean – 67.63		SD – 30.18								
DW of roots	Tall	>38							*	
	Semi tall	18 – 38	*	*	*	*	*			
	Dwarf	<18						*		*
Mean – 27.71		SD – 10.35								
DW of shoot	Tall	>90							*	
	Semi tall	40 – 90	*	*	*	*	*	*		
	Dwarf	<40								*
Mean – 65.08		SD – 24.60								
Stomata	Tall	>106					*		*	
	Semi tall	78 – 106		*	*	*		*		*
	Dwarf	<78	*							
Mean – 92.08		SD – 13.93								
PXR	Tall	>0.7								
	Semi tall	0.5 – 0.7	*	*	*	*	*	*	*	

	Dwarf	<0.5								*
Mean – 0.59		SD – 0.09								
Bark percentage	Tall	>15			*	*				
	Semi tall	11 – 15	*	*			*		*	*
	Dwarf	<11						*		
Mean – 13.24		SD – 2.21								
Phenol	Tall	>55	*					*		
	Semi tall	41 – 55		*	*		*		*	*
	Dwarf	<41				*				
Mean – 47.77		SD – 7.48								
Total score	Tall		16	13	4	27	2	2	15	0
	Semi tall		34	38	48	24	49	47	37	6
	Dwarf		3	4	1	2	2	4	0	47

4.6. MOLECULAR STUDIES

4.6.1. Isolation of total genomic DNA

4.6.1.1. Stage of leaf for genomic DNA extraction

Among the three types of leaves used for genomic DNA extraction, immature green leaf was found ideal for extracting good quality DNA. The DNA extracted from immature and brown leaf was degraded and the quality was very low while that from the mature green one was contaminated with the chlorophyll.

4.6.1.2. Protocol for genomic DNA extraction

Since the polysaccharide content and phenol were interfering with the DNA extraction, three procedures *viz.*, CTAB method (Rogers and Bendich, 1994), Porebski *et al.* (1997) , DNA extraction kit 'Origin' (Origin Diagnostics and Research, Kerala), were tried along with modifications in each as detailed in materials and methods. Out of this the best procedure which yielded good quality DNA in high quantity was from the modified CTAB method.

4.6.1.3. DNA quantification and checking quality

Gel electrophoresis and spectrophotometer method showed that the DNA is of good quality and in sufficient quantity for doing the RAPD analysis (Plate 4). Quantification of the extracted genomic DNA is given in the Table 26.

Table 26. Quantification of DNA

Cultivar	260/280 ratio
V_1 Bappakai	1.82
V_2 Chandrakaran	1.43
V_3 Creeping	1.80
V_4 Kalapady	1.61
V_5 Kurukkan	1.38
V_6 Muvandan	2.06
V_9 Rumani	1.88
V_{10} Vellaikolamban	1.73

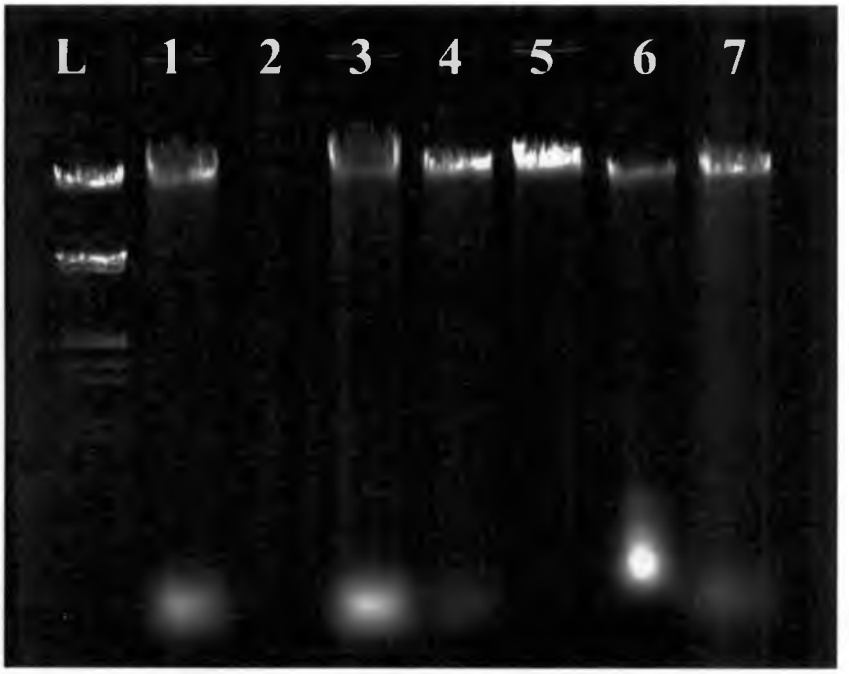
4.6.2. Primer screening

Based on the amplification pattern, 10 primers were selected from the 30 primers screened using one DNA sample of the cultivar Kalapady (Plate 5). The selected primers and their sequence are given in Table 5. The primers selected were, OPA 10, OPA 11, OPA 17, OPM 20, OPX 01, OPX 08, OPX 17, RPI 01, RPI 03 and RPI 04.

4.6.3. Amplification of genomic DNA using selected primers

OPX 01

Twenty four amplicons were obtained from the primer of which, one was monomorphic and 23 were polymorphic (Plate 6). A specific band of 4268 bp was found in Creeping whereas Kalapady was having three specific bands (3500, 1904 and 450 bp). A band with a size of 1375 bp was identified for Kalapady and Creeping. In Muvandan and Creeping 947 bp band was lacking. Only Chandrakaran and Muvandan had 831 bp band. A band with a size of 800 bp was present only in Kalapady and Creeping. In Vellaikolamban, 700 bp band was



L- Molecular weight marker 1. Bappakai (V_1) 2. Chandrakaran (V_2) 3. Creeping (V_3) 4. Kalapady (V_4) 5. Kurukkan (V_5) 6. Muvandan (V_6) 7. Rumani (V_9)

Plate 4. Genomic DNA

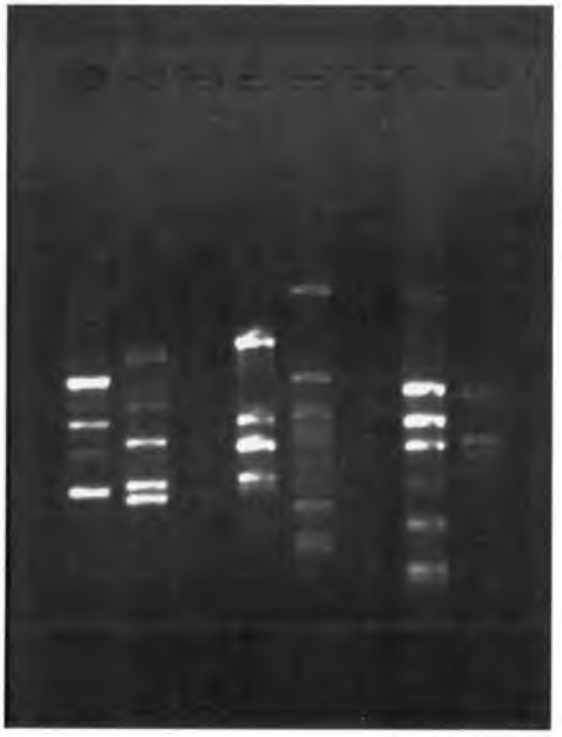


Plate 5. Primer Screening

absent, which was present in all other genotypes. For Bappakai and Kurukkan two bands were found in common, at 375 bp and 275 bp. A band at 210 bp was only found in Kurukkan.

OPX 08

Among the 12 bands amplified by OPX 08, two were monomorphic and 10 were polymorphic (Plate 7). Chandrakaran and Muvandan were not amplified by the primer. Three bands were lacking in Kurukkan and Vellaikolamban (2027, 800 and 600 bp), which were present in the rest of the genotypes. Band with a size of 1100 bp was absent in both Kalapady and Bappakai. Only in Kalapady, 1000bp band was absent. 900 bp band was detected only for Kalapady and Bappakai. In Rumani a specific band at 530 bp was detected.

RPI 04

The primer amplified nineteen polymorphic bands (Plate 8). A band with a size of 2027 bp was present in Kalapady and Creeping. Only Muvandan was lacking the band of 1450 bp size whereas Kalapady was lacking the band of 1375 bp. In Bappakai and Kurukkan two bands were found in common *viz.* 1100 and 350 bp size which were absent in others. Only in Creeping, 1000 bp sized band was absent. 750 bp sized band was only found in Chandrakaran and Bappakai. Bappakai and Vellaikolamban contained a band of 700 bp size. Chandrakaran and Vellaikolamban lacked 500 bp band, which were present in all other genotypes. A specific band of 400 bp size was found in Vellaikolamban. In Bappakai specific band was found at 275 bp size.

OPA 11

Among the 22 bands amplified by the primer OPA 11, one was monomorphic and 21 were polymorphic (Plate 9). The first band above 1000 bp was present only in the cultivars Bappakai and Creeping. Rumani lacked the fourth band above 1000 bp. Seventh band above 1000 bp and a low molecular

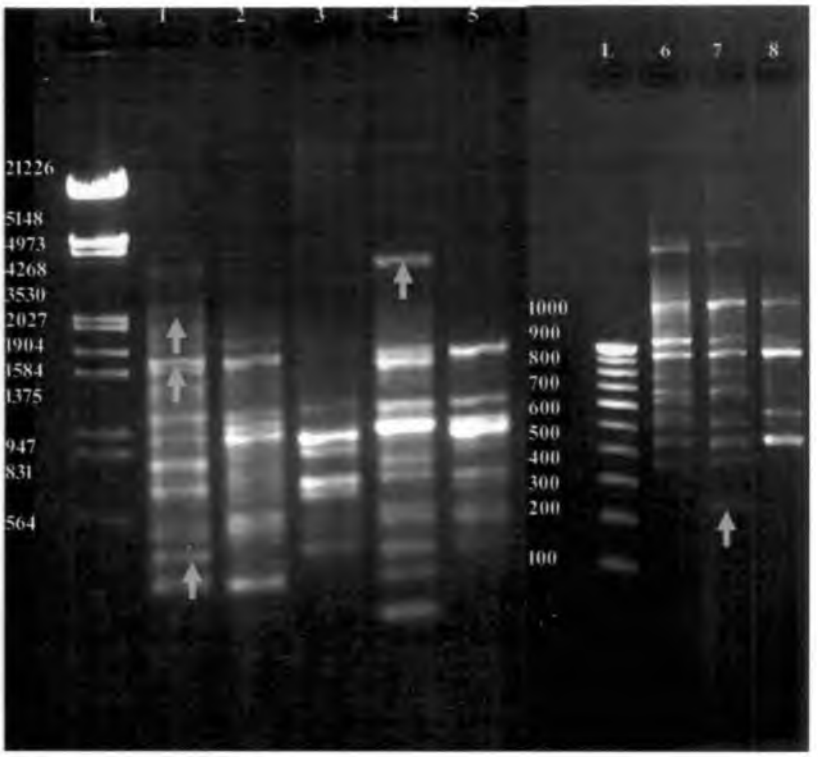


Plate 6. Amplification by OPX 01

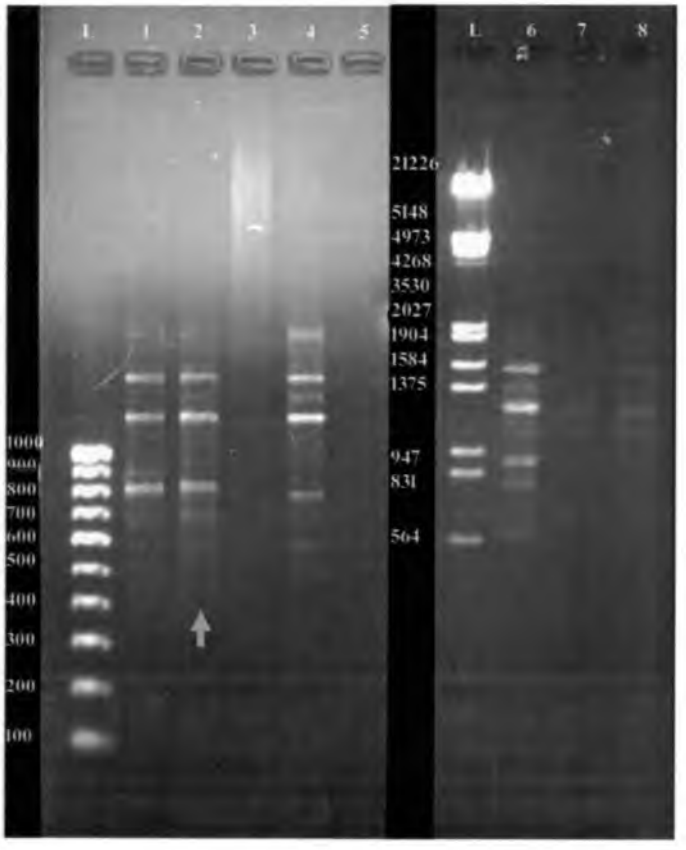


Plate 7. Amplification by OPX 08

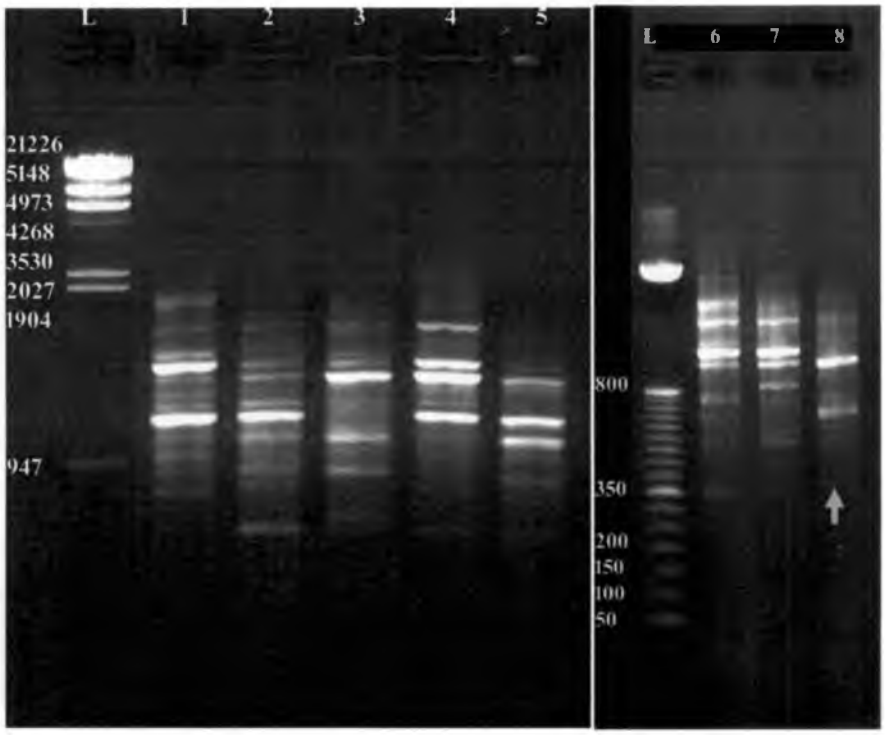


Plate 8. Amplification by RPI 04

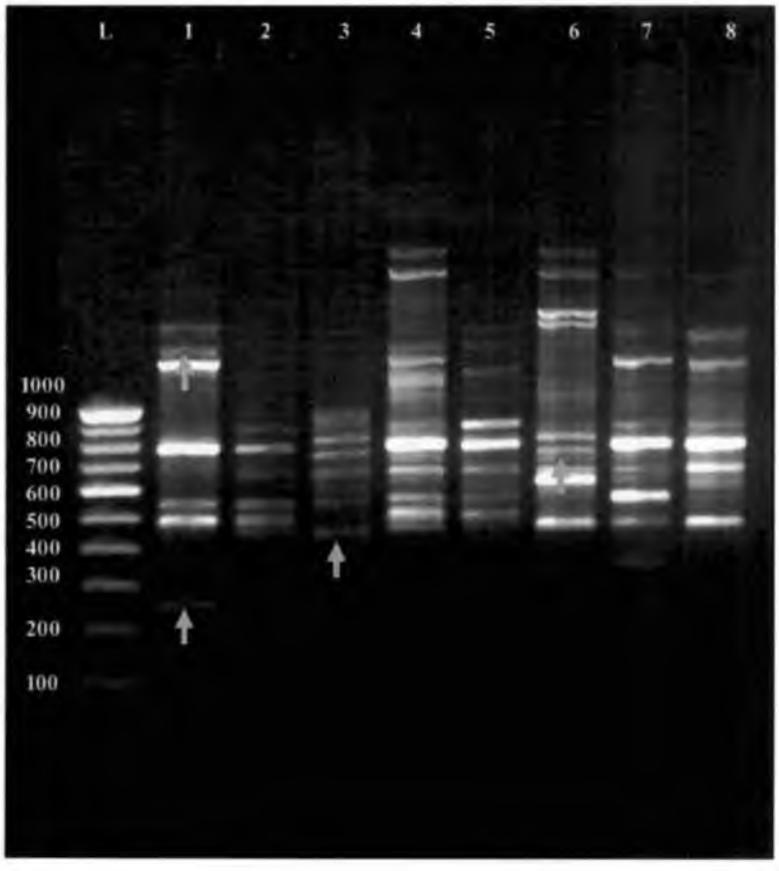


Plate 9. Amplification by OPA 11

weight band of 250bp were specific for the cultivar Kalapady. Rumani and Chandrakaran were lacking the eighth band above 1000 bp. A band of 900 bp was present in Rumani and Bappakai. A specific band of 750 bp size was found in Bappakai. All the cultivars were having the 700 bp sized band except Kalapady. A 450 bp sized band was observed in Chandrakaran.

OPA 10

Twelve bands with 4 monomorphic and 8 polymorphic bands were detected (Plate 10). Muvandan was not amplified by the primer. The first and third bands above 1000 bp were specific to the cultivar Bappakai. 800 bp band was absent in Kalapady. Kalapady and Vellaikolamban lacked 700 bp sized band, which was present in all other genotypes. A band of size 350 bp was only found in Rumani. 300 bp sized band was specific to Chandrakaran.

OPA 17

Only 11 polymorphic bands were detected (Plate 11). Chandrakaran and Muvandan were not amplified by the primer. The first band above 1000 bp and 800 bp bands were only found in Creeping and Bappakai. The second band above 1000 bp was absent in Kalapady and Rumani. Third band above 1000 bp was absent in Kalapady. 1000 bp band was absent in Vellaikolamban which was present in all other genotypes. 650 bp band was only found in Kalapady and Bappakai. A band of 600 bp size was found in Kalapady and Vellaikolamban. Vellaikolamban lacked the 320 bp sized band.

OPM 20

Among the 20 bands detected 3 were monomorphic and the remaining 17 were polymorphic (Plate 12). The second band above 1000 bp was absent only in Muvandan. Fifth band above 1000 bp was only present in Creeping. Seventh band above 1000 bp was present in creeping and Bappakai. Eighth band above 1000 bp was lacking in Muvandan, which was present in all other genotypes.

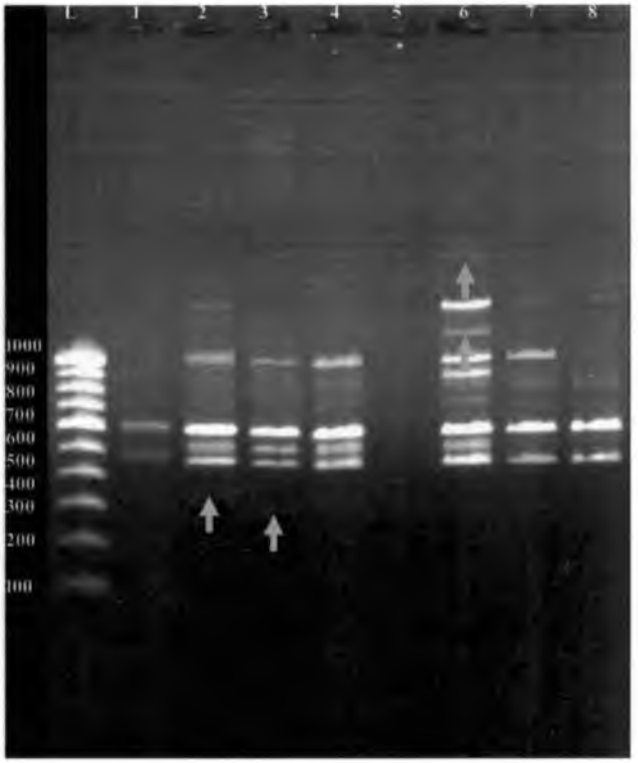


Plate 10. Amplification by OPA 10

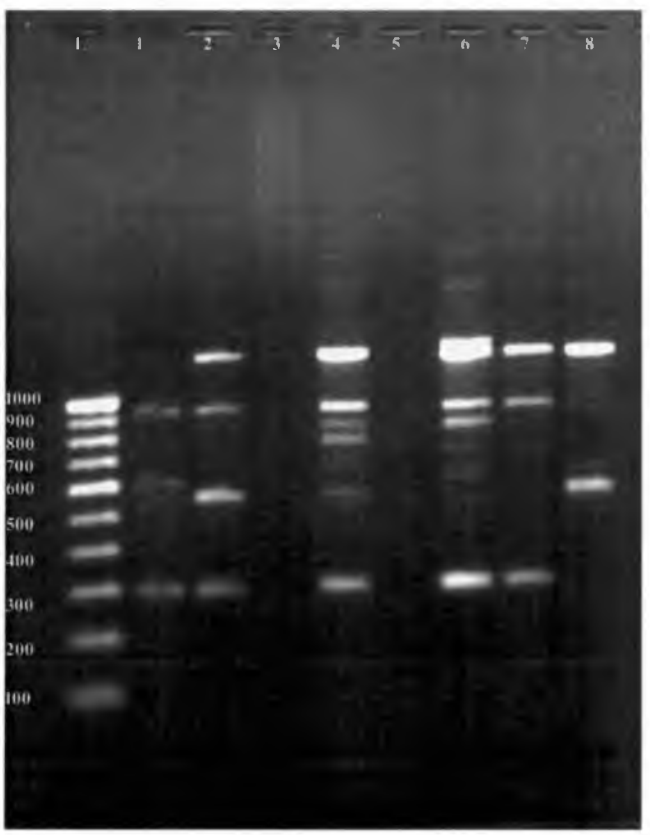


Plate 11. Amplification by OPA 17

Both in Muvandan and Chandrakaran the ninth band was absent. Rumani contained a specific band at 1000 bp. In Rumani and Chandrakaran 700 bp band was absent. 420 bp band was present only in Rumani and Vellaikolamban while 210 bp band was present only in Chandrakaran and Vellaikolamban.

OPX 17

Twelve bands were amplified by primer OPX 17, out of which 2 were monomorphic and 10 polymorphic (Plate 13). The first band above 1000 bp was present only in Kurukkan and Vellaikolamban. The second band above 1000 bp was only found in Bappakai and Kurukkan. The fourth band above 1000 bp was absent in Chandrakaran which was present in all other genotypes. Only Kalapady and Muvandan contain 950 bp and 800 bp bands. 600 bp band was found only in Chandrakaran whereas a band of 400 bp size was found only in Creeping and Bappakai.

RPI 01

Among the 20 bands amplified, one was monomorphic and 19 were polymorphic (Plate 14). Muvandan was not amplified by the primer. Fifth band above 1000 bp was found only in Creeping. The sixth band above 1000 bp was specific for Bappakai. Chandrakaran lacks the seventh band above 1000 bp. Vellaikolamban and Creeping lacks 800 bp band. 700 bp band was absent in Kalapady and Creeping. Only in Creeping and Vellaikolamban a band of size 620 bp was found. Chandrakaran and Vellaikolamban lacked 480 bp as well as 400 bp band. 425 bp band was absent in Bappakai and Kurukkan. Only in Creeping the band 375 bp was absent whereas 225 bp band was absent in Chandrakaran and Kalapady.

RPI 03

Twenty amplicons were detected using this primer. Among them 2 were monomorphic and 18 were polymorphic (Plate 15). The first band above 1000 bp

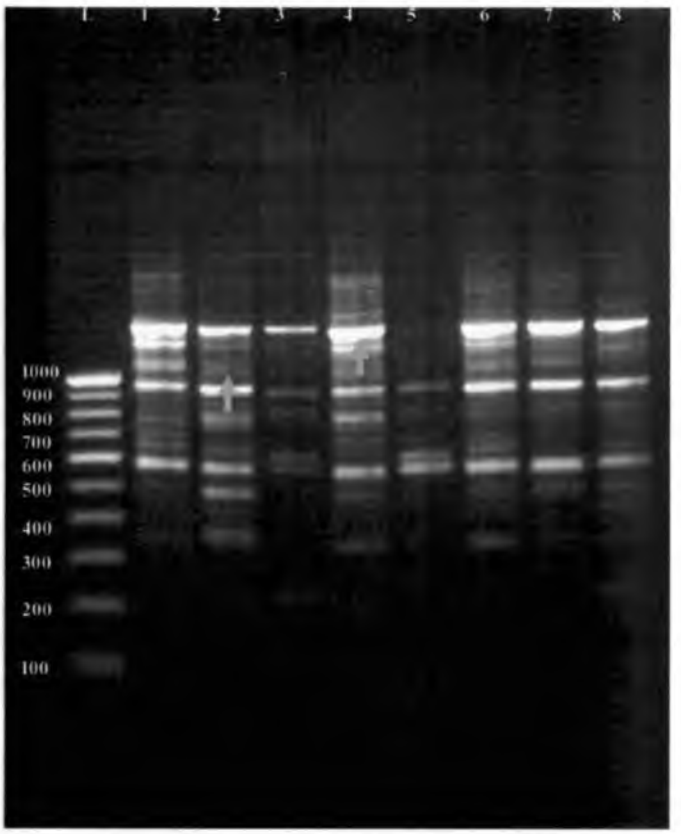


Plate 12. Amplification by OPM 20

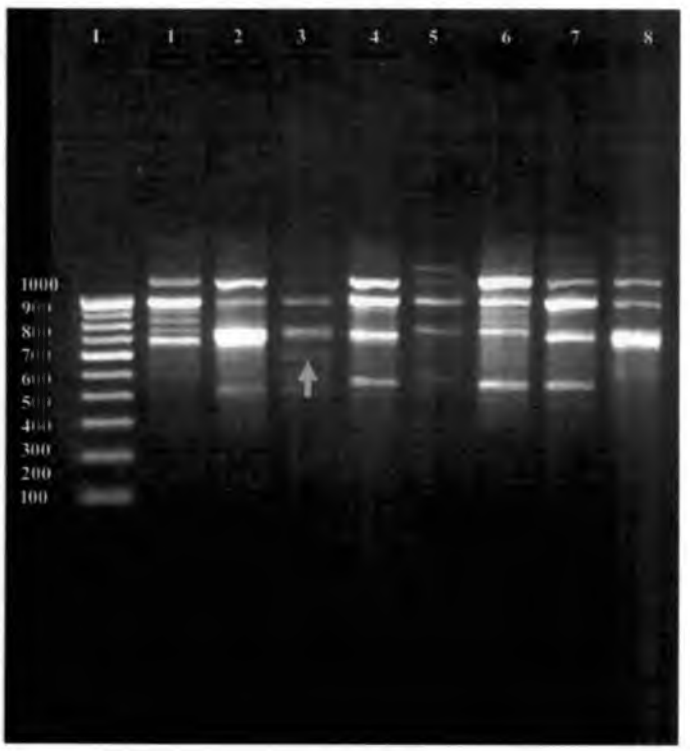


Plate 13. Amplification by OPX 17

was specific to Creeping. The second band above 1000 bp was found only in Creeping and Bappakai. Third band above 1000 bp was present in Bappakai and Kurukkan while the fourth band was found in only Kurukkan. Sixth band above 1000 bp and two low molecular weight bands of size 500 and 350 bp were present only in Kalapady. Eighth band above 1000 bp was absent in Kalapady and Muvandan, which were present in all other genotypes. 1000 bp band was only present in Muvandan. Only Creeping and Vellaikolamban contained 800 bp band. 750 bp band was present both in Muvandan and Bappakai. Kalapady lacked 600 bp as well as 400 bp bands. 575 bp sized band was present only in Rumani. Rumani and Muvandan lacked 475 bp sized band. 450 bp sized band was specific to Vellaikolamban.

4.6.1.1. Genetic diversity analysis

The selected ten primers produced 172 bands out of which only 16 were monomorphic bands. These bands were used for drawing dendrogram using the software DaRwin5 (Version: 5.0.158). The tree contained three major clusters (Plate 16). The varieties Chandrakaran, Rumani and Kalapady formed a cluster (Cluster 1). The second cluster consisted of Vellaikolamban, Kurukkan and Bappakai whereas Muvandan and Creeping formed the third cluster.

The first cluster branched out into two with Chandrakaran and Rumani forming one group and Kalapady formed another. Even though Rumani and Chandrakaran were clustered together, Chandrakaran showed more genetic diversity than Rumani.

The second cluster also branched into two sub clusters with Vellaikolamban and Kurukkan in one cluster and Bappakai forming the other. Vellaikolamban was more diverse than the other two varieties.

In the third cluster, Muvandan was more diverse from Creeping.

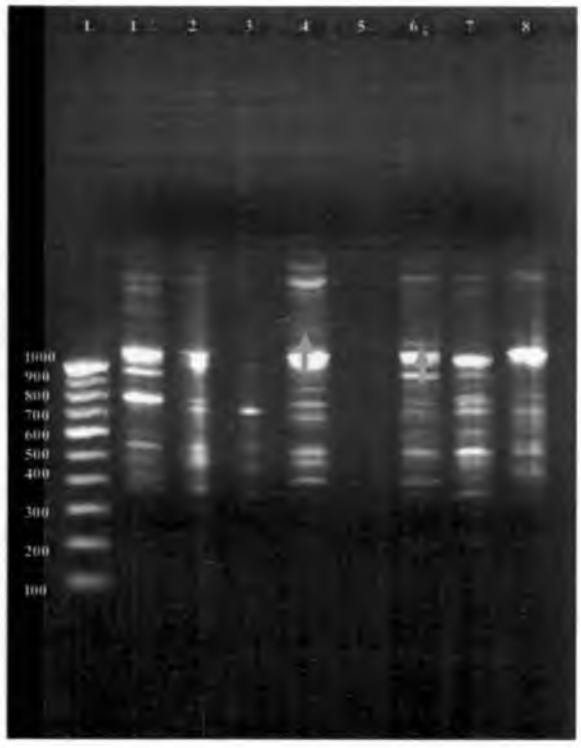
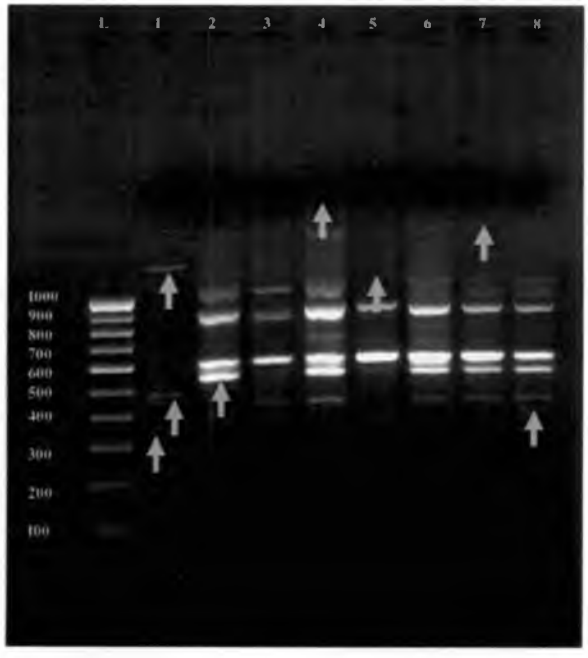


Plate 14. Amplification by RPI 01



L- Molecular weight marker 1. Kalapady (V_4) 2. Rumani (V_9)
Irakaran (V_2) 4. Creeping (V_3) 5. Muvandan (V_6) 6. Bappakai (V_1)
7. Kurukkan (V_5) 8. Vellaikolamban (V_{10})

Plate 15. Amplification by RPI 03

From the dissimilarity coefficient Table (Table 27) the highest dissimilarity was found between Muvandan and Chandrakaran followed by Muvandan and Kalapady. The least dissimilarity was between Kurukkan and Bappakai followed by Vellaikolamban and Kurukkan.

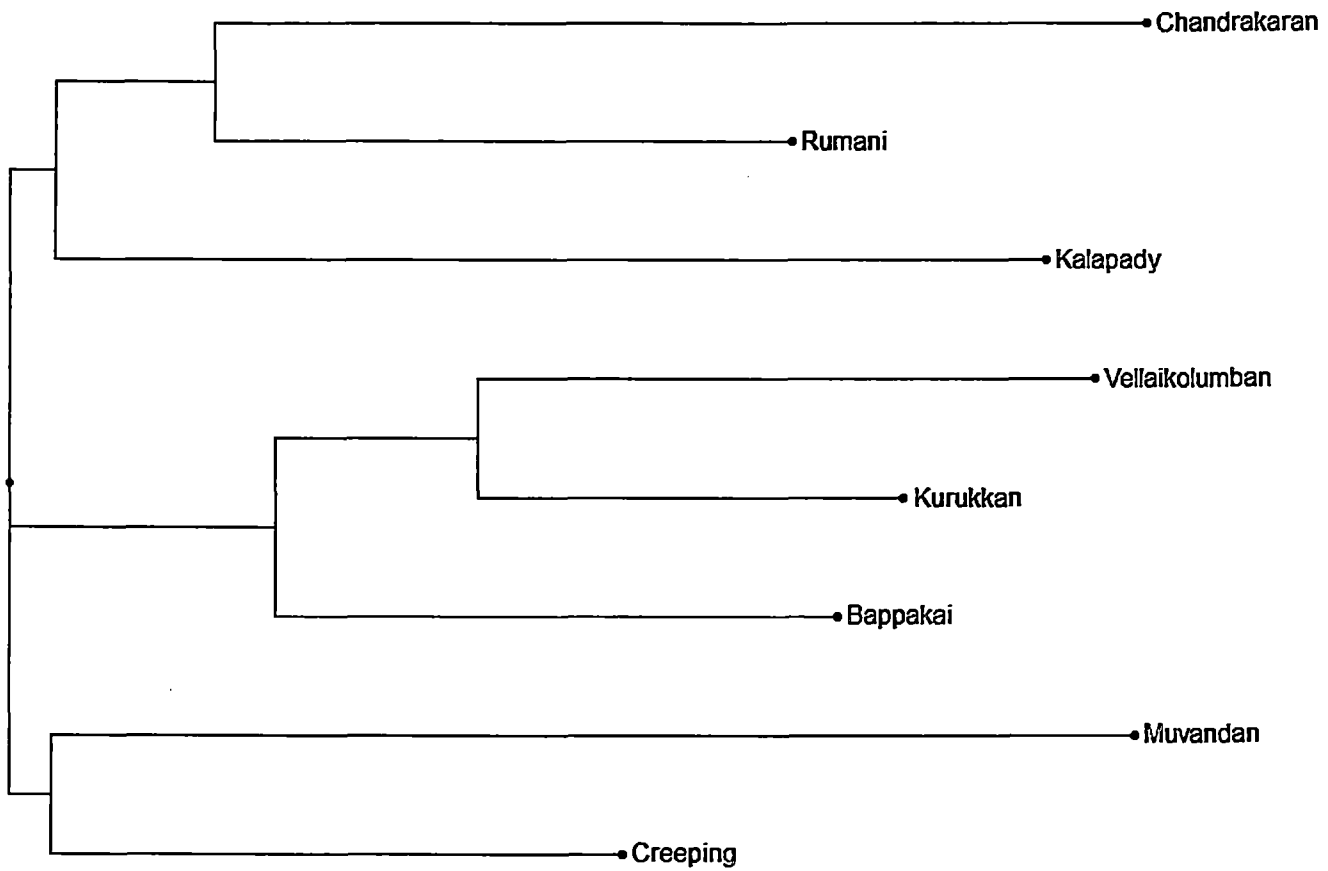


Plate 16. Dendrogram based on ten primers

Table 27. Dissimilarity coefficient between the eight varieties

Variety	Kalapady	Rumani	Chandrakaran	Creeping	Muvandan	Bappakai	Kurukkan
Rumani	0.377						
Chandrakaran	0.492	0.344					
Creeping	0.396	0.279	0.432				
Muvandan	0.500	0.415	0.511	0.376			
Bappakai	0.422	0.383	0.450	0.281	0.473		
Kurukkan	0.423	0.413	0.458	0.365	0.465	0.233	
Vellaikolamban	0.475	0.452	0.463	0.396	0.483	0.350	0.237

DISCUSSION

5. DISCUSSION

The investigation on "Marker assisted characterization of mango (*Mangifera indica* L.)" was conducted for the early selection of plants based on markers linked to the short stature. Dwarfness is an important character of fruit trees. Identification of characters associated with dwarfness will help in the selection dwarf plants/ dwarfing rootstocks/ in breeding programme of new varieties.

5.1. MORPHOLOGICAL CHARACTERS

5.1.1. Plant height

Among the eight varieties included in the study, Vellaikolamban recorded the least height of 37.13 cm followed by Muvandan (61.2 cm). Similar results were reported by Murti and Upreti (2003), Rao and Reddy (2005) and Srivastav *et.al.* (2009).

5.1.2. Number of leaves

The number of leaves was more in Kalapady and Rumani. Vellaikolamban recorded less number of leaves. Muvandan and Bappakai recorded more number of leaves than Vellaikolamban, Chandrakaran and Kurukkan. The number of leaves showed high positive correlation with the plant height. This confirms the earlier findings (Murti and Upreti, 2003; Srivastav *et al.*, 2009).

5.1.3. Leaf – length, width and area

Leaf length was more in Kalapady and Rumani. Shortest leaf was observed in Vellaikolamban. The leaf length showed high positive correlation with the plant height.

Leaf width was more in Bappakai and least width was recorded for Vellaikolamban. The height is highly correlated with the leaf width.

The least value for total leaf area was recorded by Vellaikolamban. Plant height showed high positive correlation with the total leaf area. Similarly in the study conducted by Murti and Upreti (2003) Vellaikolamban recorded 7.21cm^2 leaf area during 120 days after transplanting. Abirami *et al.* (2011) also reported less leaf area in Vellaikolamban (19.4cm^2).

Positive correlation between leaf area and plant height was also reported by Agarwal (1986) in trifoliate orange and Kurian and Iyer (1997) and Muralidhara *et al.* (2014) in mango.

5.1.4. Internodal length

Internodal length showed high positive correlation with the plant height from fourth month onwards. The lowest internodal length was recorded by Vellaikolamban (3.2cm) and highest by Kalapady (10.27cm). In the experiment conducted by Srivastav *et al.* (2009) least internodal length was shown by Vellaikolamban.

Positive correlation between internodal length and plant height was also found in the studies by Iyer and Subramanyam (1986) in mango, Wang and Faust (1987) in apple and Lim and Hawa (2007) in papaya.

5.1.5. Root number and length

Root number as well as root length was lowest in Vellaikolamban. Both parameters had positive correlations with the plant height. Srivastav *et al.* (2009) recorded lowest root length of 28.2cm in Vellaikolamban. Shaban (2010) also found positive correlation between the plant height and root length in mango.

5.1.6. Dry matter content of root and shoot and total dry matter production

Dry matter content of root was more in Rumani (46.67g) and less in Muvandan (15g). The dry weight of root and stem was positively correlated with plant height but the correlation was only significant with the plant height during

fourth, fifth and twelfth month. The dry weight of stem was less in Vellaikolamban (26.67g) and highest in Rumani (108.33g). It was having high positive correlation with the plant height. Singh *et al.* (1986) reported that the dry matter content had positive correlation with the plant height.

5.2. ANATOMICAL CHARACTERS

5.2.1. Stomatal density

The highest stomatal count was recorded for Rumani and Kurukkan. Stomatal density was having a weak positive correlation with height and the correlation was not significant. According to the classification using stomatal density, Kurukkan and Rumani were classified as tall while the cultivar Bappakai as dwarf but the dwarf cultivar Vellaikolamban and all other varieties were classified as medium tall. Compared to other morphological, anatomical and physiological characters, similar trend could not be obtained in the case of Vellaikolamban to classify it as a dwarf rootstock. Results with similarity were reported by Srivastava *et al.* (1980), Iyer and Kurian (1992), Kurian and Iyer (1997), Reddy *et al.* (2003), and Abirami *et al.* (2011).

5.2.2. Phloem – xylem ratio

Vellaikolamban recorded the maximum phloem – xylem ratio of 0.78. The phloem – xylem ratio was negatively correlated with the plant height. Iyer and Kurian (1992) reported that the induced dwarf trees of mango variety Alphonso treated with paclobutrazol showed negative correlation between the plant height and phloem to xylem ratio. Rashdey *et al.* (2014) reported that in mango, vigorous cultivars contains high xylem and low phloem percentage.

Similar relationship was found in apple in the dwarfing rootstock M_9 by several scientist *viz.* Beakbane and Renwick (1936), Beakbane and Thompson (1939), Beakbane (1941) and Wang and Faust (1987). In other fruit crops like guava (Saeed *et al.*, 2010) and olive (El Said *et al.*, 2013) also reported the negative correlation with the plant height and phloem to xylem ratio.

5.2.3. Bark percentage

Bark percentage was positively correlated with the height and the correlation was weak and non significant. Lowest bark percentage was found in Muvandan. Contradictory results were found by Majumdar *et al.* (1972), Mukherjee and Das (1980), Singh *et al.* (1986) and Abirami *et al.* (2011) in mango.

In other fruit crops like apple, as reported by Beakbane and Thompson (1939), dwarfing rootstocks had a bark to wood ratio of 1 – 2.3. In guava, the bark percentage was negatively correlated with the plant height, as reported by Saroj *et al.* 1997.

5.3. PHYSIOLOGICAL CHARACHERS

5.3.1. Phenol content

Highest phenol content was found in Kalapady (58.2mg/g) followed by Vellaikolamban (53.33mg/g). Phenol content was negatively correlated with the height and the correlation was weak and non significant.

Marie and Parameswaran (2001) reported the similar results, high phenol content in Vellaikolamban and Kalapady and the lowest in Muvandan and Chandrakaran.

Study conducted by Murti and Upreti (2003) recorded the least phenol content in Vellaikolamban (59.1mg/g) during 120 days after transplanting. Abirami *et al.* (2011) recorded 27.4mg/g phenol in Vellaikolamban and found a negative correlation with the height.

Negative correlation between plant height and phenol content was reported by Babu *et al.* (1985) and Kurian *et al.* (1994).

5.3.2. Relative Water Content(RWC)

All the eight varieties showed no significant difference with respect to RWC.

This might be due to the high rainfall received during the growth period; i.e. no water stress was found, compared to studies conducted by Murti and Upreti (2003) in Bangalore and by Abirami *et al.* (2011) in New Delhi. Correlation between growth and RWC was not significant in studies conducted by Abirami et.al. (2011) and further studies were suggested by Murti and Upreti (2003) to know the role of leaf water potential in regulating vigour.

5.4. CLASSIFICATION OF MANGO VARIETIES

Eight varieties were classified as tall, semi tall and dwarf based on morpho-anatomical and physiological characters. Vellaikolamban with a total score of 47 came under the dwarf group. This result confirms the earlier findings of in terms of less plant height and high phloem-xylem ratio and high phenol content, as reported by Singh *et al.* (1986) in Vellaikolamban, Iyer and Kurien (1992) in chemically induced dwarf trees of Alphonso and by Murti and Upreti (2003) in Vellaikolamban respectively.

5.5. MOLECULAR STUDIES

Studies for identification of molecular markers associated with dwarfness have been reported in other crops like apple (Ying *et al.*, 2002), rubber (Venkatachalam *et al.*, 2004), pear (Yike *et al.*, 2008) etc., though such reports are not available for mango. However, RAPD marker related to other characters such as the polyembryony (Lopez *et al.*, 1997) and differential flowering behaviour (Damodaran *et al.*, 2007) in mango have been carried out earlier.

In the present study, the selected ten random decamer primers produced a total of 172 bands out of which only 16 were monomorphic. The RAPD analysis

brought out the extent of genetic relationship between the cultivars under study. The total genetic variability between the 8 genotypes is high as indicated by the good number of polymorphic bands (156) exhibited by the selected 10 primers. Among the ten primers, highest number of total bands (24) and highest number of polymorphic bands (23) were produced by OPX 01. In the experiment conducted in mango by Lopez *et al.* (1997) using 13 RAPD primers, 109 reproducible amplicons were reported. Similarly only 157 bands were obtained from 42 primers in the experiment conducted by Simi *et al.* (2013).

In the work conducted by Radha *et al.* (2015) on molecular characterization of popular mango cultivars of Kerala, the varieties Kalapady and Vellaikolamban fell under same cluster.

The primers were also efficient in amplifying specific regions in each cultivar (Table 28), which may be useful in characterisation of a particular genotype. Kalapady, which is classified as tall cultivar in the present study (based on the morphological and physiological observations) has the highest number of specific bands (8) from three primers. It is also found that primer RPI 03 is able to amplify three specific bands for the tall cultivar Kalapady and one specific band for Vellaikolamban, which is classified as dwarf in the present study. Hence, RPI 03 may be considered as a useful primer for distinguishing between tall and dwarf types.

The primers RPI 04 and RPI 03 amplified specific bands in Vellaikolamban at 450bp and 400bp respectively. However, since the intensity of the bands was comparatively low, these bands have to be further validated by amplifying the DNA from more number of dwarf genotypes. Lopez *et al.* (1997) also found a specific band for the polyembryonic cultivar using the primer OPM12, a size of 550 bp. Similarly the primers OPX9, OPX10, OPF4 and OPC2 produced specific bands with a size ranging 200 – 300 bp in multiple flowering clones (Damodaran *et al.*, 2007). The bands were absent in the single flowering clones.

If the amplicons for Vellaikolamban are found to be associated with dwarfness, they may be eluted and sequenced to develop a SCAR (Sequence Characterized Amplified Region) marker for dwarfness in mango.

From the cluster analysis, the least dissimilarity was showed between Bappakai and Kurukkan followed by Kurukkan and Vellaikolamban. The highest dissimilarity was between Muvandan and Chandrakaran followed by Muvandan and Kalapady.

From the experiment conducted on "Marker assisted characterization of mango (*Mangifera indica* L.)" the characters such as leaf length, width and area, internodal length, length and number of roots, dry matter content and phloem – xylem ratio can be used for the classification of dwarf plants.

In the molecular studies the primer RPI 03 amplified several bands which distinguish between the tall (Kalapady) and dwarf (Vellaikolamban). It can be used as a marker for dwarfness after making the further clarifications.

Table 28. Specific bands identified for different varieties

Cultivar No.	Name of cultivar	Number of specific amplicons	Primer	Approximate size of the amplicons
V_1	Bappakkai	4	OPA 11	750bp
			OPA10	>1000bp two bands
			RPI 01	>1000bp
V_2	Chandrakaran	3	OPA 11	450bp
			OPA 10	300bp
			OPX 17	600bp
V_3	Creeping	4	OPX 01	4268bp
			OPM 20	>1000bp
			RPI 01	>1000bp
			RPI 03	>1000bp
V_4	Kalapady	8	OPX 01	3500bp,1904bp,450bp
			OPA 11	>1000bp, 250bp
			RPI 03	>1000bp,500bp,350bp
V_5	Kurukkan	2	OPX 01	210bp
			RPI 03	>1000bp
V_6	Muvandan	1	RPI 03	>1000bp
V_9	Rumani	4	OPX 08	350bp
			OPA10	350bp
			OPM 20	1000bp
			RPI 03	575bp
V_{10}	Vellaikolamban	2	RPI 04	400bp
			RPI 03	450bp

SUMMARY

6. SUMMARY

The experiment entitled "Marker assisted characterization of mango (*Mangifera indica* L.)" was conducted during 2013 – 2015 at the College of Agriculture, Padannakkad. Objective of the study was to identify the markers associated with the dwarfness in mango *viz.* morphological, anatomical, physiological and molecular (RAPD). A pot culture experiment was laid out in completely randomized design (CRD) with eight genotypes. Each genotype was replicated thrice. The varieties included were V_1 (Bappakai), V_2 (Chandrakaran), V_3 (Creeping), V_4 (Kalapady), V_5 (Kurukkan), V_6 (Muvandan), V_9 (Rumani) and V_{10} (Vellaikolamban).

The morphological characters recorded other than the plant height in the present study were number of leaves, leaf length, width of leaves, average leaf area, total leaf area, internodal length, number of roots, root length, dry matter content of shoots and roots. The least plant height was recorded in Vellaikolamban while the maximum was in Kalapady. The lowest number of leaves was recorded by Vellaikolamban and it had a high positive correlation with the plant height. The width, length as well as area of the leaf were lowest in Vellaikolamban; all these parameters showed a high positive correlation with its plant height. The least internodal length (3.2 cm) was recorded in Vellaikolamban. Number as well as length of root was positively correlated with the height. The dry matter content of stem was more correlated with the plant height than the root. The dry matter accumulation in stem was less in Vellaikolamban (26.67g) and more in Rumani (108.33g).

Among the anatomical characters, stomatal density showed a weak positive correlation with the plant height. The phloem xylem ratio was negatively correlated with the plant height. Vellaikolamban recorded the highest phloem xylem ratio of 0.78. The bark percentage was positively correlated with the plant height, but the correlation was weak and non significant.

In the case of physiological parameters like phenol content and relative water content, the correlations were not significant. .

The morphological observations plant height, number of leaves, leaf length, width of leaves, average leaf area, total leaf area, internodal length, dry matter of shoot and dry matter of root were highly correlated with the plant height and could be used for the early detection of dwarf plants. These characters showed a positive correlation with the plant height.

The anatomical characters except phloem – xylem ratio showed weak correlations with the plant height. Phloem – xylem ratio was negatively correlated with the plant height and the correlation was strong. It is a good marker for the identification of dwarfness.

Physiological parameters *viz.* phenol content and RWC might not be used for the classification of dwarf genotypes. The phenol content was negatively correlated with the plant height, but it was a weak marker and the RWC was not significant between the varieties.

Eight varieties were classified as tall, semi tall and dwarf based on various morphological, anatomical and physiological characters. Vellaikolamban with a total score of 47 was classified as dwarf. Bappakai, Chandrakaran, Creeping, Kurukkan, Muvandan and Rumani were classified as semi tall and Kalapady as tall.

Specific bands with a size of 450 bp and 400 bp were identified in Vellaikolamban by RAPD analysis using the primers RPI 03 and RPI 04 respectively. Further studies are required to confirm whether it corresponds to the dwarf character.

Future line of work

- The present study was a preliminary work for the identification of dwarf specific markers. Detailed studies are required for the further clarification of the markers.
- Screening of more number of dwarf mango varieties using more number of RAPD primers including the primers RPI 03 and RPI 04.
- Development of SCAR markers from the dwarf specific bands
- Screening of dwarf mango genotypes using co-dominant markers such as SSR marker

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Marker assisted characterization of mango (*Mangifera indica* L.)

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ABSTRACT

The experiment entitled "Marker assisted characterization of mango (*Mangifera indica* L.)" was conducted during 2013 – 2015 at the College of Agriculture, Padannakkad, with an objective to identify markers associated with dwarfness in mango. Different morphological, anatomical, physiological and molecular (RAPD) parameters were included in the study. The experiment was laid out in completely randomized design (CRD) with eight varieties , i.e. Bappakai (V_1), Chandrakaran (V_2), Creeping (V_3), Kalapady (V_4), Kurukkan (V_5), Muvandan (V_6), Rumani (V_9) and Vellaikolamban (V_{10}).

The morphological characters studied were plant height, number of leaves, leaf length, width of leaves, average leaf area, total leaf area, internodal length, number of roots, root length, dry matter of shoot and dry matter of root. The least plant height was recorded in V_{10} (Vellaikolamban) while the maximum was in V_4 (Kalapady). The number of leaves was less in V_{10} (Vellaikolamban) and it had a high positive correlation with the plant height. The lowest width, length as well as leaf area were also recorded by Vellaikolamban and all the parameters showed high positive correlations with the plant height. The internodal length showed a positive correlation with the plant height and the lowest was recorded by Vellaikolamban (3.2 cm). Number as well as length of root was positively correlated with the height. Dry matter accumulation in the stem was less in Vellaikolamban (26.67g) and more in Rumani (108.33g) and the dry matter content of stem and roots were positively correlated with height.

From the analysis of anatomical characters, stomatal density showed a weak positive correlation with the plant height. The phloem – xylem ratio was negatively correlated with the plant height and Vellaikolamban recorded the maximum phloem – xylem ratio of 0.78. The bark percentage was positively correlated with the plant height but the correlation was weak and non significant.

Among the physiological parameters, phenol content was negatively correlated with the plant height. But the correlation was weak and non significant. The relative water content was found to be not significant.

Based on various morpho-anatomical and physiological characters, Vellaikolamban was classified as dwarf.

From the molecular studies using RAPD marker, specific bands with a size of 450 bp and 400 bp were identified in Vellaikolamban using the primers RPI 03 and RPI 04 respectively. These bands might be associated with the dwarf character in mango.