MORPHOMOLECULAR CHARACTERIZATION AND EVALUATION OF *Pandanus* spp.

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DECLARATION

I hereby declare that this thesis entitled **"Morphomolecular characterization and evaluation of *Pandanus* spp."** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that this thesis entitled **"Morphomolecular characterization and evaluation of *Pandanus* spp."** is a record of research work done independently by **Smt. T. Thangaselvabai (2001-22-02)** 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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Dedicated to

My former Guide
Dr. G. Sreekandan Nair

&

My Loving Father
Sri. K. Thiraviam

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CONTENTS

LIST OF TABLES

LIST OF TABLES CONTINUED

LIST OF TABLES CONTINUED

LIST OF FIGURES

LIST OF FIGURES CONTINUED

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

µl	–	Micro litre
µm	–	Micro molar
AC	–	Activated charcoal
AFLP	–	Amplified Fragment Length Polymorphism
BA	–	Benzyl adenine
BAP	–	Benzyl aminopurine
bp	–	Base pair
CD	–	Critical difference
cm	–	Centimetre
CRD	–	Completely Randomized Design
DNA	–	Deoxy ribonucleic acid
dNTPs	–	Deoxy nucleotide
EDTA	–	Ethylene diamino tetra acetic acid disodium salt
GA	–	Genetic Advance
GA_3	–	Gibberellic acid
GCV	–	Genotypic coefficient of variation
H^2	–	Heritability
IAA	–	3- indole acetic acid
IBA	–	3-Indole butyric acid
KCl	–	Potassium chloride
kg	–	Kilogram
KIN	–	Kinetin
M	–	Molar
m	–	Metre
$MgCl_2$	–	Magnesium chloride
ml	–	Millilitre
MS	–	Murashige and Skoog (1962)
1/2 MS	–	Half strength Murashige and Skoog

LIST OF ABBREVIATIONS CONTINUED

NAA	–	α - Naphthalene acetic acid
NaCl	–	Sodium chloride
ng	–	Nanogram
NS	–	Not significant
OD	–	Optical density
PCR	–	Polymerase chain reaction
PCV	–	Phenotypic coefficient of variation
PM	–	Pico Molar
PMBBC	–	Plant Molecular Biology and Biotechnology Centre
PVP	–	Poly vinyl pyrrollidone
RAPD	–	Random Amplified Polymorphic DNA
RFLP	–	Restriction fragment length polymorphism
SCAR	–	Sequence characterized amplified region
SE	–	Standard error
SFAC	–	Small Farmer's Agri-business Consortium
SSR	–	Simple sequence repeats
STMS	–	Sequence tagged micro satellite sites
TAE	–	Trisacetic acid EDTA
TE	–	Tris HCL-EDTA
Tris-HCL	–	Tris (hydroxy methyl) amino methane hydrochloride
TSS	–	Total soluble solids
UPGMA	–	Unweighted pair group method arithmetic average

Introduction

1. INTRODUCTION

Pandanus or screwpine is one of the under exploited dominant plant species in the coastal vegetation of India. The plant form thickets along seashore, banks of rivers, ponds, canals etc. It is known as *ketaki* in Sanskrit, *kewda* or *keora* in Hindi, Marathi and Gujarathi, *kiya* in Oriya and Bengali, *mogali* in Telegu, *kaitha* in Malayalam and *thazha* in Tamil. It belongs to the family Pandanaceae, which is the sole member of the order Pandanales in the monocotyledoneae (Stone, 1976). Over 500 species are known in this largest genus *Pandanus*, of which 36 species have been recorded in India (CSIR, 1966b).

Pandanus has a tropical distribution extending from Western Africa to Eastern Polynesia. The most common species in India is *Pandanus fasicularis,* which is native to South and Peninsular South East Asia. In India, it is widely distributed in coastal districts of Orissa, Andhra Pradesh, Tamil Nadu, Kerala, Gujarat and some districts of Uttar Pradesh.

The plant constitutes the backbone of the local economy by way of providing the raw material for perfume, cottage and pharmaceutical industries. These industries will provide high profit margin with low capital investment. *Pandanus* is ecologically very important since its root system binds soil and checks soil erosion (Panda *et al.*, 2001).

The male inflorescence is highly valued for the fragrance emitted by the stamens and the spathes. Valuable attar and oil extracted from the male inflorescence are the most popular perfumes used in India since ancient times. They blend well with all types of fancy perfumes and used in scenting clothes, lotions, soaps and cosmetics, hair oils and agarbatti. The attar and oil are also used for flavouring various foods, sweets, syrups, soft drinks and tobacco. The fruits from female inflorescence are used for the extraction of alcohol.

Leaves of *Pandanus* are good paper making material and the cured leaves are used as raw material for mat weaving enterprise. Various fancy articles *viz.*, cordages,

hats, baskets, handbags etc. are prepared from the leaves. The root and stem fibre are also employed in cottage industries for making various articles (SFAC, 1997). Different parts of *Pandanus* are also known for their use in traditional medicines of Ayurveda, Siddha and Unani. Traditional knowledge about this crop is presented in Appendix I.

Though *Pandanus* is widely distributed in coastal parts of India, its commercial exploitation is limited in Kollam and Thrissur districts of Kerala and Ganjam district of Orissa. The total area under *Pandanus* in Kerala is about 6,033 ha and the production of mats from the leaves per year is 134.41 lakhs (SFAC, 1998). The area in the state of Orissa is about 4800 ha with in an aerial distance of 8 to 10 km from the seacoast (Misra *et al.*, 1998). The share of *Pandanus* attar in the total attar trade is more than 50 per cent amounting to Rs.45 to 50 crores (Rao, 2000).

Pandanus is highly polymorphous and has been described under several names. It includes numerous varieties and forms. Although, the wild growing population of *Pandanus* has a great measure of variation in morphological and qualitative characters, a detailed study of these variants and the edaphic, climatic and other factors responsible for this variation is still lacking. For utilizing these species and for the selection of superior strains a critical characterization and evaluation based on their morphology, anatomy and qualitative traits is essential.

The biosphere remains in a continued interaction with changing geosphere and permits acquiring novel genetic traits, many of which may be even undetectable at a morphotypic level. With the advent of molecular markers, a new generation of markers has revolutionized the entire scenario of biological sciences. DNA based molecular markers have acted as versatile tools and have found their own position in various fields like taxonomy, physiology, embryology, genetic engineering etc.

The discovery of PCR (Polymerase Chain Reaction) is a landmark in this effort and proved to be an unique process that brought about a new class of DNA profiling markers. This facilitated the development of marker based gene tags, map

based cloning of agronomically important genes, variability studies, phylogenic analysis, marker assisted selection of desirable genotypes etc. These DNA markers offer several advantages over traditional phenotypic markers, as they provide data that can be analysed objectively (Khanuja *et al.*, 1998). Random Amplified Polymorphic DNA analysis technique holds great promise for genome analysis and detects high levels of polymorphism (Vuylsteke *et al.*, 1998). These studies will be helpful for the effective exploitation of the diversity in the genus *Pandanus*.

Generally *Pandanus* flowers are collected from the natural growing populations since it takes nearly four to five years for flowering. Hence for eliminating the long juvenile phase and for the mass multiplication of the crop, standardization of suitable propagation technique is highly essential.

Despite the potential of this species as a source of raw material for perfume, cottage and pharmaceutical industries, meagre information is available regarding the genetic variability, selection of elite genotypes and their rapid mass multiplication through different propagation techniques. Though the leaves are utilized in cottage industries for mat weaving purpose, no systematic and scientific study is conducted regarding the curing of leaves. Hence the present investigation was carried out with the following objectives:

1. To assess the genetic diversity at inter and intra species level among the *Pandanus* genotypes collected from different locations of Kerala and Tamil Nadu based on morphological, anatomical and molecular markers.
2. To study the variability between male genotypes of *Pandanus* based on morphological, anatomical and biochemical characters.
3. To quantify the genetic distance between the male genotypes of *Pandanus* based on multivariate analysis (D^2 statistic).
4. To select best genotypes for higher foliage and flower yield of better quality.
5. To standardize the propagation techniques for rapid mass multiplication.
6. To standardize the curing technology of leaves in order to obtain quality fibre and mat.

Review of Literature

2. REVIEW OF LITERATURE

Pandanus exhibits wide variation in nature with regard to vegetative characters, floral morphology and biochemical constituents. Knowledge on the pattern of genetic variation enables the breeder to know the magnitude of genetic variation available within them for selection and crop improvement. Morphological, biochemical, anatomical and molecular characterizations by using a wide range of characters and molecular markers are highly needed in *Pandanus* for the selection of superior genotypes with higher yield and better quality.

Published reports on *Pandanus* regarding variability are scanty, hence review of relevant works on some related medicinal and aromatic plants are reviewed in this chapter.

2.1 MORPHOLOGICAL VARIABILITY

Morphological characters have been the main criteria by which plant genotypes have been identified and it is also employed to assess the variability between genotypes. Most of the crop plants are essentially classified into varieties on the basis of characters such as growth habit, leaf and floral morphology.

Jagadev *et al.* (1993b) conducted survey in the natural plantations of *Pandanus* in Orissa, India and based on morphological variability present, selected six morphotypes, which exhibited distinct variation in 19 important characters, particularly flower bearing, weight, colour and aroma of flower and in adaptability to location, land type and soil texture. Flower production, the most important economic trait was maximum in dark green small thorny type followed by dark green large thorny type and very poor in dwarf type, weight of flower was highest in thornless type whereas aroma of flower was strongly scented in dark green large thorny type.

Alikhan and Muthusamy (1969) spotted out five morphological variants in *Jasminum auriculatum* and these differed in important floral characters like diameter of open flower, weight of flower bud, corolla tube length, fruit set and hetero style.

In *Jasminum sambac* a total of 15 accessions were studied for variability in morphological traits, with particular reference to distinguishing characters of economic value to the flower trade. There were marked differences among them in respect of length of pedicel, length of corolla tube and size and shape of flower bud, which are important characters of economic value in flower trade (Alikhan *et al.*, 1970). Considerable morphological variation in vegetative and floral characteristics of *Jasminum auriculatam* and *J. grandiflorum* from different states of India was reported by Rao and Muthuswami (1972); Muthuswamy *et al.* (1972); Rao and Divakar (1981).

Indiresh and Farooqi (1990) observed variations in pin and thrum type of open pollinated progenies of *Jasminum auriculatum.* For most of the morphological and floral characters studied, thrum type flowers are superior to pin types.

Wide variation for leaf and inflorescence morphology was reported in *Ocimum basilicum* germplasm collections (Darrah 1974; Borodkin and Girenke 1982)

Singh *et al.* (2002a) observed significant morphological variations for plant height, girth, inter nodal length, leaf and inflorescence characters of 14 accessions of *O. basilicum* and based on their morphological features, categorized them into ten morphotypes that were easily distinguishable.

Wetzel *et al.* (2002) observed morphological variations in 257 accessions of *Ocimum* including species, varieties and sub species. In general the leaves showed very high variation in terms of shape, size and colour.

Appreciable variation in vegetative and floral morphology was recorded in the population of single and double types of tuberose by Nambisan and Krishnan (1983).

Nazarenko (1985) studied the floral morphology of oil-bearing rose types and observed greatest variation for number of flowers per plant. Slightly less variation in flower weight, the frequency of double flowers and essential oil content was noticed.

Sethi and Maheshwari (1985) recorded significant variation for plant height, leaf width and leaf length in 63 populations of *Cymbopogon martinii* collected from Madhya Pradesh and Maharashtra.

Shylaraj and Thomas (1992) observed few accessions with entirely different morphological attributes and aroma, while screening the germplasm bank of *Cymbopogon flexuosus* at the Aromatic and Medicinal Plants Research Station, Odakkali, Kerala.

Jagadev *et al.* (2001) observed lot of variations in relation to plant height, colour of stem and inflorescence, leaf sheath and node in palmarosa.

Popova and Peneya (1987) reported significant variation for 14 morphological characters in 15 wild, one diploid and two tetraploid populations of chamomile (*Chamomila recutita*).

A study of 30 varieties and forms of diverse ecological and geographical origin of coriander revealed differences in number of rosette leaves, stem length, leafiness, umbel structure and fruit size. The varieties and forms studied were divided into six morphological groups (Rzhanova and Romanyak, 1987).

Komarova and Shasilova (1988) studied the morphological diversity occurred in *Anethum graveolens* belonging to eleven geographical regions of USSR and observed specific variation in leaf size, length and number of terminal segments of the leaves and in the structure of the seed and inflorescences.

Baghdadi *et al.* (1989) recorded inter and intra specific variation in morphological characters of the leaves, spines, flowers and in plant height of *Lycium schweinfurthii* and *L. shawii.*

Brophy *et al.* (1994b) identified two morphotypes of *Murraya paniculata* namely big leaved and small leaved types. They differ greatly in their growth habit, plant height, leaf, flower and fruit size, pedicel length and odour etc. Enormous variability was found for plant height, leaf length, leaflet numbers, main stem thickness, and branches per plant in diverse origin germplasm lines of curry neem *(Murraya koenigii)* by Lal *et al.* (2001b).

Prakash and Krishnan (1994) observed variation in various accessions and inter varietal hybrids of *C. forskohlii* at different stages of growth.

Lal *et al.* (1999) recorded considerable amount of natural variability in 18 morphologically distinct accessions of vetiver grass for nine metric traits, *viz.*, plant height, tillers per plant, leaf width, root depth, root girth, fresh and dry root yield, oil content and oil yield whereas, Lal (2000) observed variability in morphometric traits of 45 indigenous and exotic collections of vetiver.

Padmesh *et al.* (1999) conducted morphological scoring of 35 accessions of *Andrographis paniculata* for a set of characters like average plant height, number of branches per plant, average length of fruits, average number of seeds per fruit and weight of seeds. The data revealed moderate distribution of variation in observed characters.

Six accessions of itching beans *(Mucuna pruriens)* collected from Tamil Nadu and Kerala were evaluated for agro botanical traits like plant height, leaf area number of branches, pod length, pod weight, seed weight and seed recovery percentage and significant variation was observed among them (Vadivel and Janardhanan, 2000). According to Bayram (2001) sufficient variability was present for the traits like plant height, yield of fresh

herbage, and drug herbage in Anatolia sage *(Salvia fruiticosa* Mill.*)* collected from 17 different location in Turkey.

D' Antuono *et al.* (2002) evaluated 85 populations of sage (*Salvia officinalis* L.) based on morphological and biochemical characters. Very high variability was observed among individual plants for all characters.

Ojeda *et al.* (2001) evaluated the variability among natural populations of peperina (*Minthostachys mollis)* an aromatic species from Argentina and observed substantial variability in plant height and leaf characteristics.

Geetha and Maiti (2002) observed considerable morphological variability in *Chlorophytum borivilianum* collections maintained at the National Research Centre for Medicinal and Aromatic plants.

Fifteen selections of rosemary germplasm were evaluated for morpholigical, phenological and essential oil composition in Italy by Mulas *et al.* (2002) and high variation for habit, vigour, leaf size and phenology was observed.

Paisooksantivatana *et al.* (2002) observed morphological variation in relation to habitats of *Curcuma alismatifolia* in Thailand.

Bhatia *et al.* (2003) found distinguishing morphological characters in certain Indian species of *Swertia.*

Fifteen accessions of *Asparagus* species maintained in glass house conditions at CIMAP were evaluated for morphological characters estimating phenotypic diversity. Remarkable variation was observed at inter and intra specific level in terms of plant height and habit forms *viz.*, climbing herbs, short herbs, dense shrubs and short shrubs (Shasany *et al.*, 2003).

2.2 BIOCHEMICAL / QUALITATIVE VARIABILITY

The *Pandanus* perfume industry has great potential for expansion due to the increasing global demand of fragrance and flavour materials. It necessitates the study of variation in oil yield and quality and selection of superior clones for higher oil recovery and good quality perfume production.

2.2.1 Essential oil of *Pandanus*

The essential oil yield from male flowers of *Pandanus odoratissimus* ranged from 0.015 to 0.035 per cent and the major constituent was methyl ether of β - phenyl ethyl alcohol (65 to 80 per cent) which is responsible for the characteristic aroma of the flowers (Deshpande, 1938; Dhingra *et al.*, 1954).

According to Dhingra *et al.* (1954) the other constituents identified from the *Pandanus* essential oil were dipentene 8.31 per cent, d – linalool 18.70 per cent, phenyl ethyl acetate 3.48 per cent, citral 1.82 per cent and acids as caproic acid 0.49 per cent and stearoptene 0.20 per cent.

Sadagopal (1959) obtained oil content of 0.1 to 0.3 per cent from the male flowers of *Pandanus fasicularis* and determined its physico-chemical properties. The major constituent of oil (65 to 80 per cent) was methyl ether of β-phenyl ethyl alcohol. Other minor constituents were dipentene, α-linalool, pheneyl ethyl alcohol, citral and some terpene esters.

According to Nigam and Ahmad (1992) the yield of essential oil of *Pandanus odoratissimus* was 0.03 per cent by hydro distillation of its flowers. On the basis of gas chromatography the oil was found to contain 74.67 per cent of phenyl ethyl methyl ether, phenyl ethyl alcohol (16.23 per cent) and phenyl ethyl acetate (2.76 per cent) as the major constituents along with some other trace constituents.

Capillary gas chromatography of essential oil from *Pandanus* flowers showed presence of five major components, methyl ether of β-phenyl ethyl alcohol (70 per cent) terpinene 4 – ol (17 per cent) α - terpineol (18 per cent),

y–terpinene (1.5 per cent) and cineole (10.8 per cent). Phenyl ethyl alcohol and phenyl ethyl acetate were found in traces (Maheswari, 1995).

From gas chromatographic and GC–MS analysis of the essential oil of *Pandanus odoratissimus*–methyl ether of β - phenyl ethyl alcohol (PEME) and Terpinene–4–01 were identified as major compounds (Bisht *et al.*, 1997; Misra and Rao, 1997; Rao, 2000).

The methoxyl values of essential oil of *Pandanus odoratissimus* was found to be in the range 15.4 t to 18 per cent. The hydroxyl values varied between 0.9 to 2.27 per cent, the total alcohol content calculated as terpinene –4-ol varied in the range 8.0 to 22.0 per cent (Misra and Rao, 1998).

2.2.2 Essential oil of *Ocimum* spp.

Ocimum kilimandscharicum Guerke yields light yellow coloured oil with a strong odour of camphor. The camphor content varies from 61.0 to 80.5 per cent. Leaves contain the maximum amount of oil and camphor followed by flower. Stems contain only minute quantities of oil and camphor (CSIR, 1966a).

Components of essential oil of *Ocimum kilimandscharicum* grown in Rwanda showed 62 per cent 1, 8 – cineole, indicating the occurrence of a new chemotype of the species concerned (Ntenzurubanza *et al.*, 1984).

Analysis of the essential oil of *Ocimum kilmandscharicum* was done by Charles and Simon (1992). Seventeen constituents were identified in oil obtained from leaves and flowers, the composition of which were quite similar with linalool as the major constituent.

Ocimum canum Sims. yields a volatile oil with methyl cinnamate, d-camphor and citral as the principal constituents (Xaasan and Raxmann, 1981).

Studies on the percentage composition of the essential oil of *Ocimum americanum* from Ethiopia was done by Asfaw and Abegaz (1989). The plant yielded linalool (15.35 per cent), camphor (15.1 per cent) and

terpinen–4-ol (17.65 per cent). It exists in three chemotypes *viz.*, linalool type, camphor type and citral type (Gulathi, 1989; Vimalan *et al.*, 1989).

Ocimum gratissimum L. contains thymol and eugenol as the principal component and exists in three chemotypes *viz.*, eugenol, thymol and citral type (Gulathi, 1989).

According to Kaladharan *et al.* (1990) the flower oil of *Clocimum* tends to have a relatively lower eugenol content than leaf oil. Bhattacharya *et al.* (1996) conducted gas chromatographic analysis of oils of *Ocimum gratissimum* and *Ocimum sanctum* grown under semi arid tropical climate of Andhra Pradesh and observed limonene (1.98 per cent), eugenol (87.68 per cent), β - caryophyllene (1.84 per cent) and β- selinene (2.82 per cent) as the major compounds. According to Pino *et al.* (1996) thymol was the main constituent in leaf and flower oils of *O. gratissimum* grown in Cuba.

Studies on the composition of the essential oil of *Ocimum gratissimum* from Bangladesh was done by Yusuf *et al.* (1998). Forty two components were identified that represents 97 per cent of the whole oil. The main component was thymol followed by β - terpinene and P – cymene.

Vimalan *et al.* (1989) reported seven chemotypes of *O. basilicum* based on the variation in oil composition. It has been utilized as a chemical index for fine classification.

Lawrence (1992) made extensive studies on basil and on the basis of chemical composition of the oil, recognized four distinct chemotypes.

Suchorska – Tropilo and Osinska (2001) analysed five forms of sweet basil from Germany, Romania, Hungary and Egypt for morphological, developmental and chemical variations. The oil content varied from 0.10 to 0.55 per cent and on the basis of the chemical composition of oil, all forms, with the exception of the Egyptian one, was classified as the European type.

Singh *et al.* (2002a) recorded highly significant variations in the oil content and essential oil composition of sweet basil. Oil content in the flowering tops on fresh weight basis varied from 0.25 to 0.70 per cent.

Eight genotypes belonging to seven *Ocimum* species varied greatly for herb and oil yield (Verma *et al.*, 1998a). The composition of essential oils from the *Ocimum* gene bank collections of Germany showed enormous variability (Kruger *et al.*, 2002). Wetzel *et al.* (2002) observed variation in essential oil content and composition of 257 accessions of *Ocimum* including species, varieties and subspecies.

2.2.3 Essential oil of *Mentha* spp.

Punia *et al.* (1987) screened four species of mint for their oil content. Kokini and Vokou (1989) analysed oil content of wild populations of *Mentha spicata* and the oil content ranged from 0.3 to 2.2 per cent. Four different chemotypes were distinguished, characterized by large proportions of linalool, piperitone oxide, carvone–dihydro carvone and pulegone–methone–iosmethone.

Thirteen genotypes belonging to five *Mentha* spp. were evaluated for herb and oil yield (Verma *et al.*, 1998b). High range of variability was observed for herb yield, oil content (0.37 to 0.65 per cent) and oil yield (74.1 to 209. 7 tonnes per hectare).

Kumar *et al.* (1999b) screened superior genotypes of menthol mint (*Mentha arvensis)* for high yield of herbage and essential oil under late cropping conditions of the sub tropical Indo Gangetic plains.

Variation in essential oil content and its composition between cultivars and species of *Mentha* was observed by Bahl *et al.* (2000). The oil content was high in *Mentha arvensis* cultivars and the essential oil of *M. spicata* was rich in carvone.

Kumar *et al.* (2000) studied the essential oil profiles of Indian menthol mint cultivars at different stages of growth and found variation in oil

content and its composition with regard to different cultivars at different stages of growth.

Six cultivars of menthol mint *(Mentha arvensis)* were examined by Sushilkumar *et al.* (2000) for their essential oil content and their terpenoid profiles. The results revealed that the different cultivars were comparable with regard to the oil content and contents of eight important terpenoids of the oil.

2.2.4 Essential Oil of Other Aromatic Crops

Akimov and Rabotyagov (1987) observed biochemical heterogeneity based on essential oil content in *Lavandula angustifolia* and *L. latifolia.*

Three cultivars of *Lavandula hybrida* were compared for essential oil content (Marotti *et al.*, 1989). It was found that, cultivar Abrialis had the highest oil content (1.71 per cent) and oil yield (174.4 kg/ha) although its inflorescence and flower yields were the lowest.

Mallavarapu *et al.* (2000) analysed essential oils of lavender *(Lavendula angustifolia* Mill.*)* produced in Kashmir and Kodaikanal by GC method and identified 44 constituents representing 97.7 per cent of the oil from Kashmir and 37 constituents representing 98.2 per cent of the oil from Kodaikanal. Quantitative variation in the constituents was observed from the oils of these two locations.

The existence of various chemical types of *Cinnamomum tamala* in different regions of the North West Himalayas were identified by Bradu and Sobti (1988).

Cinnamomum parthenoxylon variants growing in North East India were studied by Baruah and Nath (2000). Leaves and stem bark of both the variants contained essential oil ranging from 3 to 5 per cent and 0.2 to 0.5 per cent respectively. The compound 1,8 – cineole was detected as the major oil

component in one variant, while linalool was the major oil component in the other variant, indicating the status of the individual variants as chemotypes.

Kennedy *et al.* (2000) evaluated five cinnamon accessions for essential oil content and recorded variation among them. Sel.44 recorded highest leaf oil, while Sel. 189 recorded highest bark oil.

Pathania *et al.* (1988) observed greatest variation for curcumin content (0.28 to 8.76 per cent) while examining 23 varieties of *Curcuma longa* for fourteen agronomic and quality characters.

Garg *et al.* (1999) examined 27 accessions of *Curcuma longa* and major constituents of the oil. The oil content varied between 0.16 per cent of 1.94 per cent on fresh weight basis. Based on the composition of the essential oil the accessions were classified into two groups.

Martonfi and Cernaj (1989) found variation in the essential oil composition of 63 *Teucrium chamaedrys* populations and the variation encountered was due to environmental factors rather than genetic factors.

Five accessions of davana (*Artemisia pallens* Wall.) were evaluated for their oil content of flower heads and herbage. The oil content ranged from 0.24 to 0.37 per cent in flower heads and 0.09 to 0.11 per cent in herbage (Farooqi *et al.*, 1990).

Quantitative and qualitative differences were observed in the essential oil content and composition of *Artemisia absinthium* accessions from the same geographical location (Nin *et al.*, 1995).

Kuriakose (1990) analysed 15 *Cymbopogan flexuosus* types and recorded high oil recovery from the white types (0.439 to 0.731 per cent) and low from the light red and red types, except the light red type 19 which had an oil recovery of 1.5 per cent.

While comparing five commercial *Cymbopogon* species *viz.*, *Cymbopogon martinii* var. Motia, *C. citratus, C. pendulus, C. flexuosus and C. winterianus* for herb and oil yield, the performance of *C. martinii* var.

Motia was significantly higher than *C. pendulus* and *C. flexuosus* (Rahman *et al.*, 1992).

In a comparative evaluation trial with palmarosa, jamrosa and *C. flexuosus* accession IW. 455, IW.455 excelled in herbage, oil, geraniol yield and oil recovery percentage over jamrosa and palmarosa (Shylaraj and Thomas, 1992).

Jagadev *et al.* (2001) observed variation in total oil yield and oil content of inflorescence of nine types of palmarosa (*Cymbopogon martinii*).

Studies conducted by Lal (2002) revealed that, selection based on higher fresh biomass yield followed by oil content will definitely lead to increased oil yield of better quality in Java citronella.

Abegaz *et al.* (1993) found variation in physical and chemical composition of essential oil extracted from leaves and flowers of wild and cultivated forms of *Lippia adoensis.* Fourteen compounds representing 88 to 95 per cent of the oil from the cultivated plants and 16 compounds constituting 68 to 86 per cent of the oil from wild plants were identified.

Juliani *et al.* (2002) assessed the essential oil variability of *Lippia junelliana* collected from 16 different sites of Argentina and detected four chemotypes based on the variability in oil content and oil composition. The oil was dominated by monoterpenes with low amount of sesquiterpenes and phenyl propanoids.

Brophy *et al.* (1994a) observed quantitative and qualitative differences in essential oil of *Asteromyrtus* spp. and it could be used to differentiate the species.

According to Brophy *et al.* (1994b) two morpho types of *Murraya paniculata* differ quantitatively in volatile leaf oil composition.

Butcher *et al.* (1994) reported significant variation in essential oil yield and its composition of 11 populations of *Melaleuca alternifolia.* Trees from north western populations produced a terpinolene rich oil while eastern

population produced an oil with either terpinen-4-ol or 1-8-cineole as the major component.

Homer *et al.* (2000) studied the composition and yield of essential oils from 615 populations of *Melaleuca alternifolia* and identified six chemotypes.

Kokini *et al.* (1994) found variation in essential oil content of *Origanum vulgare* with respect to different geographical locations in Greece.

Misra *et al.* (1994) observed wide variations in the chemical composition of essential oil of *Coleus forskohlii* genotypes. Patil *et al.* (2001) observed variation in essential oil content of six diverse genotypes of *C. forskohlii.*

Significant differences in essential oil yield and its component traits was observed in 12 cardamom clones by Korikanthimath *et al.* (1999b).

Bayram (2001) observed variation in essential oil content and the most important oil component (15.96 to 75.50 per cent) of 17 diverse populations of Anatolia sage (*Salvia fruiticosa* Mill.) in Turkey.

D'Antuono *et al.* (2002) observed variation in essential oil composition of 85 populations of sage (*Salvia officinalis* L.).

According to Ojeda *et al.* (2001) there was significant variation in essential oil content of peperina (*Minthostachys mollis)* natural populations. The pulegone content ranged from 3.90 to 65.10 per cent.

Mulas *et al.* (2002) observed variation in essential oil content (1.18–4.07 per cent) and components among the genotypes of rosemary germplasm in Italy.

Bhatia *et al.* (2003) conducted chemotypic comparision of certain *Swertia* species and found that, each *Swertia* species possesses a unique chemical profile for xanthones and bitters.

2.3 ANATOMICAL VARIABILITY

Adaptation to different environmental conditions has resulted in extreme modifications of organs particularly those of the vegetative body. In order to get detailed information about the population, diagnostic characters other than gross morphology are strongly needed. Root and leaf anatomical characters have been used extensively in taxonomic and phylogenetic studies because they are very informative and reliable.

Micro morphological characters of the leaf surface have been used widely in taxonomic and phylogenetic studies. Several leaf epidermal characters associated with systematic consideration includes number of stomata, type of stomata, morphology of trichomes, leaf thickness, cuticle etc.

Manian *et al.* (2002) studied the root, stem and leaf anatomical features of *Pandanus tectorius* and observed that the lamina of the leaf is 570 to 580 nm thick with even and glabrous surface. Structurally the lamina is bilateral and amphistomatic. They also noticed the occurrence of raphides in palisade tissue and presence of one or two sub epidermal layers in the lamina.

Jarvie and Koerniati (1986) observed considerable variation in leaf anatomical characters of 11 cultivated and wild populations of *Syzygium aromaticum* from Indonesia. Three groups were distinguished, reflecting three overlapping centres of diversity.

Olowokudejo and Nyananyo (1990) examined the epidermal morphology of the species *Khaya senegalensis, K. grandifoliola, K. anthotheca* and *K. ivorensis* and observed striate, reticulate or verrucose cuticle and isodiametric or irregular epidermal cells. Anticlinal walls varied in thickness and their position were marked by raised ridges or shallow irregular grooves. All taxa exhibited anomocytic stomata which were either located superficially or slightly sunken. Stomatal size and index varied for the four species.

Variation is total plant pubescence and essential oil content was studied in *Origanum vulgare* in Greece. In respect to glandular and non-glandular trichomes, *O.vulgrace* plants were differentiated into six distinct geographic climatic groups (Kokini *et al.*, 1994).

Sherlija *et al.* (1998) studied the anatomy of rhizomes of four commercially important species of *Curcuma viz., C. longa, C. aromatica, C. amada* and *C. zedoaria.* Though all the species basically had similar anatomical characters, variations were noticed in the number and arrangement of primary and secondary vascular bundles, orientation of endodermoid layer, number and shape of starch grains and curcumin cells.

Characteristics of the foliar epidermis of the 13 *Hyocyamus* species occurring in Iran were studied. Variation in the most useful anatomical characters like stomatal occurrence, stomatal index, pattern of anticlinal wall density and type of trichomes were observed (Ghahreman *et al.*, 1999).

Stern and Judd (1999) compared the anatomy of 17 species of *Vanilla* and found enormous variation in the occurrence of uniseriate hypodermis, crystals in the foliar epidermis, raphide bundles in the mesophyll tissue and size of the stomatal apparatus.

Baruah and Nath (2000) studied micro morphological characters namely the epidermal and venation character of the two variants of *Cinnamomum parthenoxylon* and recorded no remarkable variation in quantitative data for the variants. In qualitative characters, the stomata in both the variants were hypostomatic, paracytic, randomly distributed and irregularly oriented. The epidermal cells were tetragonal to polygonal in nature.

Muthuraman and Sampath (2000) studied the pharmacognostic anatomy of siddha drug sarkaraivembu (*Scoparia dulcis* L.) and identified, undifferentiated mesophyll cells of the leaf, glandular trichomes with one stomata celled stalk and multi cellular globose head, anomocytic stomata

with wavy anticlinal walls and dense cylinder of secondary xylem and phloem and secondary xylem consists of libriform fibres and small angular vessels.

The characteristic features of the leaf epidermal morphology of *Amaranthus dubius*, A. *hybridus*, *A. lividus*, *A. spinosus*, *A. tricolor* and *A. viridis* were investigated for the systematic characterization of these taxa. The results showed significant variation in the architecture of the epidermal cells, possession of paracytic stomata and calcium oxalate crystals in the lamina (Edeoga and Otoide, 2001).

According to Edeoga (2001) eight wild species of *Dioscorea* could be differentiated from each other based on the difference in foliar epidermal cells, trichomes, vascular bundle structure and stomatal size, shape and index.

Baruah and Nath (2002) determined the taxonomic status of two chemotypes each of *Litsea cubeba* and *Clausena heptaphylla* based on physico–dermal structures of the leaves. Both the chemotypes of *L. cubeba* (cineole – citral and linalool – citronellol type) showed paracytic stomata. However, those of the linalool citronellol type are irregularly oriented and are higher in number compared to those of the cineole – citral type. The stomata in both chemotypes of *C. heptaphylla* are anomocytic and non significant variations in the qualitative data of their stomatal features were observed.

The morphology of epidermal cell walls, nature and number of epidermal cells per unit area, stomatal frequency, trichome length, and stomatal index were identified as useful parameters for the differentiations of twelve species of Zingiberaceae (Gogoi *et al.*, 2002).

Carelli *et al.* (2003) examined the presence of vascular bundle sheath cells and its relation to photosynthetic pathway and leaf anatomy in six species of the genus *Coffea*. The results showed that, in all genotypes, the

vascular bundle was surrounded by a layer of cells with numerous chloroplasts in a centrifugal position.

Nartunai *et al.* (2003) examined the leaf anatomy of *Cyphostemma setosum* and found that, the upper and lower epidermis are made up of polygonal, straight and thin walled cells with anomocytic stomata. Glandular trichomes are found with multi cellular stalk and obovoid head. Raphides are common in mesophyll tissue. Vascular strands are more prominent in the midrib than in the lamina.

2.4 MOLECULAR MARKERS

Traditionally, majority of the taxonomic and variability studies have been conducted based on morphological, anatomical and physiological features. In view of the advancement of scientific techniques which are capable of critically analyzing the biological material at molecular level, the potential of molecular tools for taxonomic and variability refinement can be very well visualized. The macro molecules which can be studied for generating distinct molecular profiles of an individual include DNA, RNA, proteins, lipids and carbohydrates. The techniques of RAPD (Random Amplified Polymorphic DNA), DFP (DNA Finger Printing), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism) etc., are being utilized practically in various plant species for such molecular marking. The important application of molecular markers include genome mapping and gene tagging, taxonomic studies, cultivar identification, genetic diversity analysis, sex differentiation, genetic fidelity analysis etc.

2.4.1 Protein Markers

The use of protein markers is based on protein polymorphism. These markers code for specific proteins and they can be visualized through gel electrophoresis.

Electrophoretic analysis of seed proteins from seven induced morphological mutants and their parental line of *Nigella sativa* revealed a wide range of variation among genotypes for band number and intensity. In a quantitative analysis, buffer soluble and acid soluble protein contents ranged from 0.0084 to 0.0189 (g/ dry seed) 0.0069 to 0.0111 respectively, most of the mutants having higher contents than the parental line (Datta *et al.*, 1987).

Agarwal and Kaul (1993) conducted seed protein analysis in eight varieties of dill (*Anethum graveolens)* by gel electrophoresis and found significant variation between varieties in seed protein content which ranged from 10.8 to 23.8. Proteins were also qualitatively different between varieties.

Tseng *et al.* (1993) conducted protein profile analysis from leaves of four species of *Aralia* and detected 454, 326, 847 and 351 numbers of proteins from *A. spinosa, A. elata, A. racemosa and A. nudicaulis* respectively. Cluster analysis via the unweighted pair group method revealed that, *A. spinosa* is more closely related to *A. elata* than to *A. racemosa* and *A. nudicalis.*

Bank *et al.* (1995) observed low levels of genetic variation within species and high degree of differentiation between species for four morphologically distinct populations of *Aloe ferox* and one population of *A. marlothii* through gel electrophoresis and the variation was assessed at 22 protein coding loci.

According to Sharma *et al.* (2000b) protein markers could not delineate *Podophyllum hexandrum* populations into region specific groups.

Singh *et al.* (2002c) determined the protein profile of different genotypes of *Catharanthus roseus* by SDS-PAGE gel electrophoresis analysis and found variation in total protein content among the six genotypes.

2.4.2 Isozyme Markers

Phylogenetic relationship of *Allium* species and their intra generic, intra specific differentiation and grouping by chloroplast DNA sequence coding and restriction enzyme analysis was reported by Havey (1991); Linne *et al.* (1996); Klass (1998).

Pooler and Simon (1993) employed morphological and isozyme assay method for intra specific classification of 110 garlic cultivars.

Mabb and Klass (1995) studied the intra specific differentiation of garlic (*Allium sativum* L.) by isozyme and RAPD markers.

Isozyme markers were reported to be used in rose for varietal identification (Kim and Byrne, 1996; Walker and Werner, 1997) and classification (Kim and Byrne, 1994).

Seven early flowering and two unidentified *Curcuma* species was studied for isoenzyme polymorphism by PAGE. Based on the polymorphism phylogenetic relationships were compared (Apavatjrut *et al.*, 1999).

Alloenzyme analysis and RAPD were used to evaluate the genomic polymorphism and heterozygosity of ginseng (*Panax ginseng*) from three extent natural populations in Russia and the results revealed very low levels of polymorphism and hetrozygosity (Zhuravlev *et al.*, 1999).

Sebastian *et al.* (2000) observed wide variation among cultivated types of *Piper nigrum* through isoenzyme analysis.

High diversity was observed from cultivated and wild populations of *Curcuma alismatifolia* in Thailand by alloenzyme polymorphism (Paisooksantivatana *et al.*, 2001).

The genetic variation among six accessions of *Baccharis myriocephala* was investigated using multivariate methods, morphological descriptors and isoenzymatic characters (Castro *et al.*, 2002).

Low levels of genetic variability was observed between seventeen populations of *Swertia perennis* by isozyme analysis (Lienert *et al.*, 2002).

Peroxidase activity was assayed in six genotypes of *Catharanthus roseus* by Singh *et al.* (2002c).

Allozyme analysis among 109 ginseng populations was conducted by Korean *et al.* (2003). He observed low level of genetic variability among the ginseng populations.

Lopes *et al.* (2003) observed high polymorphism in eight accessions of *Polygonum punctatum* by isoenzyme analysis.

Though isozyme markers provide the basis for relatively simple genetic analysis, the small number of consistently resoluable loci limits their utility. Moreover, isozymes are influenced by stages of development and the tissue used for extraction.

2.4.3 DNA Markers

Among the molecular markers used, DNA markers are more suitable and ubiquitous to most of the living organisms (Joshi *et al.*, 1999).

2.4.3.1 Restriction Fragment Length polymorphism (RFLP)

In this method, the genomic DNAs are cleaved using restriction endonucleases followed by fractionating the fragments electrophoretically and then detecting the fragments containing homologous sequences by hybridizing them to specific DNA probes. RFLP technique provides a potentially infinite number of variable markers for the development of genetic linkage maps.

Ballard *et al.* (1996) identified and constructed the genomic map of rose cultivars using random amplified polymorphic DNA (RAPD) and RFLP markers.

Mess *et al.* (1998) included 29 species of *Allium* and seven species of related genera in a pylogenetic study using amplified chloroplast DNA, PCR – RFLP.

PCR –RFLP successfully differentiated medicinal species *Codonopsis pilosula, C. langshen, C. modesta and C. nervosa* var. *macrantha* from two adulterants *Campaumoea javanica and Platycodon grandiflorus* (Zhao *et al.*, 1999).

PCR – RFLP analysis produced very different finger prints between different ginseng types and this method will be able to authenticate the concerned *Panax* species (Young *et al.*, 2001).

The RFLP probes are locus specific, resulting in an easy to screen co – dominant behaviour. However, it is relatively labour intensive, expensive and involves the use of radioactive chemicals, which are harmful to the users.

2.4.3.2 Random Amplified Polymorphic DNA (RAPD)

This method was first developed by Welsh and McClelland (1990) and Williams *et al.* (1990). This method utilizes single short oligo nucleotide primers of arbitrary sequence for the amplification of DNA segments distributed randomly throughout the genome, using polymerase chain reaction (PCR). In this reaction the primers of arbitrary sequence bind to perfect or imperfect sites in the genomic DNA, so that a subset of them will lie in inverted orientation to each other. As a result a number of bands are amplified through the action of DNA polymerase. The reaction products are conveniently analysed on agarose gels. Since this technique is being adopted in the present study, more literature in this aspect is reviewed in Medicinal and Aromatic crops.

2.4.3.2.1 RAPD for Genome Mapping and Gene Tagging

Plant improvement, either by natural selection or through the efforts of breeders, has always relied upon creating, evaluating and selecting the

right combination of alleles. With the use of molecular markers it is now a routine to trace valuable alleles in a segregating population and mapping them. Once mapped, these markers are efficiently employed in tagging several individual traits that are extremely important for a breeding programme like yield, disease resistance, stress tolerance, seed quality, secondary metabolite production etc.

Debener *et al.* (1996) reported that RAPD markers were used for the construction of chromosome linkage maps in *Rosa.* Debener and Mattiesch (1999) used RAPD and RFLP markers to construct the first linkage maps of rose genome. A total of 305 RAPD and AFLP markers were analysed in a population of 60 F_1 plants.

Grothe *et al.* (2001) characterized salutaridinol 7-0 acetyl transferase which involved in morphine biosynthesis in opium poppy.

Peng *et al.* (2002) conducted studies to analyse the sequences of the nuclear ribosomal RNA small subunit gene (18 Sr RNA) and the chloroplast mat K gene of crude drug patchouli to provide molecular evidence for the identification of patchouli drug.

Straka and Nothnagel (2002) conducted linkage analysis and genetic map construction in *Papaver somniferum* based on molecular and morphological markers. RAPD and AFLP technique were employed and 125 molecular markers were detected, 77 as AFLP and 48 as RAPD markers.

2.4.3.2.2 Genetic Diversity Analysis

Campose *et al.* (1994) studied the genomic relationship among lotus species based on random amplified polymorphic DNA.

Mabb and Klass (1995) studied the intraspecific differentiation of garlic *Allium sativum* by isozyme and RAPD markers, whereas, Bradley *et al.* (1996) studied the usefulness of RAPD markers for determining the distribution of genetic variability within and among 20 taxonomic units of Australian garlic cultivars.

The relationship of common onion to different types of shallot was investigated by RAPD markers and morphological traits (Dennequin *et al.*, 1997).

Shasany *et al.* (2000) observed low level of diversity among 23 accessions of *Allium sativum* from different geographical part of India through RAPD profiling.

Aloisi *et al.* (1996) studied the molecular polymorphism in botanical and ancient roses via RAPD analysis using 10-mer Operon primers and observed lot of variation.

The genetic diversity of the recognized varieties of *Asparagus* was carried out using RAPD markers (Khandka *et al.*, 1996; Raimondi *et al.*, 2001). Shasany *et al.* (2003) detected significant diversity among *Asparagus recemosus* (51.7 per cent) accessions and also within species (48 per cent).

Renou *et al.* (1997) studied the genetic variability in the genus *Pelargonium* using RAPD markers and found enormous variability among the *Pelargonium* genotypes.

Comparitive studies of four elite genotypes of *Vetiveria zizanioides* through RAPD analysis showed a wide range of variability among them (Shasany *et al.*, 1998). According to Lal *et al.* (2003) 40 per cent diversity was observed among 51 accessions of vetiver through RAPD profiling.

Elite and popular cultivars of *Cymbopogon martinii* were examined for genomic variability through RAPD, enzyme and SDS-PAGE protein polymorphisms (Sangwan, *et al.*, 2003). They observed wide range of molecular diversity among the cultivars in all type of analysis.

Padmesh *et al.* (1999) observed high genetic variability among 35 accessions of *Andrograrphis paniculata* collected from different phyto geographical regions.

Genetic diversity within and among population of *Afgekia sericea*, a rare medicinal species of Thailand was investigated using RAPD–PCR assays

(Prathepha and Baimai, 1999). The results revealed population polymorphism within populations ranged from 38.4 and 60.3 per cent.

Diversity among three female varieties of *Piper longum* was noticed through RAPD analysis (Philip *et al.*, 2000).

RAPD profiles for band similarity indices clearly differentiated five of the *Mentha arvensis* accessions from the rest of the accessions belongs to other species of the genus *Mentha* (Khanuja *et al.*, 2000).

RAPD profiles distinguished eight peppermint accessions of different geographical origin. However, only limited polymorphism was observed among the most widely grown pepper mint and scotch spearmint cultivars (Fenwick and Ward, 2001).

Shasany *et al.* (2002b) assessed the genetic diversity of *Mentha spicata* germplasm through RAPD analysis and observed high level of polymorphism.

Polymorphism between important cultivars belonging to different species of *Jasminum* was detected (Mukundan, 2000) using RAPD technique. The results revealed wide diversity between the species and cultivars.

Saikia *et al.* (2000) detected low level of polymorphism and close clustering with small molecular distances in *Taxus wallichiana* accession from North East India using RAPD analysis.

Darokar *et al.* (2001) examined narrow genetic base among various geographically distinct accessions of *Bacopa monnieri* by RAPD analysis. Low level of polymorphism was observed and the range of similarity was 0.8 to 1.0.

RAPD analysis was conducted in two morphologically distinct varieties of *Digitalis* namely *D. minor* var. *minor* (pubescent) and *D. minor* var. Palavi (glabrous) and found restricted population variability (Sales *et al.*, 2001).

Wagner *et al.* (2001) determined the genetic relationship between improved population of chamomile for (-)-∝ bisabolol content through RAPD and AFLP marker techniques. Genetic similarity was estimated between 0.52 and 0.91 for RAPDs and 0.58 and 0.79 for AFLPs and in a cluster analysis genotypes were grouped according to their origin.

Zhang *et al.* (2001) applied RAPD technique to distinguish *Lycium barbarum* from other closely related species of the same genus and obtained distinctive DNA finger prints corresponding to different *Lycium* species and higher similarity values between intra species than inter species.

Arnholdt-Schmitt (2002) found significant polymorphic genotypes while characterizing *Hypericum perforatum* accessions by RAPD technique.

Klocke *et al.* (2002) conducted DNA finger printing studies through RAPD analysis in three progenies of majoram pollinator lines and found heterogeneity among them.

According to Youliang *et al.* (2002) high polymorphism was observed in germplasm resources of *Herba houltyniae* in China. The 92 accessions were grouped into 14 clusters based on RAPD markers.

Aga *et al.* (2003) observed 75 per cent similarity while analysing C*offea arabica* gene pool in Ethiopia.

Genetic analysis performed with RAPD markers in *Glycyrrhiza glabra* revealed that the coefficient of similarity among populations ranged from 0.70 to 0.95. The data showed that the groups were not necessary related with their geographical origin (Agrimonti *et al.*, 2003).

RAPD analysis provided inter and intra specific variations in *Aloe* species (Darokar *et al.*, 2003).

RAPD analysis was used to access genetic diversity in basil (Vieira *et al.*, 2003). Means of genetic similarities with in *Ocimum* spp showed that, the domesticated species showed the lowest similarity.

Genetic diversity estimates of five population of *Changium smyrnioides* through RAPD analysis from China indicated that 51.2 per cent of the total diversity was among the populations and 48.8 per cent was the diversity within populations (Xin *et al.*, 2003).

2.4.3.2.3 Cultivar Identification

Torres *et al.* (1993) differentiated rose cultivars by comparing DNA banding patterns. RAPD and RFLP banding patterns were utilized to identify and differentiate 22 cultivars of rose (Ballard *et al.*, 1996).

Khandka *et al.* (1996) studied the polymorphism exhibited between *Asparagus* cultivars by random amplified polymorphic DNA.

RAPD markers were utilized in crops *viz.*, *Ocimum basilicum* var. Kusumohal (Kumar *et al.*, 1999a), *Mentha piperata* var. Pranjal (Dwivedi *et al.*, 2001a), *Mentha gracilis* cv. *cardiaca* var. Pratik (Dwivedi *et al.*, 2001b), *Mentha arvensis* var. Saksham (Khanuja *et al.*, 2001), *Catharanthus roseus* var. Prabal (Dwivedi *et al.*, 2001c) and *Panax ginseng* var Aizuk-111 (Tochika-Komatsu and Asaka, 2001) to identify and define the uniqueness of the particular variety from other varieties of their respective species.

Nine accessions of three species of the medicinal plant genus *Scutellaria* were analysed by RAPD in an effort to distinguish between members of these three species. RAPD markers produced species specific bands and this could be useful for the future identification of members of the three species (Hosokawa, 2000).

Two accessions of *Mentha spicata viz.*, CIMAP C 33 and CIMAP C 32 were distinguished clearly from each other by RAPD analysis (Khanuja *et al.*, 2000).

Different cultivars of *Pelargonium* species were identified through RAPD markers (Lesur *et al.*, 2000).

Three genotypes of *Pelargonium graveolens* namely Algerian, Kelkar and Bourbon were distinguished at genetic level at early growth stage itself through the genotype specific RAPD profiles (Shasany *et al.*, 2002a).

Pradeepkumar *et al.* (2001) analysed 24 accessions including 13 land races and 9 advanced cultivars of pepper through RAPD technique and found cultivar specific bands and this could be successfully utilized for cultivar identification in pepper.

To identify ginseng plants at DNA level, RAPD and PCR–RFLP analyses were conducted within *Panax* species. Similarity coefficients among the DNA of ginseng plant were very low ranging from 0.197 to 0.491 by RAPD analysis (Young *et al.*, 2001).

RAPD analysis distinguished different *Panax* spp. as well as the morphological variants of *P. notoginseng* by producing cultivar specific band (Cui *et al.*, 2003).

RAPD analysis distinctly differentiated yellow and blue *Aconitum* species and also separated *A. paniculatum* from all populations belonging to *A. napellus* group (Fico *et al.*, 2003).

2.4.3.2.4 Taxonomic Studies

Molecular taxonomy is the fast emerging branch of biosystematics today for precise and finer analysis to establish phylogenetic relationships among plant species. The rate of evolution of specific markers certainly discloses the type of classification that the ecotypes or forms or subspecies need. Further more these studies hold a great promise for revealing more about the pattern of genetic variation within species or forms.

Millan *et al.* (1996) analysed 19 species of rose using RAPD markers. Specific banding pattern was observed and that grouped plants belonging to the same species in to different botanical sections. Dendrograms constructed showed a good correlation with previous classifications based on morphological and karyological studies.

Taxonomy of 22 wild species of rose was analysed using isoenzymes and RAPD markers (Moreno *et al.*, 1996).

In a typical case of defining *Eucalyptus granticola,* whether it is a relict species or hybrid of existing species, through RAPD analysis, the data showed, *E. granticola* is 40 per cent similar to *E. rudis*, and 35 per cent similar to *E. drummondii*, whereas *E. rudis* and *E. drummondii* are 25 per cent similar among themselves. The RAPD data in combination with the morphological data revealed that, *E. granticola* is a hybrid between *E. rudis* and *E. drummondii* and not a relict species (Rosetto *et al.*, 1997).

The genus *Scaevola* which was initially misclassified by Linnaeus was reclassified by Swoboda and Bhalla (1997) through RAPD analysis.

While analysing the two subspecies in *Plantago major* through RAPD analysis Wolff and Richards (1998) identified some intermediate forms.

According to Khanuja *et al.* (2000) *Mentha gracilis* var. *cardiaca* showed a high similarity to *M. spicata* and *M. arvensis,* which themselves showed a greater distance in RAPD analysis indicating that *M. gracilis* var. *cardiaca* might have evolved as a natural hybrid between *M. arvensis* and *M. spicata.*

Calculation of genetic distance, based on the number of shared bands through RAPD analysis indicated that *M. spicata* is more closely related to *M. gracilis* than to *M. piperita* (Fenwick and Ward, 2001).

While analyzing 15 accessions of *Asparagus* spp. through RAPD technique Shasany *et al.* (2003) found that two of the accessions from *A. racemosus* were associated with morphologically unrelated species.

RAPD markers were used to study inter specific variation among six species of the genus *Digitalis.* The species relationships revealed were fully consistent with those previously obtained using morphological affinities. The hybrid *D. exelsior* seems to have stronger affinity to the section *Digitalis* than to *Grandiflora* (Nebauer *et al.*, 2000).

2.4.3.2.5 Genetic Fidelity Analysis

Plants regenerated by somatic embryogenesis from callus culture were subjected to RAPD analysis in five garlic cultivars (Al-Zahim *et al.*, 1999). Certain changes were observed in the RAPD profiles of the regenerents of different cultivars, suggesting the existence of somoclonal variants.

RAPD analysis was done by Babu (2000) to assess the genetic stability in tissue culture derived black pepper plants. Monomorphic banding pattern was observed for the tissue culture regenerents, compared with their respective source plants.

Genetic fidelity of *Tylophora indica* plants regenerated via somatic embryos was tested through RAPD analysis and the results showed genetic homogeneity and true to type nature of the regenerants (Jayanthi and Mandal, 2001).

The regenerated plantlets of *Plumbago* species appeared morphologically similar to the mother plants and no variation was observed among the regenerated plants through RAPD markers (Bout, 2002).

2.43.2.6 Sex Differentiation

Sex differentiation is one of the most useful applications of the molecular markers. Male and female plants do not show any sex specific morphological difference until flowering and molecular markers are very useful to detect the sex at early stage of growth. Perennial dioecious plants having long juvenile phase will take nearly 4 to 6 years to show sex discrimination and under such situation the use of molecular markers are more pronounced.

According to Banerjee *et al.* (1999) the appearance of two consistent RAPD bands in male genotype, suggesting the male associated nature of the DNA markers in dioecious *Piper longum.*

The sex specific DNA markers could potentially be employed to determine the sex of nutmeg seedlings. Out of 20 random primers screened

using RAPD PCR one primer OPE-11, elicited a female sex specific DNA amplification product and these findings offer the exciting possibility of being able to determine sex at seedling stage (Shibu *et al.*, 2000).

RAPD markers successfully distinguished between sexual and apomictic offspring in *Hypericum perforatum* (Steck *et al.*, 2001).

2.4.3.3 Amplified Fragment Length Polymorphism (AFLP)

This technique is also called "Selective Restriction Fragment Amplification". It is a combination of RFLP and PCR used for obtaining highly informative fingerprints. The technique involves restriction of the DNA and ligation of oligonucleotide adapters, selective amplification of sets of restriction fragments and gel analysis of the amplified fragments.

Morphological descriptors, quantitative, phytochemical analyses and DNA finger printing using AFLP were utilized to define the extent of variation existing between Hawaiian cultivars of *Piper methysticum* and extremely narrow genetic base was observed among the accessions (Lebot *et al.*, 1999).

Mace *et al.* (1999) studied species relationship and genetic distance within the tribe *Daturae* for 47 accessions of over 12 species. The results of AFLP were found to be consistent with previous results based isoenzyme and morphological analysis.

Lesur *et al.* (2000) compared molecular methods namely RFLP, RAPD, SCAR (Sequence Characterised Amplified Regions), STMS (Sequence-Tagged Micro-Satellite markers) and AFLP in order to select appropriate technique for cultivar identification in 23 *Pelargonium* cultivars. AFLP and STMS technique were reported to be the best methods for cultivar identification because of their minimum requirement of template DNA and high reproductability.

AFLP was successfully employed to detect genetic relationships and variability among 90 hop *(Humulus lupulus)* cultivars and the clustering

revealed two main clusters reflecting the two main clusters reflecting the two main sources of origin (Seefelder *et al.*, 2000).

AFLP markers were used to identify rose verities and also to assess the intra varietal variability (Zhang *et al.*, 2000).

According to Carolin *et al.* (2002) AFLP markers differentiated *Papaver* species and assessed the genetic fidelity between *in vitro* cell lines of *Papaver bracteatum* and their mature plants from callus culture.

AFLP finger printing was employed to differentiate or to identity *Actaea racemosa* from its closely related species *A. pachypoda, A. cordifolia* and *A. podocarpa* (Zerega *et al.*, 2002).

RAPD and AFLP profiling of *Aloe* germplasm (*Aloe vera, A. perryii, A. orborescens, A. saponaria)* were carried out for estimating the extent of diversity and establishing phylogenetic relationship (Darokar *et al.*, 2003). The pattern of phylogeny remained parallel to RAPD pattern, and the degree of diversity revealed by AFLP showed zooming effect over RAPD analysis.

Genetic diversity of 62 *Mentha* accessions from different geographic origins representing five species and three hybrids was assessed through AFLP method and the analysis supports taxonomic classification established among *Mentha* species by conventional methods (Gobert *et al.*, 2003).

Lal *et al.* (2003) conducted genetic diversity analysis using AFLP technique in 14 accessions of *Vetiveria zizanioides* and obtained 65 per cent divergence due to polymorphism with nine primer combinations.

According to Taviani *et al.* (2003) AFLP markers were able to differentiate and group the populations of *Chamomilla recutita* and it was comparable with grouping based on essential oil composition.

2.5 GENETIC VARIABILITY, CORRELATION AND DIVERGENCE

Genetic variability and divergence are of great interest to the plant breeder as they play a vital role in forming a successful breeding programme. Genetic divergence among them was quantified by multivariate analysis. The concept of a measure of divergence between population was first introduced and further developed by Mahalanobis (1936). The multivariate analysis (D^2) is a powerful tool to measure genetic divergence within a set of genotypes (Murthy and Arunachalam, 1966).

The relationship of yield to its attributing morphometric characters is of great importance for effective selections. Therefore while making selection the inter dependence between characters based on simple correlation has to be considered. The mere selection, based on the yield as such is not effective. The path coefficient analysis is most suited to know about the direct and indirect effects of characters on yield and to identify the major traits that determine yield and for the selection of best genotypes.

Correlation and regression of flower production and its seven component traits in *Pandanus fasicularis* was studied by Jagadev *et al.* (1993a). The results revealed that, flower production per plant was significantly and positively correlated with leaf width (0.93) and negatively with flower weight (-0.81). Regression study indicated that an increase of one cm in leaf width increased flower production by 15.09 per plant, while an increase of one g in flower weight and 1 mm in leaf thickness decreased the flower production by 0.18 and 34.78 per plant, respectively. However, regression analysis indicated that only leaf width had recorded the maximum significant value (15.09) out of seven independent variables studied. Therefore, selection indices based on broad leaved plants and flowers with least weight are most important factor.

Genetic variability, correlation and path analysis in vetiver (*V. zizanioides*) indicated that, the number of tillers, root length, root yield per plant and oil content were the major contributing characters towards the

oil yield (Ramanujam and Kumar, 1963; Singh *et al.*, 1998; Gurmani *et al.*, 2004).

Lal *et al.* (1999) conducted D^2 analysis of pooled divergence among 18 vetiver accessions and grouped them into six clusters. Plant height, oil yield, tillers per plant, root length, root girth, oil content, were the important contributors to the total divergence.

Correlation studies conducted in turmeric cultivars indicated that, weight of primary, secondary and mother rhizomes had direct effect on yield. The genotypic correlation coefficients were in general higher than the phenotypic correlation coefficients, thus revealing strong association at genotypic level between the characters (Mohanty, 1979; Reddy, 1987; Shanmugasundaram *et al.*, 2001).

Variability studies of turmeric (*Curcuma longa*) genotypes revealed that, the characters *viz.*, fresh rhizome yield, number of primary and secondary rhizomes per plant, weight of primary and secondary rhizomes per plant exhibited high variability, heritability and appreciable genetic advance. Number of leaves per clump, leaf area, leaf area index, number of primary and secondary fingers had positive strong association with rhizome yield at both genotypic and phenotypic levels (Hazra *et al.*, 2000; Jana *et al.*, 2001; Narayanpur and Hananashetti, 2003; Singh *et al.*, 2003a).

Genotypic co efficient of variation, heritability and genetic advance were studied in ginger genotypes (Ali *et al.*, 1994; Yadav, 1999a; Singh and Mittal, 2003) The genotypic coefficient of variation was high for length and weight of secondary and primary rhizomes and rhizome yield per plant. High heritability coupled with high genetic advance as percentage mean was observed for plant height, leaf length, suckers per plant, number of mother and secondary rhizomes, weight of primary rhizome and rhizome yield per plant.

Singh *et al.* (2000b) conducted genetic diversity analysis in 16 genotypes of ginger and grouped them into 3 clusters. Significant association of essential oil content with rhizome yield per plant and per cent oleoresin content was observed.

Citral type strains of *Ocimum americanum* collected from different geographical regions of the world were subjected to a detailed chemical and genetic investigation by Pushpangadan *et al.* (1979). The results revealed a wide range of genetic variability for oil content with in the species.

Variation and correlation studies in *Ocimum viride* were conducted by Pal *et al.* (1987). The results revealed a wide range of genetic variability for most of the traits. The total oil yield was positively correlated with leaf yield and oil content.

Eight genotypes belonging to seven species of *Ocimum* varied greatly for herb and oil yield. High heritability and high genetic gain for oil yield, fresh herb yield and oil content was observed (Verma *et al.*, 1998a).

Variability and correlation studies in Palmarosa (*C. martinii*) revealed that, selection with taller plants, more number of spikelets and leaves and oil content in inflorescence would lead to higher total oil yield. However, oil content in inflorescence was the most effective selection criterion for improvement (Gupta and Trivedi, 1984; Jagadev *et al.*, 2001).

Dhar *et al.* (1985) studied genetic variability for oil yield per plant, oil quality and leaf traits from 24 wild forms of *Cymbopogon jwarancusa* and the results revealed a wide range of variability for most traits and more than 50 per cent heritability for all traits. Total oil yield per plant was positively and significantly correlated with total fresh and dry leaf yield per plant. Percentage oil in leaves showed a negative correlation with these traits.

Kulkarni and Rajagopal (1986) studied the broad and narrow sense heritability for leaf yield, leaf width, tiller number and oil content in East Indian lemongrass. They observed that, average broad sense heritability

estimates for leaf yield, leaf width, tiller number and oil content. Oil content was not significantly correlated with any of the other traits.

Variability studies conducted among *Cymbopogon flexuosus* strains revealed that, plant height, tiller number, leaf area, and oil and geraniol content per plant were positively correlated with oil yield. Heritability estimates were close to 100 per cent for all these characters (Patra *et al.*, 1987; Sharma *et al.*, 2000a).

Lal *et al.* (2001a) examined genetic variability in thirty accessions of Java citronella (*Cymbopogon winterianus*) and four accessions of Ceylon citronella (*C. nardus* var. *nardus*). For most of the traits examined genetic correlations were found to be larger than phenotypic correlations. Oil herbage yield and citronellol content of the oil were negatively correlated with the citronellyl acetate content.

Singh *et al.* (2001) carried out genetic divergence analysis among 43 clones of lemongrass (*Cymbopogon flexuosus)* and grouped them into nine clusters.Genetic divergence analysis was conducted among 34 accessions of citronella Java assembled from different states of India and Sri Lanka based on fourteen quantitative and qualitative traits (Lal *et al.*, 2002b). Based on their values, all the 34 accessions were grouped into seven clusters such that the genotypes within a cluster have smaller D^2 values among themselves than those belonging to different clusters.

High heritability and genetic advance were observed in *Corundum sativum* genotypes for plant height, umbels per plant and seeds per umbel (Jindla *et al.*, 1985; Mehta and Patel, 1985; Yadav, 1999b).

Information on genetic divergence for 11 yield components of 29 USA and 171 diverse Indian *Corundum sativum* genotypes were studied using D^2 analysis and the genetic divergence was not related to geographical distribution (Bandari and Gupta, 1993).

Genetic association and distances were assessed in 15 Indian accessions of coriander, on the basis of seven economic plant traits including essential oil content and oil yield. The results of genetic association and D^2 analysis revealed that the variation in seed yield was the most prominent force accounting for both variations in oil yield and genetic distance in the material (Singh *et al.*, 2002b).

Correlation and path coefficient analysis in *Papaver somniferum* revealed that, the opium weight per plant was positively correlated with plant height, stem diameter, capsule number and weight per plant (Shukla and Khanna, 1987; Saini *et al.*, 1999; Shukla *et al.*, 2003; Singh *et al.*, 2003b).

Farooqi *et al.* (1990) found genetic variation among the genotypes of davana (*Artemisia pallens* Wall.) for plant height and oil content in the herbage, with 52.8 per cent and 90.7 per cent estimated heritability.

Gopal *et al.* (1990) conducted correlation studies in three types of cardamom and reported that panicles per plant, capsule fresh weight per plant, nodes per panicle and inter nodal length within the panicle were useful characters in selection of cardamom for yield improvement.

Korikanthimath *et al.* (1999a) studied the correlation of 5 yield components in the yield of 12 elite cardamom clones and observed positive correlation of the yield components with yield.

Oil yield showed significant and positive correlation with herb yield and oil content of rose scented geraniam (Rao *et al.*, 1993).

Multivariable analysis techniques were used to study the intra specific variability of *Thymus morderi* and *Thymus antoniae*. Two chemotypes of *T. morederi* was identified having different sesquiterpene compositions (Canigueral *et al.*, 1994).

Dwidedi *et al.* (1999) conducted variability studies among 26 genotypes of *Catharanthus roseus* for total alkaloid content and found significant variation for all the traits studied.

Mishra *et al.* (2001) observed large difference among 32 accessions of periwinkle collected from different geographical areas of India, Madagascar, Singapore and Malaysia. The multivariate analysis allowed classification of the accession into five to seven morphologically and genetically distinct groups.

Genetic variability, correlations, broad sense heritability and genetic advance of 39 diverse strains of *Asparagus officinalis* were studied by Pandita and Bhan (1999). They observed moderate to high coefficient of variation, fairly high heritability coupled with high genetic advance.

Lal *et al.* (2000) studied the nature and extent of variability, associations of German chamomile for eight economic traits. The genotypic correlation was higher than phenotypic correlations for most of the traits.

Twenty two morphometrically diverse genotypes of *Andrographis paniculata* evaluated for genetic variability revealed considerable amount of genetic variability among them (Misra *et al.*, 2000).

The results of the path analysis of the essential oil related characters of *Mentha* spp. indicated that the percentage of leaf essential oil and leaf length had a high direct effect on the flower essential oil yield (Nodoushan *et al.*, 2001).

Srivastava *et al.* (2003) studied intra specific variation in four widely cultivated cultivars of Japanese mint *(Mentha arvensis)*. Oil content showed significantly positive correlations with yield traits like leaf and stem fresh and dry weights.

Germplasm collection of celery (*Apium graveolens* L.) involving 30 accessions, representing a broad spectrum of variability to as subjected to D^2 statistic for assessing the nature and degree of inter and intra specific divergence. Based on six characters the accessions could be grouped in five clusters. Maximum divergence was exhibited between cluster one and two (Choudhary *et al.*, 2002).

Lal *et al.* (2002a) investigated genetic divergence among 52 genetic stocks of shankhapushpi (*Convovulus microphyllus*) and quantified them by multivariate analysis for eight most economic traits. All the 52 accessions were grouped into eight clusters had smaller D^2 values among themselves than those belonging to different clusters.

Bhagat and Jadeja (2003) evaluated 13 indigenous germplasm accessions of safed musli (*Chlorophytum borivilianum)* and noticed significant differences between genotypes for all the characters except proteins.

Mathur *et al.* (2003) characterized 16 accessions of *Centella asiatica.* These 16 accessions were found to harbour considerable variation and they were dispersed into 9 clusters on the basis of 18 quantitative traits.

Palai *et al.* (2003) conducted variability studies in hybrid tea roses and the results revealed highly significant differences for most of the biometric characters. High coefficient of variation both at genotypic and phenotypic level coupled with high heritability and genetic advance was observed for thorn density, plant height, and petal number.

2.6 PROPAGATION TECHNIQUES

Generally *Pandanus* starts flowering after four to five years of planting. Identification of suitable propagule for getting economic yield within a short span is highly essential. Since there is limited reports on different propagation methods of *Pandanus*, work from some of the medicinal aromatic plants, forest trees and related crops are reviewed.

2.6.1 Seed

Propagation by seeds is the major method by which plants reproduce in nature and it is one of the most efficient and widely used propagation methods for cultivated crops. The seedling plants, whether originating sexually or asexually (apomixis), have a characteristic life cycle that contrasts to the life cycle of a vegetatively propagated plant.

Seed propagation involves careful management of germination conditions and knowledge about the requirements of individual kinds of seeds. A germination test will be helpful in indicating the necessity for any pre germination treatment. Owing to the presence of tough seed coat the germination percentage of *Pandanus* seeds as such is very low indicating the requirement of pre germination treatments. Seed germination and seeding growth are regulated by hormones and nutrients, the exogenous application of which have positive influence on many seeds.

The possibility of propagation of *Pandanus* through seeds was reported by Singh *et al.* (2000a).

Application of 6 benzyl aminopurine or gibberellic acid proved effective in breaking the dormancy in seeds of *Rosa arvensis* (Jackson and Blundell, 1963).

Seed germination of *Rosa dumetorum* var. Laxa was improved by GA_3 (10, 100 or 1000 mg l^{-1}) or BA (10 or 100 mg l^{-1}) applied before incubation at 20^0C for four weeks followed by 5^0C for four weeks (Foster and Wright, 1983).

Treatment of seeds with sulphuric acid for 1-2 hours before sowing was found to improve the germination in rose seeds (Yadav *et al.*, 1998).

Iyer *et al.* (1978) observed good germination of 75-90 per cent in pineapple seeds under intermittent mist.

For enhancing germination in pineapple, seeds should be treated with concentrated sulphuric acid for 30 to 60 seconds prior to sowing (Bose, 1991).

Enhancement of germination and seedling growth has been influenced by growth regulators such as IAA and KNO_3 at lower concentrations, GA_3 at higher concentration and CCC at low concentration in *Cassia obtusifolia* (Singh and Murthy, 1987); coumarine, maleic hydraxide and CCC in *Cassia sophera* (Takur, 1989); IAA and IBA in *Picea smithiana* (Singh, 1990); and

GA_3 (200 ppm), CCC (200-400 ppm) and IAA (200 ppm) in *Azadirachta indica* (Kumaran *et al.*, 1994).

The chemicals such as, potassium chloride, potassium dihydrogen phosphate, potassium nitrate, diammonium phosphate and cow dung solution were also found to be beneficial in *Acacia nilotica* (Palani *et al.*, 1995).

According to Amodu *et al.* (2000) seeds immersed in hot water (5 min.) and in sulphuric acid (17.5 min.) recorded the highest percentage of germination of 79.0 and 67.9 per cent respectively in *Leucaena leucocephala.*

Seed treatment with 200 ppm of gibberellic acid (GA_3) was found to be effective in improving germination and survival percentage of *Aconitum heterophyllum* (Singh *et al.*, 2000c).

Seed germination studies were conducted in medicinal plant species like *Glycyrrhiza glabra, Psoralea cordifolia, Rauvolfia serpentina, Chlorophytum borivilianum* and *Withania somnifera.* Dormancy breaking treatments like concentrated H_2SO_4 treatment, scratching with course sand paper and GA_3 treatment were found effective in *Glycyrrhiza, Psoralea* and *Withania somnifera* respectively (Verma *et al.*, 2000).

Suryawanshi *et al.* (2001) obtained maximum germination of 92 per cent at 20^0C in *Withania somnifera* while seeds of Dawana showed maximum germination at 30^0C without any seed treatment. The seeds of *Cassia angustifolia* gave maximum germination after soaking it in water for 24 hours and germinating at 20^0C. Treating the seeds with 25 per cent nitric acid for 10 minutes and germination at 30^0C was found ideal for *Ocimum pallens.* For wild brinjal seed treatment of 12.5 per cent nitric acid for 15 minutes followed by 1000 ppm GA_3 for 24 hours with alternate temperature of 20 to 30^0C was ideal.

According to Gupta (2002) GA_3 at 5 to 30 ppm level was very effective in overcoming seed dormancy in *Ocimum* species. Also salts like KCl and KNO_3 were found to be effective in inducing significant increase in

seed germination capacity, germination velocity index and seedling vigour index.

Chakaraborthy *et al.* (2003) studied the germination behaviour of *Basilicum polystachyon* and found pretreatment with 100 ppm GA_3 for 24 hours and germination under light and dark was beneficial.

Tewari *et al.* (2001) observed significant improvement in percentage germination with GA_3 and NAA in *Allium cepa.* Higher dose of all plant growth regulators showed negative effect. Hence lower dose (10 ppm) was recommended than higher doses (50 to 100 ppm).

Gopikumar (2002) reported that, treating the seeds with concentrated sulphuric acid for 5 minutes proved to be the best seed treatment in *Prosopis cineraria, Paraserianthus falcataria* and *Leucaena leucocephala* in terms of germination, while boiling water treatment for 5 minutes produced superior results in *Ceiba pentandra.*

Seed germination percentage in *Leptadaenia reticulata* an endangered species of medicinal value, improves with 25 per cent (2 min.) and 50 per cent H_2SO_4 (1 min.) pretreatment and hot water treatment (55 to 60^0C) for 2 and 5 minutes. Application of IBA (25 ppm) induced seedling growth. Maximum root/shoot length can be achieved by germinating the seeds in total darkness (Kasera *et al.*, 2002).

Masilamani and Dharmalingam (2002) observed beneficial effects of GA_3 250 ppm on the germination of three months old silver oak seeds.

Sharma and Punam (2002) conducted germination studies in germplasm of three economically important nitrogen fixing tree species of Himalayas *viz., Acacia catechu, Alnus nitida and Dalbergia sissoo* with cold water soaking and acid treatment.

According to Thakur *et al.* (2002) soaking the seeds of *Grewia optiva* in dilute sulphuric acid (N/10 H_2SO_4) for 36 hours was the most effective method for breaking seed dormancy under glass house conditions.

Saikia and Nath (2003) reported 92.5 per cent germination in the seeds of *Cinnamomum impressinervium* treated with 500 ppm solution of GA_3 after depulping them with luke warm water.

2.6.2 Vegetative Propagation

Mass propagation by vegetative means helps to maintain the superiority and uniformity of specific clones. Apart from that, the major economy vegetative propagation comes from the elimination of the juvenile phase and shortening the time to reach reproductive maturity.

2.6.2.1 Pandanus

According to Dutta *et al.* (1987) propagation by stem cuttings and suckers were found to be effective in the production of flowers within a period of three to four years after planting in *Pandanus.*

For the identification of suitable planting material in *Pandanus*, five types of planting materials were raised and observations on plant survival, height, girth, number of new leaves and aerial roots developed per plant were recorded (Jagadev *et al.*, 1993b).

The results revealed that during initial 90 days, the survival was significantly higher in ground sucker and crowns (>80 per cent), whereas success was very low in stem cuttings (<30 per cent). At later stage (240 and 720) DAP, a significant high survival was noticed in ground suckers over crown of flowering plants. The values of other vegetative traits were highest for ground suckers followed by crown of flowering plants.

Propagation by offsets and division of suckers was reported by SFAC (1997). Plant regenerated by means of offsets began to yield leaves from second year onwards, whereas leaves were available after 2 to 3 years in the other cases.

Rao (2000) recommended propagation by branch or limb cuttings and aerial roots in *Pandanus.*

Singh *et al.* (2000a) reported the feasibility of multiplication of *Pandanus* through suckers.

2.6.2.2 Pineapple

Pineapple is usually propagated by vegetative methods. Naik (1949) suggested that suckers were preferred in South India because they produced crops in 18 months as compared to two years when other planting materials were used.

Evans (1952) suggested splitting of the crown into eight vertical section before planting. Topper (1952) recommended pineapple propagation by suckers, slips crown and segmented stem under Jamaican condition. Nambiar (1955) reported success in propagating pineapple from stem bits.

The use of suckers, slips, crown and green stump as planting material was also suggested by Teaotia and Pandey (1962). Teaotia and Pandey (1966) recorded vigorous growth and high yield from slips and the performance of suckers was next to slip. Wang and Kwong (1966) reported promising results with slips, 330 to 380 g in weight.

Suckers were found to produce higher percentage of flowering and early fruiting (Py, 1964; Senewiratne, 1964). Reynhardt and Dalldorf (1968) concluded that, slips are the best planting material. Gaillard (1969) found that, the weight of the slips markedly influenced the productivity of pineapple.

Chadha *et al.* (1974) in their trial to compare various type and sizes of planting materials namely crowns, slips and suckers found the superiority of slips and suckers as planting material in comparison to crowns. Both in suckers and slips, larger planting material resulted in more vigorous plants. The best planting material was however, found to be slips ranging from 300 to 450 g in weight.

In an experiment with five different planting materials *viz.*, tops, slips, butts and aerial and ground suckers, Bourke (1976) found that aerial and

ground suckers were the first to bear flowers, followed by slips and tops and lastly the butts came to flowering.

Kew pineapple raised form side suckers, slips, crowns or stem disc cuttings were assessed for plant height, number of leaves per plant, days from planting to fruit maturity, percentage of fruiting, fruit weight, percentage of edible fruit, crown weight and length, number of slips and suckers per plant and fruit chemical composition. Plants raised from slips followed by those raised from side suckers gave the best results with regard to yield, fruit quality, number of days to maturity and sucker production (Nandy *et al.*, 1982). Ahmed and Mohan (1985) studied the size and type of planting material on growth yield and quality of pineapple and recorded highest yield in plant propagated by suckers of 501 to 750 g.

2.6.2.3 Banana

Eastwood (1949) studied the performance of suckers, butts, pieces and eyebuds as different planting materials and recommended sword suckers as the best planting material.

In an experiment on propagating materials, Bhan and Mazumdar (1956) concluded that butts and bits were equally good planting materials when quick multiplication was desired.

In a trail for the selection of planting material, Srivastava (1963) found that the sword suckers were more vigorous producing bigger and heavier bunches in 11 months than the water suckers doing in more than 15 months.

Sharma and Roy (1974) studied the performance of different planting materials like whole corm, corm portions and suckers. They found that suckers were the first to flower.

In an investigation with planting materials of banana, Rodriguez and Irizarry (1979) found that the sword suckers of the common dwarf and Moricongo cultivars gave higher yield compared to water suckers.

Of seven types of planting material studied the offshoot with entire pseudostem attached was found best on account of the shortness of the growth cycle and bunch weight (Sosa and Nava, 1984).

2.6.2.3 Orchids

Trails indicated that most of the sympodial orchids could be propagated by division of large clumps and orchids like *Phalaenopsis*, *Oncidium*, *Dendrobium* etc., produce off shoots or keikies which could be removed from the parent plant and used as propagating materials (Bose and Bhattacharjee, 1972; Arora *et al.*, 1975; Bose and Bhattacharjee, 1980).

Use of terminal cuttings for propagation in monopodial orchids was suggested by Arditti (1981).

2.6.2.4 Anthurium

Propagation of *Anthurium* by one, two, three, leaved terminal cuttings and single node cuttings rooted under intermittent mist was reported by Kunisaki and Sagawa (1971).

Division of off shoots with aerial roots found to be the best propagating material in *Anthurium*. Plants obtained through this method flower early in comparison with those from cuttings or other methods (Bhatt and Desai, 1998).

2.6.3 Micropropagation through Enhanced Release of Axillary Buds

The application of tissue culture technique to the regeneration and commercial mass multiplication has become an important alternative for more conventional propagation procedures for a wide range of plant species. Shoot tip culture is the most common *in vitro* method for commercial multiplication. It commences growth rapidly and contains more axillary buds.

Since *Pandanus* multiplies slowly by conventional propagation methods *viz.*, seeds, cuttings, suckers and offsets, micro propagation through enhanced release of axillary buds will be beneficial for rapid multiplication.

2.6.3.1 Pineapple

2.6.3.1.1 Explants

Sita *et al.* (1974) obtained successful meristem culture of pineapple using explants from 1 to 1.5 month old slips.

Pineapple plantlets were obtained from crown axillary leaf bud on a Murashige and Skoog's medium (Pannetier and Lanaud, 1975). Mathews and Rangan (1979) cultured lateral buds from the crown of pineapple. Zepeda and Sagawa (1981) were able to propagate by *in vitro* culture of axillary buds from crown of mature fruits.

2.6.3.1.2 Growth Regulators

Pannetier and Lanaud (1975) obtained plantlets on MS medium containing only kinetin.

Zepeda and Sagawa (1981) were able to obtain plantlets in MS medium with 25 per cent coconut water and BA. Hirimburegama and Wijeshighe (1992) observed bud development in the presence of either Kinetin or BA and greater proliferation of shoots with 10^{-6} M BA and 10^{-6} M IAA.

According to Deva *et al.* (1997) cultures were initiated on MS I medium fortified with 0.1 mg NAA and 1.0 mg BA l^{-1} and multiplied on MS II medium supplemented with higher concentration of BA.

Aghion and Beauchesne (1960) obtained poor rooting on transferring plantlets to a medium with 2 mg l^{-1} auxin, while Sita *et al.* (1974) noticed cluster of roots in meristem explants grown on a medium with 1mg dm^{-3} NAA.

Mathews *et al.* (1976) could induce rooting with low auxin levels (0.1 to 0.4 mg dm^{-3}). It appears that very little or even no auxin is necessary for root formation in pineapple.

2.6.3.2. Banana

2.6.3.2.1. Explant

Ma and Shii (1972, 1974) reported *in vitro* adventitious bud formation in banana from shoot apex culture. The applicability of excised shoot tip culture technique to a number of *Musa* clones was assessed by Vessey and Rivera (1981); Bower and Frazer, (1982); Doreswamy *et al.* (1983); Mante and Tepper (1983); Swamy *et al.* (1983); Banerjee and De Langhe (1985); Vuylsteke and De Langhe (1985); Bhaskar (1991).

2.6.3.2.2 Growth Regulators

In vitro cultured apical meristem slices of banana produced shoots in MS media containing 3.5 mg l^{-1} BA and 160 to 200 mg l^{-1} adenine sulphate (Mante and Tepper, 1983). Rapid shoot multiplication of dessert banana clones was stimulated by BA 5 mg l^{-1} (Cronauer and Krikorian, 1984).

Jarret *et al.* (1985) established shoot tip cultures of two cooking banana, Saba and Pelipita (AAB) on a modified MS medium supplemented with BA 5 mg l^{-1}.

MS medium supplemented with 5 mg l^{-1} BA and 2 mg l^{-1} IAA produced adventitious buds from meristem culture (Sun, 1985). Fitchet and De Winnaar (1987) succeeded in the rapid multiplication of banana by tissue culture using MS basal medium supplemented with IAA, NAA, kinetin, activated charcoal and sodium phosphate.

Rodriguez *et al.* (1987) stated that high concentration of BA (5 mg l^{-1}) is necessary for intense proliferation of shoots. According to Raut and Lok hande (1989) banana variety Basrai formed multiple shoots on MS medium

supplemented with 7 to 10 mg l^{-1} BA where as, MS medium with 5 mgl^{-1} BA produced single shoots.

In shoot tip culture of banana on an average, each explant released 11 axillary shoots when the media contained NAA 1.0 ppm and BA 10 ppm or BA 10 ppm. Out of the two cytokinins tried (BA and kinetin), BA was found to be the most efficient one for the induction of axillary shoots in culture (Bhaskar, 1991).

Bekheet and Saker, (1999) developed an efficient medium for *in vitro* propagation of banana cv. William, Grande Naine and Maghraby. Shoot cultures were established on MS medium supplemented with 2 mg BA l^{-1}. Highest number of proliferated shoots was recorded with BA 6 mg l^{-1}.

Shoot tips of diploid, triploid and tetraploid banana genotypes were multiplied in MS medium fortified with 5.0 mg BA l^{-1} (Oliveira *et al.*, 1999). Trujilio *et al.* (1999) succeeded *in vitro* multiplication of *Musa* clones using cytokinin concentration ranging from 5 to 15 mg l^{-1}.

Shoot tips excised from healthy suckers of banana varieties Dwarf Cavendish, Amrutha Pani, Tella Chakkerakeli and Robusta were established on MS medium supplemented with 6 mg l^{-1} BAP, 2 mg l^{-1} IAA and 200 mgl^{-1} adenine sulphate. Shoot proliferation was in MS medium with reduced BA concentration of 4 mg l^{-1} (Vani and Reddy, 1999).

According to Mante and Tepper (1983) *in vitro* developed shoot lets generated roots on media with sucrose 1 to 1.5 per cent and either NAA (0.1 to1 mg l^{-1}) or IBA (2 to10 mg l^{-1}).

In banana both IBA (0.5 to 2 mg l^{-1}) and NAA (0.02 to 2 mg l^{-1}) have been reported to induce rooting of shoots and embryoids (Krikorian and Cronauer, 1984; Banerjee *et al.*, 1987).

Transfer of axillary shoots to hormone free medium resulted in rapid and extensive root formation (Jarret *et al.*, 1985). Rooting was induced on MS medium with 2 mg IBA l^{-1} (Raut and Lokhande, 1989).

Among different types of auxin used for rooting of banana shoots, NAA was more effective than IAA or IBA (Bekheet and Saker, 1999). Vani and Reddy (1999) reported that *in vitro* shoots were rooted on MS medium containing 2 mg l^{-1} BAP and 2 mg l^{-1} IAA.

The addition of activated charcoal at 0.025 per cent to the basal rooting medium resulted in the initiation of maximum number of roots (Krikorian and Cronauer, 1984; Bhaskar, 1991). According to Vani and Reddy (1999) addition of 0.1 per cent activated charcoal to the rooting media resulted in the initiation of more number of roots.

2.6.3.2.3 Planting out and Acclimatization

Bhaskar (1991) reported that 90 per cent of planted out plantlets survived when the plantlets after removal from the rooting media were kept in distilled water for eight hours and then for another eight hours in MS liquid medium of $1/10^{th}$ strength and then planting out into the potting mixture covered with moistened plastic cover for four weeks.

2.6.3.3 Orchid

2.6.3.3.1 Explant

Morel (1960) obtained plantlets with free of infection by culturing meristem tips of virus infected *Cymbidium.* Higher percentage of apical buds of *Aranthera* cv. James Storie produced protocorm like bodies with in a shorter period than the axillary buds (Widiastoety, 1986).

Shoot meristems of *Cymbidium ensifolium* and *Cymbidium goeringii* formed PLBs with in four to six months. When the PLBs were divided and subcultured, they formed new bright green rosettes of PLBs within a month (Wang, 1988).

Sharma and Prakash (1996) reported that shoot tips obtained from *in vitro* raised seedlings of *Dendrobium chrysanthum* produced PLBs 60 days after culturing and shoot formation occurred in 90 days. PLB formation was observed from the shoot tips of *Oncidium* by Bagde and Sharon (1997).

Shoot apices responded well to culture establishment by enhanced release of axillary buds in *Dendrobium* and *Aranthera* (Kuriakose, 1997).

2.6.3.3.2 Growth Regulators

BA at lower concentrations induced growth of shoots from protocorms of *Cymbidium* (Fennesbech, 1972). Kim and Kako (1982) found that the addition of BA encouraged PLB formation and shoot development. The greatest number of shoots in *Dendrobium* was obtained by enriching the medium with BA at 5 mg l^{-1} (Kukulczanka and Wojciechowska, 1983).

Shoot growth of *Cymbidium* hybrid was the greatest, when explants were cultured on medium containing BA at 10 mg l^{-1} for 10 days and then transferred to a medium containing 0.5 mg l^{-1} BA (Paek *et al.*, 1989).

Sounderrajan and Lokeswari (1994) observed better shoot multiplication of *Dendrobium* cv. Madame Pompadour, when the medium was supplemented with BA at 0.5 mg l^{-1} and NAA at 0.1 mg l^{-1}.

Trails with different combinations of BA and NAA tried, BA 2.5 mgl^{-1} and NAA 1.0 mg l^{-1} produced maximum number of vigorous shoots (18.33) in *Dendrobium* cv. Sonia and BA 5 mg l^{-1} + NAA 1mg l^{-1} produced maximum number of shoots per culture in *Aranthera* cv. Annie Black (Kuriakose, 1997).

Multiple shoot production of *Dendrobium* hybrids using shoot tip and axillary buds was the best with NAA and BAP each at 3 mg l^{-1} (Devi and Laishran, 1998). Pathania *et al.* (1998) observed better response on culturing shoot tips of *Dendrobium* cv. Sonia in medium supplemented with NAA at 10 μM and BA at 5 μM or NAA at 2 μM and KIN at 10 μM.

Protocol for *in vitro* rapid clonal multiplication of *Dendrobium* cv. Sonia was investigated by Pathania and Sehgal (1999). Maximum establishment of cultures occurred on MS medium containing 2 μM NAA + 5 μM BAP or 2 μM NAA + 10 μM kinetin. MS medium supplemented

with 2 μM NAA + 6 μM BAP + 4 μM paclobutrazol was optimum for shoot multiplication.

Plant let formation in *Oncidium* cv. Gower Ramsey was more in medium with NAA 0.5 mg l^{-1} and BA at 5 mg l^{-1} (Santana and Chapparro, 1999). The establishment of the explants, number of PLBs and shoot lets per explant were higher in the combination of BAP 2.0 mg l^{-1} and NAA 1.0 mgl^{-1} in *Dendrobium* cv. Sonia (Ramsundar *et al.*, 2000).

Kukulczanka and Wojciechowska (1983) reported more number of roots in medium supplemented with 1.75 mg l^{-1} NAA and 1.75 mg l^{-1} IBA in *Dendrobium*. The addition of 2 mg l^{-1} IBA to the media was necessary for the root formation of *Phalaenopsis* hybrid and *Dendrobium* 'Miss Hawaii' (Nuraini and Shaib, 1992).

IBA at 0.1 mg l^{-1} was the best for producing many long rooted shoots in *Dendrobium* (Lim *et al.*, 1993). Mujib and Jana (1994) observed induction of roots in *Dendrobium* 'Madame Pompadour' in the presence of NAA at 0.1 mg l^{-1}.

Culturing shoots of *Dendrobium* in rooting medium supplemented with NAA 0.1 mg $l^{-1}$was found to be better for rooting *in vitro* (Kuriakose, 1997).

In vitro rooting of *Dendrobium* cv. Sonia was favoured by medium supplemented with IBA 1.0 mg l^{-1} or NAA 1.8 mg l^{-1} (Pathania *et al.*, 1998).

Rooting of plantlets of terrestrial orchid *Anoectochilus* was achieved by adding activated charcoal 0.2 per cent to the medium (Ganga Prasad *et al.*, 2000).

2.6.3.3.3 Planting Out and Acclimatization

Acclimatization was the best with peat moss showing maximum stem diameter and plant height although perlite was found to be the best for increasing number of roots (Lim *et al.*, 1993). Hardening the *in vitro*

plantlets in a green house with misting facility recorded cent per cent survival (Kuiakose, 1997).

2.6.3.4 Anthurium

2.6.3.4.1 Explant

Kunisaki (1980) obtained higher yields of viable cultures of *Anthurium andreanum* with the use of small explants of vegetative buds.

Shoot tips from *in vitro* grown seedlings was used as explants for the enhanced release of axillary buds (Sreelatha, 1992).

2.6.3.4.2 Growth Regulators

MS medium supplemented with 15 per cent coconut water + BA 0.2 mg l^{-1} was found to be ideal for shoot proliferation in *Anthurium (*Kunisaki, 1980*). In vitro* shoot production from shoot tips of Anthurium was effectively stimulated by BA at 0.2 or 0.5 mg l^{-1} (Geier, 1985).

Maximum number of shoots were observed in a medium containing one fourth strength of MS major nutrients and full strength of minor nutrients supplemented with kinetin 2.0 mg l^{-1} as well as BA 1.0 mg l^{-1} (Sreelatha, 1992). Chen *et al.* (1995) proved that BA 1 mgl^{-1} had the best inductive effect on *in vitro* shoot formation of *Anthurium.*

Kinetin 1.5 mg l^{-1} + IAA 3.0 mg l^{-1} was found to be most promising for shoot proliferation and produced a maximum of 13.49 shoots (Thomas, 1996). MS basal medium supplemented with BAP (0.8 ml l^{-1}), vit. B_5 (0.5 mg l^{-1}), IAA (0.1 mg l^{-1}), PVP (200 mg l^{-1}) and coconut water (150 mg l^{-1}) was found to be best for multiple shoot production (4.66 shoots per explant) in *Anthurium andreanum* cv. Agnihogtri (Mahanta and Paswan, 2001).

Leffring and Soede (1979) reported that kinetin 0.2 mg l^{-1} produced optimum number of shoots where as BA and 2-iso pentenyl adenine produced less number of shoots in *Anthurium.*

According to Geier (1990) rooting of shoots may occur spontaneously when cultures are allowed to stand for long periods under illuminations.

Half strength MS medium supplemented with 0.1 ppm NAA was best for inducing root formation in *Anthurium* (Chen *et al.*, 1995). The highest rooting percentage (80 per cent) was observed in MS basal medium supplemented with 1.0 mg l^{-1} of IAA (Mahanta and Paswan, 2001).

Ajithkumar (1993) observed that, *in vitro* plantlets of *Anthurium* with at least 2.5 to 3 cm length with 3 to 4 leaves and roots had the highest survival rate (100 per cent) irrespective of media and containers.

2.7 LEAF CURING

In Kerala, about two lakh farm families are involved in mat weaving process utilizing the cured leaf strips of *Pandanus*. Quality mats always fetches higher price. It improves consumer preference as well as the financial status of the rural poor those who are preparing the mats. Hence for the improvement of quality attributes standardization of proper curing technology of the leaf is very much essential.

Green leaves after dethorning and splitting into convenient widths were boiled in clean water for 30 minutes to get bleached. Immediately on taking out of boiled water, the splits were shade dried for over night and sun dried (SFAC, 1997).

According to Panda *et al.* (2001) green leaves plucked from the middle portion of the plant after trimming off the spine, made into bundles and dipped in boiling water for 30 minutes. They are then left in cold water for about two days and sun dried.

Materials and Methods

3. MATERIALS AND METHODS

The study on "Morphomolecular characterization and evaluation of *Pandanus* spp". was carried out at the Department of Plantation Crops and Spices and the Department of Plant Biotechnology, College of Agriculture, Vellayani during the year 2002 to 2004, with the view of estimating the genetic diversity at inter and intra species level among 30 genotypes of *Pandanus* selected from different locations of Kerala and Tamil Nadu based on morphological, anatomical and molecular markers and also to study the genetic variability and divergence among 22 male genotypes of *Pandanus*. Investigations were also conducted for the rapid multiplication of the crop through different propagation techniques in order to obtain economic yield with in a short span. Standardization of leaf curing technology was also undertaken to obtain quality mats.

3.1 MORHO ANATOMICAL AND MOLECULAR CHARACTERIZATION

3.1.1 Materials

Survey was conducted in natural growing plantations in different locations *viz.*, Thiruvananthapuram, Kollam districts of Kerala and Kanyakumari district of Tamil Nadu during 2002 to 2004 and 30 genotypes of *Pandanus* belonging to the species *P. fasicularis, P. sanderi, P. pacificus, P. amaryllifolius* and *P. stellatus* of the family Pandanaceae (Table 1) were selected to assess the variability present with regard to growth, yield and quality characters.

3.1.2 Methods

For recording morphological observations, ten matured bearing plants were selected in each location with uniform size and spread and were labeled. From the labeled plants three plants were selected randomly and such three replications were used for the study.

Table 1. Details of *Pandanus* genotypes selected for the study

Sl. No.	Genotype No.	Selection sites	Description of morphotypes
1.	PF 1	Agasteeswaram, Kanyakumari	Long spiny, yellow inflorescence type
2.	PF 2	Kovalam, Kanyakumari	Long spiny, yellow inflorescence type
3.	PF 3	Atchankulam, Kanyakumari	Long spiny, yellow inflorescence type
4.	PF 4	Kalvizhai, Kanyakumari	Long spiny, yellow inflorescence type
5.	PF 5	Nallur, Kanyakumari	Grayish green leaved, short spiny, white inflorescence type
6.	PF 6	Thovazhai, Kanyakumari	Long spiny, yellow inflorescence type
7.	PF 7	Parakkai, Kanyakumari	Grayish green leaved, short spiny, white inflorescence type
8.	PF 8	Chothavizhai, Kanyakumari	Long spiny, yellow inflorescence type
9.	PF 9	Thakkalay, Kanyakumari	Female red fruited type.
10.	PF 10	Thottiode, Kanyakumari	Short spiny, dull white inflorescence type.
11.	PF 11	Alloor, Kanyakumari	Long spiny, yellow inflorescence type
12	PF 12	Eraniel, Kanyakumari	Grayish green leaved, short spiny, white inflorescence type
13.	PF 13	Thirunanthikarai, Kanyakumari	Glossy green leaved, short spiny, white inflorescence type
14.	PF 14	Kollankode 1, Kanyakumari	Female yellow fruited type.
15.	PF 15	Kollankode II, Kanyakumari	Glossy green leaved, short spiny, white inflorescence type.
16.	PF 16	Kollankode III, Kanyakumari	Long spiny, yellow inflorescence type

Table 1. Continued

Sl. No.	Genotype No.	Selection sites	Description of morphotypes
17.	PF 17	Vellayani, Thiruvananthapuram	Glossy green leaved, short spiny, white inflorescence type.
18.	PF 18	Karamana, Thiruvananthapuram	Long spiny, yellow inflorescence type
19.	PF 19	Kazhakutam, Thiruvananthapuram	Short spiny, dull white inflorescence type.
20.	PF 20	Kollam	Short spiny, dull white inflorescence type.
21.	PF 21	Chavara I, Kollam	Female red fruited type.
22.	PF 22	Chavara II, Kollam	Grayish green leaved, short spiny, white inflorescence type
23.	PF 23	Karunagapally, Kollam	Short spiny, dull white inflorescence type.
24.	PF 24	Thazhava I, Kollam	Long spiny, yellow inflorescence type
25.	PF 25	Thazhava II, Kollam	Short spiny, dull white inflorescence type.
26.	PS 26	Vellayani, Thiruvananthapuram	Long green and yellow variegated leafy type
27.	PP 27	Vellayani, Thiruvananthapuram	Glossy green thick leaved, ornamental type
28.	PA 28	Vellayani, Thiruvananthapuram	Musky odoured leaves, culinary type
29.	PSt 29	Vellayani, Thiruvananthapuram	Small green and yellow variegated leaf, potted ornamental herb
30.	PSt 30	Vellayani, Thiruvananthapuram	Small green and white variegated leaf, potted ornamental herb

3.1.2.1 Morphological Characters

Observations on vegetative characters were recorded and the mean values were taken.

3.1.2.1.1 Stem growth Characters

3.1.2.1.1.1 Height of the Plant

Height of the plant was measured from the base of the plant to the tip of the plant and recorded in metres.

3.1.2.1.1.2 Girth of Stem

Girth of the stem was recorded at 30 cm above the ground level and expressed in metres.

3.1.2.1.1.3 Number of Primary Branches

The number of primary branches produced per plant was recorded.

3.1.2.1.1.4 Number of Crowns or Tufts

Number of crowns or tufts produced per plant was recorded.

3.1.2.1.1.5 Number of Ground Suckers

Number of ground suckers produced per plant was recorded.

3.1.2.1.1.6 Number of Prop Roots

Number of prop roots produced per plant was recorded.

3.1.2.1.2 Leaf Growth Characters

For recording leaf growth characters ten leaves were selected randomly from each plant and the mean values were calculated.

3.1.2.1.2.1 Number of Leaves per Crown

Three crowns were selected randomly from each plant and the number of leaves per crown was recorded.

3.1.2.1.2.2 Leaf Colour

The colour of the leaves were observed visually and recorded.

3.1.2.1.2.3 Leaf Length

The length of the leaf blade was measured from the base to the tip of the leaf and expressed in centimetres.

3.1.2.1.2.4 Width of Leaf

Maximum width of the leaf was measured in centimetres.

3.1.2.1.2.5 Leaf Weight

Weight of the leaf from each plant was recorded and expressed in grams.

3.1.2.1.2.6 Leaf Yield per Plant

The total leaf yield from each plant was recorded in kilograms.

3.1.2.1.2.7 Stiffness of the Leaf

Stiffness of the leaf was observed by hand feeling and recorded.

3.1.2.1.3 Spine Characters

For recording spine characters, ten leaves were selected randomly from each plant and the mean values were calculated.

3.1.2.1.3.1 Number of Spines per 10 cm Length of Leaf Margin

Number of spines in 10 cm length of leaf margin was recorded.

3.1.2.1.3.2 Spine Colour

Spine colour was observed visually and recorded.

3.1.2.1.3.3 Angle of Spine Projection

The angle of spine projection was measured by protractor and expressed in degrees.

3.1.2.1.3.4 Spine Length

The spine length of three randomly selected spines from each leaf was observed under microscope using standardized ocular micrometer and recorded in millimetres.

3.1.2.2 Anatomical Characters

3.1.2.2.1 Leaf Thickness

Free hand cross sections of three randomly selected leaves were observed under microscope and the thickness was measured using ocular micrometer and recorded in millimetres.

3.1.2.2.2 Number of Stomata in the Adaxial Surface of the Leaf

A thin film of quick fix was applied over three randomly selected leaves. The thin film was removed after few minutes and the number of stomatal impressions was counted using a microscope (10x magnification). Three accurately measured paper bits of $1mm^2$ were placed inside the eyepiece and the stomatal density in that particular area was counted and recorded.

3.1.2.2.3 Number of Stomata in the Abaxial Surface of the Leaf

The number of stomata in the abaxial surface was observed and recorded as above.

3.1.2.3 Molecular Markers (RAPD)

3.1.2.3.1 Isolation of Genomic DNA

The genomic DNA was isolated based on modified Murray and Thompson (1980) method after making some necessary changes. About 500 mg of tender

emerging leaf material was collected in the morning hours between 8 and 9 am. It was washed in running tap water followed by one per cent polyvinyl pyrollidone (PVP) solution and in distilled water for four to five times after chopping the leaves coarsely. Then the chopped leaf bits were wiped off completely with tissue paper and transferred to a sterilized pre-chilled porcelain mortar and were ground well to a fine powder in liquid nitrogen. The powder was then transferred to a 15 ml polypropylene centrifuge tube containing 5 ml of pre-warmed CTAB extraction buffer with the help of sterile spatula (extraction buffer: 2 per cent (2w/v) CTAB (Hexa decyl Trimethyl Ammonium Bromide), 100 mM Tris.HCL, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 0.2 per cent mercaptoethanol). One ml of one per cent PVP solution was also added along with the sample. Then the samples were incubated at 60°C for 30 minutes in water bath with occasional mixing by gentle swirling. After 30 minutes the samples were taken and kept at room temperature for 10 minutes. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed by inversion and centrifuged at 15,000 rpm for 10 minutes at 5°C temperature. The aqueous phase was taken with a wide-bore pipette and transferred to a clean centrifuge tube and with this equal volume of phenol chloroform isoamyl alcohol was added and centrifuged for 10 minutes at 5°C in 15000 rpm. The upper phase was collected once again and extracted with chloroform: isoamylalcohol (24:1). Then with the aqueous phase, 0.1 volume of 3 M sodium acetate (pH 4.8) and 2/3 volume of isopropanol were added. It was mixed by quick gentle inversion to precipitate the DNA. Then the precipitated DNA was pelleted by centrifugation at 10, 000 rpm for 5 minutes at a temperature of 5°C. The supernatant was decanted carefully and the DNA pellet was washed with 70 per cent cold ethanol and air dried. Then it was dissolved in 50-100 TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) and stored at 4°C.

All the materials used in the preparation and storage of reagents including reagent bottles, conical flasks, centrifuge tubes, spatula, glass rods, funnels and tips of micro pipettes were washed with labolin solution, rinsed with distilled water and autoclaved for 45 minutes before use. Phenol used was

saturated and equilibrated using Tris buffer and pH adjusted to 8.0. The protocol was given in Appendix II.

3.1.2.3.2 Quantification of DNA

The quantification of DNA was carried out with the help of UV-vis spectrophotometer (Spectronic Genesys 5).

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

Amount of the DNA (μg/μl) = A_{260} x 50x dilution factor / 1000

Where A_{260} is the absorbance at 260 nm and A_{280} is the absorbance at 280 nm.

The quality of the DNA could be judged from the ratio of the OD values recorded at 260 nm and 280 nm. The A_{260}/A_{280} ratio between 1.5 and 2.0 indicates best quality of DNA.

3.1.4.4 Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out in a horizontal gel electrophoresis unit. Required amount of agarose was weighed out (0.9 per cent for visualizing the genomic DNA and 1.4 per cent for visualizing the amplified products) and melted in 1xTAE buffer (0.04 M Tris acetate, 0.001 M EDTA, pH 8.0) by boiling. After cooling to about 50°C ethidium bromide was added to a final concentration of 0.5μg ml^{-1}. The mixture was then poured to a pre set template with appropriate comb. After solidification of the agar, the comb and the sealing tapes were removed and the gel was mounted in an electrophoresis tank. The tank was loaded with 1 x TAE

buffer, so that it just covered the entire gel. Required volume of DNA sample and gel loading buffer (6X loading dye with 40 per cent sucrose, 0.25 per cent bromophenol blue) were mixed. Each well was loaded with 20 µl of sample. One of the wells was loaded with 5.0 µl of PCR molecular weight marker along with required volume of the gel-loading buffer. Electrophoresis was performed at 75 volts until the loading dye reached ¾th length of the gel. The gel was visualized using an ultraviolet visible (UV-Vis) transilluminator.

3.1.2.3.3 Polymerase Chain Reaction

DNA amplification was done using sixty arbitrarily designed decamer primers (Operon Inc, CA, USA.) adopting the procedure of Lim *et al.* (1999) with required modifications.

Polymerase chain reactions were carried out in a volume of 25 µl containing 2.5 µl 10 X buffer (10 mM Tris HCI pH 9.0, 1.50 mM Mg Cl_2, 50 mM KCl and 0.01 per cent gelatin), 10 pM primer, 250 µM each of deoxynucleotides (dNTPs), 0.2 units of Taq DNA polymerase and 20 ng of genomic DNA. Amplifications was performed in a programmable Thermal Controller (MJ Research, Inc.) for an initial denaturation at 95^{0}C for one minute, followed by 35 cycles of denaturatioin at 95^{0}C for one minute, annealing at 35^{0}C for two minutes and extension at 72^{0}C for two minutes. A final extension at 72^{0}C for 10 minutes was included after the last cycle. Finally the products of amplification were cooled to 4^{0}C. A negative control containing sterile water instead of template was included in each reaction set.

The DNA fragments produced and the PCR molecular weight marker were visualized in a 1.4 per cent agarose gel electrophoresis, stained with ethidium bromide and photographed with the help of gel doc system. The RAPD bands were represented as '+' for presence and '-' for absence. The PCR was repeated twice in order to confirm the

reproducibility. The amplified products of four primers alone used for further analysis since it produced amplification for most of the genotypes.

3.1.3 Statistical Analysis

3.1.3.1 Morpho anatomical Characters

The data collected were tested for the homogeneity of error variances and subjected to statistical analysis in CRD with three replications to test the significance of genotypic differences among the 30 genotypes of *Pandanus*. Analysis of Variance (ANOVA) technique was applied and the mean, variance and standard error were estimated (Panse and Sukhatme, 1985).

3.1.3.2 Molecular Markers (RAPD)

The reproducible bands were scored for their presence (+) or absence (-) for all the genotypes included in the study. Based on the presence or absence data score value was given as one for presence of band and zero for absence of band. A genetic similarity matrix was constructed using the Jaccard's coefficient method (Jaccard, 1908).

Formula:

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

a = number of bands present in both the genotypes in a pair

b = number of bands present in the first genotype but not in the second one

c = number of bands present in the second but not in the first

Based on the similarity coefficient the association between the genotypes was computed with the help of a software package NTSYS (version 2.02).

Using the similarity coefficient values between the genotypes, a dendogram was constructed following the Unweighted Pair Group Method with arithmetic Average (UPGMA). Similarity between the 30 genotypes of *Pandanus* was assessed from the dendrogram.

3.2 GENETIC VARIABILITY AND DIVERGENCE

3.2.1 Materials

Twenty two male genotypes of *Pandanus* belonging to the species *P. fasicularis* were utilized for the variability and divergence studies.

3.2.2 Methods

As per experiment I, three plants were selected randomly per replication for recording observations on vegetative and floral characters.

3.2.2.1 Vegetative Characters

Stem, leaf and spine growth characters were recorded as per experiment I.

3.2.2.2 Inflorescence Characters

For recording inflorescence characters, five inflorescences from each plant were selected and the mean values calculated.

3.2.2.2.1 Number of Inflorescence

Number of inflorescences produced per plant for a period of one year was recorded.

3.2.2.2.2 Length of the Inflorescence

Length of the inflorescence was measured from the base of the inflorescence stalk to the tip of the spathe and expressed in centimetres.

3.2.2.2.3 Girth of the Inflorescence

Girth of the inflorescence was measured in the middle portion of the inflorescence and expressed in centimetres.

3.2.2.2.4 Weight of the Inflorescence

Inflorescence weight was recorded in grams.

3.2.2.2.5 Number of Spathes per Inflorescence

Number of spathes in each inflorescence was counted and recorded.

3.2.2.2.6 Number of Androecious Fesicles per Inflorescence

Number of androecious fesicles in three randomly selected inflorescences were counted and recorded.

3.2.2.2.7 Colour of the Spathe

Spathe colour was observed visually and recorded.

3.2.2.2.8 Shelf life of the Inflorescence

Number of days the inflorescence retaining its freshness was observed and recorded.

3.2.2.2.9 Fragrance of the Inflorescence

Fragrance of the inflorescence was recorded by sensory feeling.

3.2.2.2.10 Quality Characters

Essential oil

The materials used for estimation of essential oil was fresh flower collected from their respective variant plant types with in 12 to 20 hours after the emergence of the inflorescence. The method adopted for the estimation of essential oil was hydro distillation, for two hours using Clevenger distillation apparatus. Since the oil recovery was poor, the distillate collected was repeatedly re distillated with fresh flowers. The finally collected distillate was transferred to a separating funnel and thoroughly mixed with ether. While mixing the essential

oil was separated and it was collected carefully after draining the distillate. The oil recovery was expressed in percentage (V/W) on wet weight basis.

3.2.2.3 Anatomical Characters

Leaf thickness and number of stomata both in the adaxial and abaxial surfaces of the leaf were recorded as per experiment I.

3.2.3 Statistical Analysis

The data collected were subjected to the following statistical analysis after testing the homogeneity of error variances.

Analysis of Variance (ANOVA) technique was used to test the significance of genotypic differences among the 22 genotypes. Mean, variance, standard error and coefficient of variation were estimated. The character associations were estimated through correlation coefficients and path coefficient analysis using Analysis of Covariance (ANACOVA) technique (Panse and Sukhatme, 1985).

The methodology employed in the estimation of the parameters is given below. For the two characters x and y measured on 'g' genotypes in completely randomized design with 'r' replications, the variance/ co-variance analysis (ANACOVA) is as follows

3.2.3.1 Analysis of variance /co-variance

Source	df	Mean square		
		X	Y	XY
Between genotypes	(g-1)	Gxx	Gyy	Gxy
Error	(r-1) (g –1)	Exx	Eyy	Exy

3.2.3.2 Estimates of Components of Variance and Co-variance

	Genotypes	Environment	Phenotype
X	$\sigma^2_{gx} = \frac{G_{xx} - E_{xx}}{r}$	$\sigma^2_{ex} = E_{xx}$	$\sigma^2_{px} = \sigma^2_{gx} + \sigma^2_{ex}$
Y	$\sigma^2_{gy} = \frac{G_{yy} - E_{yy}}{r}$	$\sigma^2_{ey} = E_{yy}$	$\sigma^2_{py} = \sigma^2_{py} + \sigma^2_{ey}$
XY	$\sigma_{gxy} = \frac{G_{xy} - E_{xy}}{r}$	$\sigma_{exy} = E_{xy}$	$\sigma_{pxy} = \sigma_{gxy} + \sigma_{exy}$

3.2.3.3 Coefficient of Variation

Phenotypic and genotypic coefficient of variation (PCV and GCV) for a trait x were estimated as

$$GCV = \frac{\sigma_{gx}}{\overline{X}} \times 100$$

$$PCV = \frac{\sigma_{px}}{\overline{X}} \times 100$$

Where,

σ_{gx}: genotypic standard deviation

σ_{px}: Phenotypic standard deviation

$\overline{X}$: Mean of the character under study

3.2.3.4 Correlation Analysis

The correlation coefficients (phenotypic, genotypic and environmental) between two characters denoted as X and Y were worked out as follows

$$\text{Genotypic correlation } (\gamma_{gxy}) = \frac{\sigma_{gxy}}{\sigma_{gx} \times \sigma_{gy}}$$

$$\text{Phenotypic correlation } (\gamma_{pxy}) = \frac{\sigma_{pxy}}{\sigma_{px} \times \sigma_{py}}$$

$$\text{Environmental correlation } (\gamma_{exy}) = \frac{\sigma_{exy}}{\sigma_{ex} \times \sigma_{ey}}$$

3.1.3.5 Heritability and Genetic Advance

Heritability (H^2) in broad sense was estimated as the proportion of heritable components of variation (Jain, 1982).

$$\text{Heritability coefficient } (H^2) = \frac{\sigma_{gx}^2}{\sigma_{px}^2} \times 100$$

$$\text{Genetic advance as percentage of mean (GA)} = \frac{kH^2\sigma_{px}}{\overline{x}} \times 100$$

Where k is the selection differential whose value = 2.06 if five per cent selection is to be practiced (Miller *et al.*, 1958).

3.2.3.6 Path Coefficient analysis

Path analysis is applied to identity relatively important component characters (which are the independent variables) of a dependent variable on the basis of their direct and indirect effects and it helps the plant breeder to lay emphasis on component characters during selection. The solution of the matrix equation

$$\underset{\sim}{A}\,\underline{B} = \underline{C}$$

Where $\underset{\sim}{A}$ is the genotypic inter-correlation matrix with respect to independent variables, $\underline{B}$ is the column vector of path coefficients and $\underline{C}$ is the column vector of genotypic correlation co-efficient between the dependent and independent variables. Vector $\underline{B}$ provides estimates of path coefficients, which means the direct effect of the independent variable on the dependent the independent variable, and also the indirect effect of each independent variable on dependent variable through other variables. Residual variation which could arise from unknown and uncontrollable factor was also estimated using vector $\underline{B}$ (Dabholkar, 1992).

The direct and indirect effects were calculated and classified into very high (>1), high (0.30 to 0.99), moderate (0.20 to 0.29), low (0.10 to 0.19) and negligible (0.00 to 0.09) (Lenka and Mishra, 1973).

3.1.3.7 Mahalanobis (D^2) Analysis

Mahalanobis D^2 (1936) technique was applied to cluster the 22 *Pandanus* genotypes for the i^{th} and j^{th} genotypes, the D^2 value is computed as

$$D^2 = \sum_{i=1}^{k} (Xil - Xjl)^2$$ where k is the number of characters

The genotypes were grouped into several clusters based on these D^2 values by Tocher's method of clustering (Rao, 1952).

3.3 PROPAGATION

3.3.1 Seeds

3.3.1.1 Materials

Seeds were extracted from the fully matured and ripe fruits of female genotype PF 14 and washed thoroughly to remove the pulp and fleshy material adhering to them. The seeds those were floating in water were discarded and the filled seeds utilized for pre germination treatments.

3.3.1.2 Experimental Design and Layout

The experiment was laid out in a Completely Randomized Design (CRD) with nine treatments and three replications. Thirty seeds were sown under each treatment.

Treatment details

T_1 Control (as such sowing of seeds with out any special treatments)

T_2 Soaking in water for 48 hours

T_3 Treating in hot water for 30 minutes at 70°C

T_4 Treating in GA_3 100 ppm for 30 minutes

T_5 Treating in con. H_2SO_4 for 5 minutes

T_6 Soaking in cow urine (diluted with equal volume of water) for 12 hours

T_7 Soaking in diluted cow urine and cow dung slurry for 12 hours

T_8 Soaking in diluted cow urine for 12 hours followed by GA_3 100 ppm for 30 minutes

T_9 Mechanical scarification of the seeds

After treatment the seeds were sown in poly bags containing pot mixture and kept under shade net house and watered regularly. Three plants were selected randomly in each replication and utilized for recording growth observations.

3.3.1.2.1 Growth Parameters

Shoot and root growth parameters were recorded at $4^{th,}$ 8^{th} and 12^{th} months of sowing.

3.31.2.1.1 Germination Percentage

Number of seeds germinated under each treatment was recorded and the percentage of germination was calculated.

3.3.1.2.1.2 Number of days Taken for the Germination of Seeds

Number of days for the germination of seeds under various treatments were recorded.

3.3.1.2.1.3 Height of the Seedling

Height of the seedling due to various treatment effects were recorded at different growth stages and expressed in centimetres.

3.3.1.2.1.4 Girth of the Seedling

Stem girth of the seedlings under different treatments at different growth stages were recorded in centimetres.

3.3.1.2.1.5 Number of Leaves

Number of leaves was recorded in different treatments at different growth stages.

3.3.1.2.1.6 Length and Width of the Leaf

Length and width of the longest leaf was measured in different treatments at different growth stages and expressed in centimetres.

3.3.1.2.1.7 Number of Primary Roots

Number of primary roots was recorded in each treatment.

3.3.1.2.1.8 Length and Girth of the Roots

Length and girth of the longest primary root was recorded at different growth stages in different treatments and expressed in centimetres.

3.3.1.2.1.9 Root Spread

The roots were spread over a marked paper and the root spread was measured at its broadest part and expressed in centimetres.

3.3.1.3 Statistical Analysis

The data generated from the various treatments were subjected to statistical analysis in completely randomized design as per Panse and Sukhatme (1985).

3.3.2 Vegetative Propagation

3.3.2.1 Planting Material

The following four types of planting material were collected from the flowering plants of genotype PF 12 and planted in the nursery area of the Department of Plantation Crops and Spices. The experimental site was located at an altitude of 29 m above MSL and at 8° N latitude and 76° E longitude.

T_1 Terminal cuttings with crown

T_2 Limb cuttings without crown

T_3 Aerial suckers

T_4 Ground suckers

The experiment was laid out in Randomized Block Design (RBD) with four replications and each type of planting material was considered as one treatment. The population maintained per treatment was 16. The soil type of the experimental site was red loam. The following observations were recorded to evaluate the performance of different types of planting material.

3.3.2.2 Methods

The following growth observations were recorded at 3^{rd}, 6^{th}, 9^{th}, 12^{th}, 15^{th}, and 18^{th} months after planting to evaluate the performance of different type of planting material.

3.3.2.2.1 Establishment Percentage

The percentage of establishment of different types of planting material was recorded.

3.3.2.2.1 Number of Days for Sprouting

The number of days taken for the sprouting of different types of planting material was recorded.

3.3.2.2.3 Plant Height and Girth

Plant height and girth were measured and expressed in centimetres.

3.3.2.2.4 Number of Leaves

Number of leaves produced in each planting material was recorded.

3.3.2.2.5 Length and Width of Leaf

The maximum length and width of the middle leaf was recorded in centimetres.

3.3.2.2.6 Weight of the Leaves

The total weight of leaves produced in each type of planting material was recorded in grams.

3.3.2.2.7 Number of Suckers and Prop Roots

Number of suckers and prop roots produced in each type planting material was recorded.

3.3.2.3 Statistical Analysis

The data generated in this experiment was subjected to statistical analysis in randomized block design as per Panse and Sukhatme (1985).

3.3.3 Micropropagation through Enhanced Release of Axillary Buds

3.3.3.1 Explant Establishment (Stage I)

3.3.3.1.1 Collection and Preparation of Explants for Culturing

Shoot tip explants were collected from the suckers of healthy plants. The suckers after separation from the mother plant were de topped to a size of 5 cm in length retaining a small portion of the underground stem. The explants after collection were immediately taken to the laboratory and washed thoroughly in tap water to remove all the dirt and soil particles adhering to them. Then the outer whirls of matured leaves were removed and the shoot tip explants were washed with 5 per cent PVP solution followed by distilled water for 4 or 5 times. Further sterilization procedures were carried out under perfect aseptic conditions in a Thermadyne, laminar airflow chamber. The explants were subjected to surface sterilization using different chemicals as given below:

3.3.3.1.2 Surface Sterilization

T_1 Mercuric chloride 0.06 per cent for 5 minutes

T_2 Mercuric chloride 0.06 per cent for 7 minutes

T_3 Mercuric chloride 0.06 per cent for 10 minutes

T_4 Mercuric chloride 0.08 per cent for 5 minutes

T_5 Mercuric chloride 0.08 per cent for 7 minutes

T_6 Mercuric chloride 0.08 per cent for 10 minutes

T_7 Mercuric chloride 0.1 per cent for 5 minutes

T_8 Mercuric chloride 0.1 per cent for 7 minutes

T_9 Mercuric chloride 0.1 per cent for 10 minutes

Observations on the percentage of culture survival (as exhibited by the retention of the green colour), percentage of cultures contaminated and percentage of cultures showing explant death were made on five explants per treatment after three weeks of culturing.

The explants after surface sterilization were rinsed with sterile double distilled water for four times. Then placed over sterile filter paper kept in sterile petridish for drying.

3.3.3.1.3 Basal Medium of Culture

In order to study the morphogenetic response of *Pandanus* explants in culture, half strength basal MS medium (Murashige and Skoog, 1962) was tried. The composition of the medium is given in Appendix III. This was supplemented with cytokinins and auxins at various concentrations as detailed below:

Explant : Shoot tip

Growth hormones : BA and NAA (3 x 3 combinations)

T_1 BA 0.5 mg l^{-1} and NAA 0.2 mg l^{-1}

T_2 BA 0.5 mg $l^{-1}$and NAA 0.3 mg l^{-1}

T_3 BA 0.5 mg l^{-1} and NAA 0.4 mg l^{-1}

T_4 BA 1.0 mg l^{-1} and NAA 0.2 mg l^{-1}

T_5 BA 1.0 mg l^{-1} and NAA 0.3 mg l^{-1}

T_6 BA 1.0 mg l^{-1} and NAA 0.4 mg l^{-1}

T_7 BA 1.5 mg l^{-1} and NAA 0.2 mg l^{-1}

T_8 BA 1.5 mg l^{-1} and NAA 0.3 mg l^{-1}

T_9 BA 1.5 mg l^{-1} and NAA 0.4 mg l^{-1}

T_{10} Half MS medium without growth hormones

$CuSO_4$ 100 ppm and 0.7 per cent PVP were also added to the media to avoid contamination and discolouration of the media and explant.

Observations on the percentage of culture establishment, number of days taken for culture establishment and number of shoots per culture were made after four weeks of culturing.

The chemicals used for preparing the culture medium were of analytical grade from Sisco Research Laboratories or Merck. Standard procedures were followed for the preparation of the media. Stock solutions of major and minor nutrients were prepared first by dissolving the required quantity of chemicals in distilled water and stored under refrigerated conditions in amber coloured bottles. The stock solution of major nutrients was prepared fresh in every four weeks and that of vitamins, amino acids and phytohormones were prepared fresh in every week.

Specific quantities of the stock solution of chemicals and phytohormones were pipetted out into a 1000 ml beaker. For half MS medium the macronutrient concentration was reduced to half. Sucrose and inositol were added fresh and dissolved. Then the volume was made up to 1000 ml by adding double distilled water. The pH of the solution was adjusted using an electronic pH meter using 0.1N HCl / NaOH. Agar was added to the medium and final volume made up exactly to one litre. The solution was then melted at a temperature of 90 to 95°C in hot air oven or in gas stove. The medium was then poured hot to the sterilized culture vessels and tightly closed with non absorbant cottonwool plugs.

In order to ensure aseptic condition of the medium, the containers plugged with cotton were autoclaved, for 15 to 20 minutes at 1.06 kg /cm^2 pressure at 121°C temperature. After sterilization, the culture vessels were immediately transferred to an air conditioned culture room.

3.3.3.1.4 Inoculation of Explants

All the inoculation operations were carried out under perfect aseptic conditions in a Thermadyne laminar airflow cabinet. The cotton wool plug of the culture vessel was removed and the vessel neck was first flamed over a burner kept in the chamber. The sterile explants were quickly transferred to the culture vessels containing suitable culture medium, using sterilized forceps. The neck of the culture vessel was once again flamed and quickly closed with cotton plug. The culture vessels were then transferred to a culture room, where the explants

were incubated at a temperature of 27 ± 2°C. Artificial illumination was provided using cool white fluorescent lamps, photoperiod was fixed as 16 h per day.

3.3.3.2 Induction of Axillary Shoots and Rapid Shoot Multiplication (Stage II)

3.3.3.2.1 Basic Proliferation Medium

Full strength MS medium was used for the induction of axillary shoots. The explants were shoots from established culture. The concentration of the growth regulating substances *viz.*, cytokinins and auxins were as detailed below:

T_1 BA 2 mg l^{-1} and NAA 0.6 mg l^{-1}

T_2 BA 2 mg l^{-1} and NAA 0.8 mg l^{-1}

T_3 BA 2 mg l^{-1} and NAA 1.0 mg l^{-1}

T_4 BA 3 mg l^{-1} and NAA 0.6 mg l^{-1}

T_5 BA 3 mg l^{-1} and NAA 0.8 mg l^{-1}

T_6 BA 3 mg l^{-1} and NAA 1.0 mg l^{-1}

T_7 BA 4 mg l^{-1} and NAA 0.6 mg l^{-1}

T_8 BA 4 mg l^{-1} and NAA 0.8 mg l^{-1}

T_9 BA 4 mg l^{-1} and NAA 1.0 mg l^{-1}

Observations on percentage of culture developing shoots, number of shoots produced per culture, length of the longest shoot and number of leaves produced per shoot were made on five explants after four weeks of culturing.

Coconut water 200 ml was added as medium supplement and as carbon source sucrose 30 g was added.

3.3.3.3 In vitro Rooting and Acclimatization (Stage III)

3.3.3.3.1 *In Vitro* Rooting

3.3.3.3.1.1 Basal Rooting Medium

Half strength MS medium with sucrose at the concentration of 20 g per litre was used as rooting medium. Shoots of 2 to 3 cm length with

five to six leaves excised from shoot proliferating cultures were utilised as explants. The different auxins and their levels tried for rooting of *Pandanus* shoots are the following:

T_1 NAA 0 and 2 mg l^{-1} IBA

T_2 NAA 0 and 4 mg l^{-1} IBA

T_3 NAA 1 mg l^{-1} and 2 mg l^{-1} IBA

T_4 NAA 1 mg l^{-1} and 4 mg l^{-1} IBA

T_5 NAA 2 mg l^{-1} and 2 mg l^{-1} IBA

T_6 NAA 2 mg l^{-1} and 4 mg l^{-1} IBA

T_7 NAA 3 mg $l^{-1}$and 2 mg l^{-1} IBA

T_8 NAA 3 mg l^{-1} and 4 mg l^{-1} IBA

Observations on the percentage of cultures showing root initiation, number of roots produced per shoot, nature of roots and the number of days taken for root initiation were made on five cultures after four weeks of culturing.

3.3.3.3.1.2 Rooting media supplements (Activated Charcoal)

Activated charcoal (AC) at the concentration of 0.1 per cent, 0.2 per cent, 0.3 per cent, 0.4 per cent, 0.5 per cent and 0.6 per cent, were added to the rooting media containing half strength MS mineral salts with growth regulators *viz.*, NAA 1 mg l^{-1} and IBA 2 mg l^{-1} .The treatment details are:

T_1 NAA 1 mgl^{-1} and IBA 2mgl^{-1} + AC 0.1 per cent
T_2 NAA 1 mgl^{-1} and IBA 2mgl^{-1} + AC 0.2 per cent
T_3 NAA 1 mgl^{-1} and IBA 2mgl^{-1} + AC 0.3 per cent
T_4 NAA 1 mgl^{-1} and IBA 2mgl^{-1} + AC 0.4 per cent
T_5 NAA 1 mgl^{-1} and IBA 2mgl^{-1} + AC 0.5 per cent
T_6 NAA 1 mgl^{-1} and IBA 2mgl^{-1} + AC 0.6 per cent

Observations on the percentage cultures showing root initiation, number roots produced per shoot and the number days taken for root initiation were made on five cultures after four weeks of culturing.

3.3.3.3.2 Acclimatization

In order to acclimatize the *Pandanus* plantlets produced *in vitro*, the rooted plantlets with 3 to 5 leaves and 5 to 10 roots were taken out from the culture vessels with the help of forceps. Then the agar adhering to the roots were completely removed by washing with distilled water and planted in plastic pots containing sterile sand. The pots were covered with ventilated polythene covers and kept under shade net house.

Observations were made on ten plants on the percentage of plantlet survival after one month of planting out.

3.3.3.4 Field Planting and Evaluation (Stage IV)

After one month of acclimatization, the plants were planted out in earthen pots of size 20 cm height and 15 cm diameter containing ordinary non sterile potting mixture (1 sand: 1 soil: 1 red earth: 1 dried and powdered cattle manure) and kept under shade net house with regular watering.

Observations were recorded on plant survival, plant height, number of leaves per plant after 3 and 6 months after planting out.

3.3.3.5 Statistical Analysis

The data generated from the various experiments were subjected to statistical analysis in completely randomized design, wherever necessary as per Panse and Sukhatme (1985).

3.4 STANDARDIZATION OF CURING TECHNOLOGY OF LEAVES

3.4.1 Material

Young and partially matured leaves harvested from genotype No. PF12 was used for different curing treatments.

3.4.2 Methods

Harvested leaves were subjected to dethorning by tearing off leaf margins and midribs. After that the leaves were split into strips of 0.8 to1cm width and cured as per the treatment schedule as detailed below:

T_1 Sun drying of leaf splits as such without any treatment

T_2 Shade drying of leaf splits with out any treatment

T_3 Boiling in clean water for 30 minutes and sun drying

T_4 Boiling in clean water for 30 minutes and shade drying

T_5 Soaking in clean water for 48 hours and sun drying

T_6 Soaking in clean water for 48 hours and shade drying

T_7 Boiling in 10 per cent turmeric water for 30 minutes and sun drying

T_8 Boiling in 10 per cent turmeric water for 30 minutes and shade drying

T_9 Boiling in water for 30 minutes and sun drying and after that treating with synthetic dyes

For treatment 9 the leaf strips were first cured as per treatment 3 and again treated with synthetic dyes of different colours with a view of value addition.

The leaf strips after curing was given to the mat weavers for weaving according to different treatments. The art of weaving was performed by criss cross weaving of the alternately arranged leaf strips in opposite directions till the required size was obtained. Care was taken

while weaving to strengthen the edges. After weaving, the mats were distributed to 10 persons. A five point scale scoring sheet was formulated and the evaluation panel members were asked to score the products in terms of colour, texture, eye appeal, durability and over all acceptability. Durability was assessed after a period of two years. Scorecard is presented in Appendix IV.

3.4.3 Statistical Analysis

The data generated in this experiment was subjected to statistical analysis in completely randomized design with three replications as per Panse and Sukhatme (1985).

Results

4. RESULTS

The results of the present investigation, "Morphomolecular characterization and evaluation of *Pandanus* spp." are presented in this chapter.

4.1 MORPHO ANATOMICAL AND MOLECULAR CHARACTERIZATION

Thirty genotypes of *Pandanus* consisting of different species, male and female lines were evaluated for the genotypic differences existing among them based on morphological, leaf anatomical and molecular markers.

4.1.1 Morphological Characters

Analysis of variance revealed significant differences between the genotypes for characters *viz.*, stem, leaf and spine growth characters.

4.1.1.1 Stem Growth Characters

The growth performance of the 30 genotypes of *Pandanus* with respect to the stem growth characters are presented in Table 2.

The plant height ranged from 0.55 to 6.53 m. Genotype PF 14 recorded the highest plant height of 6.53 m and it was on par with PF 3, PF 5, PF 7, PF 13, PF 15, PF 17, PF 18 and PF 21. Genotype PSt 30 recorded the lowest plant height of 0.55 m and it was on par with PP 27 and PSt 29.

Regarding plant girth, the highest value of 0.35 m was recorded by genotype PF 7 and the lowest value of 0.03 m was recorded by genotype PSt 30. The number of primary branches varied from 1.00 to 11.66. The highest value was with genotype PF 11 (11.6) and the lowest value was with genotypes PP 27, PA 28, PSt 29 and PSt 30 (1.00).

The number of crowns per plant was highest (57.66) in genotype PF 11 and it was followed by PF 3 (54.30). The least number of crowns per plant was recorded by PF 14 (9.33) and it was on par with PF 13, PF 15 and PF 17 (10.66, 10.66 and 12.00 respectively).

Table 2. Stem growth characters of 30 genotypes of *Pandanus*

Genotype No.	Plant height (m)	Plant girth (m)	Number of primary branches	Number of crowns	Number of ground suckers	Numbers of prop roots
PF 1	6.00	0.31	9.00	47.00	14.00	31.33
PF 2	5.16	0.28	7.33	36.33	6.33	35.66
PF 3	6.20	0.31	11.33	54.30	13.33	36.00
PF 4	4.30	0.28	8.66	37.00	5.00	53.66
PF 5	6.43	0.32	6.33	24.66	10.66	27.33
PF 6	6.00	0.31	10.33	51.66	11.00	38.00
PF 7	6.50	0.35	8.00	32.00	9.00	39.66
PF 8	4.00	0.27	10.66	39.33	3.66	51.33
PF 9	4.90	0.26	7.00	30.33	20.00	9.66
PF 10	5.03	0.27	5.00	25.00	24.30	8.30
PF 11	6.03	0.30	11.66	57.66	10.00	32.00
PF 12	5.93	0.32	7.33	28.00	13.66	32.00
PF 13	6.30	0.31	2.66	10.66	32.66	18.30
PF 14	6.53	0.32	2.33	9.33	33.33	14.00
PF 15	6.43	0.31	2.66	10.66	33.66	15.33
PF 16	4.30	0.27	10.66	40.66	10.60	45.00
PF 17	6.46	0.33	3.00	12.00	34.60	15.30
PF 18	6.06	0.32	11.33	48.33	12.33	32.00
PF 19	5.56	0.28	4.66	23.33	23.00	10.00
PF 20	5.10	0.27	4.66	23.00	23.66	11.33
PF 21	6.20	0.31	7.66	32.66	15.33	23.00
PF 22	5.83	0.30	7.66	31.66	14.66	24.66
PF 23	5.26	0.29	5.66	26.66	31.33	12.33
PF 24	6.03	0.29	11.00	48.33	11.33	29.60
PF 25	5.86	0.31	11.00	50.66	8.00	21.00
PS 26	3.46	0.34	3.00	23.00	1.00	39.00
PP 27	1.00	0.19	1.00	19.00	50.00	19.30
PA 28	1.90	0.13	1.00	24.00	15.30	19.33
PSt29	0.63	0.05	1.00	30.00	9.60	5.60
PSt30	0.55	0.03	1.00	25.00	8.66	6.66
SEd	0.2345	0.0150	1.0611	4.2374	4.2120	3.4437
CD (0.05)	0.469**	0.030**	2.123**	8.476**	8.425**	6.889**

The genotype PF 27 produced the highest number of ground suckers (50.00) followed by PF 17 (34.60) while, genotype PS 26 produced the least number of ground sucker (1.00).

With respect to the prop roots the highest number was recorded by genotype PF 4 (53.66) followed by PF 8 (51.33). The lowest number of prop roots (5.60) was recorded by PSt 29.

4.1.1.2 Leaf and Spine Growth Characters

Differences in the leaf and spine growth characters of 30 genotypes of *Pandanus* are presented in Table 3.

The number of leaves per crown varied from 14.60 to 106.60. The genotype PF 14 recorded the highest number of (106.60) leaves followed by PF 13 and PF 17 (102.33). The least value was recorded by the genotype PSt 29 (14.60) and it was on par with PSt 30 (17.00).

With respect to leaf length, the highest value was recorded by PF 12 (238.00 cm) which was on par with PF 10 (228.00 cm). The lowest leaf length of 29.00 cm was recorded by PSt 29 and it was on par with PSt 30 (34.00 cm).

Leaf width ranged from 1.00 cm to 13.00 cm. The genotype PP 27 recorded the highest leaf width of 13.0 cm and it was significantly superior to all other genotypes. Lowest leaf width of 1.00 cm was recorded by genotype PSt 29 and PSt 30 and it was followed by PA 28 (3.00 cm).

The highest leaf weight was recorded by genotype PP 27 (51.60 g) which was significantly superior to other genotypes. Lowest leaf weight of 1.00 g was recorded by PSt 29 and PSt 30.

Leaf yield per plant varied from 0.42 to 88.20 kg. The maximum value was recorded by genotype PF 21 (88.20) and it was on par with PF 22, PF 25, PF 7 and PF 12 (87.36, 80.08, 78.65, and 78.26 kg respectively). The minimum leaf yield was recorded by genotype PSt 29 and PSt 30 (0.42 kg).

Number of spines in 10 cm length of lamina, recorded the maximum value (34.00) by the genotype PP 27 and the minimum (6.00) by PSt 29.

Table 3. Leaf and spine growth characters of 30 genotypes of *Pandanus*

Genotype No	Number of leaves/ crown	Leaf length (cm)	Leaf width (cm)	Leaf weight (g)	Leaf yield per plant (kg)	Number of spines in 10 cm length of lamina	Angle of spine projection in degrees	Spine length (cm)
PF1	54.00	145.00	5.36	25.66	52.70	7.00	60	0.53
PF 2	45.66	124.00	4.70	23.33	33.26	7.33	55	0.56
PF 3	57.00	150.00	5.00	24.33	70.80	7.00	60	0.56
PF 4	32.00	73.00	4.10	12.33	14.20	9.00	45	0.80
PF 5	64.00	196.00	7.16	43.33	66.04	12.33	63	0.30
PF 6	54.66	151.00	5.16	27.00	70.20	6.66	60	0.66
PF 7	64.33	200.00	7.00	41.66	78.65	12.00	65	0.35
PF 8	44.66	78.00	4.33	15.60	25.74	8.60	45	0.76
PF 9	60.00	206.00	7.33	40.60	72.00	11.33	70	0.36
PF 10	66.66	228.00	7.16	41.66	65.60	12.66	70	0.33
PF 11	51.33	151.00	5.10	23.65	65.55	8.60	55	0.60
PF 12	65.65	238.00	7.16	43.35	78.26	12.60	70	0.31
PF 13	102.33	203.00	7.10	43.30	43.00	12.00	80	0.28
PF 14	106.60	203.00	6.76	43.35	41.02	12.60	80	0.27
PF 15	101.30	208.00	7.10	43.30	13.00	13.30	80	0.29
PF 16	43.33	73.00	4.33	15.00	25.80	7.00	45	0.76
PF 17	102.30	200.00	6.93	43.00	52.63	13.60	80	0.26
PF 18	49.33	135.00	5.16	25.00	60.00	7.66	60	0.58
PF 19	69.00	205.00	7.16	45.00	70.38	13.00	70	0.35
PF 20	74.00	205.00	7.00	44.00	74.80	13.00	70	0.32
PF 21	75.00	216.00	7.50	42.30	88.20	12.30	70	0.30
PF 22	78.00	215.00	7.83	40.00	87.36	13.30	70	0.34
PF 23	72.00	206.00	7.00	40.00	74.88	12.60	70	0.31
PF 24	51.30	148.00	4.80	22.30	52.80	7.66	55	0.80
PF 25	79.00	218.00	7.30	44.00	80.08	12.33	70	0.32
PS 26	51.60	162.00	6.00	35.00	40.25	21.00	85	0.24
PP 27	35.60	90.00	13.00	51.60	33.25	34.00	85	0.17
PA 28	58.00	66.00	3.00	7.00	21.92	-	-	-
PSt 29	14.60	29.00	1.00	1.00	0.42	6.00	60	0.20
PSt 30	17.00	34.00	1.00	1.00	0.52	6.70	60	0.20
SEd	5.0925	8.2651	0.3260	1.8792	6.455	0.8735	5.9628	0.0496
CD(0.05)	10.186**	16.533**	0.652**	3.759**	12.432**	1.7472**	11.928**	0.099**

The angle of spine projection was very narrow in PS 26 and PP 27 (85°) and it was on par with PF 13, PF 14, PF 15 and PF 17. Wide angle of spine projection was recorded in PF 4, PF 8 and PF 16 (45°).

The highest spine length of 0.80 cm was recorded by genotype PF 4 and PF 24 while the lowest spine length of 0.17 cm was recorded by PP 27. Variability in plant characters is depicted in Plate 1 and 2.

4.1.2 Anatomical Characters

Mean performance with regard to leaf anatomical characters in thirty genotypes of *Pandanus* are presented in Table 4.

Analysis of variance revealed significant differences for characters like leaf thickness and number of stomata in the abaxial surface of the leaf. Number of stomata in the adaxial leaf surface did not reveal any significant differences among the thirty genotypes.

Leaf thickness varied from 0.20 to 0.93 mm. The highest leaf thickness of 0.93 mm was recorded by genotype PP 27 and the lowest value of 0.20 mm was recorded by PSt 29 and PSt 30.

The number of stomata in the abaxial leaf surface ranged from 4.33 to 13.66 per mm^2. The highest number of stomata (13.66 per mm^2) observed in genotype PF 9 and it was on par with PF 10 (13.33 per mm^2), PF 5 (12.66 per mm^2), PF 7 (12.33 per mm^2) and PF 19 (12.33 per mm^2). The lowest number of stomata was observed in genotype PF 8 (4.33 per mm^2) and it was on par with PF 4 (5.00 per mm^2).

4.1.3 Molecular Markers (RAPD)

4.1.3.1 DNA Isolation

Genomic DNA extracted based on modified Murray and Thompson (1980) protocol was poor quality with brown colour. Addition of one ml of 0.1 per cent PVP along with the extraction buffer improved the quality of the DNA. The purity and yield of the DNA are presented in Table 5.

Table 4. Leaf anatomical characters of 30 genotypes of *Pandanus*

Genotype No.	Leaf thickness (mm)	Number of stomata in abaxial surface (mm^2)	Number of stomata in adaxial surface (mm^2)
PF1	0.50	9.33	1.30
PF 2	0.48	9.00	0.66
PF 3	0.49	8.66	1.00
PF 4	0.58	5.00	0.33
PF 5	0.58	12.66	0.33
PF 6	0.49	9.00	1.00
PF 7	0.58	12.33	0.33
PF 8	0.60	4.33	0.66
PF 9	0.55	13.66	1.33
PF 10	0.54	13.33	1.33
PF 11	0.57	8.66	0.33
PF 12	0.60	12.00	0.33
PF 13	0.57	9.33	0.66
PF 14	0.58	8.66	0.33
PF 15	0.58	9.33	0.66
PF 16	0.60	6.00	0.33
PF 17	0.56	9.00	1.33
PF 18	0.52	7.66	0.33
PF 19	0.54	12.33	1.33
PF 20	0.55	11.66	1.00
PF 21	0.59	11.66	1.00
PF 22	0.59	9.66	1.33
PF 23	0.55	10.33	2.66
PF 24	0.50	7.00	0.00
PF 25	0.55	10.00	0.33
PS 26	0.41	6.60	0.33
PP 27	0.93	6.00	1.66
PA 28	0.35	6.33	0.00
PSt 29	0.20	7.33	0.00
PSt 30	0.20	7.00	0.00
SEd	0.0120	0.8119	0.6992
CD(0.05)	0.024**	1.624**	1.399 NS

Table 5. Quality and quantity of DNA of 30 genotypes of *Pandanus*

Genotype No.	OD value A_{260} nm	OD value A_{280} nm	Quality	Quantity of DNA $\mu g\ \mu l^{-1}$
PF 1	0.080	0.048	1.66	2.40
PF 2	0.079	0.048	1.64	2.37
PF 3	0.027	0.015	1.80	0.81
PF 4	0.043	0.023	1.87	1.29
PF 5	0.072	0.048	1.50	2.16
PF 6	0.036	0.024	1.50	1.08
PF 7	0.016	0.010	1.60	0.48
PF 8	0.015	0.009	1.66	0.45
PF 9	0.014	0.008	1.75	0.42
PF 10	0.031	0.019	1.63	0.93
PF 11	0.029	0.019	1.53	0.87
PF 12	0.032	0.018	1.78	0.96
PF 13	0.035	0.020	1.75	1.05
PF 14	0.043	0.026	1.65	1.29
PF 15	0.035	0.021	1.67	1.05
PF 16	0.045	0.025	1.80	1.35
PF 17	0.037	0.022	1.68	1.11
PF 18	0.040	0.022	1.82	1.20
PF 19	0.024	0.014	1.72	0.72
PF 20	0.027	0.017	1.59	0.81
PF 21	0.024	0.014	1.71	0.72
PF 22	0.022	0.013	1.69	0.66
PF 23	0.036	0.024	1.50	1.08
PF 24	0.029	0.019	1.53	0.87
PF 25	0.021	0.012	1.75	0.63
PS 26	0.019	0.011	1.72	0.57
PP 27	0.022	0.013	1.69	0.66
PA 28	0.029	0.019	1.53	0.87
PSt 29	0.011	0.007	1.54	0.33
PSt 30	0.016	0.010	1.60	0.48

PF 1

PF 11

PF 4

PF 13

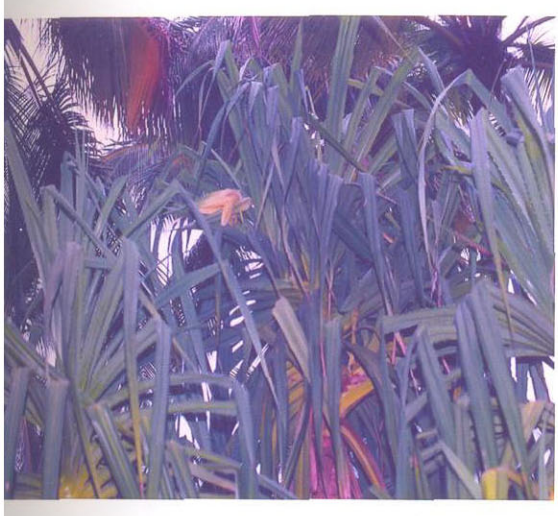
PF 12

PF 18

Plate 1 Variability in plant characters of *Pandanus*

PF 15

PF 5



PF 2

PF 7

PF 16

PF 10

Plate 1. Continued

PF 9

PF 14

PF 3

PSt 29

PSt 30

Plate 1. Continued

PF 17

PA 28

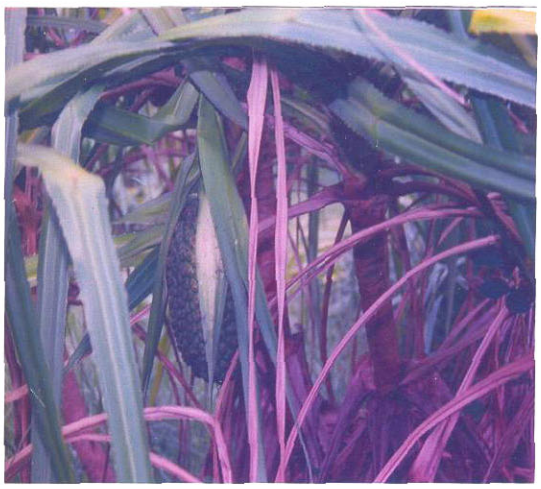
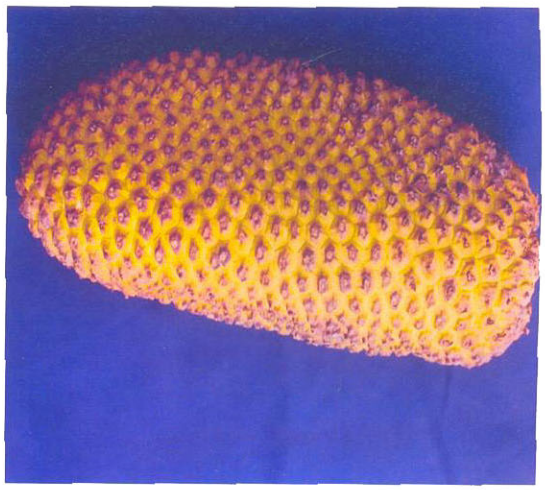
PF 22

PF 6

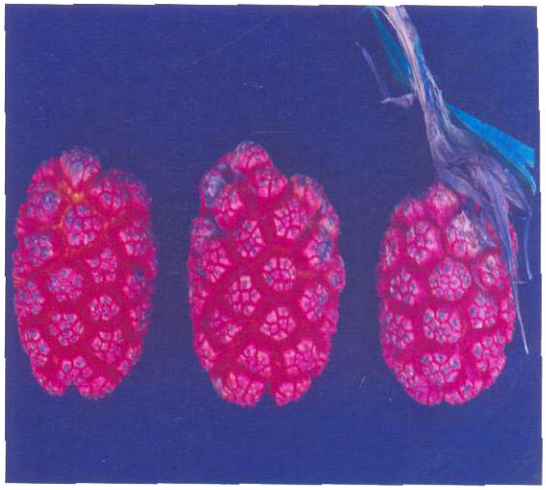
PS 26

PP 27

Plate 1. Continued

PF 14 Fruit

PF 9 Fruit


PF 21 Fruit

Plate 2. Variability in fruit characters of *Pandanus*

The purity and yield of DNA were good when fresh unfurled tender leaves were used. The purity (OD value of A_{260}/A_{280}) value ranged from 1.50 to 1.87 with the mean of 1.66. The highest purity value of 1.87 was obtained by genotype PF 4 and it was followed by PF 18 (1.82), PF 16 (1.80) and PF 3 (1.80). The lowest value of 1.50 was obtained by genotype PF 23, PF 5 and PF 6. The average genomic DNA yield of 30 genotypes of *Pandanus* was 0.98 $\mu g\ \mu l^{-1}$ and it ranged from 0.33 to 2.40 $\mu g\ \mu l^{-1}$. The highest DNA yield (2.40 $\mu g\ \mu l^{-1}$) was recorded by genotype PF I followed by PF 2 (2.37 $\mu g\ \mu l^{-1}$) and the lowest yield of 0.33 $\mu g\ \mu l^{-1}$ was recorded by PSt 29.

The electrophoretic assay of DNA samples using agarose gel electrophoresis (1.4 per cent) revealed that the integrity of the DNA samples were good, without any smearing. Infact, the DNA samples were in high molecular weight of 2 kb in size and un degraded and uncontaminated by RNA. The buffer used (1 x TAE buffer 0.04 M Tris acetate, 0.001 M EDTA, pH 6.0) was found to be good for the separation of the bands.

4.1.3.2 Polymerase Chain Reaction

The 25 µl reaction mixture consisted of 2.5 µl 10 x buffer (10 m M Tris HCl, pH 9, 1.50 mM Mg Cl_2, 50 mM KCl and 0.01 per cent gelatin), 10 pM primers 250 mM each of dNTP's, 0.2 units of Taq DNA polymerase and 20 ng of DNA gave good amplification. The programme consisted of an initial denaturaion at 95°C for one minute followed by 35 cycles of denaturation at 95°C for one minute and annealing at 35°C for two minutes and extension at 72°C for ten minutes. The amplification products were cooled to 4°C after the reaction.

Sixty primers belonging to kit A, kit B and kit C were screened for the PCR amplification. Among the 60 primers screened only 46 primers yielded amplification products (Table 6 and 7). The total number of bands ranged from 1.0 to 9.0. There was no amplification with the primers OPA-03, OPA-05, OPA-11, OPA-17, OPA-19, OPB-01, OPB-05, OPB-16, OPC-02, OPC-05, OPC-06, OPC-11, OPC-12 and OPC-16 for the DNA of *Pandanus* genotype PF 11. A total

Table 6. Primer associated banding patterns with the DNA of *Pandanus* genotype PF 11 using 40 primers belonging to kit A and kit B of Operon Inc, CA, USA

Sl. No.	Primers	Number of faint bands	Number of intense bands	Total number of bands
1	OPA-01	3	1	4
2	OPA-02	1	1	2
3	OPA-03	0	0	0
4	OPA-04	2	1	3
5	OPA-05	0	0	0
6	OPA-06	1	1	2
7	OPA-07	2	0	2
8	OPA-08	2	2	4
9	OPA-09	1	2	3
10	OPA-10	4	1	5
11	OPA-11	0	0	0
12	OPA-12	3	1	4
13	OPA-13	4	1	5
14	OPA-14	2	0	2
15	OPA-15	2	1	3
16	OPA-16	1	0	1
17	OPA-17	0	0	0
18	OPA-18	1	0	1
19	OPA-19	0	0	0
20	OPA-20	2	1	3
21	OPB-01	0	0	0
22	OPB-02	1	1	2
23	OPB-03	2	1	3
24	OPB-04	1	2	3
25	OPB-05	0	0	0
26	OPB-06	1	1	2
27	OPB-07	2	2	4
28	OPB-08	1	1	2
29	OPB-09	1	2	3
30	OPB-10	1	1	2
31	OPB-11	4	4	8
32	OPB-12	3	4	7
33	OPB-13	4	2	6
34	OPB-14	2	2	4
35	OPB-15	1	1	2
36	OPB-16	0	0	0
37	OPB-17	2	2	4
38	OPB-18	4	3	7
39	OPB-19	2	1	3
40	OPB-20	5	4	9

of 143 RAPD marker bands (average of 3.10 bands per primer) were produced by these primers and out of these 143 RAPD markers, 103 (72 per cent) were polymorphic and 40 were monomorphic.

The highest number of RAPD markers were produced by OPB-20 (9 scorable bands) followed by OPB-11 (8 scorable bands), OPB-12 (7 scorable bands), OPB-18 (7 bands) and OPB-13 (6 bands). The four primers such as OPB-11, OPB-12, OPB-18 and OPB-20 produced the highest number of intense and polymorphic bands were selected for the DNA amplification from the 30 genotypes of *Pandanus*. The PCR reaction was repeated twice and the reproducibility was confirmed. These primers amplified 41 scorable RAPD marker bands with an average of 10.2 bands per primer. Of these 41 markers, 35 were polymorphic and six were monomorphic. The number of bands resolved per amplification was primer dependent and varied from a minimum of eight to a maximum of eleven. The nucleotide sequence of these primers and the number of informative RAPD markers amplified by each primer are given in Table 8 and Fig.1.

A total of eleven scorable bands were produced by the primer OPB-11 (Fig. 2 and Plate 3) with two monomorphic bands. The genotype PS 26 recorded the highest number of bands (9.00) followed by PA 28 (7.00). Least number of bands (4.00) were produced by PF 5, PF 7, PF 12 and PF 22. Other genotypes recorded five bands each except PF 9, PF 10, PF 9, PF 20 and PF 25 which produced six bands each.

The primer OPB-12, produced a total of eight scorable bands with two monomorphic bands (Fig.3 and Plate 4). Genotype PS 26 and PA 28 recorded the maximum number of bands (7 bands each) and PF 5, PF 7, PF 9, PF 10, PF 13, PF 14, PF 15, PF 19, PF 20, PF 21, PF 22, PF 23 and PF 25 and PF 27 yielded the minimum 4 bands each. The rest of the genotypes yielded five bands each.

The primer OPB-18 yielded eleven scorable bands (Fig.4 and Plate 5) and among the eleven marker bands two bands were monomorphic for all the genotypes. PF 8 recorded the highest number (9) of bands and PF 5, PF 7, PF 10,

Table 7. Primer associated banding patterns with the DNA of *Pandanus* genotype PF 11 using 20 primers belonging to kit C of Operon on Inc., USA

Sl. No.	Primers	Number of faint bands	Number of intense bands	Total number of bands
1	OPC-01	1	0	1
2	OPC-02	0	0	0
3	OPC-03	1	1	2
4	OPC-04	2	2	4
5	OPC-05	0	0	0
6	OPC-06	0	0	0
7	OPC-07	1	0	1
8	OPC-08	2	0	2
9	OPC-09	0	1	1
10	OPC-10	1	1	2
11	OPC-11	0	0	0
12	OPC-12	0	0	0
13	OPC-13	2	0	2
14	OPC-14	2	1	3
15	OPC-15	2	0	2
16	OPC-16	0	0	0
17	OPC-17	1	1	2
18	OPC-18	1	1	2
19	OPC-19	1	1	2
20	OPC-20	2	0	2

Table 8. Nucleotide sequence of primers used and total number of informative RAPD markers amplified by them in the 10 *Pandanus* genotypes

Primer	Nucleotide sequence	Number of informative RAPD marker
OPB 11	GTAGACCCGT	11
OPB 12	CCTTGACGCA	8
OPB 18	CCACAGCAGT	11
OPB 20	GGACCCTTAC	11

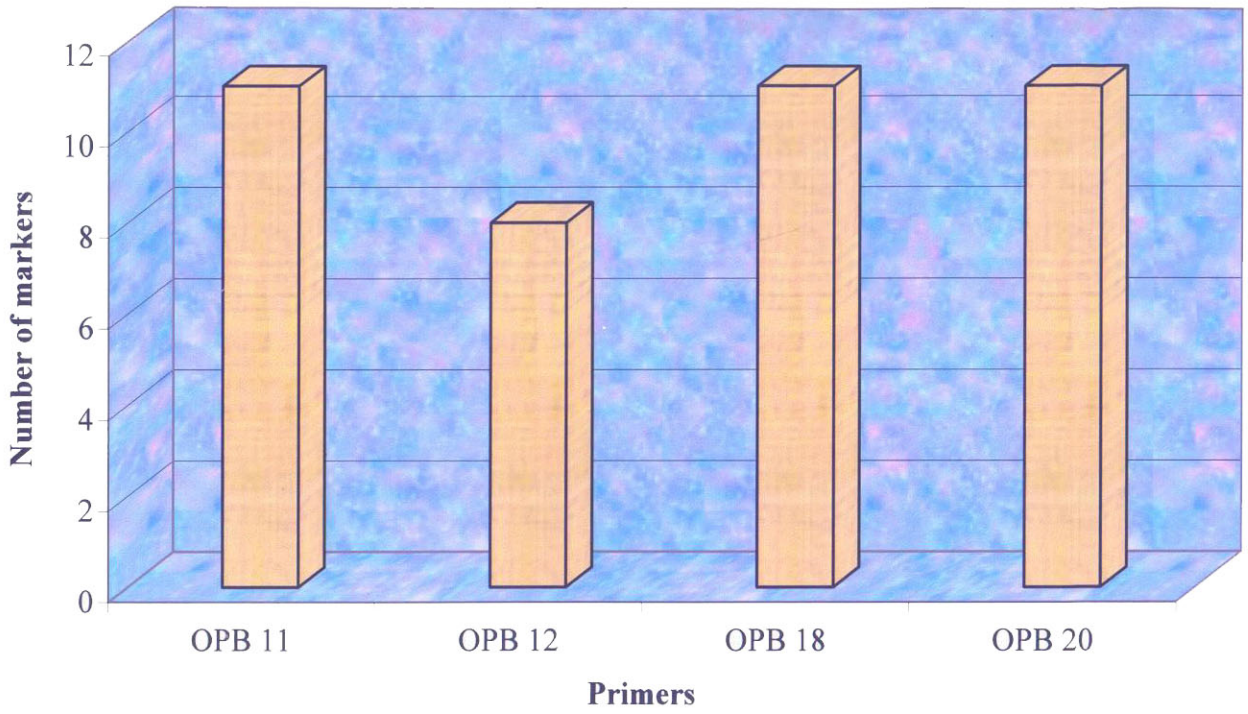


Fig. 1. Total number of informative RAPD markers amplified by the four primers in 30 genotypes of *Pandanus*

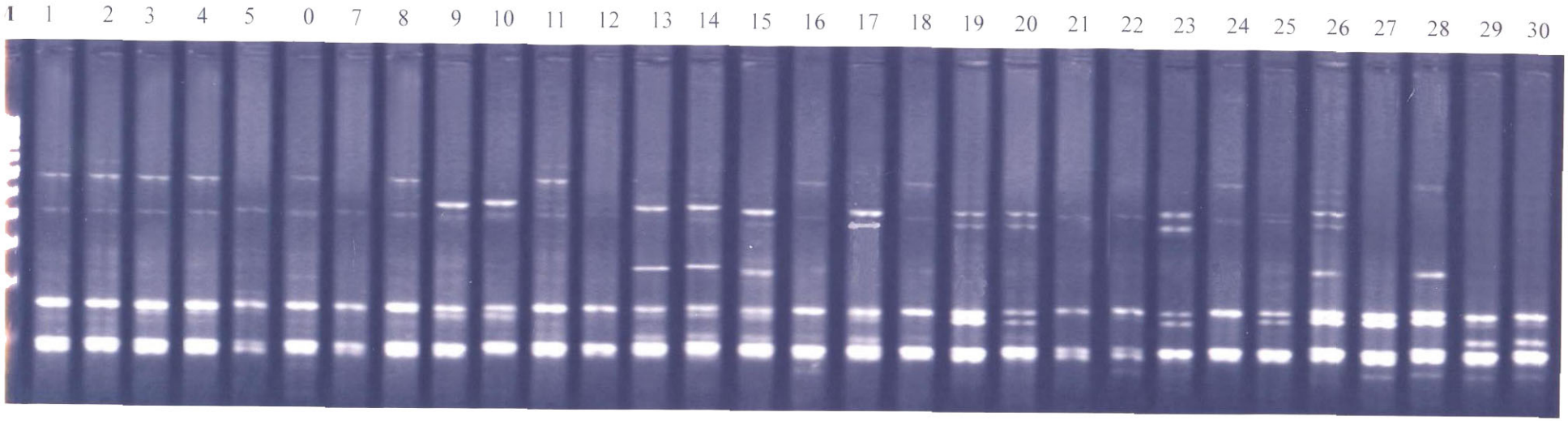
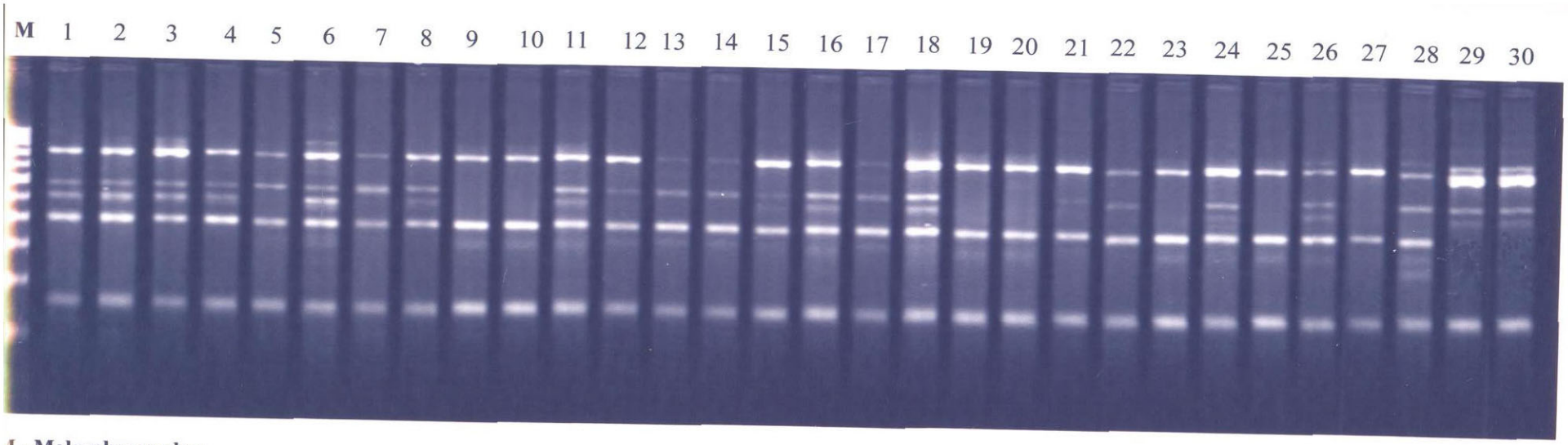


Plate 3. Amplification profile of the DNA of 30 *Pandanus* genotypes with the primer OPB-11

OPB11	PF 1	PF 2	PF 3	PF 4	PF 5	PF 6	PF 7	PF 8	PF 9	PF 10	PF 11	PF 12	PF 3	PF 14	PF 15	PF 16	PF 17	PF 18	PF 19	PF 20	PF 21	PF 22	PF 23	PF 24	PF 25	PS 26	PP 27	PA 28	PSt 29	PSt 30
M 1	1	1	1	1	0	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	1	0	0
M2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
M3	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	1	0	0	0	0
M4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
M5	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0
M6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M7	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	1	1	1	0	0
M8	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1
M9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
M11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1

Fig. 2. Representation of the amplification profile of the DNA of 30 genotypes of *Pandanus* with the primer OPB-11

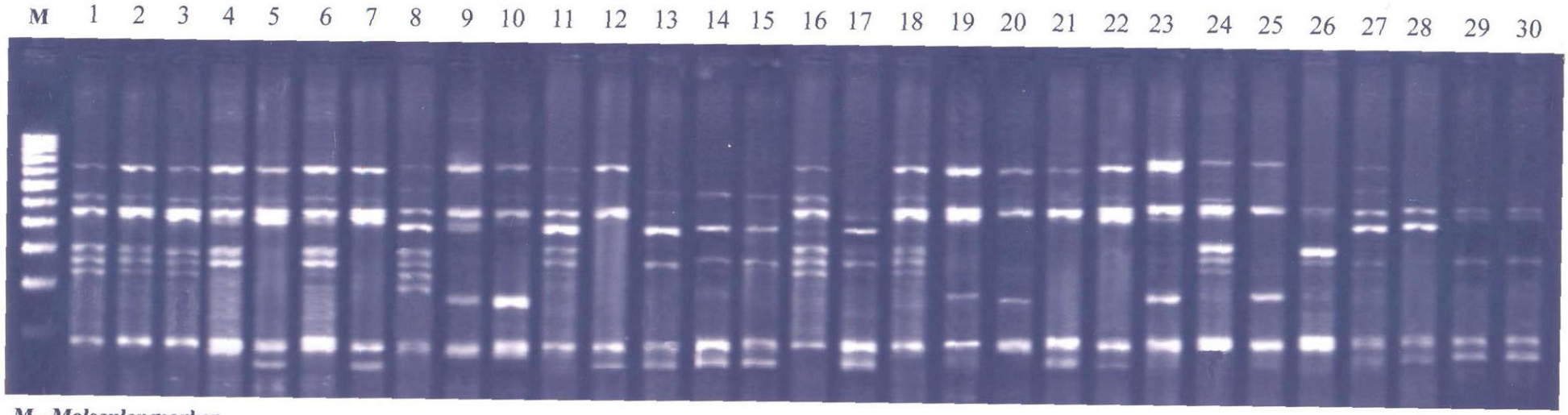


M - Molecular marker

Plate 4. Amplification profile of the DNA of 30 *Pandanus* genotypes with the primer OPB-12

OPB 12	PF 1	PF 2	PF 3	PF 4	PF 5	PF 6	PF 7	PF 8	PF 9	PF10	PF 11	PF 12	PF 13	PF 14	PF15	PF 16	PF 17	PF 18	PF19	PF 20	PF21	PF22	PF23	PF24	PF25	PS26	PP27	PA 28	PSt 29	PSt 30
M 1	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	1
M2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M3	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	0	0	1	1	0	1	0	1	0	1	1	1
M4	1	1	1	1	0	1	0	1	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0	1	0	1	0	0	1	1
M5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
M6	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	1	0	1	0	0
M7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
M8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Fig. 3. Representation of the amplification profile of the DNA of 30 genotypes of *Pandanus* with the primer OPB-12



M - Molecular marker

Plate 5. Amplification profile of the DNA of 30 *Pandanus* genotypes with the primer OPB-18

OPB 18	PF 1	PF 2	PF 3	PF 4	PF 5	PF 6	PF 7	PF 8	PF 9	PF 10	PF 11	PF 12	PF 13	PF 14	PF 15	PF 16	PF 17	PF 18	PF 19	PF 20	PF 21	PF 22	PF 23	PF 24	PF 25	PS 26	PP 27	PA 28	PSt 29	PSt 30
M 1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	1	1	1	1	1	1	0	1	0	0	0
M2	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
M3	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
M4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M5	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	1	1	1
M6	1	1	1	1	0	1	0	1	0	0	1	0	1	1	1	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0
M7	1	1	1	1	0	1	0	1	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	1	0	1	1	0	1	1
M8	1	1	1	1	0	1	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0
M9	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	0	0	0
M10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
M11	0	0	0	0	1	0	1	0	0	0	0	1	1	1	1	0	1	0	0	0	1	1	0	0	0	0	0	1	1	1

Fig. 4. Representation of the amplification profile of the DNA of 30 genotypes of *Pandanus* with the primer OPB-18

PF 12, PF 17, PF 19, PF 21, PF 22, PF 26 and PA 28 recorded the lowest number (4 bands each) of bands.

Eleven scorable marker bands were obtained by primer OPB-20 (Fig.5 and Plate 6) on amplification. Genotypes PF 5, PF 7, PF 12, PF 13, PF 14, PF 15, PF 17, PF 21 and PF 22 yielded the maximum of seven bands each. PA 28 recorded the minimum of one band.

4.1.3.3 Data Analysis

The banding pattern for each primer was scored by visual observation. The amplification product or the marker bands in each position was recorded as positive for presence and negative for absence. Based on the presence or absence data, score value was given as one for presence of band and zero for absence of band. Genetic similarity matrix was constructed using the Jaccard's coefficient method (Table 9) with the score values of RAPD marker bands.

The highest similarity coefficient value of 1.000 was obtained between the genotypes PF 1, PF 2, PF 4, PF 18 (yellow inflorescence types) PF 7, PF 12, PF 22 (grayish green leaved and white inflorescence type) PF 9, PF 20, PF 25 (dull white inflorescence type) PSt 29 and PSt 30 (*P. stellatus*). The lowest similarity coefficient value of 0.333 was obtained between the genotype pairs of PF 23 and PSt 29 and PF 23 and PSt 30.

The yellow inflorescence types such as PF 1, PF 2, PF 3, PF 4, PF 6, PF 8, PF 11, PF 16, PF 18 and PF 24 showed maximum similarity among them. Within these types 100 per cent similarity was recorded between PF 1, PF 2, PF 4 and PF 18.

Similarly the grayish green leaved and white inflorescence types such as PF 7, PF 12 and PF 22 showed cent per cent similarity among them. Dull white inflorescence types *viz.*, PF 9, PF 20 and PF 25 showed cent per cent similarity between them and the genotypes belonging to the species *P. stellatus* (PSt 29 and PSt 30) also revealed cent per cent similarity among them. Long glossy green leaved and white inflorescence types such as PF 13, PF 14 and PF 15 showed

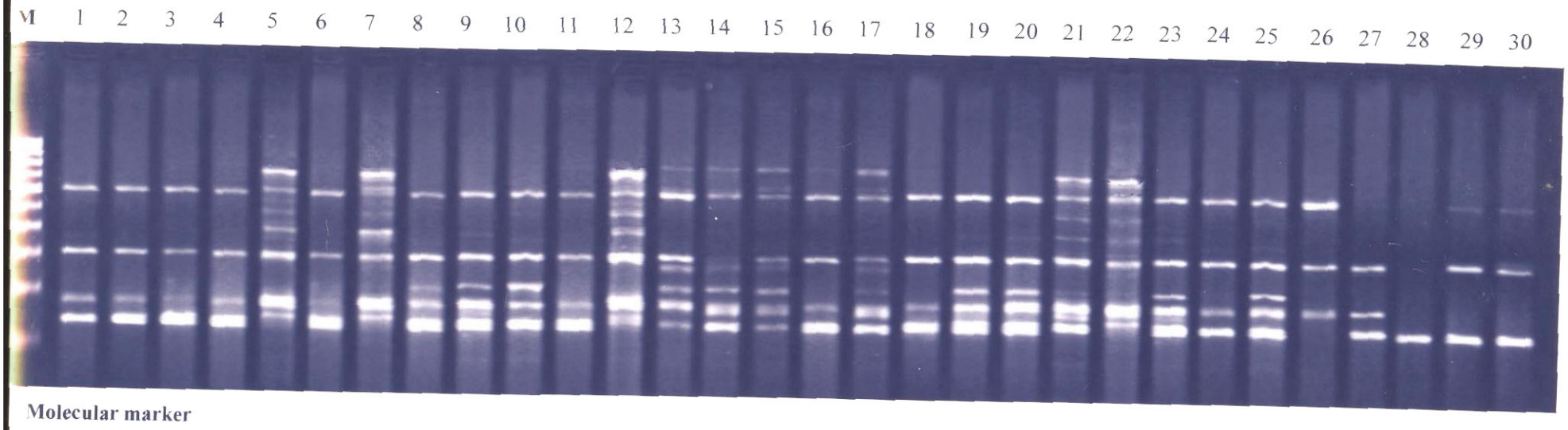


Plate 6. Amplification profile of the DNA of 30 *Pandanus* genotypes with the primer OPB-20

OPB 20	PF1	PF2	PF3	PF4	PF5	PF6	PF7	PF8	PF9	PF10	PF11	PF12	PF13	PF14	PF15	PF16	PF17	PF18	PF19	PF20	PF21	PF22	PF23	PF24	PF25	PS26	PP27	PA28	PST29	PF30
M 1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
M2	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
M3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1
M4	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
M5	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
M6	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
M7	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
M8	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1	0	1	0	1	1	0	0	1	0	1	0	0	0	0	0
M9	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
M10	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
M11	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1

Fig. 5. Representation of the amplification profile of the DNA of 30 genotypes of *Pandanus* with the primer OPB-20

Table 9. Similarity matrix of 30 genotypes of ***Pandanus*** **based on the Jaccard's similarity index**

1	1.000																													
2	1.000	1.000																												
3	0.909	0.909	1.000																											
4	1.000	1.000	0.909	1.000																										
5	0.481	0.481	0.429	0.481	1.000																									
6	0.792	0.792	0.870	0.792	0.464	1.000																								
7	0.538	0.538	0.481	0.538	0.900	0.519	1.000																							
8	0.833	0.833	0.760	0.833	0.448	0.800	0.500	1.000																						
9	0.519	0.519	0.464	0.519	0.500	0.500	0.500	0.593	1.000																					
10	0.538	0.538	0.481	0.538	0.520	0.519	0.520	0.556	0.950	1.000																				
11	0.955	0.955	0.955	0.955	0.464	0.833	0.519	0.800	0.500	0.519	1.000																			
12	0.538	0.538	0.481	0.538	0.900	0.519	1.000	0.500	0.500	0.520	0.519	1.000																		
13	0.536	0.536	0.483	0.536	0.519	0.467	0.519	0.552	0.500	0.519	0.517	0.519	1.000																	
14	0.536	0.536	0.483	0.536	0.519	0.467	0.519	0.552	0.500	0.519	0.517	0.519	1.000	1.000																
15	0.571	0.571	0.517	0.571	0.500	0.500	0.500	0.586	0.483	0.500	0.552	0.500	0.957	0.957	1.000															
16	0.955	0.955	0.955	0.955	0.464	0.833	0.519	0.800	0.500	0.519	1.000	0.519	0.517	0.517	0.552	1.000														
17	0.556	0.556	0.500	0.556	0.538	0.483	0.538	0.517	0.519	0.538	0.536	0.538	0.870	0.870	0.833	0.536	1.000													
18	1.000	1.000	0.909	1.000	0.481	0.792	0.538	0.833	0.519	0.538	0.955	0.538	0.536	0.536	0.571	0.955	0.556	1.000												
19	0.538	0.538	0.481	0.538	0.520	0.519	0.520	0.556	0.950	1.000	0.519	0.520	0.519	0.519	0.500	0.519	0.538	0.538	1.000											
20	0.519	0.519	0.464	0.519	0.500	0.500	0.500	0.593	1.000	0.950	0.500	0.500	0.500	0.500	0.483	0.500	0.519	0.519	0.950	1.000										
21	0.577	0.577	0.519	0.577	0.857	0.556	0.950	0.536	0.538	0.560	0.556	0.950	0.556	0.556	0.536	0.556	0.577	0.577	0.560	0.538	1.000									
22	0.538	0.538	0.481	0.538	0.900	0.519	1.000	0.500	0.500	0.520	0.519	1.000	0.519	0.519	0.500	0.519	0.538	0.538	0.520	0.500	0.950	1.000								
23	0.500	0.500	0.444	0.500	0.480	0.481	0.480	0.519	0.900	0.947	0.481	0.480	0.481	0.481	0.464	0.481	0.500	0.500	0.947	0.900	0.520	0.480	1.000							
24	0.870	0.870	0.870	0.870	0.464	0.913	0.519	0.875	0.556	0.577	0.913	0.519	0.517	0.517	0.552	0.913	0.536	0.870	0.577	0.556	0.556	0.519	0.538	1.000						
25	0.519	0.519	0.464	0.519	0.500	0.500	0.500	0.593	1.000	0.950	0.500	0.500	0.500	0.500	0.483	0.500	0.519	0.519	0.950	1.000	0.538	0.500	0.900	0.556	1.000					
26	0.630	0.630	0.630	0.630	0.400	0.607	0.448	0.586	0.536	0.556	0.667	0.448	0.500	0.500	0.533	0.667	0.517	0.630	0.556	0.536	0.433	0.448	0.519	0.667	0.536	1.000				
27	0.520	0.520	0.462	0.520	0.385	0.444	0.440	0.600	0.609	0.565	0.500	0.440	0.444	0.444	0.429	0.500	0.462	0.520	0.565	0.609	0.480	0.440	0.522	0.500	0.609	0.429	1.000			
28	0.379	0.379	0.429	0.379	0.357	0.414	0.357	0.400	0.444	0.407	0.414	0.357	0.414	0.414	0.400	0.414	0.429	0.379	0.407	0.444	0.393	0.357	0.370	0.414	0.444	0.500	0.500	1.000		
29	0.500	0.500	0.560	0.500	0.370	0.538	0.423	0.519	0.407	0.370	0.538	0.423	0.481	0.481	0.519	0.538	0.560	0.500	0.370	0.407	0.462	0.423	0.333	0.538	0.407	0.464	0.522	0.542	1.000	
30	0.500	0.500	0.560	0.500	0.370	0.538	0.423	0.519	0.407	0.370	0.538	0.423	0.481	0.481	0.519	0.538	0.560	0.500	0.370	0.407	0.462	0.423	0.333	0.538	0.407	0.464	0.522	0.542	1.000	1.0

maximum similarity among them. In general, genotypes like PS 26, PP 27, PA 28, PSt 29 and PSt 30 belonging to the species *P. sanderi, P. pacificus, P. amaryllifolius* and *P. stellatus* showed maximum diversity among themselves as well as with the *P. fasicularis* genotypes (PF 1 to PF 25).

Based on the similarity coefficient value the graphic dendrogram (Fig. 6) was constructed using the NTSYS 2.02 software package. On drawing a vertical line corresponding to the similarity coefficient value of 0.72 the 30 genotypes of *Pandanus* were grouped into eight clusters, while at a coefficient value of 0.86 the 30 genotypes were grouped into 11 clusters. At a similarity value of 0.56, all the 30 genotypes were got divided into seven clusters.

While considering the eight clusters, genotypes PSt 29 and PSt 30 (*P. stellatus*) made one cluster while genotypes PA 28 (*P. amaryllifolius,* PP 27 (*P. pacificus*) and PS 26 (*P. sanderi*) were formed separate clusters each. Among the rest, genotypes PF 9, PF 20, PF 25, PF 10, PF 19 and PF 23 (*P. fasicularis* – dull white inflorescence type) made one group whereas *Pandanus fasicularis* genotypes (grayish green leaved and white inflorescence type) such as PF 5, PF 7, PF 12, PF 22 and PF 21 formed another group. Long glossy green leaved and white inflorescence type *P. fasicularis* genotypes *viz.*, PF 13, PF 14, PF 15 and PF 17 constituted one cluster and the remaining yellow inflorescence *P. fasicularis* genotypes like PF 1, PF 2, PF 4, PF 18, PF 11, PF 16, PF 3, PF 6, PF 24 and PF 8 made another cluster. This cluster was the largest one when compared to the others and consisted the maximum of 10 genotypes. The maximum genetic distance (0.491) was recorded between the genotype pair of PF 21 and PF 9 while the minimum distance (zero) was obtained between the genotype pairs of PF 1 and PF 2, PF 2 and PF 4, PF 7 and PF 12, PF 9 and PF 20 and PSt 29 and PSt 30 (Table 10).

4.2 GENETIC VARIABILITY AND DIVERGENGE STUDIES

Twenty two genotypes of *Pandanus* consisting only male lines were evaluated and the data subjected to statistical analysis for estimating genotypic,

Table 10. Genetic distance between the pairs of *Pandanus* genotypes

Sl. No.	Genotype pairs	Genetic distance
1	PF 1 – PF 2	0.000
2	PF 2 – PF 4	0.000
3	PF 4 – PF 18	0.000
4	PF 7 – PF 12	0.000
5	PF 9 – PF 20	0.000
6	PSt 29 – PSt 30	0.000
7	PF 13 – PF 14	0.043
8	PF 18 – PF 11	0.045
9	PF 20 – PF 25	0.050
10	PF 25 – PF 10	0.050
11	PF 12 – PF 22	0.050
12	PF 3 – PF 6	0.065
13	PF 11 – PF16	0.076
14	PF 16 – PF 3	0.076
15	PF 10 – PF 19	0.081
16	PF 6 – PF 24	0.094
17	PP 27 – PA 28	0.094
18	PF 14 – PF 15	0.100
19	PF 8 – PS 26	0.104
20	PF 5 – PF 7	0.111
21	PF 3 – PF 23	0.132
22	PF 24 – PF 8	0.185
23	PF 19 – PF 23	0.339
24	PF15 – PF17	0.343
25	PF 17 – PF 5	0.375
26	PF 22 – PF 21	0.441
27	PA 28 – PSt 29	0.458
28	PS 26 – PF 13	0.470
29	PP 21 – PF 9	0.491

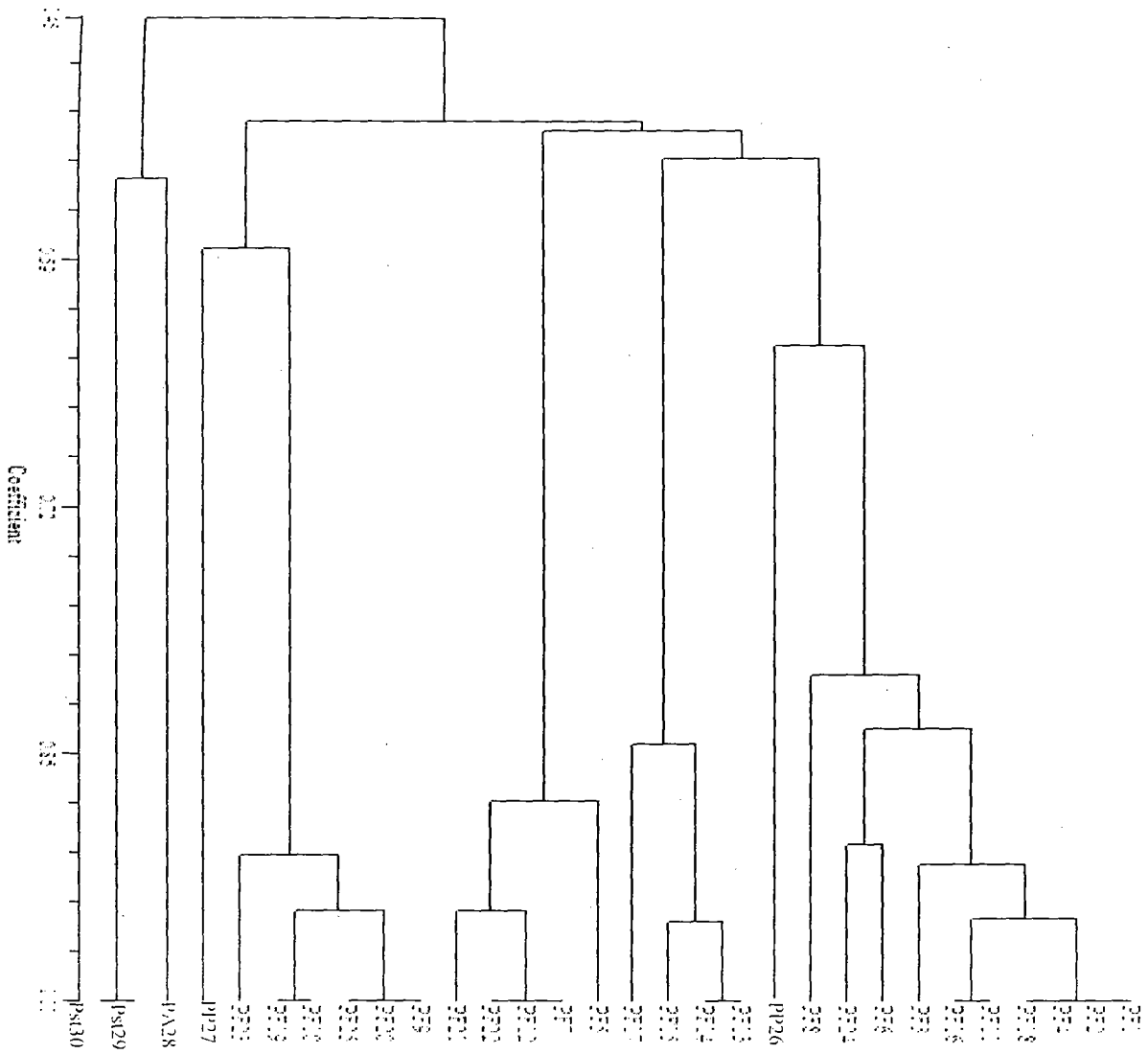


Fig. 6. UPGMA based dendrogram showing the relationship between 30 genotypes of *Pandanus*

phenotypic and environmental variability, heritability and genetic advance and correlation of characters. The results are presented.

4.2.1 Mean Performance of 22 Male Genotypes of *Pandanus*

Analysis of variance revealed significant genotypic differences for all the morphological, bio chemical and leaf anatomical characters studied.

The mean performance of the genotypes are presented in Table 11 for stem and leaf characters and for inflorescence characters in Table 12.

4.2.1.1 Stem and Leaf Characters

The average plant height was 5.67 m with a range of 4.00 m (PF 8) to 6.50 m (PF 7) and the plant girth varied from 0.27 m (PF 20) to 0.35 m (PF 7) with the mean value of 0.30 m (Table 11).

The number of crowns per plant was highest in PF 11 (57.67) and it was lowest in PF 13 and PF 15 (10.66). The mean for the number of crown per plant was observed as 34.49. The number of leaves per crown ranged from 32.00 (PF 4) to102.33 (PF 13 and PF 17) and the average was found to be 64.65.

The highest leaf length of 238.33 cm was recorded by PF 12 followed by PF 10 (228.33 cm). The lowest leaf length was observed as 73.33 cm in PF 4 and PF 16 and the average was 170.30 cm. The leaf width was 6.11 cm on an average and it ranged from 4.10 (PF 4) to 7.83 (PF 22)

Leaf weight was the highest in PF 19 (45.00 g) and it was lowest in PF 4 (12.33 g). The mean value was 32.99 g. Leaf thickness ranged from 0.48 to 0.60 mm and the average was 0.57 mm. The spine length was highest in PF 4 and PF 24 (0.80 cm) and the lowest spine length was observed as 0.30 cm in PF 5, PF 13, PF 15 and PF 17. The mean value was 0.49 cm.

4.2.1.2 Inflorescence Characters

Number of inflorescence per plant per year recorded the highest value by PF 6 (40.00) and it was followed by PF 3 and PF 11 (39.00). The lowest value of

Table 11. Mean performance of stem and leaf characters of 22 male genotypes of *Pandanus*

Genotype number	Characters								
	1	2	3	4	5	6	7	8	9
PF 1	6.00	0.31	47.00	54.33	145.00	5.37	25.67	0.50	0.53
PF 2	5.17	0.28	36.33	45.67	124.67	4.70	23.33	0.48	0.57
PF 3	6.20	0.32	54.00	57.00	143.33	5.00	24.33	0.49	0.57
PF 4	4.30	0.28	37.00	32.00	73.33	4.10	12.33	0.58	0.80
PF 5	6.43	0.33	24.67	64.00	196.67	7.17	43.33	0.49	0.30
PF 6	6.00	0.31	51.67	54.67	151.67	5.17	27.00	0.60	0.67
PF 7	6.50	0.35	32.00	64.33	200.00	7.00	41.67	0.54	0.37
PF 8	4.00	0.27	39.33	44.67	78.33	4.33	15.67	0.58	0.77
PF10	5.03	0.27	25.00	66.67	228.33	7.17	41.67	0.57	0.37
PF 11	6.03	0.30	57.67	51.33	148.67	5.17	23.67	0.60	0.67
PF 12	5.93	0.32	28.00	65.67	238.33	7.17	43.33	0.57	0.33
PF 13	6.30	0.31	10.67	102.33	203.33	7.10	43.33	0.58	0.30
PF 15	6.43	0.32	10.67	101.33	208.33	7.10	43.33	0.56	0.30
PF 16	4.30	0.27	40.67	43.33	73.33	4.33	15.00	0.52	0.77
PF 17	6.47	0.33	12.00	102.33	200.00	6.93	41.67	0.40	0.30
PF 18	6.07	0.33	48.33	49.33	135.00	5.17	25.00	0.55	0.60
PF 19	5.57	0.28	23.33	69.00	205.00	7.17	45.00	0.59	0.40
PF 20	5.10	0.27	23.00	74.00	205.00	7.00	44.00	0.55	0.33
PF 22	5.83	0.31	31.67	78.00	215.00	7.83	40.00	0.55	0.37
PF 23	5.27	0.29	26.67	72.00	206.67	7.17	40.00	0.55	0.33
PF 24	6.03	0.30	48.33	51.33	148.33	4.83	22.33	0.50	0.80
PF 25	5.87	0.31	50.67	79.00	218.33	7.33	44.00	0.55	0.33
MEAN	**5.67**	**0.30**	**34.49**	**64.65**	**170.30**	**6.11**	**32.99**	**0.57**	**0.49**
CD	**0.502**	**0.032**	**8.036**	**9.910**	**18.087**	**0.504**	**4.076**	**0.036**	**0.121**

1 Plant height (m)
2 Plant girth (m)
3 Number of crowns per plant
4 Number of leaves per crown
5 Leaf length (cm)
6 Leaf width (cm)
7 Leaf weight (g)
8 Leaf thickness (mm)
9 Spine length (cm)

19.33 inflorescences per plant per year was recorded by PF 13. The mean value was recorded as 29.49 (Table 12).

Length of the inflorescence had an average of 81.76 cm and it ranged from 39.33 cm to 115.00 cm. The highest inflorescence length was recorded by PF 23 and PF 25 (115.00 cm) and it was followed by PF 19 and PF 20 (113.33 cm). The lowest inflorescence length was recorded by PF 8 (39.33 cm).

Girth of the inflorescence ranged from 16.67 to 24.67 cm and the average was 21.06 cm. The highest inflorescence girth was recorded by PF 5 (24.66 cm) and it was on par with PF 7 and PF 10. The lowest inflorescence girth was recorded in genotypes PF 4, PF 8 and PF 16 (16.67 cm).

The inflorescence weight had an average of 212.80 g and it ranged from 71.67 to 346.67 g. The highest inflorescence weight (346.67 g) was recorded by PF 20 and it was on par with PF 10, PF 12, PF 19, PF 22 and PF 25. The least value was registered by PF 16 (71.67 g) and it was on par with PF 4 and PF 8 (86.67 and 76.67 g).

The number of spathe per inflorescence ranged from 17.00 to 17.60 with an average of 17.05. The number of androecious fesicles ranged from 13.00 to 14.00 with the mean value of 13.15. Shelf life of the inflorescence varied from the minimum of 2 days to the maximum of 4.66 days with an average of 3.06 days.

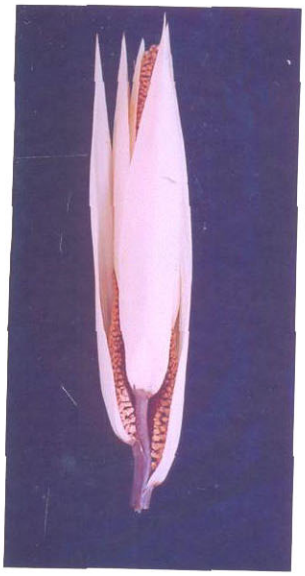
The oil content of the inflorescence ranged from 0.09 to 0.21 per cent and the mean was 0.16 per cent. The highest oil content of 0.21 per cent was obtained from PF 11 and PF 18 and it was on par with PF 1, PF 3, PF 6 and PF 24. The lowest oil content of 0.09 per cent was recorded by PF 15.

Inflorescence yield per plant per year varied from 2240.00 to 8920.00 g and the mean value was 5979.94 g. The genotype PF 10 recorded the highest inflorescence yield of 8920.00 g and it was on par with PF 7, PF 12, PF 19, PF 20, PF 22, PF 23 and PF 25 (8466.67, 8106.67, 8856.67, 8493.33, 8405.00, 8066.67, 8726.67 g respectively). The lowest inflorescence yield of 2240.00 g was

Table 12. Mean performance of inflorescence characters of 22 male genotypes of *Pandanus*

Genotype number	Characters								
	1	2	3	4	5	6	7	8	9
PF 1	34.67	63.33	20.67	17.00	13.33	4.00	153.33	0.20	5340.00
PF 2	31.00	48.33	18.33	17.00	13.00	4.00	120.00	0.17	3760.00
PF 3	39.00	63.33	21.33	17.00	13.00	4.00	153.33	0.20	5960.00
PF 4	27.00	39.33	16.67	17.00	13.00	4.00	86.67	0.13	2346.67
PF 5	26.67	100.00	24.67	17.33	14.00	2.00	300.00	0.10	7986.67
PF 6	40.00	65.00	21.00	17.00	13.00	4.00	143.33	0.20	5713.33
PF 7	29.33	100.00	24.00	17.33	13.33	2.00	290.00	0.11	8466.67
PF 8	30.67	41.00	16.67	17.60	13.00	4.33	76.67	0.13	2346.67
PF10	27.33	106.67	23.67	17.00	13.66	4.66	326.67	0.16	8920.00
PF 11	39.00	56.67	20.67	17.00	13.00	4.00	126.67	0.20	4946.67
PF 12	26.00	110.00	22.33	17.00	13.00	2.00	313.33	0.10	8106.67
PF 13	19.33	83.33	21.33	17.00	13.00	2.00	206.67	0.10	3973.33
PF 15	21.67	81.67	20.00	17.00	13.00	2.00	210.00	0.10	4513.33
PF 16	31.67	46.67	16.67	17.00	13.00	4.33	71.67	0.17	2240.00
PF 17	20.00	90.00	21.67	17.00	13.00	2.00	196.67	0.12	3906.67
PF 18	38.67	73.33	20.33	17.00	13.00	4.00	140.00	0.21	5333.33
PF 19	27.67	113.33	22.33	17.00	14.00	2.00	323.33	0.16	8856.67
PF 20	24.67	113.33	22.67	17.00	13.00	2.00	346.67	0.16	8493.33
PF 22	28.00	110.00	22.00	17.00	13.00	2.00	301.67	0.13	8405.00
PF 23	25.33	115.00	23.00	17.00	13.00	2.00	320.00	0.16	8066.67
PF 24	34.00	63.33	20.33	17.00	13.00	4.00	151.67	0.20	5128.33
PF 25	27.00	115.00	23.00	17.00	13.00	2.00	323.33	0.16	8726.67
MEAN	**29.49**	**81.76**	**21.06**	**17.05**	**13.15**	**3.06**	**212.80**	**0.16**	**5978.94**
CD	**4.642**	**9.725**	**1.487**	**0.671**	**0.496**	**0.035**	**31.859**	**0.046**	**803.133**

1 Number of inflorescence per plant
2 Inflorescence length (cm)
3 Inflorescence girth (cm)
4 Number of spathe
5 Number of androecious fesicle
6 Shelf life of the inflorescence
7 Inflorescence weight
8 Oil content
9 Inflorescence yield per plant



PF 5

PF 19

PF 20

PF 10

PF 22

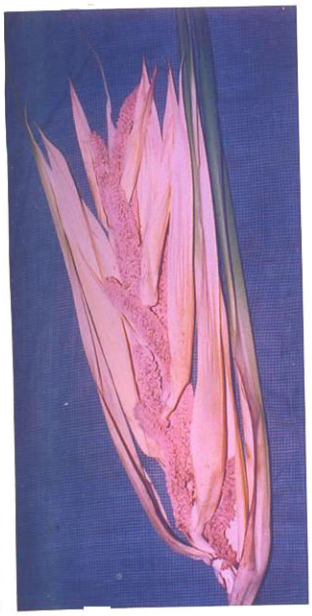
PF 3

Plate 7. Variability in inflorescence characters of *Pandanus*

PF 11

PF 23

PF 24

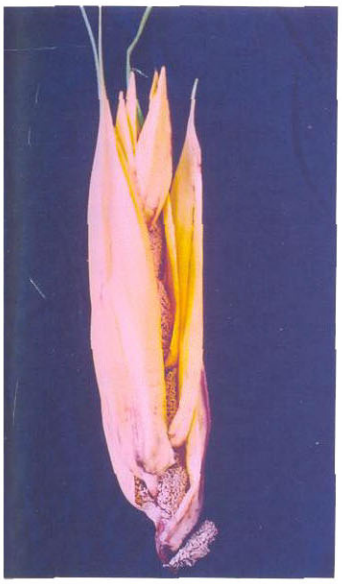


PF 25

PF 13

PF 22

Plate 7. Continued



PF 17

PF 8

PF 6 & PF 2

PF 18

PF 1

PF 16 & PF 4

Plate 7. Continued

produced by PF 16 and it was on par with PF 4 and PF 8 (2346.00 g). Variability exists in the inflorescence characters are depicted in Plate 7.

4.2.2 Variability Components

The genotypic, phenotypic, and environmental variances and the genotypic and phenotypic coefficients of variation (GCV and PCV) were estimated for fifteen characters *viz.*, plant height, plant girth, number of crown per plant, number of leaves per crown, leaf length, width, weight, thickness, spine length, number of inflorescence per plant, inflorescence length, girth, weight, oil content and total inflorescence yield per plant per year.

4.2.2.1 Stem and Leaf Characters

The genotypic and phenotypic co efficient of variation for stem and leaf characters were presented in Table 13 and Fig. 7.

Most of the characters maintained the same trend in the magnitude of variability both at phenotypic and genotypic levels.

The number of crowns per plant showed the maximum value for GCV (40.62 per cent) followed by spine length (37.10 per cent), leaf weight (34.17 per cent), leaf length (29.62 per cent), number of leaves per crown (29.46 per cent), leaf width (20.24 per cent), plant height (12.75 per cent) and leaf thickness (7.31 per cent). The GCV was lowest for plant girth (6.37 per cent).

The highest PCV was recorded for the character number of crowns per plant (43.01 per cent) as like GCV. The lowest PCV was observed for the character leaf thickness (8.25 per cent) followed by plant girth (9.03 per cent).

In general, among the stem and leaf morphological characters maximum variability was observed for number of crowns per plant followed by spine length and the least variability was noticed for plant girth and leaf thickness both at genotypic and phenotypic levels.

The difference between phenotypic and genotypic co efficient of variation was generally less for all the character studied. However,

maximum value (4.25 per cent) was observed for the character oil content and for the other characters the difference between GCV and PCV was less than that.

4.2.2.2 Inflorescence Characters

The genotypic and phenotypic variability components for inflorescence characters are presented in Table 14 and Fig. 8.

The maximum variability both at genotypic and phenotypic level (GCV and PCV) was observed for inflorescence weight (44.39 and 45.31 per cent respectively) followed by inflorescence yield per plant (38.72 and 39.57 per cent respectively). The minimum genotypic and phenotypic variability (GCV and PCV) was observed for inflorescence girth (10.63 per cent and 11.46 per cent respectively).

4.2.3 Heritability (broad sense) and Genetic Advance

The estimates of heritability and genetic advance as percentage of mean studied for the fifteen characters are presented in Table 15 and Fig. 9.

High heritability estimates, above 60 per cent, were recorded for all the characters under study except plant girth (49.71 per cent), which comes under the medium heritability category. Maximum heritability estimate was recorded for inflorescence weight (95.98 per cent), Inflorescence yield (95.76 per cent), leaf length (95.48 per cent), leaf weight (95.41 per cent), Inflorescence length (95.28 per cent), leaf width (94.24 per cent), number of leaves per crown (90.94 per cent), number of crowns per plant (89.19 per cent), inflorescence girth (86.04 per cent), spine length (86.03 per cent), plant height (84.93 per cent), number of inflorescence per plant (81.06 per cent), leaf thickness (78.76 per cent) and oil content (69.89 per cent).

Expected genetic advance as percentage of mean was used for the comparison of characters. All the characters came under high category (more than 20 per cent) as for as genetic advance is concerned except leaf thickness (13.38 per cent) and plant girth (9.25 per cent). The highest genetic advance was observed for inflorescence weight (89.59 per cent) followed by number of crowns

Table 13. Variance and coefficient of variation for stem and leaf characters of 22 male genotypes of *Pandanus*

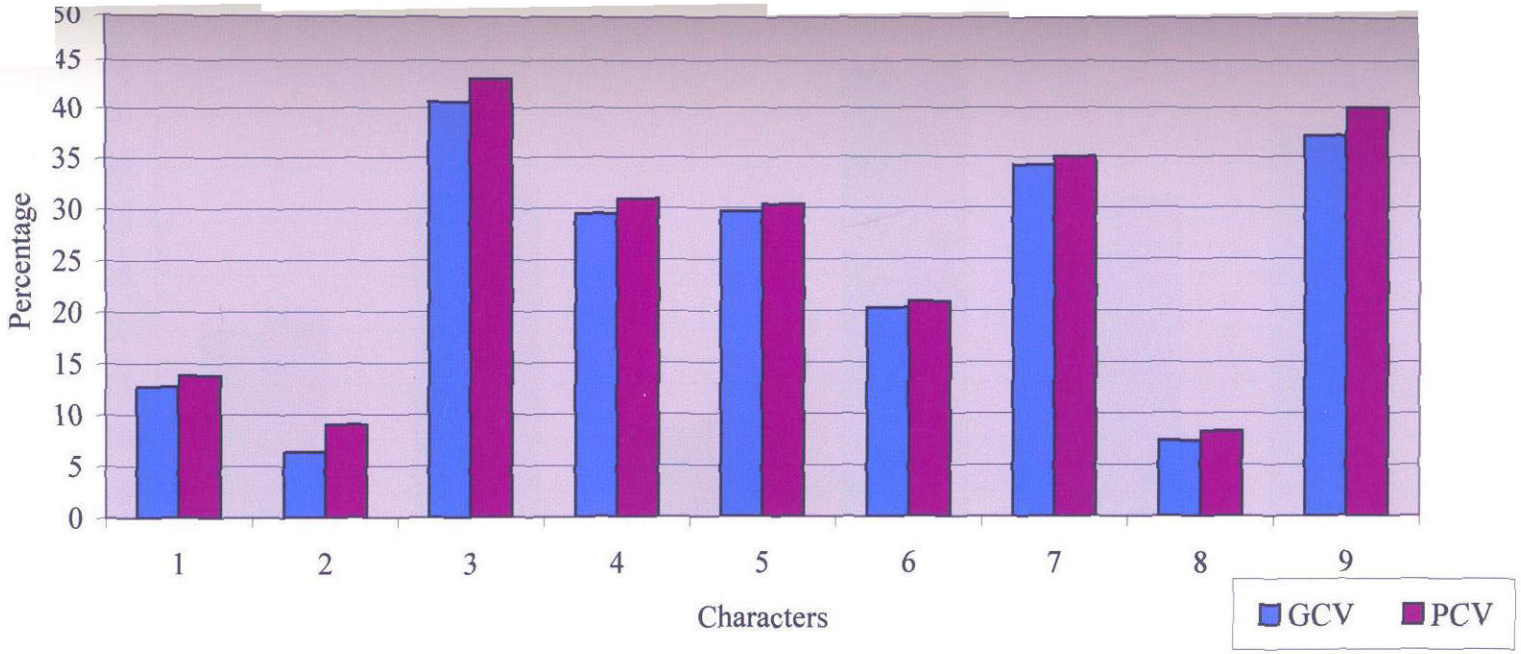
Characters	Variance			Coefficient of variation	
	GV	PV	EV	GCV	PCV
1	0.52	0.62	0.09	12.75	13.83
2	0.00	0.00	0.00	6.37	9.03
3	196.22	219.99	23.77	40.62	43.01
4	362.71	398.86	36.16	29.46	30.89
5	2544.90	2665.34	120.43	29.62	30.31
6	1.53	1.62	0.09	20.24	20.84
7	127.07	133.18	6.12	34.17	34.99
8	0.00	0.00	0.00	7.32	8.25
9	0.03	0.04	0.01	37.10	40.00

1 Plant height (m)
2 Plant girth (m)
3 Number of crowns per plant
4 Number of leaves per crown
5 Leaf length (cm)
6 Leaf width (cm)
7 Leaf weight (g)
8 Leaf thickness (mm)
9 Spine length (cm)

Table 14. Variance and coefficient of variation for inflorescence characters of 22 male genotypes of *Pandanus*

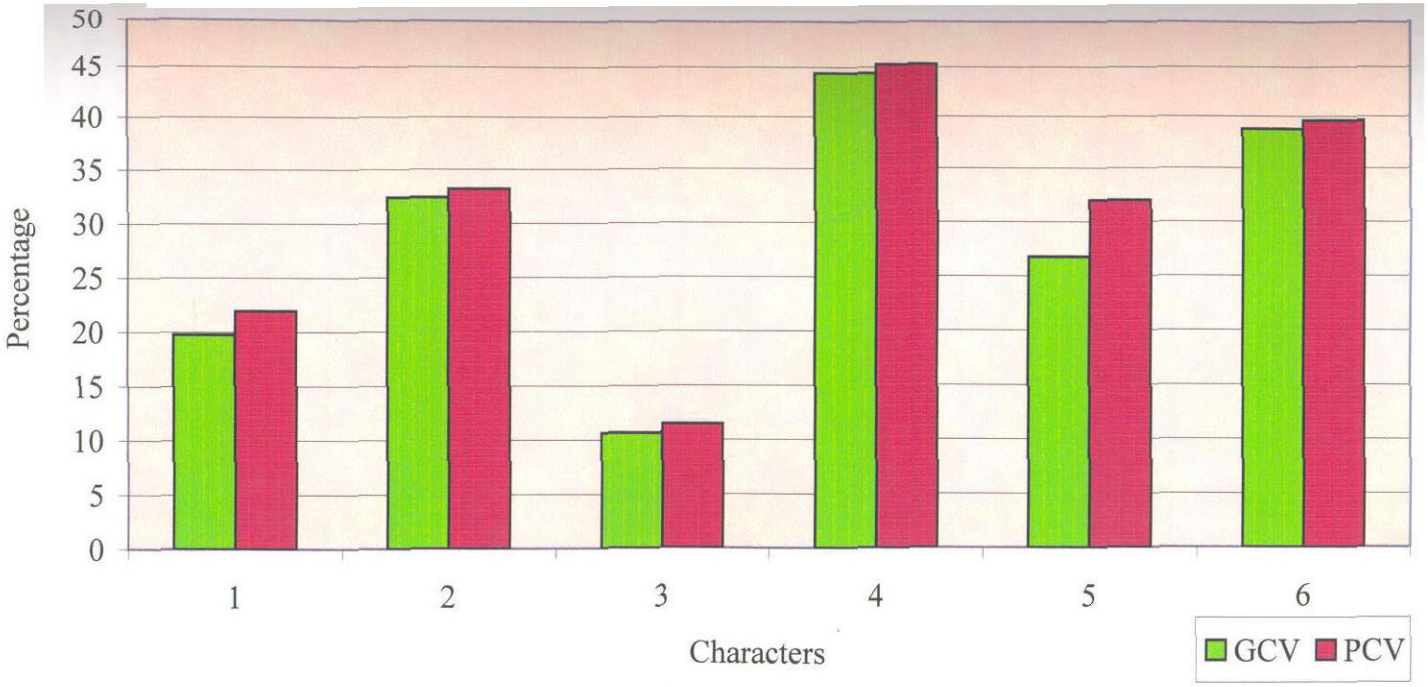
Characters	Variance			Coefficient of variation	
	GV	PV	EV	GCV	PCV
1	33.97	41.90	7.93	19.77	21.95
2	701.05	735.79	34.75	32.39	33.18
3	5.02	5.83	0.81	10.63	11.46
4	8923.77	9297.21	373.43	44.39	45.31
5	0.00	0.00	0.00	26.74	31.98
6	5360596.00	5598052.00	237456.10	38.72	39.57

1 Number of inflorescence per plant
2 Inflorescence length (cm)
3 Inflorescence girth (cm)
4 Inflorescence weight (g)
5 Oil content (v/w) (%)
6 Inflorescence yield per plant (g)



1 Plant height (m)
2 Plant girth (m)
3 Number of crowns per plant
4 Number of leaves per crown
5 Leaf length (cm)
6 Leaf width (cm)
7 Leaf weight (g)
8 Leaf thickness (mm)
9 Spine length (cm)

Fig. 7. GCV and PCV for stem and leaf characters of 22 male genotypes of *Pandanus*



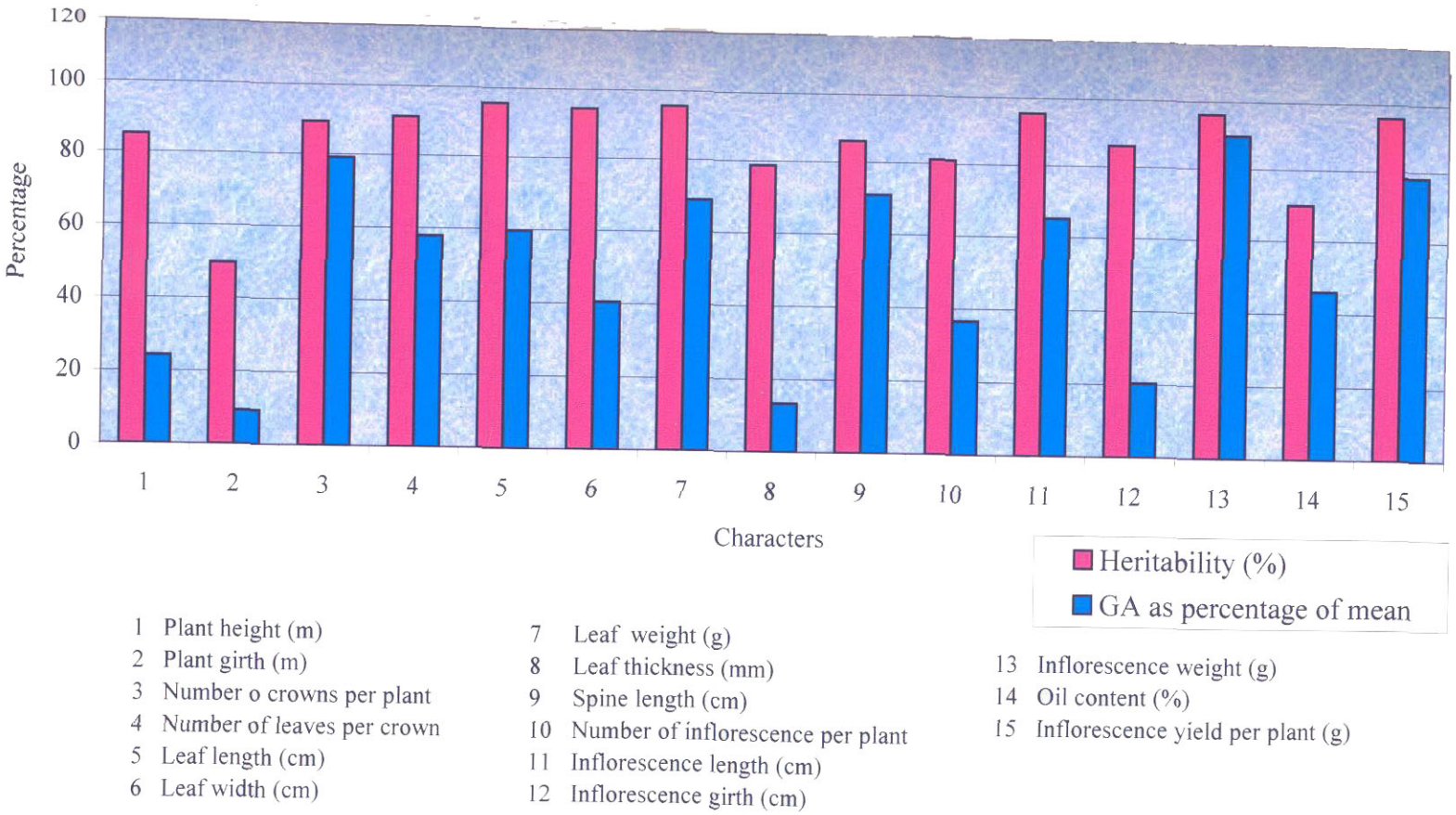
1 Number of inflorescence per plant
2 Inflorescence length (cm)
3 Inflorescence girth (cm)
4 Inflorescence weight (g)
5 Oil content (v/w) (%)
6 Inflorescence yield per plant (g)

Fig. 8. GCV and PCV for inflorescence characters of 22 male genotypes of *Pandanus*

Table 15. Heritability and Genetic advance as percentage of mean for growth characters of 22 male genotypes of *Pandanus*

Characters	Heritability (%)	GA as percentage of mean
1	84.93	24.20
2	49.71	9.25
3	89.19	79.03
4	90.94	57.87
5	95.48	59.63
6	94.24	40.47
7	95.41	68.76
8	78.76	13.38
9	86.03	70.89
10	81.06	36.66
11	95.28	65.12
12	86.04	20.32
13	95.98	89.59
14	69.89	46.04
15	95.76	78.06

1 Plant height (m)
2 Plant girth (m)
3 Number o crowns per plant
4 Number of leaves per crown
5 Leaf length (cm)
6 Leaf width (cm)
7 Leaf weight (g)
8 Leaf thickness (mm)
9 Spine length (cm)
10 Number of inflorescence per plant
11 Inflorescence length (cm)
12 Inflorescence girth (cm)
13 Inflorescence weight (g)
14 Oil content (%)
15 Inflorescence yield per plant (g)



1 Plant height (m)
2 Plant girth (m)
3 Number o crowns per plant
4 Number of leaves per crown
5 Leaf length (cm)
6 Leaf width (cm)
7 Leaf weight (g)
8 Leaf thickness (mm)
9 Spine length (cm)
10 Number of inflorescence per plant
11 Inflorescence length (cm)
12 Inflorescence girth (cm)
13 Inflorescence weight (g)
14 Oil content (%)
15 Inflorescence yield per plant (g)

Fig. 9. Heritability and genetic advance for growth characters of 22 male genotypes of *Pandanus*

(79.03 per cent), inflorescence yield (78.06 per cent) and spine length (70.89 per cent).

4.2.4 Correlation Analysis

The phenotypic, genotypic and environmental correlations were presented in Table 16, 17 and 18 and Fig. 10 and 11.

Phenotypic Correlation

Plant height was found to have significant positive correlation with plant girth, number of leaves per crown, leaf length, leaf width and inflorescence girth. But plant height was not significantly correlated with any of the other character (Table 16 and Fig.10).

Plant girth did not show significant positive correlation with any of the characters.

Number of crowns was found to have significant positive correlation with spine length, and number of inflorescence per plant. It had significant negative correlation with number of leaves.

Number of leaves per crown had significant positive correlation with plant height, leaf length, width, weight, inflorescence length and weight. But it had significant negative correlation with number of crowns per plant and spine length.

Leaf length had significant positive correlation with plant height, number of leaves per crown, leaf width, weight, inflorescence length, girth, weight and inflorescence yield per plant. It had significant negative correlation with spine length.

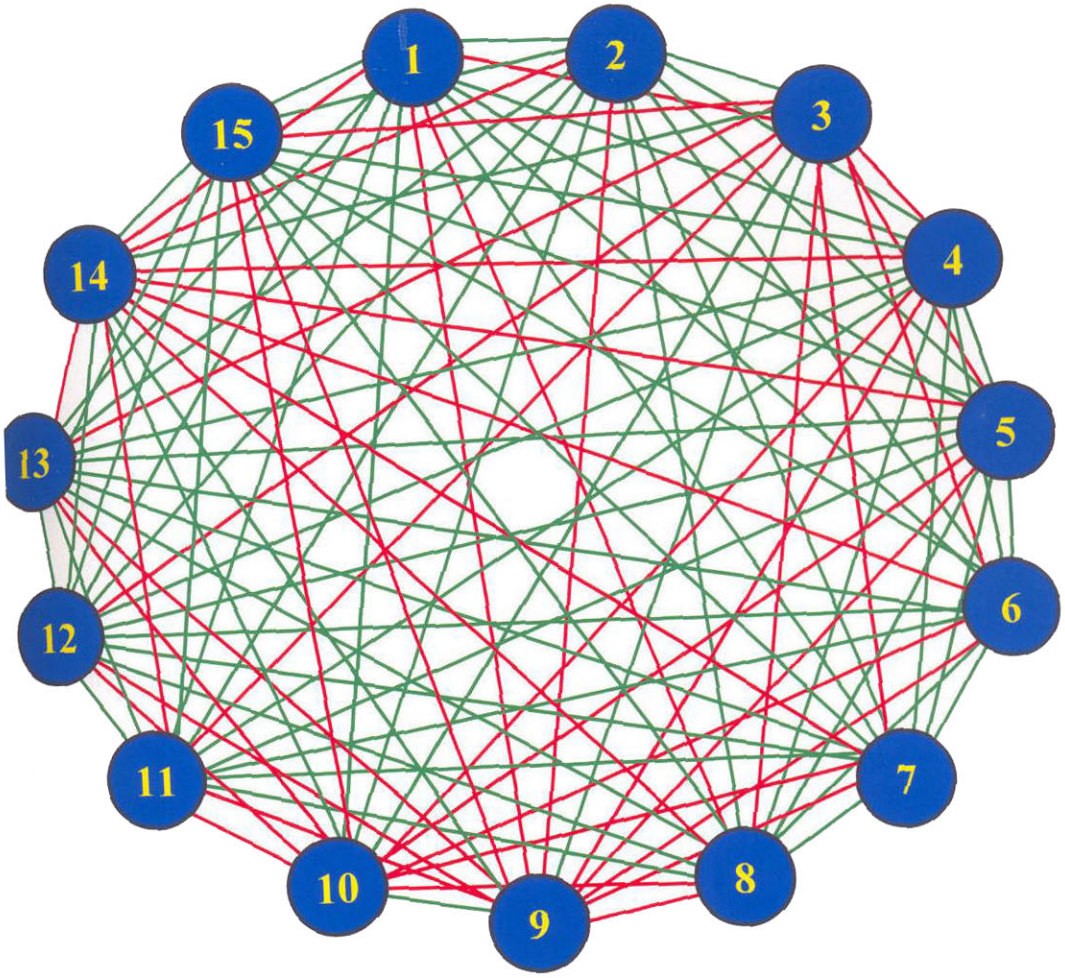
Leaf width had significant positive correlation with number of leaves per crown, leaf length, weight, inflorescence length, girth, weight and inflorescence yield per plant. Leaf width had significant negative correlation with number of crowns per plant and spine length.

Leaf weight had significant positive correlation with number of leaves, leaf length, width, inflorescence length, girth, weight and inflorescence yield per

Table 16. Phenotypic correlation coefficient for growth characters of 22 male genotypes of *Pandanus*

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.000														
2	**0.738	1.000													
3	-0.073	0.035	1.000												
4	**0.531	0.317	-0.668	1.000											
5	**0.546	0.297	-0.478	**0.724	1.000										
6	0.435	0.263	**-0.590	**0.768	**0.916	1.000									
7	0.491	0.297	**-0.600	**0.773	**0.939	**0.946	1.000								
8	-0.154	0.031	-0.447	0.349	0.243	0.408	**0.313	1.000							
9	-0.441	-0.235	**0.658	**-0.745	**-0.829	**-0.880	**-0.891	-0.328	1.000						
10	0.006	0.007	**0.834	**-0.630	-0.454	**-0.572	**-0.571	**-0.578	**0.595	1.000					
11	0.351	0.230	-0.433	**0.595	**0.899	**0.912	**0.906	0.315	**-0.801	-0.472	1.000				
12	**0.579	0.382	-0.230	0.485	**0.835	**0.771	**0.809	0.116	**-0.706	-0.195	**0.828	1.000			
13	0.289	0.159	-0.430	**0.526	**0.883	**0.891	**0.887	0.302	**-0.779	-0.480	**0.971	**0.832	1.000		
14	-0.173	-0.305	**0.570	-0.362	-0.168	-0.332	-0.290	-0.433	0.355	**0.521	-0.115	0.011	-0.096	1.000	
15	0.338	0.190	-0.108	0.293	**0.790	**0.749	**0.737	0.064	**-0.612	-0.087	**0.875	**0.852	**0.908	0.097	1.000

1 Plant height (m)
2 Plant girth (m)
3 Number of crowns per plant
4 Number of leaves per crown
5 Leaf length (cm)
6 Leaf width (cm)
7 Leaf weight (g)
8 Leaf thickness (mm)
9 Spine length (cm)
10 Number of inflorescence per plant
11 Inflorescence length (cm)
12 Inflorescence girth (cm)
13 Inflorescence weight (g)
14 Oil content (%)
15 Inflorescence yield per plant (g)



1 Plant height (m)
2 Plant girth (m)
3 Number of crowns per plant
4 Number of leaves per crown
5 Leaf length (cm)
6 Leaf width (cm)
7 Leaf weight (g)
8 Leaf thickness (mm)
9 Spine length (cm)
10 Number of inflorescence per plant
11 Inflorescence length (cm)
12 Inflorescence girth (cm)
13 Inflorescence weight (g)
14 Oil content (%)
15 Inflorescence yield per plant (g)

Fig. 10. Phenotypic correlation coefficient among the growth characters in 22 male genotypes of *Pandanus*

plant. It showed significant negative correlation with number of crowns per plant, spine length and number of inflorescences per plant.

Leaf thickness did not reveal any significant positive correlation with any of the other characters and it had significant negative correlation with number of inflorescence per plant.

Spine length had significant positive correlation with number of crowns per plant and number of inflorescence per plant. But it showed significant negative correlation with number of leaves, leaf length, width and weight.

Number of inflorescence per plant had significant positive correlation with number of crowns per plant, spine length and oil content. It had significant negative correlation with number of leaves per crown, leaf width, weight and thickness.

Inflorescence length had significant positive correlation with number of leaves, leaf length, width, weight and inflorescence girth, weight and yield. Significant negative correlation was observed with spine length.

Inflorescence girth had significant positive correlation with plant height, number of leaves per crown, leaf length, width inflorescence weight and inflorescence yield per plant. However, it showed significant negative correlation with spine length.

Inflorescence weight was found to have significant positive correlation with number of leaves per crown, leaf length, width, weight, Inflorescence length, girth, and inflorescence yield per plant. While it had significant negative correlation with spine length.

Oil content showed significant positive correlation with number of crowns per plant, spine length and number of inflorescence per plant. It did not show any significant negative correlation with other characters.

Inflorescence yield per plant was found to have significant positive correlation with leaf length, width, weight, inflorescence length, girth and weight. Inflorescence yield showed significant negative correlation with spine length.

Genotypic Correlations

Genotypic correlation among various characters are presented in Table 17 and Fig. 11. The trend of correlation was as like phenotypic correlation for almost all characters.

The character plant height was found to have significant positive correlation with plant girth, number of leaves per crown, leaf length, weight and inflorescence girth. It had significant negative correlation with spine length.

Plant girth had significant positive correlation with plant height and inflorescence girth. But it did not show significant negative correlation with any of the characters.

Number of crowns per plant showed significant positive correlation with spine length, number of flowers per plant and oil content. It had significant negative correlation with number of leaves per crown, leaf length, width, weight and thickness.

Number leaves per crown showed significant positive correlation with plant height, leaf width, weight, inflorescence length, girth and weight. It had significant negative correlation with number of crowns per plant, spine length, and number of inflorescences per plant.

Leaf length had significant positive correlation with plant height, number of leaves per crown, leaf width, weight, inflorescence length, girth, weight and inflorescence yield per plant. But it had significant negative correlation with number crowns per plant, spine length and number of inflorescence per plant.

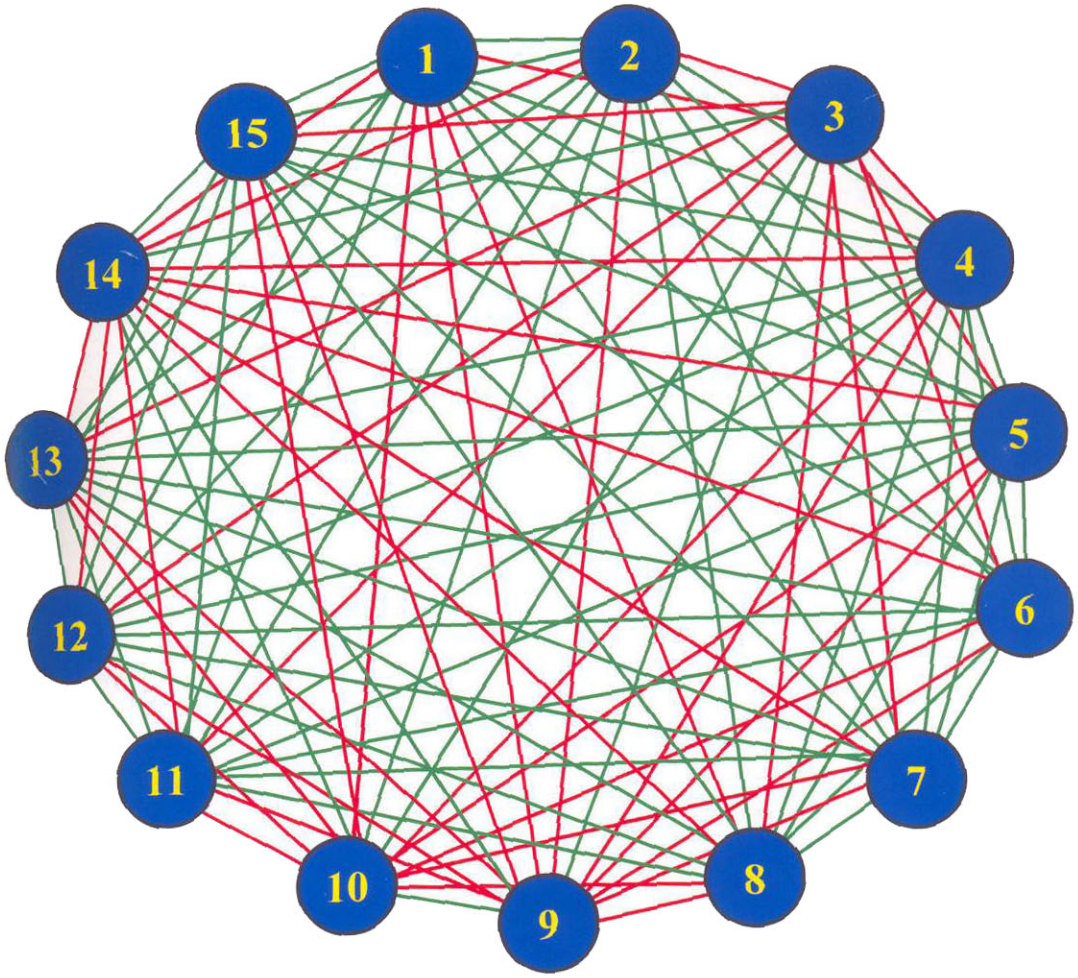
Leaf width was found to have significant positive correlation with plant height, number of leaves per crown, leaf length, weight, inflorescence length, girth, weight and inflorescence yield per plant. It had significant negative correlation with number of crowns per plant, spine length and number of inflorescence per plant.

Leaf weight had significant positive correlation with plant height, number of leaves, leaf length, width, inflorescence length, girth, weight and inflorescence

Table 17. **Genotypic correlation coefficient for growth characters of 22 male genotypes of *Pandanus***

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.000														
2	**0.936	1.000													
3	-0.132	-0.053	1.000												
4	**0.562	0.385	**-0.742	1.000											
5	**0.582	0.370	**-0.546	**0.776	1.000										
6	0.492	0.358	**-0.659	**0.811	**0.970	1.000									
7	**0.550	0.369	**-0.679	**0.833	**0.970	**0.991	1.000								
8	-0.149	0.038	**-0.502	0.374	0.292	0.467	0.365	1.000							
9	**-0.550	-0.476	**0.705	**-0.868	**-0.941	**-0.979	**-0.995	-0.413	1.000						
10	-0.024	0.038	**0.946	**-0.758	**-0.537	**-0.682	**-0.666	**-0.654	**0.717	1.000					
11	0.384	0.225	-0.496	**0.636	**0.931	**0.956	**0.935	0.332	**-0.890	**-0.513	1.000				
12	**0.652	**0.512	-0.288	**0.526	**0.905	**0.847	**0.857	0.139	**-0.821	-0.250	**0.887	1.000			
13	0.315	0.138	-0.483	**0.554	**0.916	**0.929	**0.916	0.320	**-0.869	**-0.504	**0.983	**0.889	1.000		
14	-0.236	-0.475	**0.702	-0.470	-0.225	-0.392	-0.353	-0.632	0.405	**0.691	-0.153	-0.065	-0.144	1.000	
15	0.352	0.201	-0.139	0.295	**0.814	**0.767	**0.756	0.086	**-0.677	-0.149	**0.900	**0.911	**0.929	0.114	1.000

1 Plant height (m)
2 Plant girth (m)
3 Number of crowns per plant
4 Number of leaves per crown
5 Leaf length (cm)
6 Leaf width (cm)
7 Leaf weight (g)
8 Leaf thickness (mm)
9 Spine length (cm)
10 Number of inflorescence per plant
11 Inflorescence length (cm)
12 Inflorescence girth (cm)
13 Inflorescence weight (g)
14 Oil content (%)
15 Inflorescence yield per plant (g)



1 Plant height (m)
2 Plant girth (m)
3 Number of crowns per plant
4 Number of leaves per crown
5 Leaf length (cm)
6 Leaf width (cm)
7 Leaf weight (g)
8 Leaf thickness (mm)
9 Spine length (cm)
10 Number of inflorescence per plant
11 Inflorescence length (cm)
12 Inflorescence girth (cm)
13 Inflorescence weight (g)
14 Oil content (%)
15 Inflorescence yield per plant (g)

Fig. 11. Genotypic correlation coefficient among the growth characters in 22 male genotypes of *Pandanus*

yield per plant. It had significant negative correlation with number of crowns per plant, spine length and number of inflorescence per plant.

Leaf thickness did not show significant positive correlation with any of the characters but it showed significant negative correlation with number of inflorescence per plant and oil content.

Spine length had significant positive correlation with number crown per plant and number of inflorescence per plant. But it showed significant negative correlation with plant height, number of leaves per crown leaf length, width, weight, thickness, inflorescence length, girth, weight and total inflorescence yield per plant.

Number of inflorescence showed significant positive correlation with number of crowns per plant, spine length and oil content. It showed high negative correlation with number of leaves per crown, leaf length, width, weight, leaf thickness and inflorescence length.

Inflorescence length had significant positive correlation with number of leaver per crown, leaf length, width, weight, inflorescence girth, weight and total inflorescence yield per plant. It had significant negative correlation with spine length and number of inflorescence per plant.

Inflorescence width showed significant positive correlation with plant height, plant girth, number of leaves, leaf length, width, weight, inflorescence length, weight and total inflorescence yield per plant. It had significant negative correlation with spine length.

Inflorescence weight had significant positive correlation with plant height, plant girth, number of leaves, leaf length, width, weight, inflorescence length, weight and total inflorescence yield per plant. It had significant negative correlation with spine length and number of inflorescence per plant.

Oil content showed significant positive correlation with number of crowns per plant, spine length and number of inflorescence per plant. It had significant negative correlation with leaf thickness.

Inflorescence yield per plant was found to have significant positive correlation with leaf length, width, weight, inflorescence length, girth and weight. It had significant negative correlation with spine length.

Environmental correlation

Environmental correlation coefficients are presented in Table 18.

In general, low values for correlation coefficient due to environmental effects were observed for all the characters studied. However, inflorescence yield per plant showed high positive correlation with leaf width and number of inflorescence per plant.

4.2.5 Path co-efficient analysis

Direct effect

Path coefficient analysis was done to estimate direct and indirect effects of plant height, number of crowns per plant, number of leaves per crown, number of inflorescence per plant, inflorescence length, girth, weight and oil content on total inflorescence yield per plant. The direct and indirect effects are presented in Table 19 and Fig.12.

Characters like number of leaves per crown, number of inflorescence per plant, inflorescence girth and inflorescence weight showed very high positive direct effect on total inflorescence yield per plant whereas characters such as plant height, number of crowns per plant, inflorescence length and oil content showed negative direct effect on inflorescence yield.

The characters such as number of crowns per plant (4.825) number of inflorescence per plant, (4.144) inflorescence girth (2.546) and inflorescence weight (4.582) registered very high positive direct effect on inflorescence yield per plant. Very high negative direct effect was recorded by plant height (-3.131) inflorescence length (-2.317) and oil content (-2.059). Number of crowns per plant showed moderate negative direct effect on inflorescence yield.

Table 18. Environmental correlation coefficient for growth characters of 22 male genotypes of *Pandanus*

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.000														
2	0.473	1.000													
3	0.330	0.299	1.000												
4	0.322	0.275	-0.001	1.000											
5	0.265	0.276	0.372	0.013	1.000										
6	-0.054	0.106	0.170	0.239	-0.074	1.000									
7	-0.056	0.283	0.365	-0.046	0.289	0.129	1.000								
8	-0.182	0.022	-0.168	0.236	-0.104	0.058	-0.031	1.000							
9	0.203	0.289	0.332	0.196	0.302	0.011	0.130	0.068	1.000						
10	0.156	-0.055	0.208	0.165	0.199	0.233	0.155	-0.275	-0.026	1.000					
11	0.063	0.484	0.351	0.050	0.229	0.113	0.308	0.269	0.059	-0.233	1.000				
12	0.152	0.177	0.182	0.177	0.187	0.092	0.410	0.007	0.002	0.081	0.305	1.000			
13	0.057	0.449	0.260	0.129	0.141	0.155	0.259	0.260	0.150	-0.408	0.690	0.324	1.000		
14	0.041	-0.063	0.088	0.077	0.141	-0.109	-0.012	0.144	0.203	0.003	0.086	0.298	0.196	1.000	
15	0.250	0.352	0.298	0.290	0.279	0.405	0.314	-0.113	0.034	0.486	0.350	0.337	0.403	0.038	1.000

1 Plant height (m)
2 Plant girth (m)
3 Number of crowns per plant
4 Number of leaves per crown
5 Leaf length (cm)
6 Leaf width (cm)
7 Leaf weight (g)
8 Leaf thickness (mm)
9 Spine length (cm)
10 Number of inflorescence per plant
11 Inflorescence length (cm)
12 Inflorescence girth (cm)
13 Inflorescence weight (g)
14 Oil content (%)
15 Inflorescence yield per plant (g)

Table 19. Direct and indirect effects of growth characters of 22 male genotypes of Pandanus

Characters	1	2	3	4	5	6	7	8
1	**-3.131**	0.038	2.710	-0.101	-0.889	1.659	1.444	0.485
2	0.412	**-0.290**	-3.580	3.920	1.150	-0.733	-2.212	-1.446
3	-1.758	0.215	**4.825**	-3.143	-1.474	1.339	2.540	0.968
4	0.076	-0.274	-3.660	**4.144**	1.187	-0.635	-2.307	-1.423
5	-1.202	0.144	3.069	-2.124	**-2.317**	2.258	4.506	0.315
6	-2.041	0.083	2.539	-1.034	-2.055	**2.546**	4.071	0.133
7	-0.987	0.140	2.675	-2.087	-2.279	2.262	**4.582**	0.295
8	0.738	-0.204	-2.270	2.863	0.355	-0.165	-0.658	**-2.059**

1 Plant height (m)
2 Number of crowns per plant
3 Number of leaves per crown
4 Number of inflorescence per plant
5 Inflorescence length (cm)
6 Inflorescence girth (cm)
7 Inflorescence weight (g)
8 Oil content (%)

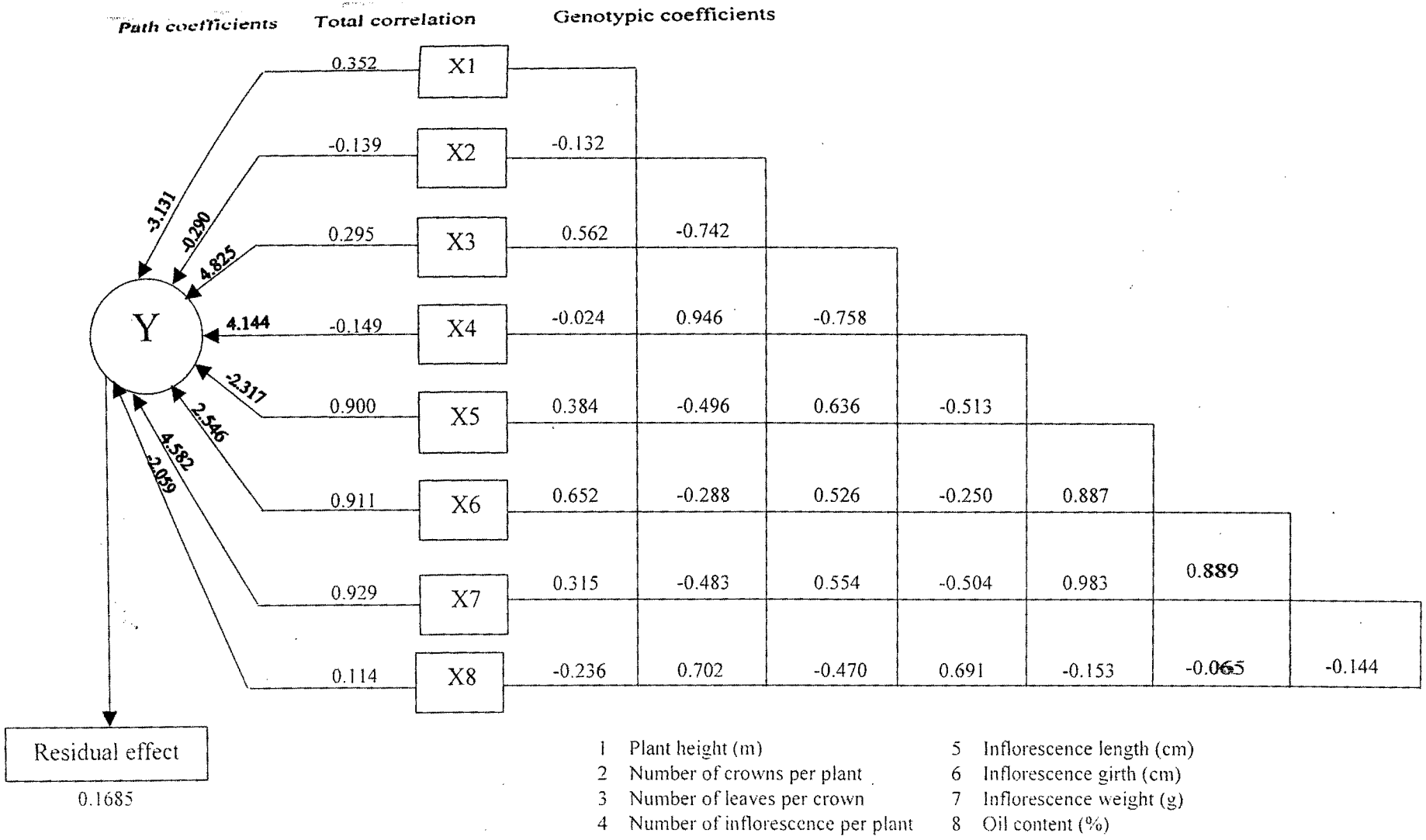


Fig. 12. Path diagram showing direct effects and interrelationship in 22 male genotypes of *Pandanus*

Indirect effect

Plant height had very high positive indirect effect on inflorescence yield per plant through number of leaves, inflorescence girth and inflorescence weight. However, it showed high positive indirect effect through oil content. It exerted high negative indirect effect on inflorescence yield per plant through inflorescence length and low negative indirect effect through number of inflorescence per plant.

Number of crowns per plant showed very high positive indirect effect on inflorescence yield through number of inflorescence per plant (3.920) and inflorescence length (1.150). It had very high negative indirect effect on inflorescence yield through number of leaves per crown, inflorescence weight and oil content.

Number of leaves per crown had very high positive indirect effect on inflorescence yield through inflorescence weight (2.540) and inflorescence girth (1.339), while it showed very high negative indirect effect through plant height, number of inflorescence per plant, and inflorescence length.

Number of inflorescence per plant revealed very high positive indirect effect on inflorescence yield through inflorescence length (1.188). But it showed very high negative indirect effect on inflorescence yield through number of leaves per crown, inflorescence weight and oil content.

Inflorescence length had very high positive indirect effect on inflorescence yield per plant via number of leaves per crown inflorescence girth (2.258) and inflorescence weight (4.506). Where as, it had very high negative indirect effect through number of inflorescence per plant and plant height.

Inflorescence girth registered very high positive indirect effect on inflorescence yield per plant through inflorescence weight (4.071) while it had very high negative indirect effect through plant height and inflorescence length and number of inflorescence per plant.

Inflorescence weight showed very high positive indirect effect on inflorescence yield through number of leaves per crown (2.675) and inflorescence

girth (2.262). It had very high negative indirect effect through number of inflorescence per plant and inflorescence length.

Oil content had very high positive indirect effect on inflorescence yield through number of inflorescence per plant (2.863). But it showed very high negative indirect effect through number of leaves per crown (-2.270).

4.2.6 Cluster Analysis

The 22 male genotypes of *Pandanus* were subjected to D^2 analysis based on the fifteen characters *viz.*, plant height, girth, number of crowns per plant, number of leaves per plant, leaf length, width, thickness, spine length, number of inflorescence per plant, length of the inflorescence, girth of the inflorescence, weight of the single inflorescence, oil content and inflorescence yield per plant. Tocher's method was applied to cluster the genotypes based on their pair wise D^2 values and as such six clusters were formed. The different clusters with their genotypes are given in Table 20.

The cluster 1 with two genotypes (PF 13 and PF 15), cluster11 with six genotypes (PF 1, PF 2, PF 3, PF 4, PF 8 and PF 16), cluster III with three genotypes (PF 10, PF 19 and PF 20) cluster IV with six genotypes (PF 5, PF 6, PF 7, PF 11, PF 12 and PF 25), cluster V with three genotypes (PF 17, PF 22 and PF 23) and the cluster VI had two genotypes namely PF 18 and PF 24.

Based on total D^2 values, the average inter and intra-cluster distances were estimated and presented in Table 21 and Table 22 and Fig.13.

The average intra cluster D^2 value varied from 6.971 (cluster I) to 427.500 (cluster VI). The inter cluster D^2 value was highest between cluster I and cluster II (958.84) and it was lowest between cluster III and cluster V (123.518).

The cluster means of the fifteen characters are presented in Table 23. Cluster I showed the highest cluster mean for plant height, number of leaves and leaf thickness. The highest cluster mean for spine length was observed in cluster II. Cluster III recorded highest cluster mean for leaf weight, inflorescence length, girth, weight and total inflorescence yield per plant.

Table 20. Group constellation of 22 male genotypes of *Pandanus* based on D^2 analysis

Cluster	Number of genotypes	Name of the genotypes
CI	2	PF 13 and PF 15
CII	6	PF 1, PF 2, PF 3, PF 4, PF 8 and PF 16.
CIII	3	PF 10, PF 19 and PF 20.
CIV	6	PF 5, PF 6, PF 7, PF 11, PF 12 and PF 25.
CV	3	PF 17, PF 22 and PF 23.
CVI	2	PF 18 and PF 24.

Table 21. Average inter and intra cluster distances ($\sqrt{D^2}$)

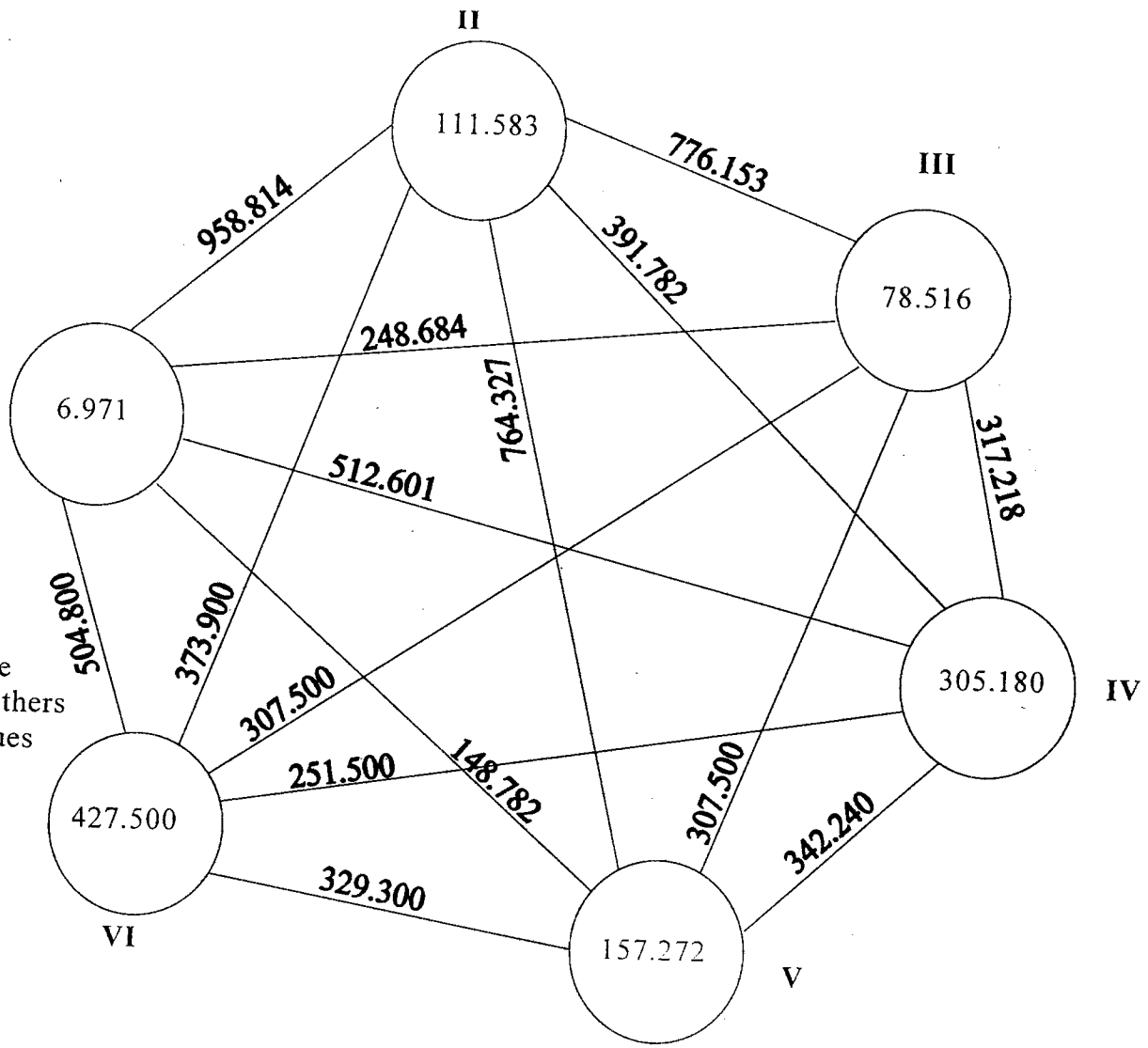
	1	2	3	4	5	6
1	2.640	30.965	15.770	22.641	12.198	22.400
2		10.593	27.860	19.793	27.646	19.300
3			8.861	17.811	11.114	17.500
4				17.469	18.500	15.800
5					12.541	18.100
6						20.600

Table 22. Average inter and intra cluster distances (D^2)

	1	2	3	4	5	6
1	**6.971**	958.814	248.684	512.601	148.782	504.800
2		**111.583**	776.153	391.782	764.327	373.900
3			**78.516**	317.218	123.518	307.500
4				**305.180**	342.240	251.500
5					**157.272**	329.300
6						**427.500**

Table 23. Cluster means of the growth characters of 22 male genotypes of *Pandanus*

Sl. No	Characters	C I	C II	C III	C IV	C V	C VI	Percentage contribution
1.	Plant height (m)	6.367	4.994	5.700	5.928	5.856	5.950	3.46
2.	Plant girth (m)	0.313	0.289	0.292	0.314	0.309	0.305	0.00
3.	No. crowns per plant	10.667	42.389	23.667	40.444	23.444	49.500	0.00
4.	No. leaves per crown	101.833	46.167	69.00	58.667	84.111	65.167	0.00
5.	Leaf length(cm)	205.833	106.333	202.222	183.667	207.222	183.333	6.49
6.	Leaf width(cm)	7.1	4.6	7.1	6.1	7.3	6.0	3.03
7.	Leaf weight (g)	43.33	19.389	44.111	33.722	40.556	33.167	6.48
8.	Leaf thickness (mm)	0.600	0.550	0.567	0.572	0.600	0.550	6.43
9.	Spine length (cm)	0.300	0.667	0.344	0.500	0.333	0.567	1.30
10.	Number of inflorescence	20.500	32.333	26.333	33.389	24.444	30.500	25.54
11.	Inflorescence length (cm)	82.500	50.333	108.889	85.278	105.000	89.167	3.03
12.	Inflorescence girth (cm)	20.667	18.389	23.222	22.000	22.222	21.667	9.96
13.	Single Inflorescence weight (g)	208.30	110.20	323.30	223.3	272.7	237.5	4.76
14.	Oil content (%)	0.100	0.167	0.156	0.167	0.133	0.200	4.33
15.	Inflorescence yield per plant (g)	4243.33	3665.55	8445.55	6914.44	6792.778	6927.50	31.17



The values in circles indicate intra-cluster D^2 values and others indicate inter-cluster D^2 values

Fig. 13. Diagrammatic representation of clustering of 22 male genotypes of *Pandanus*

Cluster IV recorded the highest mean for plant girth and number inflorescence per plant. Cluster V showed highest cluster mean for leaf length, width and thickness. Cluster VI registered highest cluster mean for number of crowns per plant and oil content.

Inflorescence yield and number of inflorescence per plant were the major contributing characters towards the divergence at inter cluster level (31.17 and 25.54 per cent respectively) and it was followed by inflorescence girth (9.96), leaf length (6.49), leaf weight (6.48), leaf thickness (6.43), inflorescence weight (4.76) and oil content (4.33). The characters like plant girth, number of crowns per plant and number of leaves per crown showed nil effect towards divergence indicating that the characters were not functionally important in differentiating the clusters.

4.3 PROPAGATION

4.3.1 Seed Propagation

The effect of physical and chemical seed treatments on the germination and growth of *Pandanus* is presented.

4.3.1.1 Germination Percentage

The effect of pre germination seed treatments on germination percentage is given in Table 24 and Fig.14.

Significant differences were noticed among the different pre germination treatments of *Pandanus* seeds on germination percentage. Soaking of the seeds in diluted cow urine (T_6), cow urine + cow dung slurry (T_7) and cow urine + GA_3 100 ppm (T_8) registered the highest germination percentage (80 per cent) followed by GA_3 100 ppm treatment T_4 (66.66 per cent) and acid treatment T_5 (50.00 per cent). The mechanical scarification treatment (T_9) and control (T_1) recorded the lowest germination percentage of 33.33 and 16.66 respectively.

4.3.1.2 Number of Days for Germination

The effect of different pre germination seed treatments on the number of days for germination is presented in Table 24 and Fig.14.

Table 24. Effect of seed treatments on the germination of *Pandanus* seeds

Treatments	Percentage seed germination	No of days taken for germination
T_1	16.6	35.33
T_2	53.3	31.33
T_3	40.0	32.00
T_4	66.6	27.33
T_5	50.0	26.00
T_6	80.0	24.33
T_7	80.0	23.33
T_8	80.00	23.33
T_9	33.33	33.33
SEd (CD0.05)	6.8493 14.3902 **	1.9116 4.0162 **

Table 25. Effect of seed treatments on the seedling height (cm) of *Pandanus* at different growth stages

Treatments	Months of sowing		
	4^{th}	8^{th}	12^{th}
T_1	11.33	18.33	32.00
T_2	12.00	20.66	42.00
T_3	9.00	18.00	37.00
T_4	15.33	24.00	43.33
T_5	12.50	21.00	40.00
T_6	18.33	30.66	50.00
T_7	18.66	31.66	51.00
T_8	20.33	33.33	54.00
T_9	9.66	19.00	31.30
SEd	0.7817	1.1498	1.8392
CD (0.05)	1.6424 **	3.6762 **	3.8641 **

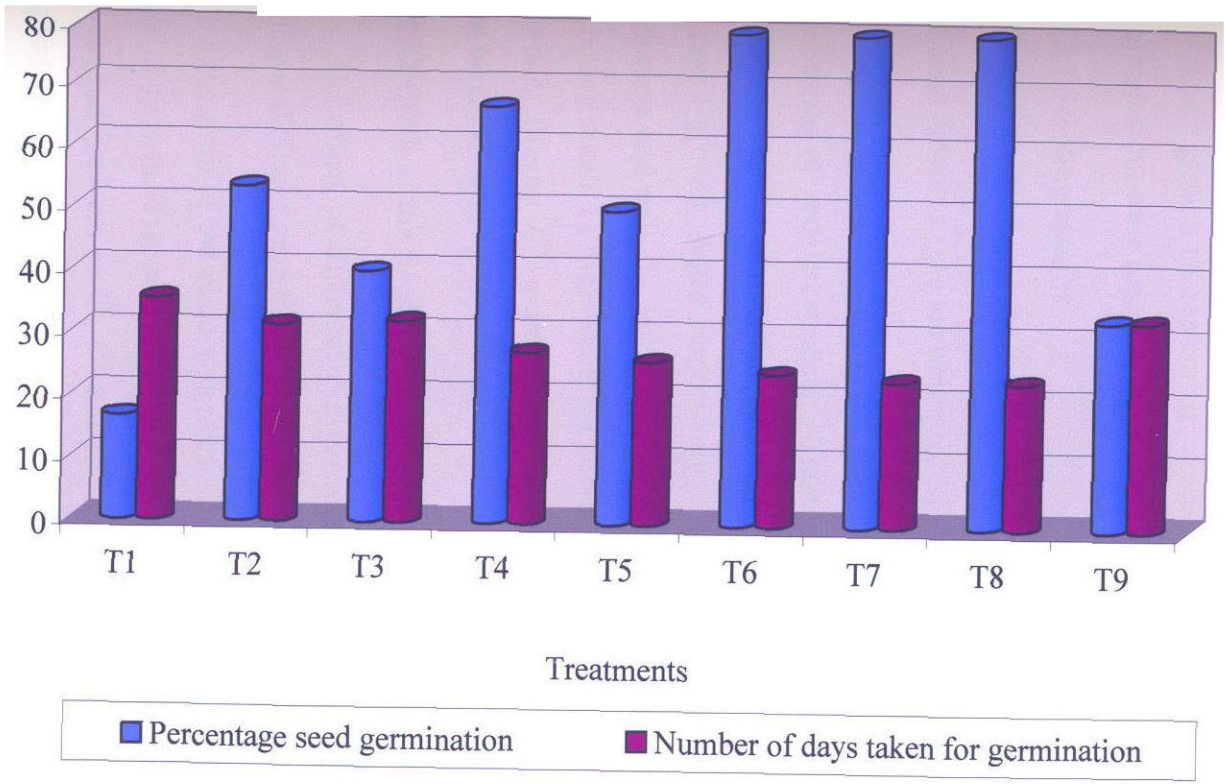


Fig. 14. Effect of seed treatments on the germination of *Pandanus* seeds

Days to germination with respect to different treatments were significant, treatment T_1 (control) took the maximum number of 35.30 days and it was on par with T_9, T_3 and T_2. The treatment T_7 and T_8 took the minimum of 23.33 days for germination and it was on par with T_6, T_5 and T_4.

4.3.1.3 Shoot Growth Characters

Shoot growth characters *viz.*, seedling height, girth, number of leaves, leaf length and width were recorded during 4th, 8th and 12th months of sowing.

4.3.1.3.1 Seedling Height

The effect of different pre germination seed treatments on seedling height at different growth stages are presented in Table 25 and Fig.15.

Height of the seedling varied significantly at various growth stages due to the influence of seed treatments. Treatment T_8 (cow urine + GA_3 100 ppm) recorded the highest plant height of 20.33, 33.33 and 54.00 cm during 4th, 8th and 12th month growth stages respectively. It was on par with T_7 during the 12th month stage and with T_6 and T_7 during 8th month growth stage. During 4th and 12th month stage T_9 registered the lowest plant height of 9.66 cm and 31.30 cm while, during 8th month stage the treatment T_1 registered the lowest value of 18.33 cm.

4.3.1.3.2 Girth of the Seedling

The effect of different pre germination seed treatments on girth of the seedling at different growth stages are given in Table 26 and Fig.16.

The girth of the plant was markedly influenced due to the treatment at all stages of growth. Treatment T_8 recorded the maximum plant girth of 2.93 cm, 4.13 cm and 8.43 cm at 4th, 8th and 12th month stages respectively. Least values for plant girth was recorded by T_1 and T_9 during 4th and 8th month (1.00 cm and 1.70 cm) and by T_9 during 12th month stage (6.00 cm).

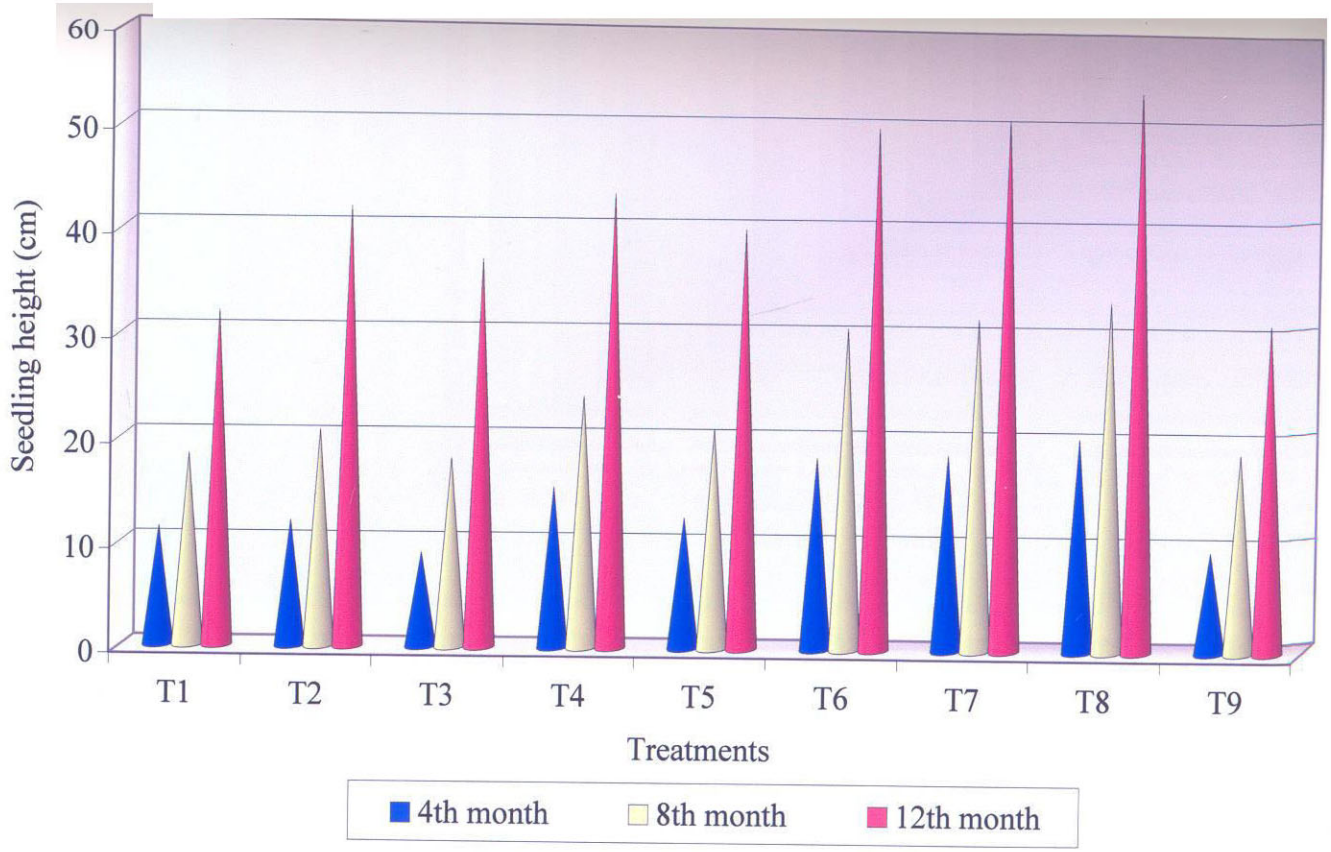


Fig. 15. Effect of seed treatments on the seedling height (cm) of *Pandanus* at different growth stages

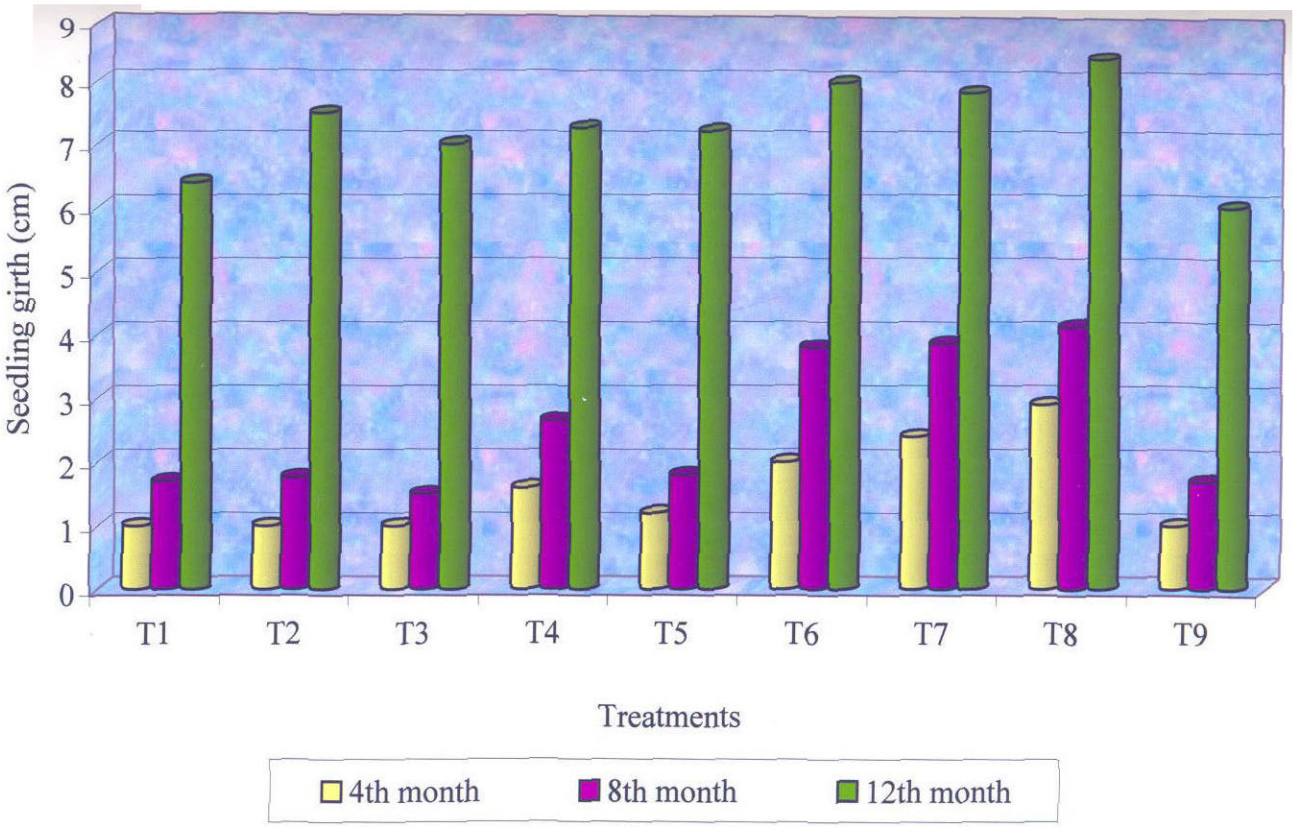


Fig. 16. Effect of seed treatments on the seedling girth (cm) of *Pandanus* at different growth stages

4.3.1.3.3 Number of Leaves

The effect of different pre germination seed treatments on number of leaves at different growth stages are given in Table 27 and Fig.17.

Number of leaves revealed significant differences among the treatments. Maximum number of leaves were produced by T_8 at all stages of growth and it was on par with treatment T_7 during 4th month stage and with T_2, T_6 and T_7 during 8th month stage. At 12th month stage maximum number of leaves (19.77) were produced by T_8 which was significantly superior to all other treatments. T_1 (control) produced a minimum number of 15.33 leaves and it was on par with T_2, T_3 and T_9 (16.00, 16.33, 15.66 leaves respectively).

4.3.1.3.4 Length of the Leaf

The effect of different pre germination seed treatments on length of the leaf at different growth stages are given in Table 28.

Length of the leaf varied significantly due to the effect of different seed treatments. During 4th and 8th month stage T_8 recorded the highest leaf length of 20.66 cm and 30.66 cm respectively, while during 12th month stage treatment T_7 recorded the highest leaf length of 41.33 cm. At 4th and 12th month stage T_9 (mechanical scarification) recorded the lowest leaf length (10.00 and 23.60 cm respectively) while at 8th month stage T_1 recorded the lowest value of 15.00 cm.

4.3.1.3.5 Leaf Width

The effect of different pre germination seed treatments on leaf width at different growth stages are given in Table 29.

Significant differences among the treatments were noticed in respect of leaf width. Maximum leaf width was observed by treatment T_8 followed by T_7 at all stages of growth. At 12th month stage T_8 recorded the maximum leaf width of 2.40 cm and it was on par with T_7 (2.36 cm). Treatments T_1, T_2, T_3, T_4, T_5 and T_9 recorded the lowest value (2.00 cm).

Table 26. Effect of seed treatments on the seedling girth (cm) of *Pandanus* at different growth stages

Treatments	Months of sowing		
	4th	8th	12th
T_1	1.00	1.70	6.40
T_2	1.00	1.76	7.50
T_3	1.00	1.50	7.00
T_4	1.60	2.66	7.26
T_5	1.20	1.80	7.20
T_6	2.00	3.80	8.00
T_7	2.40	3.86	7.83
T_8	2.90	4.13	8.43
T_9	1.00	1.70	6.00
SEd	0.0737	0.1423	0.1515
CD (0.05)	0.1548 **	0.2989 **	0.3184 **

Table 27. Effect of seed treatments on the number of the leaves of *Pandanus* seedlings at different growth stages

Treatments	Months of sowing		
	4th	8th	12th
T_1	7.00	10.66	15.33
T_2	7.66	12.66	16.00
T_3	7.66	12.00	16.33
T_4	9.00	12.00	17.00
T_5	7.00	12.00	16.77
T_6	9.00	12.66	17.60
T_7	10.33	13.66	18.00
T_8	11.00	14.33	19.77
T_9	7.00	12.33	15.66
SEd	0.7027	0.9027	0.4969
CD (0.05)	1.4764 **	1.8965**	1.0440**

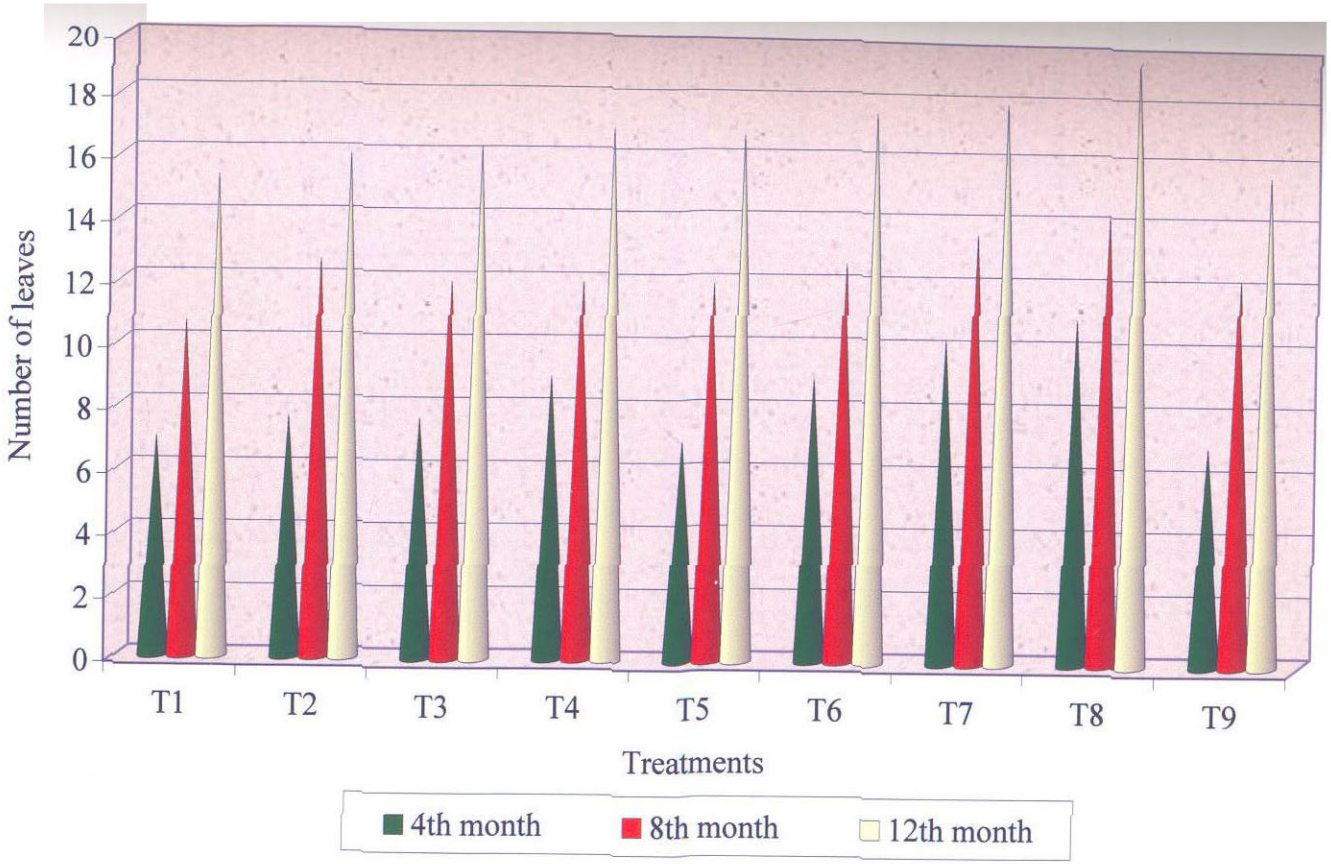


Fig. 17. Effect of seed treatments on the number of the leaves of *Pandanus* seedlings at different growth stages

Table 28. Effect of seed treatments on the leaf length (cm) of *Pandanus* seedlings at different growth stages

Treatments	Months of sowing		
	4th	8th	12th
T_1	10.33	15.00	25.66
T_2	10.33	17.33	34.00
T_3	9.66	14.66	30.66
T_4	10.33	17.33	37.33
T_5	10.00	15.00	35.00
T_6	19.66	28.00	41.00
T_7	19.33	29.33	41.33
T_8	20.66	30.66	40.66
T_9	10.00	19.33	23.60
SEd	0.6285	1.6997	1.7213
CD (0.05)	1.3205 **	3.5709 **	3.6164 **

Table 29. Effect of seed treatments on the leaf width (cm) of *Pandanus* at different growth stages

Treatments	Months of sowing		
	4th	8th	12th
T_1	0.50	1.40	2.00
T_2	0.50	1.50	2.00
T_3	0.50	1.46	2.00
T_4	0.80	1.70	2.03
T_5	0.50	1.50	2.00
T_6	1.00	1.90	2.06
T_7	1.00	1.96	2.36
T_8	1.06	2.00	2.40
T_9	0.50	0.93	2.00
SEd	0.0314	1.1054	0.0272
CD (0.05)	0.0660 **	0.2215 **	0.0572 **

4.3.1.4 Root growth Characters

Root growth characters like number of primary roots, root length, girth and root spread were recorded at 4^{th}, 8^{th} and 12^{th} months of sowing.

4.3.1.4.1 Number of Primary Roots

The influence of different pre germination seed treatments on number of primary roots at various growth stages are given in Table 30.

Significant variation was not observed in case of number of primary roots due to the influence of the seed treatments. However, maximum number of roots were produced by treatments T_8, T_7 and T_6 at 8^{th} and 12^{th} month stages. The number of primary roots at 12^{th} month stage varied from 8.0 to 9.0.

4.3.1.4.2 Root Length

The influence of different pre germination seed treatments on root length at various growth stages are given in Table 31.

Root length showed significant differences among the treatments. It varied from 13.00 to 25.30 cm during 4^{th} month stage, 24.66 to 37.00 cm during 8^{th} month stage. At all stages of growth T_8 (cow urine + GA_3 100 ppm) registered maximum values and it was on par with treatment T_7. Treatment T_3 recorded the lowest root length at all stages of growth.

4.3.1.4.3 Root Girth

The influence of different pre germination seed treatments on root girth at various growth stages are given in Table 32.

With respect to root girth, treatment T_7 registered maximum values (0.80, 1.56 and 2.56 cm) and it was on par with treatment T_6 and treatment T_8 at all stages of growth. Treatment T_3 recorded the minimum values (0.4 and 1.0 cm) at 4^{th} and 8^{th} month stage, while at 12^{th} month stage T_1 recorded the minimum value (1.86 cm).

Table 30. Effect of seed treatments on the number of roots of *Pandanus* seedlings at different growth stages

Treatments	Months of sowing		
	4th	8th	12th
T_1	3	6.33	8.33
T_2	3	6.33	8.66
T_3	3	6.00	8.00
T_4	3	6.66	8.66
T_5	3	6.00	8.33
T_6	3	7.00	9.00
T_7	3	7.00	9.00
T_8	3	7.00	9.00
T_9	3	6.33	8.66
	NS	NS	NS

Table 31. Effect of seed treatments on the root length (cm) of *Pandanus* seedlings at different growth stages

Treatments	Months of sowing		
	4th	8th	12th
T_1	17.83	25.0	32.5
T_2	21.16	28.5	37.66
T_3	13.00	24.66	31.66
T_4	22.16	28.83	41.00
T_5	20.00	26.00	35.00
T_6	23.00	33.33	43.33
T_7	23.33	36.00	45.16
T_8	25.33	37.00	15.76
T_9	16.33	25.83	33.33
SEd	1.0274	0.9061	0.8662
CD (0.05)	2.1585 **	1.9036 **	1.8199 **

Table 32. Effect of seed treatments on the root girth (cm) of *Pandanus* seedling at different growth stages

Treatments	Months of sowing		
	4th	8th	12th
T_1	0.50	1.13	1.86
T_2	0.50	1.33	2.23
T_3	0.40	1.10	1.90
T_4	0.80	1.43	2.16
T_5	0.50	1.20	2.00
T_6	0.80	1.53	2.50
T_7	0.80	1.56	2.56
T_8	0.80	1.53	2.46
T_9	0.43	1.13	1.96
SEd	0.0157	0.0567	0.0609
CD (0.05)	2.1585 **	0.1190 **	0.1279 **

Table 33. Effect of seed treatments on the root spread (cm) of *Pandanus* seedlings at different growth stages

Treatments	Months of sowing		
	4th	8th	12th
T_1	8.60	14.30	26.00
T_2	9.00	15.00	26.00
T_3	11.00	14.00	27.60
T_4	11.30	17.30	32.00
T_5	10.00	14.60	27.00
T_6	12.00	17.00	33.30
T_7	12.80	18.60	34.60
T_8	13.30	19.30	35.00
T_9	8.00	15.00	27.00
SEd	0.6236	0.8854	0.9027
CD (0.05)	1.3102 **	1.8602 **	1.8965 **

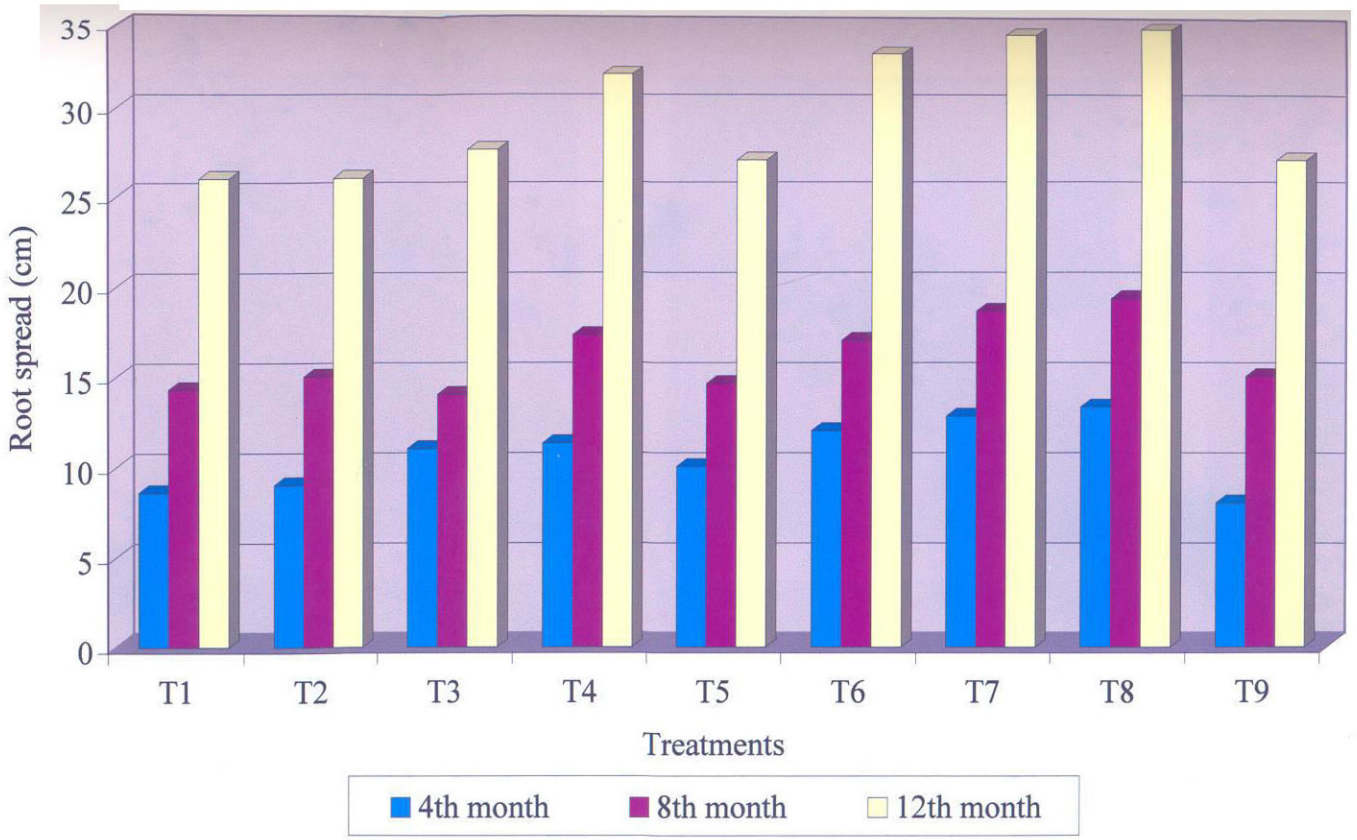


Fig. 18. Effect of seed treatments on the root spread (cm) of *Pandanus* seedlings at different growth stages

Treated seed

Germinating seedling

Shoot growth 4th month of sowing

Shoot growth 8th month of sowing

Shoot growth 12th month of sowing

Root spread 12th month of sowing

Plate 8 Seed Propagation
Effect of cow urine + GA_3 100 ppm (T_8) on seedling vigour of *Pandanus*

4.3.1.4.4 Root Spread

The influence of different pre germination seed treatments on root spread number of primary roots at various growth stages are given in Table 33 and Fig.18.

Root spread was found to vary significantly according to the treatments. The maximum root spread was recorded by T_8 at all stages of growth (13.30, 19.30 and 35.00 cm). Minimum root spread was recorded by T_9 at 4th month stage and by T_3 at 8th month stage and by T_1 and T_2 at 12th month stage.

To conclude, seed treatment with cow urine + GA_3 100 ppm (T_8), produced vigorous seedlings (Plate 8) followed by cow dung slurry + cow urine (T_7) and cow urine treatment (T_6).

4.3.2 Vegetative Propagation

The results of the study on selection of suitable vegetative propagation material for rapid multiplication of *Pandanus* are presented.

4.3.2.1 Growth Characters

Influence of different planting materials on growth parameters *viz.*, establishment percentage, number of days for establishment, plant height, girth, leaf number, leaf length, width, leaf yield per plant, sucker production per plant and prop roots per plant were recorded at 3rd, 6th, 9th, 12th, 15th and 18th months of planting (Plate 9).

4.3.2.1.1 Establishment Percentage

The effect of different planting materials on the establishment percentage is given in Table 34 and Fig.19.

Significant differences were found in the establishment percentage of different planting materials. The highest establishment (100.00 per cent) was recorded in case of ground suckers (T_4) and it was on par (93.75 per cent) with terminal cuttings with crown (T_1). The lowest establishment (68.75 per cent) was

Terminal cutting with crown

Stem cutting

Aerial sucker

Ground sucker

Plate 9. Vegetative propagation of *Pandanus*

Growth performance at 6th month of planting

Growth proformance

12th month of planting

18th month of planting

Terminal cutting with crown

12th month of planting

18th month of planting

Ground suckers

Plate 9. Continued

recorded by stem cuttings without crown (T_2) and it was on par with (75.00 per cent) aerial suckers (T_3).

4.3.2.1.2 Number of Days for Establishment

The effect of different planting materials on number of days for establishment is given in Table 34 and Fig.19.

Highly significant differences among the different planting materials were recorded for the number of days for sprouting. Ground suckers (T_3) took a minimum of 22.5 days and it was on par with treatment T_1 (terminal cuttings without crown). Stem cuttings without crown (T_2) took a maximum of 38.25 days for sprouting.

4.3.2.1.3 Plant Height

The effect of different planting materials on plant height at various growth stages are given in Table 35 and Fig. 20.

The plant height was significantly influenced due to different planting materials at all stages of growth *viz.*, 3rd, 6th, 9th, 12th, 15th and 18th month stages. Invariably at all stages of growth the highest plant height was recorded by terminal cutting with crown (T_1) followed by ground and aerial suckers (T_4 and T_3). The least value was recorded by treatment T_2 (stem cuttings without crown) at all stages of growth. At 18th month stage the highest plant height of 190.12 cm was recorded by treatment T_1 and the lowest value of 164.00 cm was recorded by treatment T_2.

4.3.2.1.4 Plant Girth

The effect of different planting materials on plant girth at various growth stages are given in Table 36.

Remarkable variation was recorded on plant girth at all stages of growth due to the influence of different planting materials. Terminal cuttings with crown (T_1) registered the maximum values of 17.75, 18.65, 20.40, 21.37, 22.50, and 23.05 cm at 3rd, 6th, 9th, 12th, 15th and 18th month stages respectively and it was

Table 34. Effect of different types of *Pandanus* planting material on establishment

Treatments	Percentage establishment	No. of days for sprouting
T_1	93.75	24.50
T_2	68.75	38.25
T_3	75.00	35.50
T_4	100	22.50
SEd	9.5470	1.8569
CD(0.05)	20.8014*	4.0458**

Table 35. Effect of various planting material on the plant height (cm) at different stages of growth of *Pandanus*

Treatments	Months of planting					
	3rd	6th	9th	12th	15th	18th
T_1	55.75	84.25	110.50	136.87	166.87	190.12
T_2	36.50	57.87	82.87	104.37	134.37	164.00
T_3	44.50	71.00	91.00	120.87	149.25	176.50
T_4	49.75	73.00	93.50	123.75	155.25	180.50
SED	2.2761	3.0221	3.5902	2.9192	5.0308	3.8325
CD (0.05)	5.1490**	6.8365**	8.1218**	6.6038**	11.3807**	8.6700**

Table 36. Effect of various planting material on the plant girth (cm) at different stages of growth of *Pandanus*

Treatments	Months of planting					
	3rd	6th	9th	12th	15th	18th
T_1	17.75	18.65	20.4	21.37	22.50	23.05
T_2	7.62	10.50	14.25	15.82	18.00	19.02
T_3	10.25	11.30	13.50	16.37	18.30	20.25
T_4	11.25	12.37	13.77	16.87	19.25	20.87
SEd	0.9095	0.8489	0.6938	0.4957	0.4765	0.2211
CD (0.05)	2.0576**	1.9203**	1.5696**	1.1213**	1.0780**	0.5002**

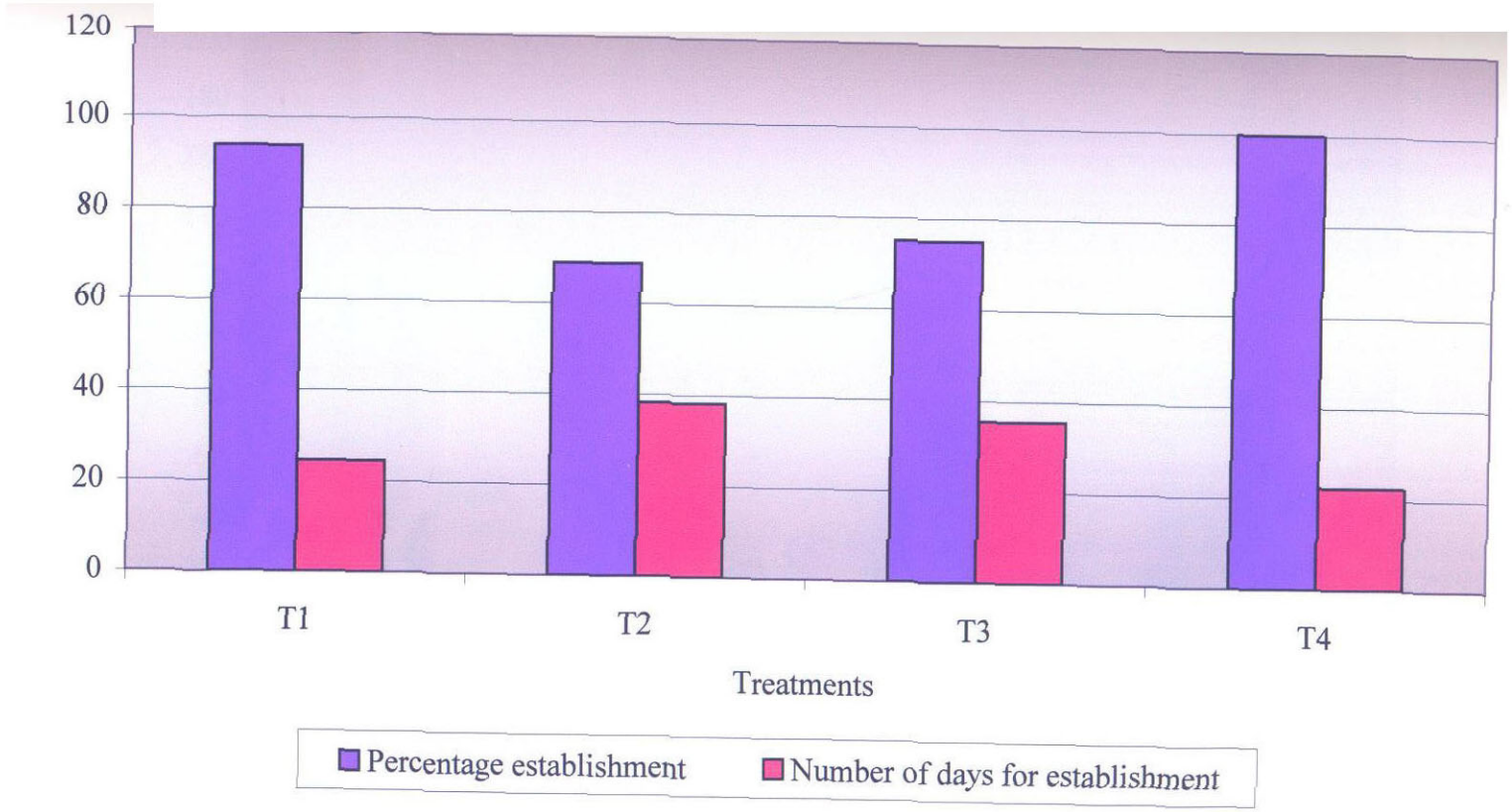


Fig. 19. Effect of different types of *Pandanus* planting material on establishment

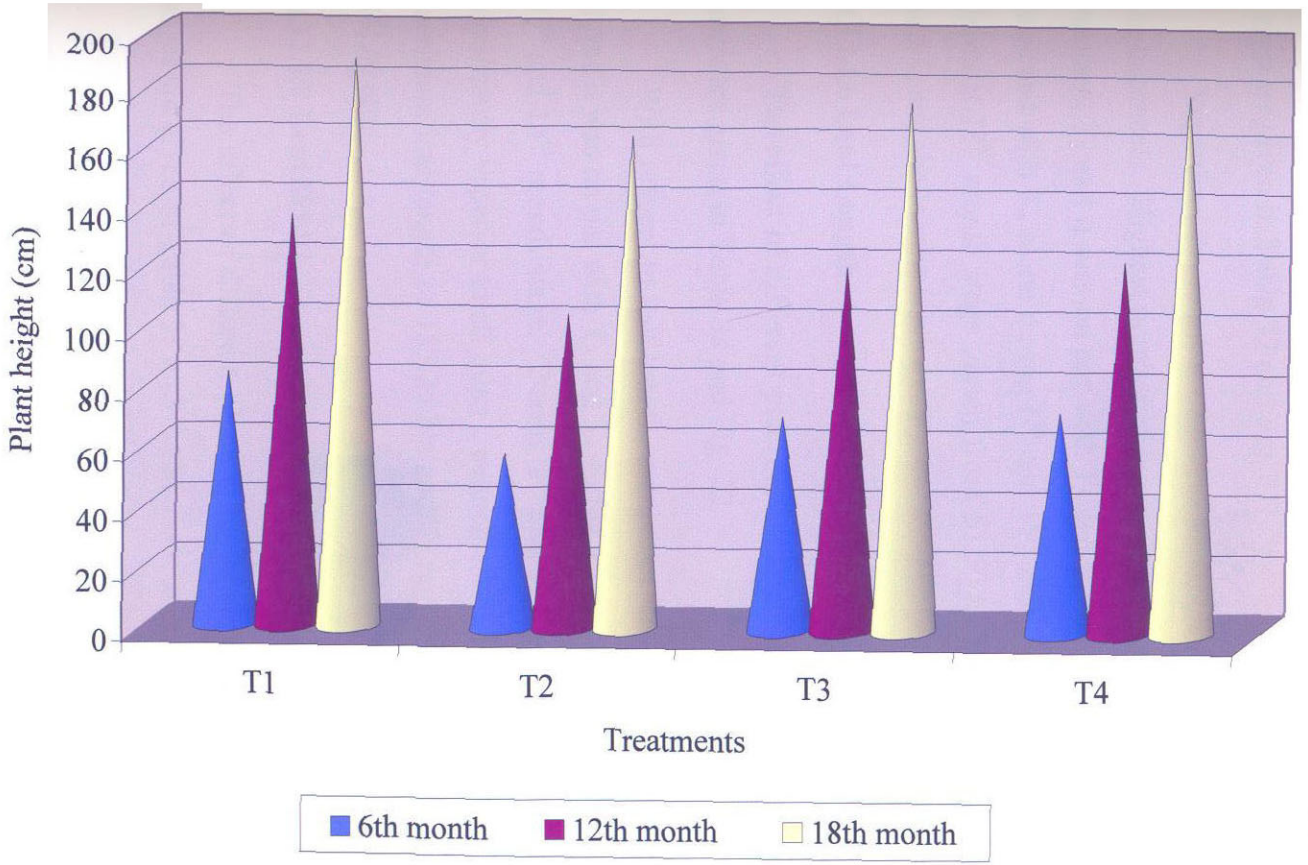


Fig. 20. Effect of various planting material on the plant height (cm) at different stages of growth of *Pandanus*

significantly superior to all other treatments at all stages of growth. Cuttings without crown registered minimum values at all growth stages.

4.3.2.1.5 Leaf Number

The effect of different planting materials on leaf number at various growth stages are given in Table 37 and Fig. 21.

Regarding the number of leaves significant difference was noticed among the treatments. Highest number of leaves were recorded at all stages of growth by treatment T_1 and it was on par with T_3 and T_4 during 3rd, 9th, 12th and 18th month stage and with T_4 alone during 6th and 15th month after planting. The number of leaves at 18th month stage varied from 63.50 to 76.00 and the highest value was with T_1 and the lowest was with T_2.

4.3.2.1.6 Leaf Length

The effect of different planting materials on leaf length at various growth stages are given in Table 38 and Fig. 22.

With respect to leaf length, maximum values were registered by treatment T_1 and it was on par with T_3 and T_4 during 3rd month stage and T_4 alone during 6th, 15th and 18th month stage. During 9th and 12th month stage T_1 was found to be significantly superior to all other treatments. Al 18th month stage T_1 recorded the maximum leaf length of 185.25 cm and T_2 registered the minimum leaf length of 161.00 cm.

4.3.2.1.7 Leaf Width

The effect of different planting materials on leaf width at various growth stages are given in Table 39 and Fig.23.

The leaf width varied significantly at all growth stages except the 12th month stage. Treatment T_1 recorded the maximum values followed by T_3 and T_4. Treatment T_2 registered minimum values at all growth stages. The highest leaf width of 5.65 cm and the lowest leaf width of 4.85 cm was recorded by treatment T_1 and T_2 respectively at 18th month growth stage.

Table 37. Effect of various planting material on number of leaves at different stages of growth of *Pandanus*

Treatments	Months of planting					
	3rd	6th	9th	12th	15th	18th
T_1	13.50	25.75	35.00	46.75	62.00	76.00
T_2	7.75	16.00	27.00	37.00	50.25	63.50
T_3	12.00	22.50	31.00	42.00	55.75	70.00
T_4	13.25	25.00	35.00	45.75	60.50	74.25
SEd	1.0992	1.248	2.5166	2.0825	1.9614	3.7680
CD (0.05)	2.4867**	2.8246**	5.6931**	4.7110**	4.4372**	8.5240**

Table 38. Effect of various planting material on leaf length (cm) at different stages of growth of *Pandanus*

Treatments	Months of planting					
	3rd	6th	9th	12th	15th	18th
T_1	44.00	62.87	113.25	136.00	160.75	185.25
T_2	34.50	48.00	86.75	111.12	131.00	161.00
T_3	41.75	56.50	89.75	121.75	148.00	177.00
T_4	43.00	59.75	92.00	122.50	156.25	179.00
SEd	2.3221	1.7263	5.1562	4.0235	4.3205	3.5059
CD (0.05)	5.2532**	3.9052**	11.6645**	9.1019**	9.7739**	7.9312**

Table 39. Effect of various planting material on leaf width (cm) at stages of growth of *Pandanus*

Treatments	Months of planting					
	3rd	6th	9th	12th	15th	18th
T_1	2.95	3.27	4.25	4.57	5.15	5.65
T_2	2.15	2.85	3.85	4.17	4.57	4.85
T_3	2.95	3.00	3.95	4.37	4.77	5.07
T_4	2.95	3.10	4.00	4.50	4.95	5.37
SEd	0.0943	0.1180	0.0965	0.1406	0.1161	0.0965
CD (0.05)	0.2133**	0.2669**	0.2182**	0.3180**	0.2625**	0.2182**

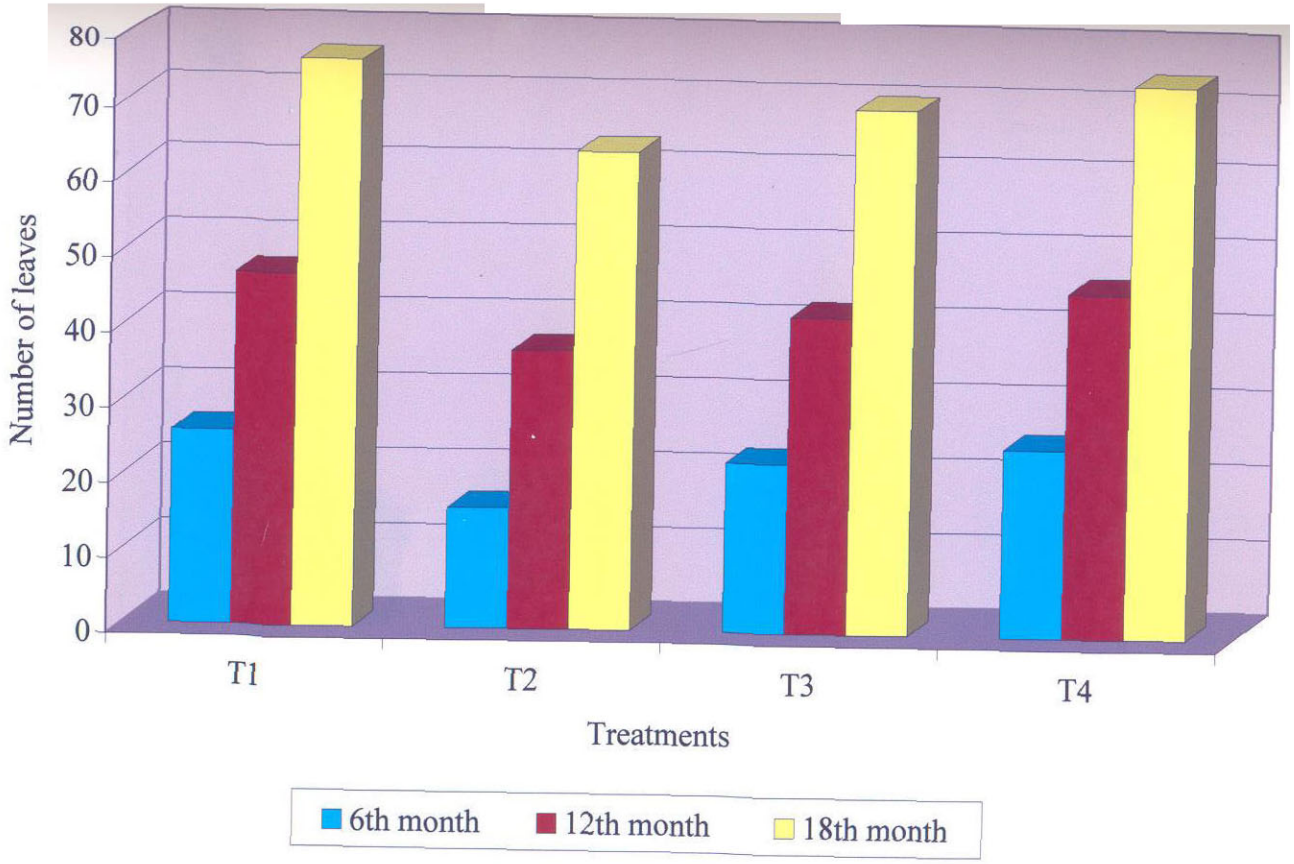


Fig. 21. Effect of various planting material on number of leaves at different stages of growth of *Pandanus*

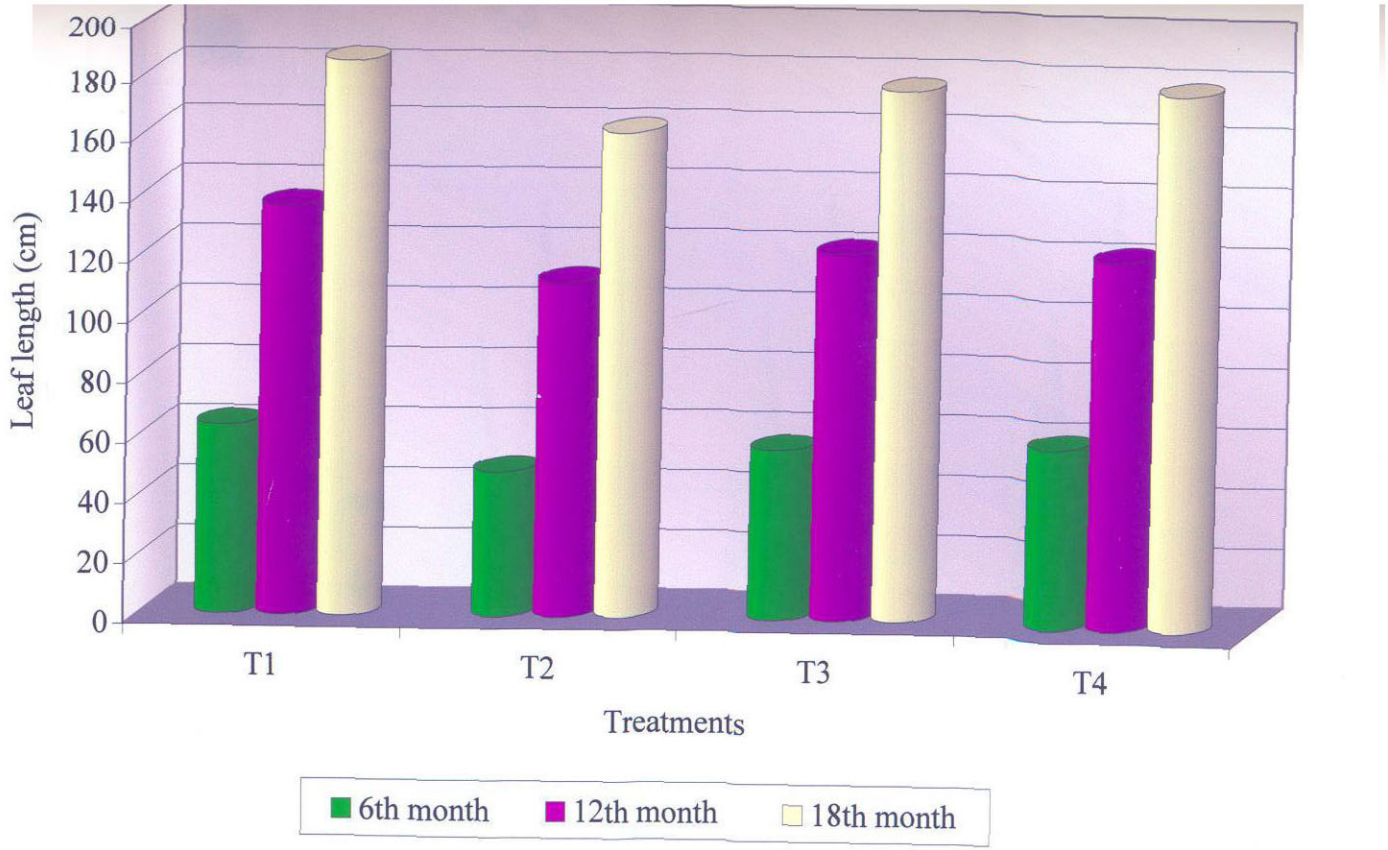


Fig. 22. Effect of various planting material on leaf length (cm) at different stages of growth of *Pandanus*

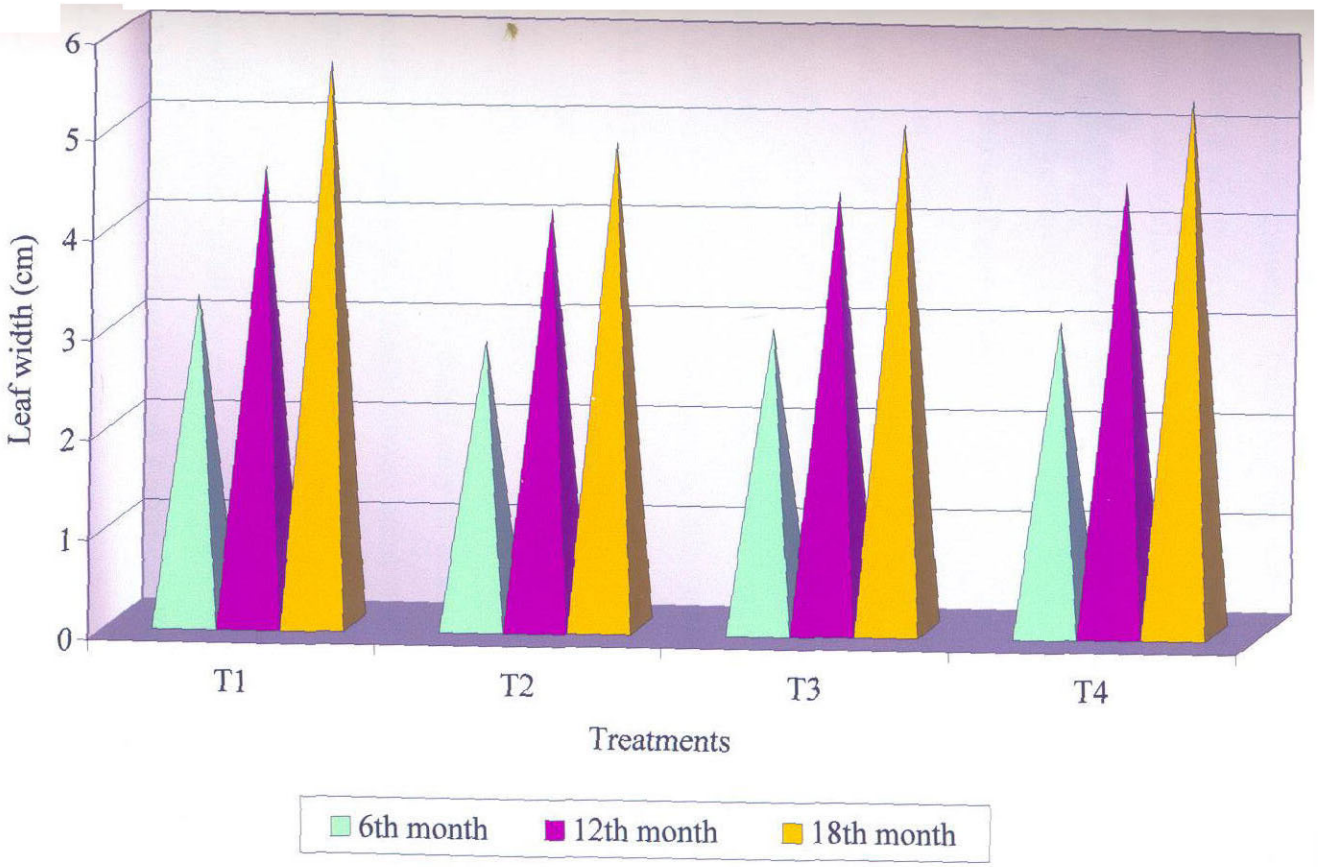


Fig. 23. Effect of various planting material on leaf width (cm) at different stages of growth of *Pandanus*

4.3.2.1.8 Leaf Yield

The effect of different planting materials on leaf yield at various growth stages are given in Table 40 and Fig.24.

The effect of planting material on leaf weight was also more pronounced at all stages of growth. Treatment T_1 was significantly superior to all other treatments at all stages of growth except 6th month stage. During 18th month stage a highest leaf yield of 2558.75 g was recorded by T_1 followed by T_4 and T_3 (2345.00 g and 2271.25 g respectively). The lowest leaf yield of 2107.50 g was recorded by treatment T_2.

4.3.2.1.9 Sucker Production

The effect of different planting materials on sucker production at various growth stages are given in Table 41.

The sucker production was not found to vary significantly among various treatments. However, during 18th month stage a maximum number of (4.50) suckers were produced by treatment T_4 followed by treatment T_3 and T_1.

4.3.2.1.10 Prop Roots

The effect of different planting materials on prop roots at various growth stages are given in Table 42.

Prop roots were produced only in treatment T_1 and T_2. Among these two treatments, T_1 produced the maximum number of roots (8.25) and T_2 produced the minimum number of roots (6.00) at 18th month stage.

In general, the treatment T_1 (terminal cuttings with crown) excelled in all growth characters at all stages of growth followed by ground suckers (T_4).

4.3.3 Micro Propagation through Enhanced Release of Axillary Buds

4.3.3.1 Explant Establishment

4.3.3.1.1 Surface Sterilization of Explants

Surface sterilization with mercuric chloride 0.08 per cent (T_5) for seven minutes was found to be very effective for surface sterilization of the shoot tip explants, as 73.33 per cent culture survival was obtained by this treatment. Even

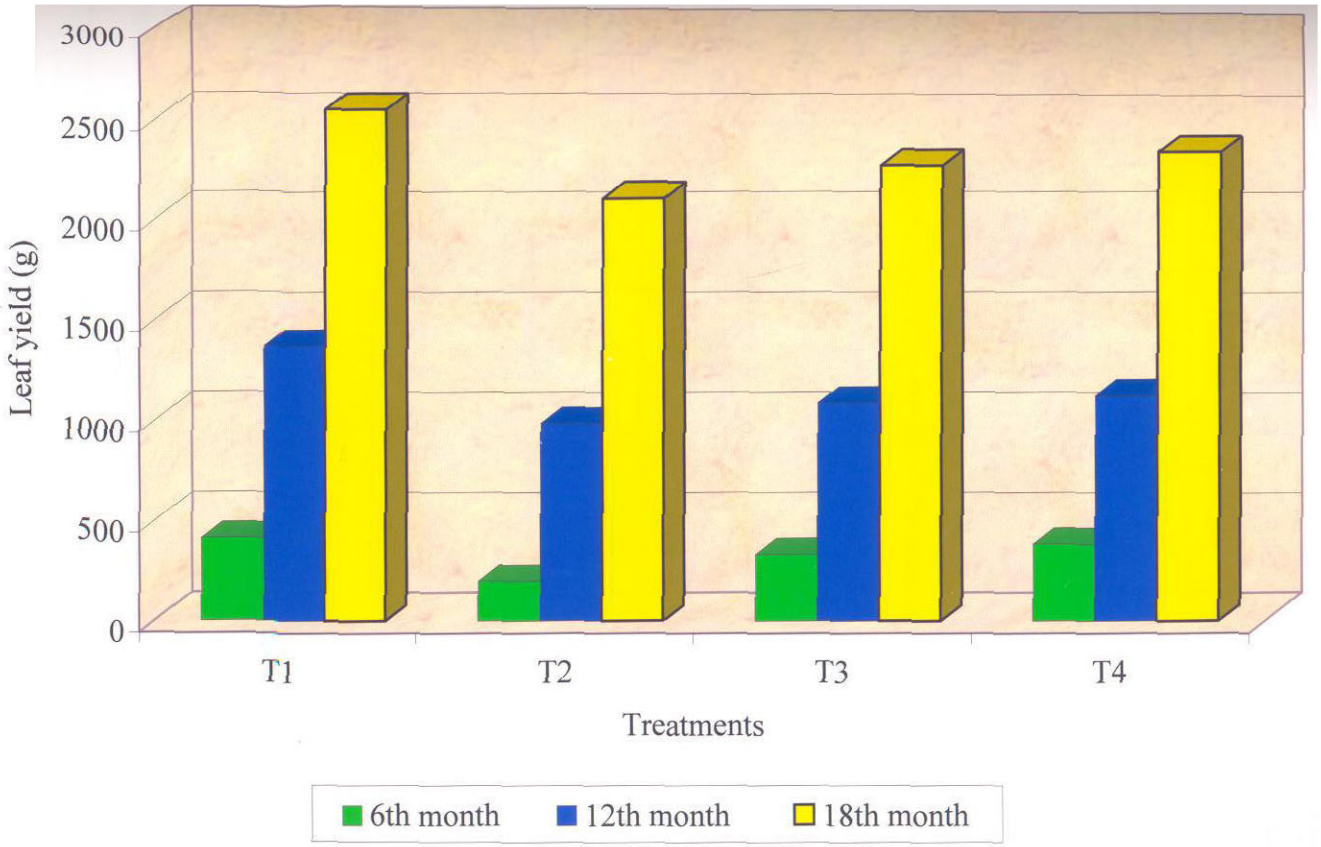


Fig. 24. Effect of various planting material on leaf yield (g) at different stages of growth of *Pandanus*

Table 40. Effect of various planting material on leaf yield (g) at different stages of growth of *Pandanus*

Treatments	Months of planting					
	3rd	6th	9th	12th	15th	18th
T_1	227.50	412.50	760.00	1371.25	1875.00	2558.75
T_2	95.00	200.00	595.00	987.00	1560.00	2107.50
T_3	204.00	332.62	623.50	1087.50	1675.00	2271.25
T_4	192.50	385.00	658.75	1125.00	1687.00	2345.00
SEd	10.0692	21.0839	24.5437	60.9041	55.3555	86.7408
CD (0.05)	22.7786**	47.6962	55.5229**	137.7777**	125.2257**	281.9074**

Table 41. Effect of various planting material on number of suckers production at different stages of growth of *Pandanus*

Treatments	Months of planting					
	3rd	6th	9th	12th	15th	18th
T_1	-	0.75	1.25	2.00	2.75	3.25
T_2	-	-	0.75	1.75	2.50	2.75
T_3	-	0.25	1.75	2.25	3.00	3.25
T_4	-	1.50	2.75	3.50	4.25	4.50
SEd		0.5652	0.9052	0.7280	0.8580	0.6038
CD (0.05)		1.2786 NS	2.0478 NS	1.7209 NS	1.9409 NS	1.3659 NS

Table 42. Effect of various planting material on number of prop roots production at different stages of growth of *Pandanus*

Treatments	Months of planting					
	3rd	6th	9th	12th	15th	18th
T_1	0.75	4.25	5.00	6.00	8.00	8.25
T_2	-	2.00	2.75	4.50	5.5	6.00
T_3	-	-	-	-	-	-
T_4	-	-	-	-	-	-
SEd	0.3385	0.3773	-	0.9052	0.8079	0.7660
CD (0.05)	0.7658	0.8535	-	2.0478	1.8277	1.7329

though sterilization treatment with mercuric chloride 0.1 per cent gave 100 per cent sterile cultures with out any microbial contamination, the survival percent age was very low due to browning of tissues (Table 43). Though the rate of explant death due to excess sterilant was zero in case of mercuric chloride 0.06 per cent, it was found to be in effective, as it resulted in very high contamination rate and lowest survival percentage. Hence, mercuric chronicle 0.08 per cent for eight minutes was selected for surface sterilization in subsequent studies.

4.3.3.1.2 Culture Establishment Medium

In order to standardize a suitable culture establishment medium for the shoot tip explants of *Pandanus,* different levels of cytokinin (BA) and auxin (NAA) were added along with MS (semi solid) medium. The results are presented in Table 44 and Fig. 25.

Establishment of the cultures occurred in all the treatment combinations. The establishment percentage was highest (80 per cent) in treatment T_3 and T_7 and lowest in treatment T_{10} involving half MS medium with out growth hormones (60 per cent). The number of days for the establishment of cultures ranged from 24 to 32 days. However, the influence of different treatments were not significant, the treatment T_7 took a minimum of 24 days and T_{10} took a maximum of 32 days for culture establishment.

Regarding the number shoots produced per culture, treatment T_9 (BA 1.5 mg l^{-1} + NAA mg l^{-1}) recorded the highest number of shoots (3.83) and it was on par with T_8 and T_7 (3.3 and 3.0) and significantly superior to other treatments. The treatment T_1 and T_{10} recorded the least number of shoots (1).

Though the number of shoots per culture was higher in treatment T_9, the treatment combination involving of BA 1.00 mg l^{-1} and NAA 0.4 mg l^{-1} (T_7) was found to be the best for initial establishment of cultures, as they showed maximum establishment of cultures with in a minimum number of days (Plate 10).

Table 43. Effect of various surface sterilization treatments on culture survival of *Pandanus* explants

Medium : ½ MS (Semi-solid and without growth hormones)
Sterilant : Mercuric chloride
Explant : Shoot tip

Treatments	Percentage of contamination	Percentage culture showed browning	Percentage of culture survival
T_1	93.30	0.00	6.70
T_2	73.30	0.00	26.70
T_3	60.00	0.00	40.00
T_4	33.30	0.00	66.70
T_5	20.00	6.70	73.30
T_6	6.70	26.70	66.70
T_7	0.00	66.70	33.30
T_8	0.00	86.70	13.30
T_9	0.00	100.00	0.00

Table 44. Effect of growth regulators (BA and NAA) on the establishment of shoot tip culture of *Pandanus*

Treatments	Percentage of establishment of cultures	Number of days for culture establishment	Number of shoots per explants
T_1	73.20	31.60	1.00
T_2	66.60	31.30	1.30
T_3	66.60	31.60	1.60
T_4	80.00	27.00	2.00
T_5	73.20	27.00	2.30
T_6	73.20	26.00	2.50
T_7	80.00	24.00	3.00
T_8	73.20	25.33	3.30
T_9	66.60	27.33	3.80
T_{10}	60.00	32.00	1.00
SEd	-	3.2897 NS	0.5270
CD (0.05)	-	6.8622	1.0994**

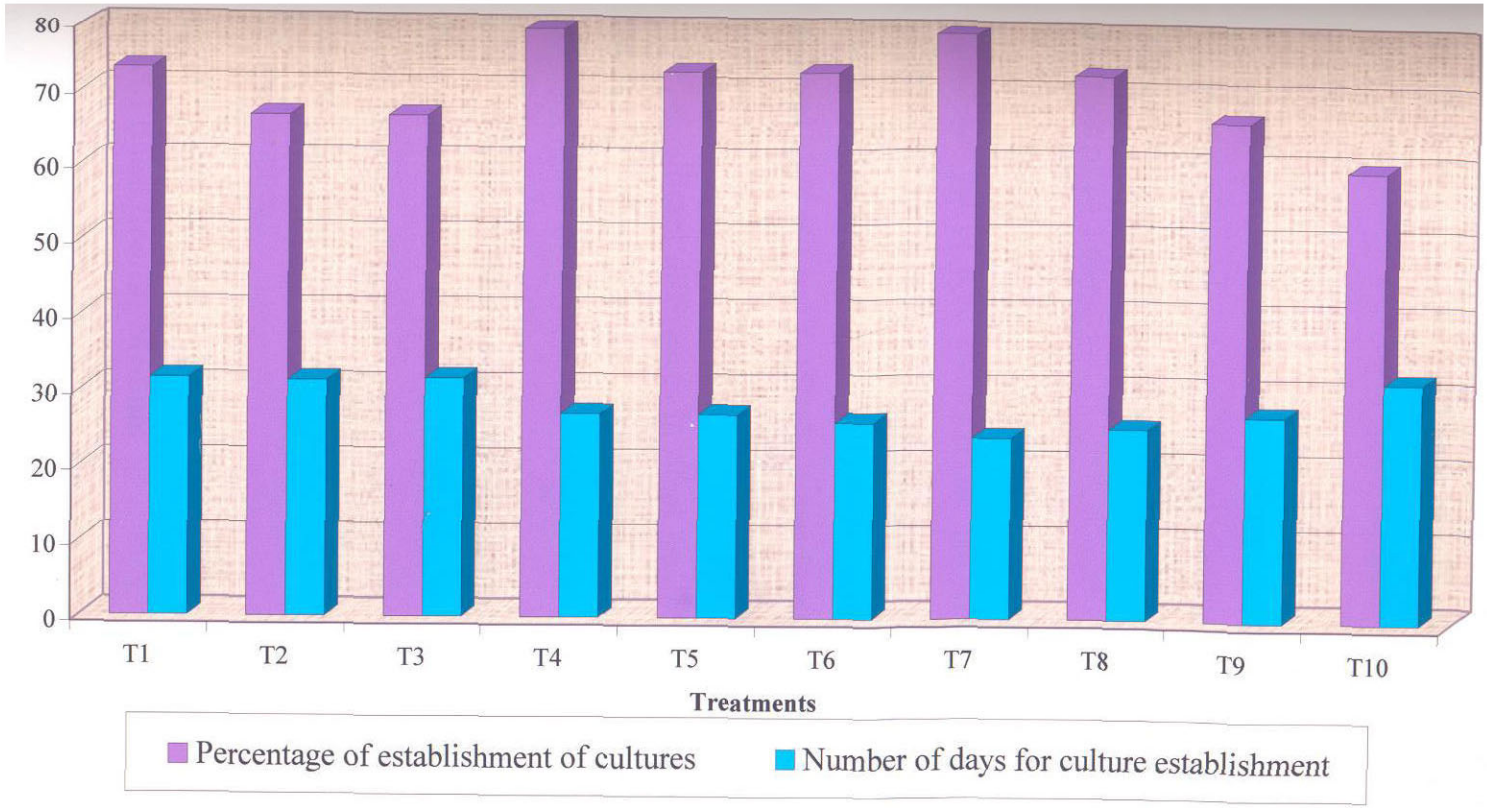


Fig. 25. Effect of growth regulators (BA and NAA) on the establishment of shoot tip culture of *Pandanus*

4.3.3.2 Induction of Axillary Buds

4.3.3.2.1 Culture Proliferation Medium

To standardize a suitable basal proliferation medium for the induction and growth of axillary shoots, different combinations of auxin (NAA) and cytokinin (BA) were used in MS (semi solid) medium. The results are given in Table 45 and Fig.26.

All the treatment combinations responded to proliferation of shoots. However, the number of days for the initiation of shoots were not significantly different, the treatment T_9, T_8 and T_7 took a minimum of 23.6 days and T_1 took the maximum of 26.3 days.

With respect to the number of shoots per culture, treatment T_9 recorded the highest number of 8.00 shoots (Plate 10) which was on par with treatment T_6 (7.30), T_7 (7.50) and T_8 (7.83) and significantly superior to all other treatments. The least number of shoots (3.93) were recorded by T_1 which was on par with treatment T_2 (4.60) and T_3 (5.16). It was found that, the increase in BA and NAA level caused a corresponding increase in the number of axillary shoots induced.

Significant differences in shoot length were recorded due to the effect of different growth regulators. The highest shoot length (4.60 cm) was resulted by treatment T_1 and the lowest shoot length (3.30 cm) was resulted by T_8 and T_9.

Number of leaves per shoot was influenced significantly by the treatments. The highest number of leaves (6.00) were recorded by treatment T_8 and T_9 and lowest number of leaves (4.00) was produced by treatment T_1 and T_2. The treatment combination involving BA 4 mg l^{-1} and NAA 1 mg l^{-1} was considered as the best treatment.

4.3.3.3 In vitro Rooting

4.3.3.3.1 Basal Rooting Medium

The effect of growth regulators like NAA and IBA on the initiation of roots of *in vitro* shoot lets of *Pandanus* in half MS medium is given in Table 46 and Fig.27.

Table 45. Effect of growth regulators (BA and NAA) on the induction of axillary shoots of *Pandanus* shoot tip culture

Treatments	Percentage of cultures produced shoot	Number of days for the initiation on of shoots	Number of shoots per culture	Length of the longest shoot	Number of leaves per shoot
T_1	86.60	26.30	3.93	4.60	4.00
T_2	86.60	26.00	4.60	4.50	4.00
T_3	86.60	26.00	5.16	4.30	4.50
T_4	93.20	25.00	6.33	4.00	5.00
T_5	93.20	24.60	6.66	4.00	5.00
T_6	93.20	24.30	7.30	3.70	5.60
T_7	93.20	23.60	7.50	3.50	5.60
T_8	93.20	23.60	7.83	3.30	6.00
T_9	93.20	23.60	8.00	3.30	6.00
SEd	-	3.1623	0.7011	0.3189	0.03768
CD (0.05)	-	6.6438	1.4731**	0.6701**	0.7916**

Table 46. Effect of growth regulators (NAA and IBA) on initiation of roots from the *in vitro* shoots of *Pandanus*

Treatments	Percentage of root initiation	Number of days for root initiation	Number of roots per shoot
T_1	93.20	26.00	2.83
T_2	100.00	25.30	3.16
T_3	100.00	24.30	3.66
T_4	100.00	22.70	4.00
T_5	100.00	21.70	4.73
T_6	100.00	21.70	5.80
T_7	100.00	23.60	6.73
T_8	100.00	24.30	7.83
SEd	-	1.8782	0.7377
CD (0.05)	-	3.9817 NS	1.5638**

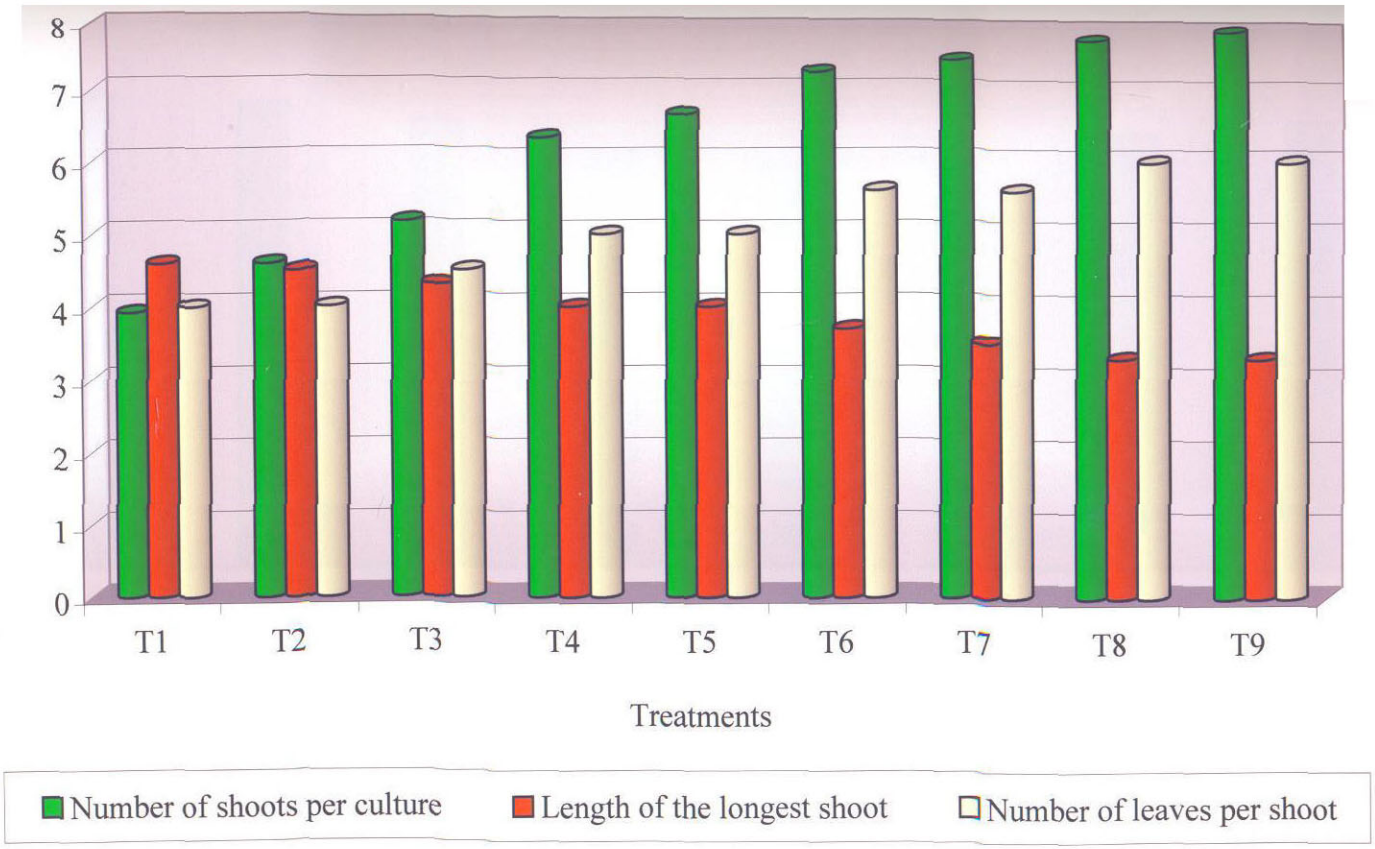


Fig. 26. Effect of growth regulators (BA and NAA) on the induction of axillary shoots of *Pandanus* shoot tip culture

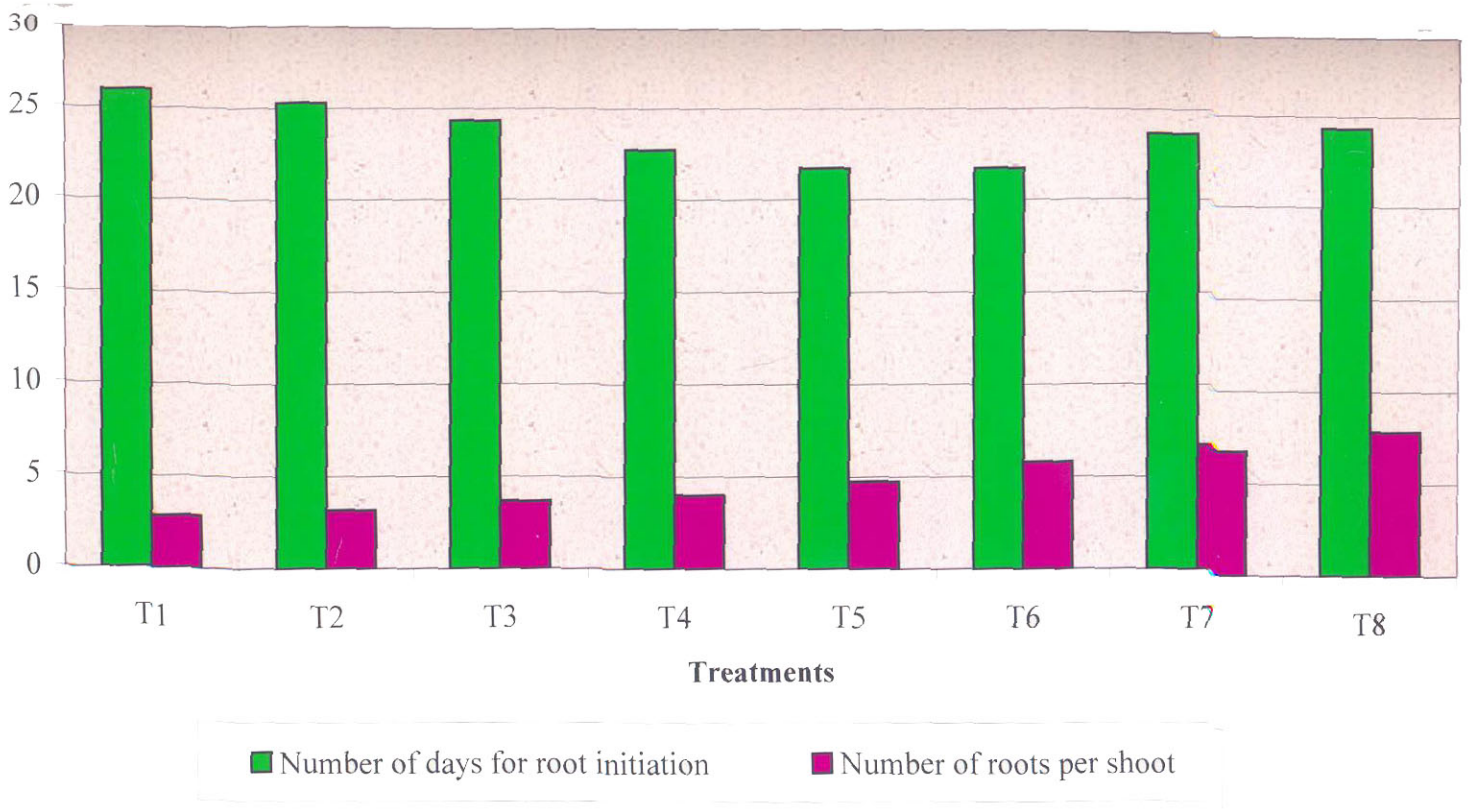


Fig. 27 Effect of growth regulators (NAA and IBA) on initiation of roots from the *in vitro* shoots of *Pandanus*

All the combinations of plant growth substances responded to the root initiation of *in vitro* shoot lets of *Pandanus*. The number of days for root initiation was not significantly different. However, treatment T_1 took the maximum number of days (26.00 days) and treatment T_5 and T_6 took the minimum (21.70) number of days for root initiation.

With respect to number of roots per shoot, significant difference was noticed. The maximum of 7.83 roots per shoot (Plate 10) was recorded by treatment T_8 (NAA 3 mg l^{-1} + IBA 4 mg l^{-1}) and it was on par with T_7 (6.73) and significantly superior to other treatments. The minimum number of roots (2.83) was recorded by T_1 (IBA 2 mg l^{-1} alone) and it was on par with T_2, T_3 and T_4.

4.3.3.3.2 Rooting Media Supplement (Activated Charcoal)

The influence of activated charcoal on the initiation of roots of *in vitro* shoot lets of *Pandanus* in half MS medium with growth hormones like NAA 1mg l^{-1} and IBA 2 mg l^{-1} is presented in Table 47 and Fig. 28.

Addition of activated charcoal (AC) to the culture medium found to influence the initiation of roots and number of roots produced per shoot. However, the number of days for the initiation of roots was not significantly different, treatment T_1 took the maximum of 22.60 days and T_5 and T_6 took the minimum of 20 days for root initiation.

While observing the number of roots produced per shoot, significant differences were noticed among the treatments. The treatment T_6 recorded the maximum of 11 roots per shoot (Plate 10) and it was on par with treatment T_5 and T_4 (10.60 and 9.30 respectively). A minimum number of 6.60 roots per shoot was registered by treatment T_1.

4.3.3.4 Planting Out and Field Evaluation

In vitro raised plantlets showed 70 per cent survival in the field (Table 48) during 3rd month of planting out while it showed 100 per cent survival during 6th month of planting. Maximum height of 12 and 22 cm was recorded during third

Table 47. Effect of activated charcoal on root initiation of *in vitro* shoots of *Pandanus*

Treatments	Percentage of root initiation	Number of days for root initiation	Number of roots per shoot
T_1	93.20	22.60	6.60
T_2	100.00	21.60	8.00
T_3	100.00	21.00	8.00
T_4	100.00	20.30	9.30
T_5	100.00	20.00	10.60
T_6	100.00	20.00	11.30
SEd	-	1.4530 NS	1.1386
CD (0.05)	-	3.1658	2.4807**

Table 48. Growth characters of *in vitro* plantlets of *Pandanus* after 3rd and 6th month of planting (Mean)

Growth Characters	Months after planting out	
	3rd	6th
Survival Percentage	70.00	100.00
Plant height (cm)	12.00	22.00
Plant girth (cm)	1.00	2.00
No. of leaves	8.00	12.00

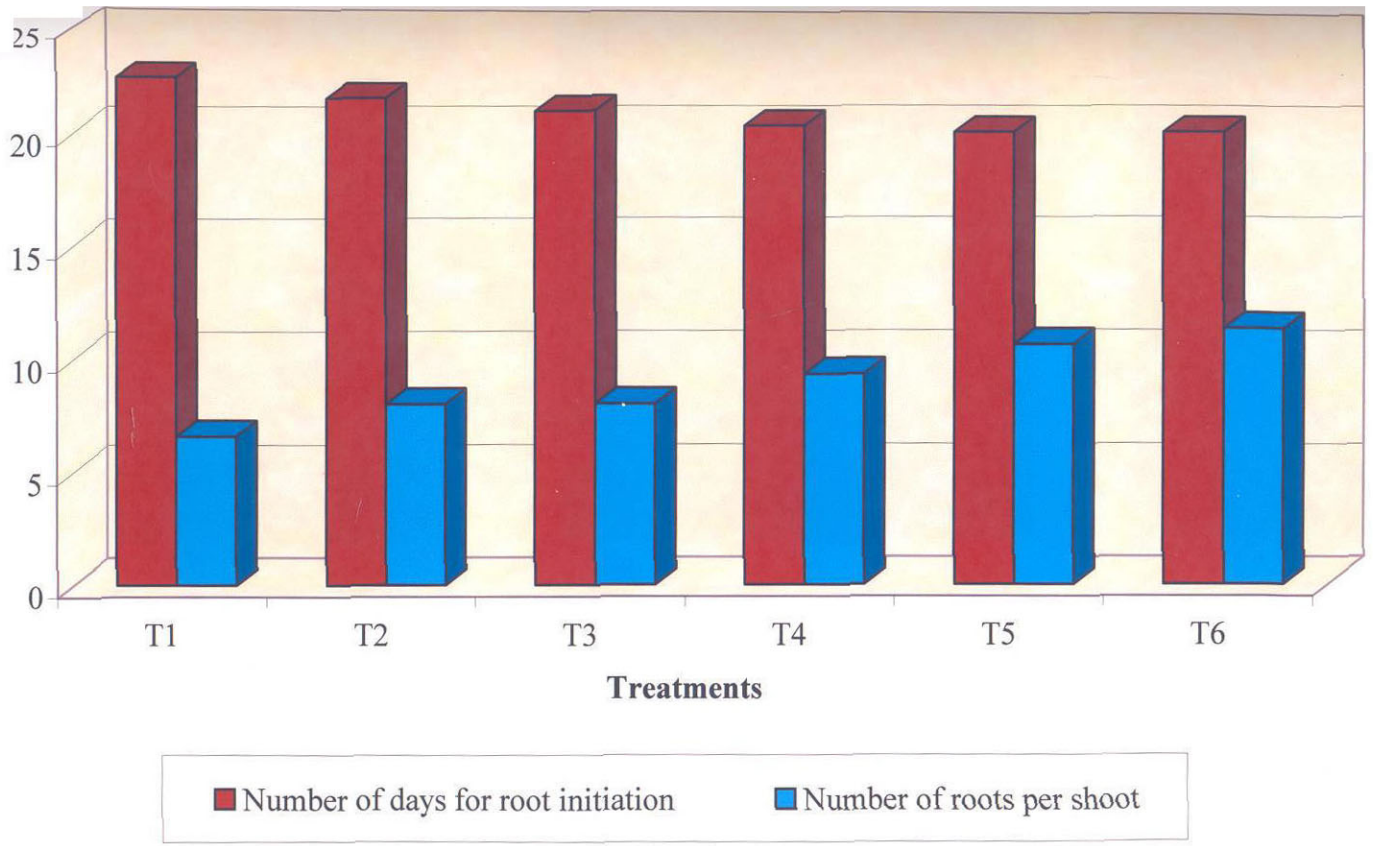
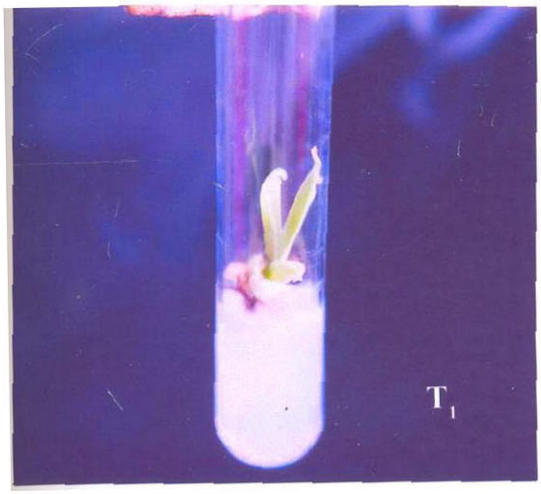
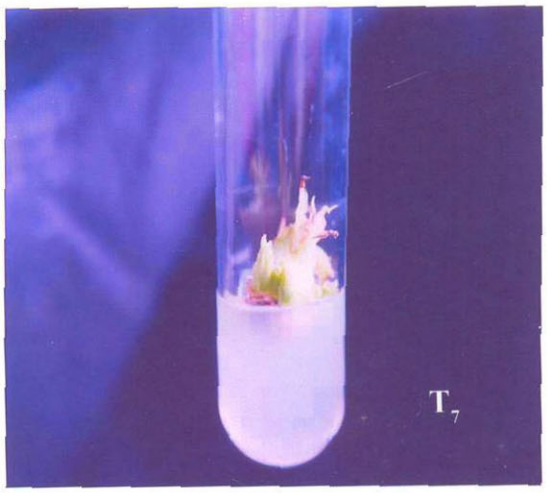


Fig. 28. Effect of activated charcoal on root initiation of *in vitro* shoots of *Pandanus*



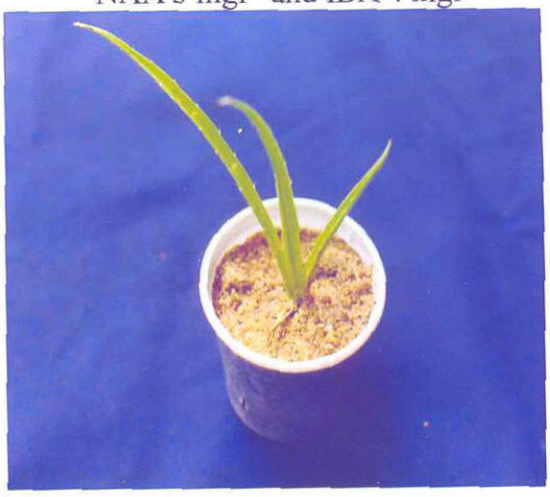


Culture establishment
BA 1 mgl^{-1} and NAA 0.4 mgl^{-1}

Induction of axillary buds
BA 4 mgl^{-1} and NAA 1 mgl^{-1}

Initiation of root
NAA 3 mgl^{-1} and IBA 4 mgl^{-1}

Initation of root
NAA 2 mgl^{-1} and IBA 2 mgl^{-1}



Ex vitro establishment

Plate 10. Micro propagation of *Pandanus*

and 6th months of field planting respectively. Increasing trend was noticed for other growth parameters like plant girth and number of leaves.

4.4 STANDARDIZATION OF LEAF CURING TECHNOLOGY

Quality attributes of *Pandanus* mats such as colour, texture, eye appeal, durability and overall acceptance with respect to different leaf curing treatments are presented (Table 49, Fig. 29 and Plate 11).

With regard to the colour of *Pandanus* mats, the mean score ranged from 2.0 to 4.2 with the corresponding score percentage from 40 to 84. A significant difference was observed between different treatments and treatment T_9 (boiling and sun drying + colouring with synthetic dyes) recorded the maximum score of 4.2 and it was on bar with T_3 (4.1) and T_5 (4.0). Treatment T_2 (shade drying of leaves without any treatment) recorded the minimum score of 2.0 and it was on par with treatment T_8 (2.1).

Considering the texture of the mats, the mean score ranged form 2.5 (50 per cent) to 4.3 (86 per cent). Significant differences were noticed among the different treatments and the treatment T_5 (soaking in water for 2 days and sun drying) recorded the maximum value (4.3) and it was on par with treatment T_3 (4.2), T_7 (4.2) and T_9 (4.2). Treatment T_2 recorded the minimum score of 2.5 and it was on par with T_1 (2.6).

The mean score obtained for eye appeal varied from 2.0 (40 per cent) to 4.4 (88 per cent). Treatment T_9 recorded the maximum score of 4.4 and it was significantly superior over other treatments. The treatment T_2 recorded the minimum score value of 2.0.

The mean score for durability ranged from 2.8 to 4.0 with the corresponding score percentage between 56 to 80. Significant differences were observed between the treatments and the treatments T_3, T_7 and T_9 recorded the maximum score value of 4.0. The treatment T_2 recorded the minimum value of 2.8 which was on par with treatment T_6 (3.0) and T_8 (3.0).

Table 49. Effect of various leaf curing treatments on quality assessment of *Pandanus* mat

(Mean score values)

Treatments	Colour	Texture	Eye appeal	Durability	Overall acceptance
T_1	2.5(50)	2.6(52)	2.8(56)	3.0(60)	2.8(56)
T_2	2.0(40)	2.5(50)	2.0(40)	2.8(56)	2.0(40)
T_3	4.1(82)	4.2(84)	4.2(84)	4.0(80)	4.4(88)
T_4	2.9(58)	3.6(72)	2.9(58)	3.5(70)	3.0(60)
T_5	4.0(80)	4.3(86)	4.0(80)	3.6(72)	4.2(84)
T_6	2.8(56)	3.7(74)	2.8(56)	3.0(60)	3.0(60
T_7	3.8(76)	4.2(84)	3.9(78)	4.0(80)	4.1(82)
T_8	2.1(42)	3.5(70)	3.0(60)	3.0(60)	3.1(62)
T_9	4.2(84)	4.2(84)	4.4(88)	4.0(80)	4.4(88)
SEd	0.1449	0.1144	0.0816	0.1286	0.1227
CD(0.05)	0.3044**	0.2403**	0.1714**	0.2702**	0.2578**

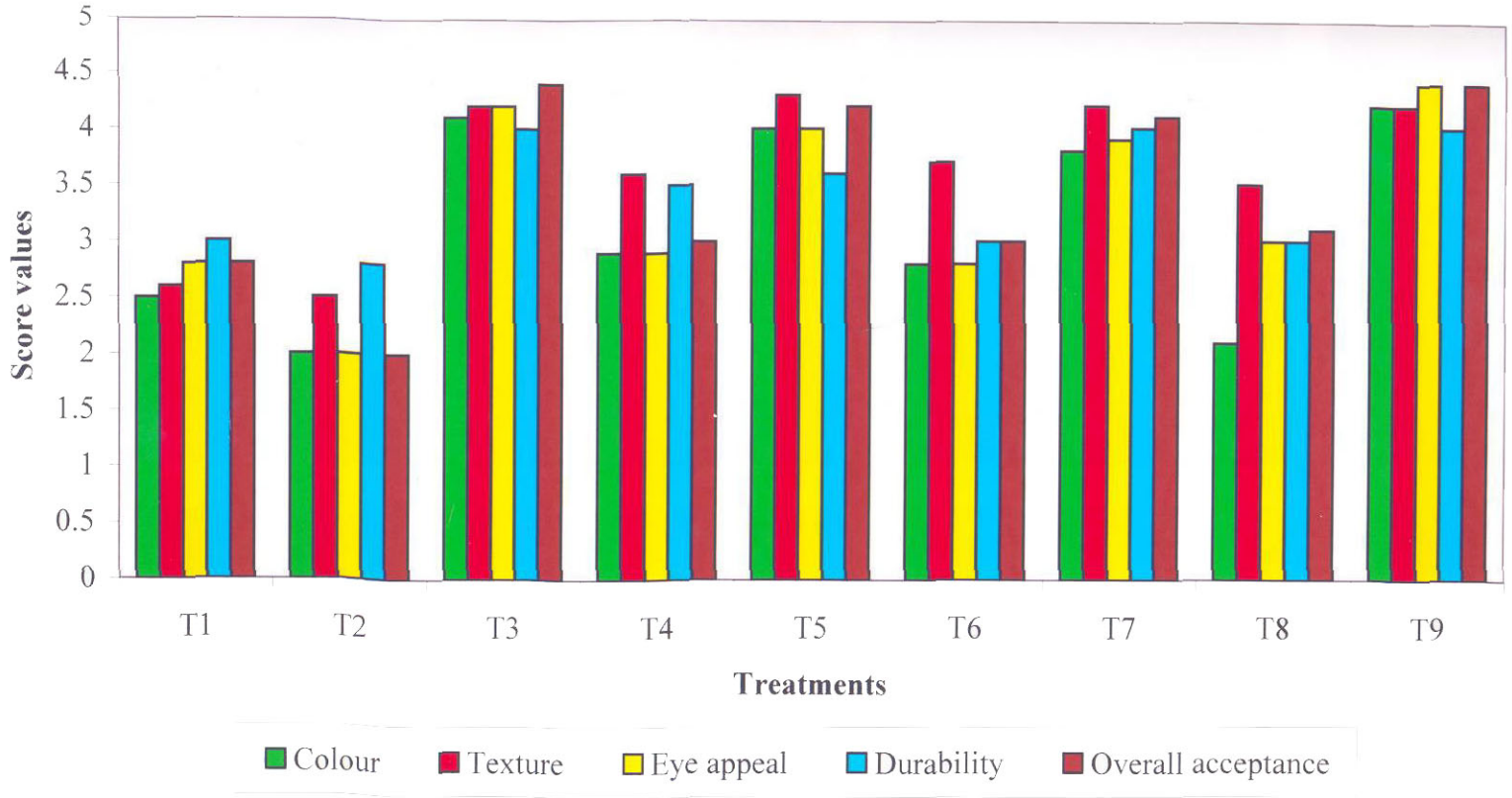


Fig. 29. Effect of various leaf curing treatments on quality assessment of *Pandanus* mat

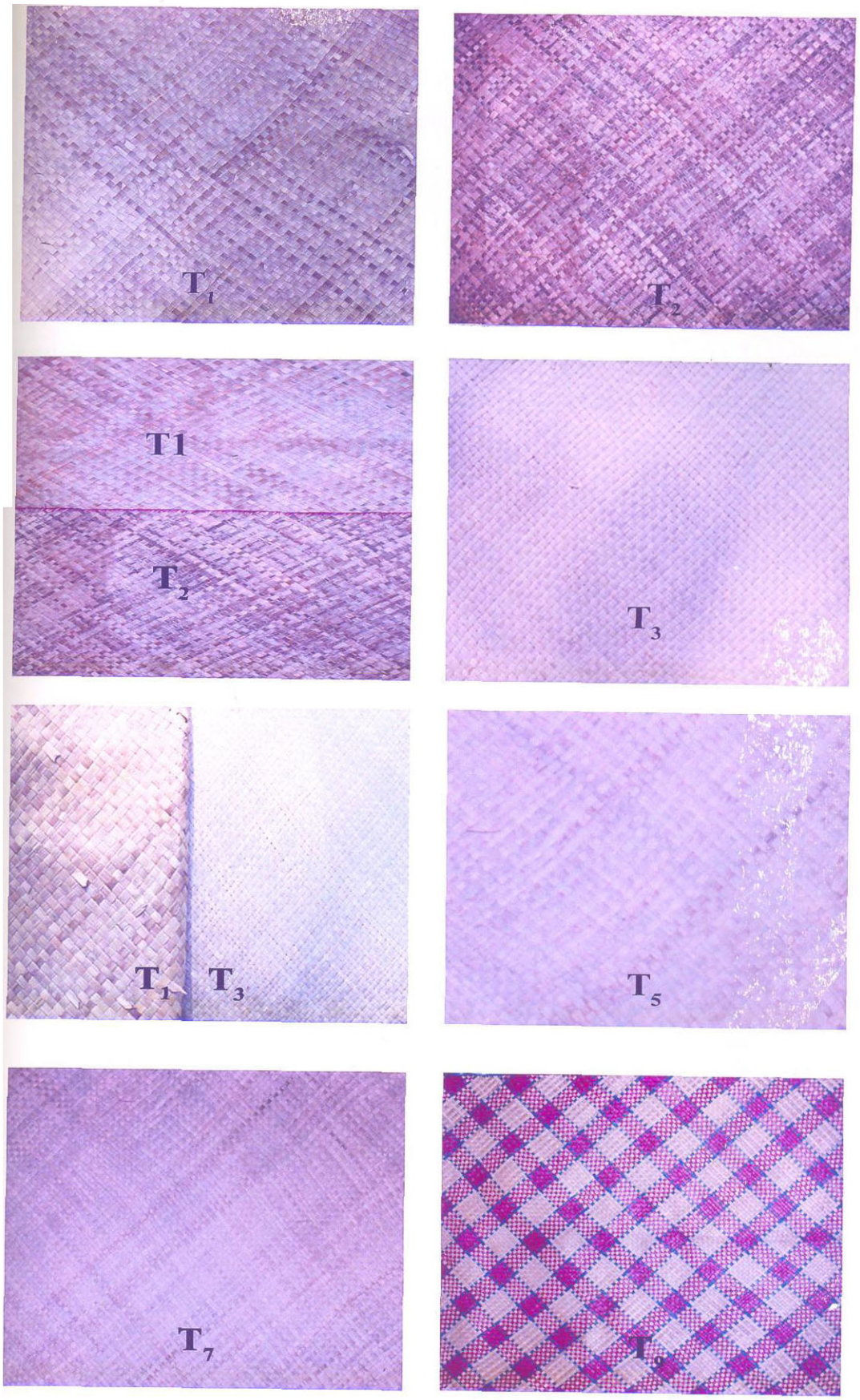


Plate 11 Effect of different leaf curing treatments on quality of *Pandanus* mats

In case of overall acceptance the mean score varied from 2.0 (40 per cent) to 4.4 (88 per cent). Significant differences were observed among the treatments and the treatment T_3 and T_9 recorded the maximum mean score of 4.4 and it was on par with T_5 (4.2). The minimum score of 2.0 was recorded by T_2 (shade drying of leaves without any treatment).

In general, the quality assessment attributes such as colour, eye appeal durability and overall acceptance were in favour of treatment T_9 and T_3 followed by treatment T_5 and T_7. Treatment T_5 excelled others in case of texture.

Discussion

5. DISCUSSION

Pandanus is one of the under exploited crops having enormous potential. Its male inflorescence has great value in perfume industries and its leaves and roots find use in cottage industries. The plant is also known for its use in traditional medicine and the ecological importance of its root system that binds soil and checks soil erosion. Unlike other crop based industries, *Pandanus* based industries usually provide high profit margin with low capital investment.

Pandanus is highly polymorphous and it includes numerous types and forms of which some of them are selected for specific uses by perfumery and cottage industries. Though the natural growing population of *Pandanus* has a great measure of variation in morphological and biochemical characters, a detailed study of these variants is still lacking.

Morphological characterization has been the major tool for the classification and identification of different genotypes since long time. But it is time consuming and subject to environmental influences. Moreover, no proper key exists in *Pandanus* for the identification of the selective genotypes. The limitations of the above classification suggest that a scheme based anatomical and molecular markers rather than morphological characters could provide more accurate assessment of diversity and systemic relationships between different *Pandanus* genotypes.

Though this crop has been utilized by perfume and cottage industries, its propagation and leaf curing techniques were not standardized scientifically till now. Hence the present study was carried out to assess the genetic variability among selected *Pandanus* genotypes through morphoanatomical and molecular markers and also to standardize the suitable propagation and leaf curing techniques.

The study was conducted as four experiments *viz.*, I. Morphoanatomical and molecular characterization of 30 genotypes of *Pandanus,* II. Variability and divergence of 22 male genotypes of *P. fasicularis* based on morphoanatomical and biochemical characters, III. Studies on standardization of propagation techniques and IV. Standardization of leaf curing technology for the preparation of quality mats.

5.1 MORPHO ANATOMICAL AND MOLECULAR CHARACTERIZATION

Morphological and agronomic traits have been extensively used to determine the relationship among plants and varieties (Ortiz *et al.*, 1998). However, morphological markers do not often reflect genetic relationships because of their interaction with the environment, epistasis and the largely unknown genetic control of the traits (Smith and Smith, 1989). In contrast, molecular markers are not influenced by environment or developmental stage of a plant, making them ideal for genetic relationship studies. Random Amplified Polymorphic DNA (RAPD) is one of the useful molecular markers for identifying different types or varieties at genotypic level. It can help to overcome the complications arising in morphoanatomical characterization. RAPD analysis has been successfully used to identify the genetic diversity in a number of crop plants (Reiter *et al.*, 1992). An attempt has been made to determine the extent of genetic diversity in thirty genotypes of *Pandanus* based on morphoanatomical and RAPD markers. In RAPD studies arbitrary primers are used to amplify random DNA sequences in the genome.

5.1.1 Morphological Characters

Morphological characters *viz.*, stem, leaf and spine characters exhibited significant variation among the 30 genotypes of *Pandanus* studied. The highest plant height was observed in genotype PF 14 (6.53 m) and the lowest was in PSt 30 (0.55 m). These results are in similarity with the findings of Sethi and Maheshwari (1985) in *Cymbopogon martinii*; Rzhanova and Romanyak (1987) in coriander; Baghdadi *et al.* (1989) in

Lycium spp.; Lal *et al.* (1999) in vetiver; Lal *et al.* (2001b) in curry neem and Shasany *et al.* (2003) in *Asparagus.* They observed significant variability in plant height among the genotypes collected from different geographical locations.

Plant girth varied significantly among the thirty genotypes of *Pandanus.* Genotype PF 7 recorded the highest plant girth of 0.35 m and it was found to be the lowest (0.03 m) in PSt 30. Nayar *et al.* (1979) and Sreerangaswamy *et al.* (1980) reported that, banana though clonally propagated showed significant variation in vegetative characters, such as plant girth, among the populations assembled from different environmental conditions. Brophy *et al.* (1994b) observed variation in plant girth among big and small leaved morphotypes of *Murraya paniculata.*

The number of branches varied from 1.00 (PP 27, PA 28, PSt 29 and PSt 30) to 11.66 (PF 11). This is in accordance with the findings of Padmesh *et al.* (1999) in *Andrographis paniculata* and Vadivel and Janardhanan (2000) in *Mucuna pruriens.* They also reported wide range of variation for number of branches per plant in *Andrographis paniculata* and *Mucuna pruriens.*

The number of crowns per plant showed remarkable variation and the mean values ranging from 9.33 (PF 14) to 57.66 (PF 11). Lal *et al.* (1999) recorded considerable amount of natural variability in 18 morphologically distinct accessions of vetiver grass for number of tillers per plant.

Significant variation was observed for number of ground suckers per plant and it varied from to 1.00 (PS 26) to 50.00 (PP 27). According to Stover and Simmonds (1987), *acuminata* banana clones produced more number of suckers compared to *balbisiana* cultivars. Shanmugavelu and Balakrishnan (1980) observed variation among banana cultivars in the number of suckers produced per plant. In the present study, the genotypes that had less number of branches and crowns produced more number of

ground suckers. Significant variation was observed among the 30 genotypes for the number of prop roots.

Number of leaves per crown varied significantly among the 30 *Pandanus* genotypes. The number of leaves recorded was the highest (106.60) in PF 14 while PSt 29 (14.60) had the lowest number. Similarly Nambisan (1972) reported variation in number of leaves among different *acuminata* banana clones.

Remarkable variation was observed among the thirty genotypes of *Pandanus* on leaf length. Genotype PF12 (238.00 cm) recorded the maximum leaf length and PSt 29 (29.00 cm) recorded the minimum leaf length. Jagadev *et al.* (1993b) observed variability in leaf length among six morphotypes of *Pandanus* and it varied from 55.00 to 70.00 cm. According to Lal *et al.* (2001b) *Murraya koenigii* showed greater variation in leaf length among diverse origin germplasm lines.

Leaf width showed significant variation among the thirty genotypes. It varied from 1.00 cm (PSt 29 and PSt 30) to 13.00 cm (PP 27). Similar variation was also observed by Jagadev *et al.* (1993 b) in *Pandanus* and Lal *et al.* (1999) in vetiver.

The thirty *Pandanus* genotypes showed wide variation among them for leaf weight. Maximum leaf weight was recorded by PP 27 (51.60 g) and least was with PSt 29 and PSt 30 (1.00 g). These results are in confirmity with the findings of Jagadev *et al.* (1993b) in *Pandanus*. According to them the leaf weight ranged from 2.50 to 4.00 g.

Leaf yield showed enormous variability and it ranged from 0.42 to 88.20 kg per plant. Genotype PF 21 recorded the highest leaf yield of 88.20 kg and the lowest leaf yield (0.42 kg) was recorded by PSt 29. Bayram (2001) reported variation among *Salvia fruiticosa* accessions with respect to fresh leaf yield.

Significant variation was also observed for spine characters *viz.*, number of spines in 10 cm length of lamina, angle of spine projection and spine length.

Number of spines varied from 6.00 (PSt 29) to 34.00 (PP 27). Highest spine length (0.80 cm) was recorded by PF 4 and PF 24 and lowest spine length (0.20 cm) was noticed in PSt 29 and PSt 30. Angle of spine projection ranged from 45° (PF 4, PF 8 and PF 16) to 85° (PP 27). Similar findings regarding spine characters were observed by Baghdadi *et al.* (1989) in *Lycicum* spp. They noticed variation in number of spines and spine length. According to Jagadev *et al.* (1993b) the number of thorns per 10 cm length of leaf margin varied from 9.50 to 15.00 and the angle of thorn projection ranged from 45^0 to 85^0 in *Pandanus*.

Genotypes belonging to *Pandanus sanderi* (PS 26), *P. pacificus* (PP 27), *P. amaryllifolius* and *P. stellatus* (PSt 29 and PSt 30) showed wide variation while comparing with *P. fasicularis* genotypes (PF 1 to PF 25). However, the variation within *P. fasicularis* genotypes were not that much wider.

Considering the above points, genomic status of the individuals might have played a vital role regarding the morphological variability among the *Pandanus* genotypes. However, the role of environment and nutrient status of the soil in different locations may not be ignored.

5.1.2 Anatomical Characters

Leaf thickness showed significant variation among the thirty genotypes of *Pandanus*. It varied from 0.20 to 0.93 mm. The highest value (0.93 mm) was recorded by PP 27 and the lowest value was recorded by PSt 29 and PSt 30 (0.20 mm). Manian *et al.* (2002) observed variation in leaf thickness of *Pandanus tectorius* accessions and it ranged from 570 to 580 nm. According to Jagadev *et al.* (1993 b) the leaf thickness of six morphotypes of *Pandanus* varied from 0.40 mm to 0.80 mm.

In the present study, significant variation regarding the number of stomata in the abaxial leaf surface was observed. It varied from 4.33 per mm^2 (PF 8) to 13.66 per mm^2 (PF 9). However, the number of stomata in the adaxial leaf surface did not show any significant variation and it ranged from 0.00 to 2.66 per mm^2.

Ghahreman *et al.* (1999) reported variation in stomatal occurrence and stomatal index of 13 *Hyocyamus* species. Edeoga (2001) observed variation in stomatal size, shape and index among eight wild species of *Dioscorea.* Stomatal frequency and index showed variation among twelve species of Zingiberaceae (Gogoi *et al.*, 2002).

Variation in anatomical characters such as leaf thickness and stomatal frequency may be due to the genetic factor or due to the influence of environment.

5.1.3 Molecular Markers (RAPD)

Isolation of genomic DNA was carried out using the modified Murray and Thompson (1980) protocol with some necessary changes in order to suit with the plant material of *Pandanus* while extraction. Usually tender leaves of *Pandanus* were rich in phenolics and this resulted in the yield of poor quality DNA which was brown in colour. Addition of 1 ml of 0.1 per cent PVP in extraction buffer along with the other reagents reduced browning and yielded good quality DNA. Similar observations were made by Nayar (2001) and Simi (2001) in red banana and it was over come by addition of 0.1 per cent PVP in extraction buffer. Fresh unfurled tender leaves were found to yield more quantity DNA. This may be due to the easy description of the tender leaves during grinding using liquid nitrogen. Use of young tender leaves on the isolation of genomic DNA with higher quantity and better quality was already reported in many crops like chrysanthemum (Scot *et al.*, 1996), roses (Vainstein and Ben-Meir, 1994), gladiolus (Pathania and Mishra, 2001), *Dendrobium* (Pillai, 2003) and banana (Simi, 2001; Rajamanickam, 2003).

The yield and quality of the DNA varied with the genotypes. The DNA yield varied from 0.33 to 2.40 $\mu g\ \mu l^{-1}$. The OD-260/280 ratio varied between 1.50 and 1.87 indicating the DNA of good quality. The variation in DNA yield and quality may be due to genetic factor or due to the chemical reaction that taking place during the extraction of DNA.

In order to identify the promising primers for RAPD analysis, sixty decamer primers (Operon Inc., USA) of kit A, kit B and kit C were screened using the DNA of *Pandanus* genotype PF 11. Out of the sixty primers screened 48 primers yielded amplification products. The total number of bands ranged from 1.00 to 9.00. The primers OPA-03, OPA-05, OPA-11, OPA-17, OPA-19, OPB-01, OPB-05, OPB-16, OPC-02, OPC-05, OPC-06, OPC-11, OPC-12 and OPC-16 did not yield any bands. Out of the 143 RAPD markers produced by the 46 primers, 103 were polymorphic (72 per cent) and 40 were monomorphic.

Bhat and Jarret (1995) suggested that the number of polymorphism is more important than the number of primers for the generation of stable phenogram. They also suggested that the number of polymorphism required to generate a stable phenetic analysis would vary with the plant material under investigation and the sequences that are amplified.

In the present study, based on the polymorphic bands obtained, four primers were selected for further evaluation. Forty one RAPD markers were scored from the four primers on amplification with the DNA of 30 genotypes of *Pandanus*. Among the 41 RAPD markers 35 were polymorphic (85.36 per cent) and six were monomorphic. The primer OPB-20 produced polymorphic bands in all genotypes.

In a similar study, Sharma *et al.* (2000b) reported that, out of the 40 primers tested for RAPD analysis, only seven primers showed polymorphism in *Podophyllum hexandrum*. According to them the amplification fragments ranged from 7 to 16 with an average of 10.9 bands per primer and the size of the amplification products varied from1 to 2 kb.

According to Shasany *et al.* (2000), 71 random primers responded to amplification and generated 2998 reproducible bands, out of which 80 per cent were polymorphic for one or other accession of garlic. RAPD analysis with 120 primers in *Pelargonium graveolens* revealed that, three primers were more informative to differentiate the three cultivars of

Pelargonium (Shasany *et al.*, 2002a). According to Li and Midmore (1999) the number of RAPD markers needed to differentiate the genotypes of a gene pool may vary with the test material. When the variation between cultivars are high, few primers will be sufficient to detect the diversity. For instance, 11 navy bean genotypes were satisfactorily distinguished by only two primers (Graham *et al.*, 1994). In the present study also, four primers *viz.*, OPB-11, OPB-12, OPB-19 and OPB-20 revealed satisfactory polymorphism among the 30 genotypes of *Pandanus*.

The difference in amplification products may be due to the difference in primer sequence, extraction protocol, DNA quality, Mg Cl_2 ion concentration, genotypic difference etc. The Mg^{2+} ion is known to affect the primer template interaction (Welsh and Mc Clelland, 1990), polymerase activity and the melting temperature of double stranded DNA.

In the present study, the similarity matrix indicated the existence of wide diversity among the *Pandanus* genotypes and the similarity coefficient value ranged from 0.333 to 1.000. The highest similarity coefficient value of 1.000 was obtained between the genotypes of PF 1, PF 2, PF 4 and PF 18 (yellow inflorescence type), PF 7, PF 12 and PF 22 (grayish green leaved and white inflorescence type), PF 9, PF 20 and PF 25 (dull white inflorescence type) and PSt 29 and PSt 30 *(P. stellatus)*. The lowest similarity coefficient value of 0.333 was obtained between the genotype pairs of PF 23 and PSt 29 and PF 23 and PSt 30.

The yellow inflorescence types such as PF 1, PF 2, PF 3, PF 4, PF 6, PF 8, PF 11, PF 16, PF 18 and PF 24 showed maximum similarity among them. Within these types cent per cent similarity was recorded between PF 1, PF 2, PF 4 and PF 18.

Similarly, the grayish green leaved and white inflorescence morphotypes such as PF 7, PF 12 and PF 22 showed cent per cent similarity among them. Dull white inflorescence types *viz.*, PF 9, PF 20 and PF 25 showed cent per cent similarity between them and the

genotypes belonging to the species *P. stellatus* (PSt 29 and PSt 30) also revealed cent per cent similarity among them. Long glossy green leaved and white inflorescence types such as PF 13, PF 14 and PF 15 showed maximum similarity among them. In general, genotypes like PS 26, PP 27, PA 28, PSt 29 and PSt 30 belonging to the species *P. sanderi, P. pacificus, P. amaryllifolius* and *P. stellatus* showed maximum diversity among themselves as well as with the *P. fasicularis* genotypes (PF 1 to PF 25).

Using the similarity matrix data of RAPD profiles, UPGMA (Unweighted Pair Group Method with Arithmetic Average) based dendrogram was constructed to represent the inter and intra specific relationships among the genotypes. On drawing a vertical line in the dendrogram along the point corresponding to the similarity coefficient value of 0.72, all the 30 genotypes of *Pandanus* were grouped into eight clusters. Genotypes PSt 29 and PSt 30 (*P. stellatus*) were completely out grouped from other genotypes and constituted one cluster. Similarly genotypes PA 28 (*P. amaryllifolius*), PP 27 (*P. pacificus*) and PS 26 (*P. sanderi)* formed separate clusters each. Among the rest, genotypes PF 9, PF 20, PF 25, PF 10, PF 19 and PF 23 (*P. fasicularis* – dull white inflorescence type) made one group while *Pandanus fasicularis* genotypes (grayish green leaved and white inflorescence type) such as PF 5, PF 7, PF 12, PF 22 and PF 21 formed another group. *P. fasicularis* genotypes (long glossy green leaved white inflorescence type) *viz.*, PF 13, PF 14, PF 15 and PF 17 were constituted one cluster and the remaining yellow inflorescence *P. fasicularis* genotypes like PF 1, PF 2, PF 4, PF 18, PF 11, PF 16, PF 3, PF 6, PF 24 and PF 8 made another cluster. This cluster was the largest one with maximum of 10 genotypes.

The clustering pattern revealed by RAPD analysis was almost in line with the morphological grouping. This finding is in conformity with the results of the RAPD studies conducted by Millan *et al.* (1996) in rose

and Nebauer *et al.* (2000) in *Digitalis*. They reported that, the grouping based on inter specific variation obtained through RAPD analysis was fully consistent with the previous grouping based on morphological affinities.

The polymorphism obtained in this study will be highly useful for finger printing as well as to determine the diversity among the *Pandanus* spp. Moreover, selection of superior genotypes for higher oil content and leaf yield of better quality based on morphological variability is possible since the diversity was confirmed at genetic level. Further studies with molecular markers like AFLP and SSR may provide more precise information regarding the diversity within the economically important and closely related *P. fasicularis* genotypes.

5.2 GENETIC VARIABILITY AND DIVERGENCE

Genetic improvement of a crop demands a thorough understanding of the genetic basis of yield and yield components of that particular crop. The variability present in the population, heritability and genetic advance are to be essentially estimated. Variations in a population arise due to the differences in the genetic make up the individuals or the environment in which they are grown. Selection is effective when there is considerable genetic variability among the individuals of a population.

5.2.1 Variability

Twenty two male genotypes of *Pandanus* showing variation in different morphological characters were evaluated. The analysis of variance showed significant differences for all the characters under study. This indicated the presence of wide range of differences between the genotypes for all these characters. It is important to know whether this variability is due to genotype or environment. Hence phenotypic, genotypic and environmental variances were estimated.

In the study, the average plant height was 5.67 m with a range of 4.00 to 6.50 m. PCV and GCV were low (13.83 and 12.75 per cent respectively). Similar to that of plant height, plant girth also showed low PCV (9.03 per cent) and GCV (6.37 per cent). The mean girth observed was 0.30 m. The number of crowns per plant showed the highest PCV (43.01 per cent) and GCV (40.62 per cent) with the mean value of 34.49. Spine length had an average of 0.49 cm with high PCV and GCV (40.00 per cent and 37.10 per cent respectively). Leaf weight showed high PCV (34.99 per cent) and GCV (34.17 per cent) with the mean value of 32.98 g.

Among the inflorescence characters the highest variability at phenotypic and genotypic level (PCV and GCV) was observed for inflorescence weight (45.31 and 44.39 per cent) with the mean of 212.80 g. Inflorescence yield showed high PCV (39.57 per cent) and GCV (38.72 per cent) and the average was 5978.94 g. Inflorescence length also had high PCV (33.18 per cent) and GCV (32.39 per cent) with the mean performance of 81.76 cm. Inflorescence girth recorded the lowest variability both at phenotypic and genotypic level. Oil content showed high PCV (31.98 per cent) and moderate GCV (26.74 per cent) with the mean oil content of 0.16 per cent. It ranged from 0.09 to 0.21 per cent among the 22 male genotypes. Variability in oil content among *Pandanus* genotypes were formerly reported by Deshpande (1938) and Dhingra *et al.* (1954). According to them the oil content ranged from 0.015 to 0.035 per cent. Sadagopal (1959) recorded oil content of 0.1 to 0.3 per cent whereas Nigam and Ahmad (1992) recorded 0.03 per cent oil from the inflorescence of *Pandanus.*

Lawrence (1992) found variation in the oil content of *Ocimum basilicum* genotypes and it varied from 0.25 to 0.70 per cent. According to Succhorska - Tropilo and Osinska (2001) the oil content of sweet basil varied from 0.1 to 0.55 per cent. Srivastava and Vasanthakumar (2004) reported wide variability among *Jasminum auriculatum* accessions for concrete yield.

Sharma *et al.* (1983) observed variability at phenotypic and genotypic levels in the clones of Java citronella for traits like plant height, tiller number, leaf characters and oil content. According to Lal *et al.* (2002b), the GCV values were

slightly less than PCV value for all the traits, namely plant height, tiller number, leaf width, herb yield and oil content in Java citronella and thereby indicating the vulnerability to environmental fluctuations. In the present study also the same trend was observed for all the characters and it remarks the role of environmental effect on phenotypic variation.

5.2.2 Heritability and Genetic Advance

The success of improvement of the characters under study depends on the heritability (broad sense) of the character and expected genetic advance under selection. Heritability coefficient gives an idea about the relative importance of the genetic and environmental components of variance in the character expression. High values of these coefficients indicate the effectiveness in the selection of phenotypically superior plants. Similarly, the magnitude of improvement in the performance of selected individuals over the population assumes importance. This is estimated through the parameter genetic advance. For easy understanding, heritability is classified as high (60 to 100), moderate (30 to 60) and low (<30) and genetic advance as high (>20), moderate (10 to 20) and low (<10) (Robinson, 1965).

Heritability along with genetic advance is more useful than heritability alone in predicting the resultant effect of selecting the best individuals (Johnson *et al.*, 1955). In this study all characters namely vegetative and floral characters showed high heritability (>60) except plant girth which showed moderate heritability.

Expected genetic advance as percentage of mean was used for the comparison of characters. As like heritability, genetic advance (GA) was also high (>20 per cent) for all the characters except leaf thickness and plant girth which showed moderate genetic advance. According to Panse (1957), the characters with high heritability and genetic advance were controlled by additive gene action and therefore amenable to genetic improvement through selection. In the present study almost all characters showed high heritability along with high

genetic advance. Hence selection of phenotypically superior plants with respect to these characters will result in a significant crop improvement.

High heritability along with high genetic advance as percentage of mean for the major characters under study were formerly reported in turmeric (Jana *et al.*, 2001; Narayanpur and Hananashetti 2003), ginger (Ali *et al.*, 1994; Yadav, 1999a; Singh and Mittal, 2003), *Coriandrum sativum* (Mehta and Patil, 1985) *Papaver somniferum* (Singh *et al.*, 2003 b), Java citronella (Lal *et al.*, 2002b) and lemongrass (Singh *et al.*, 2004).

5.2.3 Correlation

The degree of direction of the inherent association (genotypic correlation) of characters apart from the observable correlation (phenotypic correlation) between two characters are important for the simultaneous selection of characters for genetic improvement. Correlation co efficient gives an idea about the mutual relationship between various plant characters on which selection can be based for genetic improvement in yield. The presence of genotypic correlation may be either due to pleiotropic action of genes or due to linkage or more likely both. If a positive genotypic correlation was observed for a pair of characters, certainly the improvement in one character will improve the other character also, thus helping a breeder to select characters on the correlated response to selection. If the improvement of one character results a decrease in other character, will help the breeder to select the characters if necessary.

In the present study, inflorescence yield per plant had high significant positive phenotypic and genotypic correlation with leaf length, width, weight, inflorescence length, girth and inflorescence weight. It had significant negative correlation with spine length. Environmental correlation of inflorescence yield with other characters were not to the mark. However, it showed medium range of positive correlation with leaf width and number of inflorescence per plant.

Inflorescence length showed high significant positive phenotypic and genotypic correlation with number of leaves per crown, leaf length, width, weight, inflorescence length, girth and inflorescence yield per plant.

Inflorescence girth showed high significant positive phenotypic and genotypic correlation with number of leaves per crown, leaf length, width, inflorescence length, girth and total inflorescence yield per plant.

Inflorescence weight showed high positive significant correlation with number of leaves per crown, leaf length, width, inflorescence yield per plant. It had significant negative correlation with number of inflorescence per plant and spine length.

Number of inflorescence per plant showed high significant positive correlation with number of crowns per plant, spine length and oil content.

Oil content showed high significant positive correlation with number of crowns per plant, spine length and number of inflorescence per plant and negatively correlated with other traits.

This finding was in agreement with the results of Dhar *et al.* (1985) in *Cymbopogon jwarancusa*. They reported that the oil content of leaf was negatively correlated with most of the traits. From this study it was concluded that, the improvement of characters like number of leaves per crown, leaf length, width, weight, inflorescence length, girth and inflorescence weight will cause a corresponding improvement in total inflorescence yield per plant. Similarly the increase in spine length will show a corresponding increase in number of inflorescence per plant and oil content. But the increase in number inflorescence per plant will cause a corresponding decrease in flower weight.

Jagadev *et al.* (1993a) reported that, leaf width had a positive association with flower production and leaf thickness, flower weight and flower length led to decrease the total number of inflorescence per plant in *Pandanus*. According to them plant type with broad leaves, thorny margin and less flower weight was expected to produce more number of flowers.

In the present study also similar type of results were obtained for negative association between number of inflorescence and flower weight and positive correlation of spine length with number of inflorescence per plant.

5.2.4 Path Coefficient Analysis

The technique of path analysis was applied for plant selection by Dewey and Lu (1959). It measures the direct and indirect contribution of independent variables on dependent variable and the residual effects. This technique also provides information about the cause and effect situation and helps in understanding the cause of association between two variables. Direct effects and indirect effects can be classified into very high (>1), high (0.30 to 0.99), moderate (0.20 to 0.29), low (0.10 to 0.19) and negligible (0.00 to 0.09) (Lenka and Mishra, 1973). Path analysis was done considering the characters *viz.*, plant height, number of crowns per plant, number of leaves per crown, number of inflorescence per plant, inflorescence length, inflorescence girth, inflorescence weight and oil content on total inflorescence yield per plant. The present study revealed that, yield contributing characters like number of inflorescence per plant, inflorescence girth and inflorescence weight had very high positive direct effect on yield. Almost similar results were reported in vetiver (Ramanujam and Kumar, 1963; Singh *et al.*, 1998; Gurmani *et al.*, 2004). The results showed that the number of tillers per plant, root length per plant, root yield per plant and oil content were the major contributing characters towards the oil yield.

In cardamom, number of panicles per plant, capsule fresh weight per plant, nodes per panicle, and internodal length within the panicle were the yield contributing characters (Gopal *et al.*, 1990) and it had positive direct effect with yield.

Lal *et al.* (1986) observed that plant height had negative direct effect on yield of turmeric but it contributed via yield components indirectly.

According to Shanmugasundaram *et al.* (2001) the positive direct effect on rhizome yield was maximum for weight of primary rhizome followed by secondary rhizome and weight of mother rhizome, whereas plant height showed negative direct effect on yield.

Similar results regarding the very high negative direct effect of plant height on yield and its indirect contribution via yield components were observed in the present study also.

Inflorescence length had very high negative direct effect on inflorescence yield but its indirect positive contribution on inflorescence yield was through inflorescence girth and inflorescence weight.

Oil content had very high negative direct effect on yield, however it had very high positive indirect effect through number of inflorescences per plant.

In general there is high scope for improvement of *Pandanus* through selection since all the characters showed high variation along with high heritability and genetic advance. Apart from this, vegetative multiplication of this crop have an added advantage towards the selection of superior genotypes.

5.2.5 Cluster Analysis through Mahalanobis D^2 Statistics

Mahalanobis D^2 statistics gives a quantitative measure of divergence based on multiple characters. In the present study, twenty two genotypes of *Pandanus* were grouped into six clusters. The clusters were based on 15 quantitative characters, like plant height, plant girth, number of crowns per plant, number of leaves, leaf length, leaf width, leaf weight, leaf thickness, spine length, number of inflorescence per plant, length of

inflorescence, girth of inflorescence, weight of inflorescence, oil content and inflorescence yield per plant.

The greater the distance between the two clusters the greater is the divergence between genotypes belonging to the clusters and vice versa. Within a cluster, the genotypes are less divergent than those which are in different clusters. In the present study, the inter cluster distance was highest between cluster I and cluster II and it was lowest between cluster III and cluster V. The characters like inflorescence yield and number of inflorescence per plant were the major contributing factors towards the divergence followed by inflorescence girth, leaf length, leaf weight, inflorescence weight, oil content and inflorescence length. Plant girth, number of crowns per plant and number of leaves per crown showed nil effect towards divergence. Cluster I included two long glossy green leaved and white inflorescence types (PF 13 and PF 15) and it was characterized mainly by plant height, number of leaves and leaf thickness. Cluster II showed higher values for characters like spine length, number of inflorescence per plant and oil content and it contained six genotypes (PF 1, PF 2, PF 3, PF 4, PF 8 and PF 16). These six genotypes are long spiny, short leaved and yellow inflorescence types. Cluster III had three genotypes (PF 10, PF 19, PF 20) and they are long green leaved, short spiny with large dull white inflorescence. This cluster showed higher values for leaf thickness, inflorescence length, inflorescence girth, inflorescence weight and inflorescence yield per plant.

Cluster IV had higher mean values for plant girth and number of inflorescence per plant. It was grouped by six genotypes (PF 5, PF 6, PF 7, PF 11, PF 12 and PF 25) among which two belong to yellow inflorescence type (PF 6 and PF 11), three belong to grayish green thick leaved white inflorescence type (PF 5, PF 7and PF 12) and one from dull white inflorescence type (PF 25). Cluster V showed higher values for leaf length, leaf width and leaf thickness. It included three genotypes (PF 17, PF 22 and PF 23) one from long glossy green

leaved types, and the other two were from long green leaved white inflorescence types respectively. Cluster VI had two genotypes (PF 18 and PF 24) and it was characterized by number of crowns per plant and oil content. They are short leaved, long spiny and yellow inflorescence types. In general, the clusters I, II, III and VI grouped similar morphotypes whereas cluster IV and V did not follow a definite pattern with respect of particular plant character or geographical origin.

Choudhary *et al.* (2002) grouped 30 accessions of celery into six clusters by D^2 statistic. According to them the major contributing characters towards divergence were seed yield per plant, number of umbelets per umbel and oil content. In the present study also similar results were obtained and the number of inflorescence and inflorescence yield per plant were the major contributing characters towards the divergence.

Singh *et al.* (2001) reported that, tiller number, plant height, leaf length, leaf width, leaf yield, oil content and oil yield were the contributing characters of divergence in case of lemongrass and they grouped 43 clones in to nine clusters by multivariate analysis. Lal *et al.* (2002b) quantified 34 accessions of citronella Java by multivariate analysis and grouped them into seven clusters.

While considering the leaf characters *viz.*, number of leaves, leaf length, width, weight, leaf thickness and leaf yield per plant for the selection of best genotypes for leaf purpose, it was evident that the long glossy green, long grayish green and long dark green short spiny morphotypes were equally good. However, very specific selection indicated that the genotypes PF 21 and PF 22 (grayish green long thick leaved and short spiny type) and PF 25 (long dark green short spiny type) identified from Chavara and Karunagapally areas of Kollam district of Kerala are the best followed by PF 7 and PF 12 (long grayish green thick leaved type) identified from Kanyakumari district of Tamil Nadu.

Selection of best genotypes based on inflorescence yield and related characters revealed that, genotypes PF 10, PF 7, PF 12, PF 19, PF 20, PF 22, PF 23 and PF 25 are the superior ones. Except the genotype PF 7 and PF 12 (white inflorescence type) all were similar types (dull white inflorescence types). While selecting genotypes based on oil content and its fragrance, short leaved, long spiny and yellow inflorescence types such as PF 1, PF 3, PF 6, PF 11, PF 18 and PF 24 are the best. However, genotype PF 18 had the maximum oil content of 0.21 per cent.

In general the present study revealed that, the genotypes with higher inflorescence yield are inferior in oil content and genotypes with poor inflorescence yield are superior in oil content. In perfumery trade the price of the essential oil is mainly decided based on its quality. Further studies are required for selection of superior genotypes, combining these traits *viz.*, inflorescence yield, oil content and its quality.

5.3 PROPAGATION

5.3.1 Seed Propagation

In nature higher plants reproduce primarily by seeds. Seed propagation is the most efficient and economical method of propagation as long as genetic variability can be controlled within acceptable limits. In general, *Pandanus* produces apomictic seeds which do not require prior pollination and fertilization. Since the seeds are produced by an asexual process, the seedling progenies are genetically uniform. Because of this fact, seed multiplication gains importance as in case of *Pandanus* even though, the hard seed coat limits germination to a greater extent. Usually under such conditions mechanical and chemical treatments are helpful to improve germination by way of softening the seed coat and leaching out of inhibitors present in the seed coverings (Hartmann and Kester, 1989). But establishing a direct relationship between specific chemicals and germination is difficult. Nevertheless, in some specific cases where inhibitors are

present, germination can be improved or stimulated by leaching with water or removing the seed coverings or both (Norton, 1980).

Considering the above, the present investigation on propagation of *Pandanus* through seed was taken up with some pre germination seed treatments.

The effectiveness of the different seed treatments can be judged by germination percentage, germination rate, seedling growth rate and morphological appearance of the seedlings. Statements of germination percentage should involve a time element, indicating the number of seedlings produced within a specified length of time. Germination rate can be measured by number of days required to produce a given germination percentage (Hartmann and Kester, 1989).

In the present study also the above parameters were observed in order to judge the efficiency of different seed treatments. It was noticed that the germination percentage varied from 16.60 to 80 per cent due to the influence of different treatments. In general soaking of the seeds in cow urine + cow dung slurry (T_7), diluted cow urine (T_6), cow urine + GA_3 100 ppm (T_8) showed highest germination percentage (80 per cent). Sulphuric acid treatment, hot and cold water treatment and mechanical scarification treatments were also found to influence the germination percentage than the control but not to the extent of cow urine, cow dung slurry and GA_3 treatment.

The seedling growth in terms of seedling height, girth, number of leaves, length of the longest leaf, width of the leaf, number of primary roots, root length, girth, and spread at various growth stages like 4^{th}, 8^{th} and 12^{th} month of sowing had been greatly enhanced by pre soaking seed treatments. Similar to germination percentage growth parameters were also increased considerably due to cow urine, cow dung slurry and GA_3 100 ppm treatments when compared to the other treatments.

The beneficial effect of cow dung slurry in the enhancement of seed germination and seedling vigour was reported by Palani *et al.* (1995) in *Acacia nilotica.* The excellence of cow urine in the improvement of growth parameters and plant vigour was reported in *Gymnema sylvestre* by Chandrasekar *et al.* (2003). The above findings are in confirmity with the results of the present study.

The presence of growth regulating substances like auxins, gibberellins in cow urine and cow dung slurry are the major cause for the enhanced germination and growth response of the seedlings.

The effectiveness of GA_3 on the enhancement of seed germination and seedling growth was reported by Singh *et al.* (2000c) in *Aconitum heterophyllym,* Verma *et al.* (2000) in *Withania sominifera,* Suryawanshi *et al.* (2001) in wild brinjal and by Masilamani and Dharmalingam (2002) in silver oak.

The response attributed for enhanced germination and seedling growth due to GA_3 treatment are many such as diffusion of endogenous auxin and gibberellin like substances (Mathur *et al.,* 1971), antagonistic effect on the inhibitor present in the seed (Khan, 1977) and augmentation of the metabolism rate during germination (Verma and Tandon, 1988).

Rai *et al.* (1987) reported an increasing trend in the germination of *Paraserianthus falcataria* when treated with concentrated sulphuric acid for 10 minutes. According to Sur *et al.* (1987) sulphuric acid treatment enhanced germination percentage in *Leucaena leucocephala.* The effect of acid treatment in breaking hard seed coat dormancy in *Acacia* and various other leguminous species were reported by Bahuguna and Lal (1990); Bhagat (1994); Bradhan (1996); Rana and Rao (1997) and Sharma and Punam (2002). In the present study also acid scarification treatment influenced the germination percentage to 50 per cent. Generally acid treatment soften the seed coat and reduces the seed coat impermeability and this may be the probable cause for the increase in germination percentage due to acid treatment than the untreated control.

Cold and hot water treatment to the seeds of *Dalbergia sissoo* showed higher germination percentage (Stokes, 1965). Sharma and Punam (2002) observed very poor germination percentage in *Alnus nitida* with hot water treatment whereas, they obtained very good germination with cold water treatment. In the present study also similar trend was observed but the difference in germination percentage between the two treatments were not significant as in the case of the above study. In general, coldwater treatment was better as compared to hot water treatment and untreated control.

Mechanical scarification treatment recorded only 33.33 per cent germination in this particular study and it was significantly superior than control and inferior when compared with the other treatments. While considering the above point it is clear that the poor seed germination in *Pandanus* seed is not only due to the presence of hard seed coat but also due to the presence of some inhibitory substances in the seed coat.

From the study it was concluded that, any wet treatment either with cow urine or cow dung slurry or growth regulating substances or with acid or water may speed up germination in *Pandanus* seeds by way of leaching out of the chemicals or inhibitors responsible for seed coat dormancy which present in the seed coat. But while observing the subsequent growth performance of the seedlings cow urine in combination with GA_3 100 ppm was found to be the best (T_8) treatment followed by cow dung slurry treatment (T_7) and diluted cow urine treatment.

5.3.2 Vegetative Propagation

Vegetative propagation involves the reproduction process through vegetative parts of the original plant. Superiority and uniformity of specific clones can be maintained through vegetative propagation since it is a asexual process involved in the duplication of the genetic makeup of a particular individual. Moreover, the major economy in vegetative propagation comes from the elimination of the juvenile phase and

shortening the time to reach reproductive maturity. The choice of the planting material mainly depends on the performance of the plants developing from the material planted. The ease in handling and transportation of the material also count to some extent. Keeping these points in view, it was considered worthwhile to determine the appropriate type of planting material for obtaining maximum yield of quality product, as authentic information available regarding this aspect in case of *Pandanus* is very scanty.

Growth Characters

Cent per cent establishment was noticed in ground suckers (T_4) and the stem cutting without crown showed the least establishment (68.75 per cent). In general, the population stand in the field was more in case of ground suckers than stem cuttings without crown and aerial suckers. Terminal cuttings with crown ranked in between these two category. Similar results were reported by Chadha *et al.* (1974) in pineapple. According to them, establishment percentage and population stand were high for suckers when compared with slips and crown. Number of days taken for the establishment of the planting material was comparatively less for ground suckers (T_4) and terminal cuttings with crown (T_1). However it was high for stem cuttings without crown and aerial suckers. Quick and better establishment of ground suckers were probably due to the higher carbohydrate accumulation and influence of root promoting substances at the basal portion of the suckers. The better rooting of terminal cuttings may be explained by the possibility of higher concentration of endogenous root promoting substances arising in the terminal bud. There is also less differentiation in the terminal cuttings, with more cells capable of becoming meristematic (Hartmann and Kester, 1989).

Growth of the plants was measured by plant height, plant girth, number of leaves, leaf length, leaf width, leaf yield, number of suckers and number of prop roots at different stages of growth *viz.*, 3rd, 6th, 9th,

15th and 18th months of planting. In general, terminal cuttings with crown (T_1) showed superior growth performance for most of the plant characters at all stages of growth and it was followed by ground suckers (T_4). At 18th month stage terminal cuttings produced more number of leaves (76.00) with maximum mean length of 185.25 cm, mean width of 5.65 cm and total leaf yield of 2558.75 g. While ground suckers produced 74.25 leaves with mean leaf length of 179 cm, width of 5.37 cm and total leaf yield of 2345.00 g. The remarkable vegetative vigour shown by terminal cuttings with crown and ground suckers were reported earlier (Dutta *et al.*, 1987; Jagadev *et al.*, 1993b; SFAC, 1997). The pronounced growth effect of suckers as planting material in pineapple was reported by Teaotia and Pandey (1962), Bourke (1976) and Ahmed and Mohan (1985).

To conclude, terminal cuttings with crown (T_1) and ground suckers (T_4) may be utilized towards the multiplication of *Pandanus* in order to obtain maximum leaf yield within a period of 18 months of planting.

5.3.3 Micro Propagation

Pandanus is mainly propagated vegetatively through suckers and cuttings. The propagation of *Pandanus* by conventional methods are time consuming as far as the production of a large number of homogenous plants are concerned. Moreover, the rate of multiplication of *Pandanus* through this method is rather low. Tissue culture techniques ensure an extremely rapid rate of multiplication which is not season dependent and requires only a limited quantity of plant tissue as the initial explant. Tissue culture techniques can also aid in the production of disease free plants and in the cryopreservation of germplasm.

In view of the above facts, there has been an upsurge of interest in the field of rapid clonal propagation of *Pandanus* plants applying tissue culture technique. However, reports on the standardization of micro propagation techniques in *Pandanus* is still lacks. The route adopted for *in vitro* propagule multiplication was via enhanced release of axillary buds. The most remarkable advantage of this method is the genetic stability of the plantlets produced.

In micro propagation the organs and tissues are carried through a sequence of steps in which differential cultural and environmental conditions are provided. These steps have been indicated as different stages with stage I being establishment, stage II rapid multiplication of shoots through increased axillary branching, stage III rooting and hardening of *in vitro* raised plants and stage IV acclimatization and transplantation to field conditions.

Pandanus being a monocotyledonous plant, the buds in the axils are dormant due to the apical dominance shown by fast growing meristematic tip.

Surface sterilization

Microbial contamination has long been a major problem in the culture establishment of explants. Since plant parts are exposed in the field for a long time, they harbor various micro organisms and many of which penetrate in to the plant tissue resulting in systemic infection. Cultures from such tissues are hence easily contaminated (Chen and Evans, 1990).

In the present study high rate of bacterial contamination was observed since the explants were collected from field grown plants. In order to minimize the rate of contamination, the explants were subjected to various surface sterilization treatments using mercuric chloride as sterilant. Mercuric chloride 0.08 per cent for seven minutes was found to be very effective for surface sterilization. It resulted in 73.3 per cent culture survival with minimum percentage of explant mortality due to microbial contamination and browning of tissues. Enhancing the strength of mercuric chloride or extending the treatment duration resulted in still lesser rate of contamination but it increased the rate of explant mortality due to browning. Bhaskar (1991) obtained the same results while sterilizing the shoot tips of banana with mercuric chloride.

The nature and concentration of growth hormones in the culture medium are critical for *in vitro* growth and morphogenesis of plants (Skoog and Miller, 1957). In the present study, in order to standardize a suitable hormone combination for better culture establishment, BA and NAA at various

concentrations were used. When a combination of BA 1 mg l^{-1} and NAA 0.4 mg l^{-1} was added to the medium, superior results were obtained in the percentage of culture establishment, number of days taken for culture establishment and number of shoots per culture. According to Bhaskar (1991) NAA 0.5 ppm and BA 3.0 ppm were found to be the best with respect to the number of days taken for culture establishment in banana whereas, in pineapple 0.1 mg NAA l^{-1} and 1.0 mg BA l^{-1} were found to be the best for culture initiation (Deva *et al.*, 1997).

The favourable effects of axillary bud bursting and multiple shoot production by cytokinins had been demonstrated by Murashige (1974). But at higher levels, cytokinins were proved to have deleterious effect on shoot growth. Auxin added to the medium helps to nullify the suppressive effect of high cytokinin concentration on axillary shoot growth (Lundergan and Janick, 1980). In the present study, auxin (NAA) was found to influence axillary shoot induction and growth at combinations with cytokinin.

The culture proliferation medium contained NAA 1.0 mg l^{-1} and BA 4.0 mg l^{-1} released on an average of 8.00 axillary shoots. This observation was found to be in confirmation with earlier report (Bhaskar, 1991) in banana in which, it was stated that NAA 1 ppm and BA 10 ppm in the culture proliferation medium found to enhance the release of axillary buds on an average of 11.00 axillary shoots. Similar reports highlighting the beneficial effects of NAA and BA in the induction of axillary shoot was reported in *Dendrobium* cv. Sonia and in *Aranthera* cv. Annie Black (Kuriakose, 1997).

The basic phenomenon involved in the induction of axillary shoots and subsequent plantlet production *in vitro* are reported to be due to the action of plant hormones. Little is known about how hormones evoke a particular pattern of morphogenesis (Thorpe, 1980). One hypothesis is that hormone treatment starts the cells on a specific developmental pathway. The alternative view is that hormone responsive cells are already determined and that the hormones evoke the expression of the committed state. The available evidence suggests that the hormones act in both ways.

Stage 3, involves *de novo* regeneration of adventitious roots from the shoots obtained in stage 2. Half concentration of MS medium was found to influence the rooting of *Pandanus* favorably without affecting shoot growth.

Both IBA and NAA have been reported to induce rooting of shoots and embryoids in banana (Krikorian and Cronauer, 1984; Banerjee *et al.*, 1987) and in *Dendrobium* cv. Sonia (Pathania *et al.*, 1998). In the present study also, the combination of NAA and IBA was found to induce rooting of *in vitro* shootlets of *Pandanus* and on an average of 7.83 roots were produced by the rooting medium containing 3 mg l^{-1} NAA + 4 mg l^{-1} IBA.

Addition of activated charcoal (AC) to the culture medium had remarkable influence in the initiation of roots as well as in the number of roots produced per shoot. Incorporation of 0.6 per cent AC to the rooting medium had resulted in the initiation of maximum number of roots per shoot let (11) with in a period of 20 days. The favourable effects of activated charcoal in rooting had been reported by many workers in crops like banana (Wang and Huang, 1976; Bhaskar, 1991; Vani and Reddy, 1999) and Orchid (Gangaprasad *et al.*, 2000). The capacity to absorb inhibiting compounds and excessive concentration of plant growth hormones and the property to darken the medium which mimics the soil conditions are the factors proposed in favour of beneficial effects of AC in rooting (Proskauer and Berman, 1970; Wang and Huang, 1976).

Field survival of *Pandanus* plants were the highest (70 per cent) with plantlets that had developed a minimum of 3-4 leaves and 3-5 roots in sterilized pot mixture containing sand, soil and farmyard manure in equal proportions. Ajithkumar (1983) reported that, *in vitro* plantlets of *Anthurium* with at least 2.5 to 3 cm length with 3-4 leaves and roots had the highest survival rate (100 per cent). The result of the present study was also in conformity with the above finding.

5.4 STANDARDIZATION OF LEAF CURING TECHNIQUES

The leaves of *Pandanus* form the raw material for mat weaving enterprise. The mats are preferred for bedding due to its cushioning effect. They are used for laying children and preparing various fancy articles or decoratives. In Kerala, nearly about two lakh, financially very weak farm families are involved in this enterprise and their primary occupation is mat weaving only (SFAC, 1997). Normally quality mats fetch higher price and the quality of the mats lies on the colour of the mats. Bright white coloured mats are considered as good quality mats and it fetch higher price of Rs. 250 to 350 per mat when compared to poor quality mats that fetch for Rs. 60 to 100 per mat.

The curing technology of the leaf was not standardized systematically till now even though, the quality mats prepared from processed leaves fetches higher price. Hence, the present investigation on standardization of leaf curing technology was taken up with the view of preparing quality mats and thereby improving the consumer preference and farm income of rural people, who completely depends on this industry.

Regarding the colour of the mats the treatment involving boiling and sun drying of leaves and in addition to that, coloring with synthetic dyes (T_9) recorded the maximum score of 4.2 (84 per cent). This treatment was a sort of value addition to the treatment (T_3) involving boiling and sun drying of leaves. While weaving the mats, the processed and coloured leaf splits were used to make designs in the mat. Usually bright colours had more acceptance than light colours. In white background bright colours look very attractive and this may be the reason for the maximum score obtained by this particular treatment.

The texture of the mat was found to be very soft in treatment T_5 which involves soaking in water for two days and sun drying. Though the cushioning effect was very good in this particular treatment the durability

was poor when compared to the treatments T_3, T_7 and T_9. Treatment T_9 recorded maximum score for eye appeal, however, T_3, T_7 and T_9 recorded maximum score value for durability (84 per cent). Even though the treatment T_7 recorded highest score value it was not preferred much by the consumers because of its dull colour and poor appearance. In general, over all acceptability was very good for T_9 and T_3. Favourable effect of boiling and sun drying of leaf on quality enhancement of *Pandanus* mat was formerly reported by SFAC (1998) and Panda *et al.* (2001). The brightness may be because of bleaching of chlorophyll and phenolic substances due to boiling and sun drying. Generally the mats made out of shade drying treatments were of poor eye appeal and not preferred in the market.

From the studies it was concluded that, boiling and sun drying of the leaf splits is very much essential for quality improvement of the mats since the leaves of this crop are rich in phenolic substances. However, treatment T_9 and T_3 were noticed as superior with respect to quality attributes such as colour, texture, eye appeal, durability and overall acceptance of the mats. While considering the cost wise benefit also the mats made out of treatments T_3 and T_9 fetch for Rs.300 and 325 respectively as against the mats made out of the conventional method (T_1- sun drying of leaves with out boiling) which worth about Rs.60 to 80 in the market.

The male inflorescence of *Pandanus* is highly valued for the fragrance emitted by the stamens and spathes and the leaves are employed in cottage industries for weaving mats. A well established *Pandanus* plant yields nearly about 40 inflorescences per plant per year with an average weight of 100 g per inflorescence and the price of one inflorescence ranged from Rs. 5 to 7 in the local flower markets. On an average, 50 to 100 ml of essential oil could be obtained from 100 kg of inflorescence on hydro distillation and the price of one ml of essential oil is around Rs. 250.

For weaving one double layer mat, one kg of cured leaf is required. The price of 1 kg of cured leaf is around Rs. 12 to 15. The price of one finished mat is around Rs. 250 to 350 in the local markets.

Though *Pandanus* is economically important its commercial exploitation is very limited. The reasons for the under exploitation of *Pandanus* are:

1. The shortage of raw material due to the destruction of natural ecosystem and shift in cultivation of other cash crops.
2. Presence of very low quantities of aroma constituents with very high vapour pressure and water missibility.
3. Harvest risk due to presence of spines and lack of any mechanical support for harvesting and processing.
4. Unawareness of the economical and ecological importance of the plant.
5. Lack of standardized package of practices.
6. Long juvenile phase of about five years to attain blooming stage.

Awareness regarding the economical and ecological benefits that the people could derive from this plant has to be authenticated and popularized. This will help to exploit the *Pandanus* resources fully.

Pandanus being a good soil binder and saline resistant crop can suitably be taken up for raising plantations in the banks of rivers, lakes and in the coastal belts as a first barricade to check drifting of sand. This inturn will provide sufficient raw material for the expansion of the industry. Being a hardy and drought resistant crop wastelands in the rural areas can also be utilized for establishing plantations.

Genetic variability studies with molecular markers like AFLP and SSR are to be conducted for the selection of superior genotypes from the natural growing populations for quality oil and fibre yield.

Emphasis should be given to develop spineless types with desirable characters through biotechnological approaches.

Investigations are to be conducted for standardization of agronomical practices for different situations.

Mechanization of harvesting and processing techniques are to be standardized.

Strengthening of research on various post harvest management and processing aspects.

Morphology and physiology of the crop has to be studied thoroughly in order to judge the stage and time of harvest of the flowers.

Skills involved in the distillation process have to be improved to obtain highest oil recovery without loosing the aroma constituents.

Assessment of quality attributes of the perfumery product and fixation of standards for increasing their trade in both domestic and international markets.

Insecticidal and medicinal properties of the plant have to be studied.

Marketing potential at national and international level and scope for substitution of the organic material (mat) as against plastic are to be studied.

Summary

6. SUMMARY

Investigations on "Morphomolecular characterization and evaluation of *Pandanus* spp." were conducted at College of Agriculture, Vellayani with the aim of estimating the genetic diversity among *Pandanus* genotypes selected from different locations of Kerala and Tamil Nadu. The study was conducted as four experiments *viz.*, I. Morpho-anatomical and molecular characterization of 30 genotypes of *Pandanus*, II. Genetic variability and divergence studies of 22 male genotypes of *Pandanus*, III. Standardization of propagation techniques and IV. Standardization of leaf curing technology. The results are summarized in this chapter.

MORPHO ANATOMICAL AND MOLECULAR CHARACTERIZATION

Morphological characters *viz.*, plant height, girth, number of primary branches per plant, number crowns per plant, number of suckers per plant, number of prop roots per plant, number of leaves per crown, leaf length, width, weight, leaf yield, number of spines in 10 cm length of lamina, angle of spine projection and spine length revealed significant variation among the 30 genotypes of *Pandanus*.

Similarly leaf anatomical characters such as leaf thickness and number of stomata in the abaxial leaf surface showed significant differences among the 30 genotypes. The genotype PF 21 produced the highest leaf yield (88.20 kg).

The average genomic DNA yield between the 30 genotypes of *Pandanus* was 0.979 μg μl^{-1} and the quality of the DNA (A_{260}/A_{280}) was with in the range of 1.5 (PF 5, PF 6 and PF 23) to 1.89 (PF 4).

Forty eight primers belonging to kit A, kit B and kit C yielded amplification products while screening 60 primers with the genomic DNA of genotype PF 11. A total of 143 RAPD markers were produced and

among which 103 (72 per cent) were polymorphic and 40 were monomorphic. The highest number of scorable bands were produced by the primer OPB-20 (9 bands).

The four primers such as OPB-11, OPB-12, OPB-18 and OPB-20 produced highest number of intense and polymorphic bands were selected for further amplification. A total of 41 scorable RAPD bands were amplified by four primers and of these 41 RAPD markers 35 were polymorphic and six were monomorphic.

The similarity co efficient value based on Jaccard's coefficient method ranged between 0.333 and 1.000. The lowest similarity co efficient value (0.333) was between PF 23 with PSt 29 and PSt 30. Highest similarity co efficient value (1.000) was obtained between the pairs of PF 1, PF 2, PF 4 and PF 18 (yellow inflorescence type), PF 7, PF 12 and PF 22 (white inflorescence type), PF 9, PF 20 and PF 25 (dull white inflorescence type), and PSt 29 and PSt 30 (*P. stellatus* genotypes).

On drawing a vertical line in the UPGMA based dendrogram obtained from the RAPD analysis along the point corresponding to the similarity coefficient value of 0.72 all the 30 genotypes of *Pandanus* were got divided in to eight clusters.

In general, yellow inflorescence genotypes such as PF 1, PF 2, PF 3, PF 4, PF 6, PF 8, PF 11, PF 16, PF 18 and PF 24 showed maximum similarity among them and grouped in one cluster. Grayish green leaved and white inflorescence morphotypes such as PF 5, PF 7, PF 12, PF 21 and PF 22 showed highest similarity among them and they formed another cluster.

Long glossy green leaved and white inflorescence morphotypes *viz.*, PF 13, PF 14, PF 15 and PF 17 were grouped under one cluster whereas dull white inflorescence morphotypes (PF 9, PF 10, PF 19, PF 20, PF 23 and PF 25) formed another cluster.

Genotypes belonging to the species *P. sanderi* (PS 26), *P. pacificus* (PP 27), *P. amaryllifolius* (PA 28) and *P. stellatus* (PSt 29 and PSt 30) were completely out grouped from the species *P. fasicularis* (PF 1 to PF 25) and made separate cluster each, indicating the wide diversity of these species with *P. fasicularis*.

GENETIC VARIABILITY AND DIVERGENCE AMONG 22 MALE GENOTYPES OF *PANDANUS*

Stem and leaf morphological and anatomical characters such as plant height, plant girth, number of crowns per plant, number of leaves per crown, leaf length, width, weight, leaf thickness and spine length showed significant variability among the 22 male genotypes of *Pandanus*.

Similar to that of stem and leaf morphological characters, inflorescence characters *viz.*, number of inflorescences per plant, inflorescence length, girth, inflorescence weight, number of spathe, number of androecious fesicles, shelf life of the inflorescence, oil content of the inflorescence and total inflorescence yield per plant revealed significant differences among the22 male *Pandanus* genotypes.

The phenotypic and genotypic coefficient of variation (PCV and GCV) were highest for the characters *viz.*, number of crowns per plant, spine length, inflorescence weight and total inflorescence yield per plant. In general, the GCV values were slightly less than PCV values. Oil content showed highest PCV and moderate GCV.

High heritability coupled with high genetic advance was recorded for all the vegetative and inflorescence characters except plant girth and leaf thickness.

Inflorescence yield per plant had high significant positive phenotypic and genotypic correlation with leaf length, width, weight, inflorescence length, girth and inflorescence weight. It had significant negative correlation with spine length.

Path coefficient analysis revealed that, yield contributing characters like number of inflorescence per plant, inflorescence girth, weight and number of leaves per crown had very high positive direct effect on inflorescence yield while characters such as plant height, number of crowns per plant, inflorescence length and oil content showed very high negative direct effect on inflorescence yield.

D^2 analysis based on the fifteen characters *viz.*, plant height, girth, number of crowns per plant, number of leaves per plant, leaf length, width, leaf thickness, spine length, number of inflorescences per plant, length of the inflorescence, girth of inflorescence, weight of inflorescence, oil content and inflorescence yield per plant grouped the 22 genotypes of *Pandanus* into six clusters.

Cluster I with two glossy green leaved and white inflorescence genotypes (PF 13 and PF 15), cluster II with six yellow inflorescence genotypes (PF 1, PF 2, PF 3, PF 4, PF 8 and PF 16) and cluster III with three dull white inflorescence genotypes (PF 10, PF 19 and PF 20). Cluster IV with six genotypes (PF 5, PF 6, PF 7, PF 11, PF 12 and PF 25) among which three genotypes *viz.*, PF 5, PF 7and PF 12 were grayish green leaved and white inflorescence type, PF 6 and PF 11 were yellow inflorescence type and PF 25 was dull white inflorescence type. Cluster V consisted of three genotypes (PF 17, PF 22 and PF 23) among which PF 17 was glossy green leaf and white inflorescence type, PF 22 was grayish green leaf and white inflorescence type and PF 23 was dull white inflorescence type. Finally, cluster VI with two genotypes namely PF 18 and PF 24 and the both belong to yellow inflorescence type.

The major characters contributed towards divergence were inflorescence yield and number of inflorescence per plant. Characters like plant girth, number of crowns per plant and number of leaves per crown showed nil effect towards divergence. The selection of best genotypes for leaf purpose based on certain leaf characters *viz.*, number of leaves, leaf

length, width, weight and thickness indicated that, the genotype PF 21, PF 22 (grayish green leaf type) and PF 25 (long dark green and short spiny type) identified from Chavara and Karunagapally areas of Kollam district of Kerala are the superior genotypes. Selection based on the inflorescence yield and related characters revealed that the genotypes PF 10, PF 19 and PF 25 are the best. The selection for oil content and its fragrance showed that long spiny yellow inflorescence types are good. However genotype PF 18 had the highest oil content of 0.21 per cent.

PROPAGATION

Seed

The effect of different physical and chemical seed treatments on the germination and growth performance of *Pandanus* seedlings indicated that the highest germination percentage (80 per cent) was recorded by the treatment T_6 (soaking the seeds in diluted cow urine), T_7 (cow urine + cowdung slurry) and T_8 (cow urine + GA_3 100 ppm). Similar to that of germination percentage, growth characters like seedling height, girth, number of leaves, leaf length, width, root length, girth and root spread were also showed highest performance with the treatments T_8, T_7 and T_6 at 4^{th}, 8^{th} and 12^{th} month growth stages of the seedling.

Vegetative Propagation

The performance of different vegetative propagation materials *viz.*, terminal cuttings with crown (T_1), stem cuttings without crown (T_2), aerial suckers (T_3) and ground suckers (T_4) were evaluated with respect to their growth characters such as establishment percentage, number of days taken for the establishment, plant height, girth, number of leaves, length of leaf, width of leaf, leaf yield, number of suckers and number of prop roots. Terminal cuttings with crown revealed significant influence on all growth characters invariably at all stages of growth and it was followed by ground suckers. Regarding the establishment percentage and number of days

taken for the establishment, treatment T_4 (ground suckers) showed superior results (100 per cent establishment within a minimum establishment period of 22.5 days).

Micro propagation

Surface sterilization of shoot tips with 0.08 per cent mercuric chloride for seven minutes gave 73.30 per cent culture survival. The treatment combination involving BA 1 mg l^{-1} and NAA 0.4 mg l^{-1} (T_7) was found to be the best for initial establishment of cultures. For the induction of axillary buds the treatment combination with BA 4 mg l^{-1} and NAA 1 mg l^{-1} was considered as the best treatment (T_9) since it produced the maximum number of shoots (8.00).

Treatment T_8 (NAA 3 mg l^{-1} + IBA 4 mg l^{-1}) produced maximum number of roots (7.83). Addition of activated charcoal to the rooting medium was found to be beneficial regarding the initiation of roots. The treatment combination involving 0.6 per cent activated charcoal along with the growth hormones *viz.*, NAA 2 mg l^{-1} and IBA 2 mg l^{-1} (T_6) produced the maximum of 11 roots per shoot. The *in vitro* raised plantlets showed 70 per cent survival in the field.

LEAF CURING TECHNOLOGY

The effect of different leaf curing treatments on the quality attributes of the *Pandanus* mats were assessed in terms of colour, texture, eye appeal, durability and overall acceptance. Treatment T_9 (boiling and sun drying of leaves + colouring with synthetic dyes) and T_3 (boiling and sun drying of leaves) obtained highest score values for almost all the quality attributes except texture. With regard to texture treatment T_5 (soaking in water for 48 hours + sun drying) excelled the other treatments.

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*Original not seen

Appendices

APPENDIX - I

Traditional knowledge

- *Pandanus* is known for its use in the traditional medicines of Ayurveda Sidha and Unani.
- The leaves are useful in the treatment of leprosy, smallpox, syphilis, scabies, heat of body, pain, leucoderma, diseases of heart and the brain.
- The tender leaves are said to be valuable in curing diseases of the heart and the brain.
- The young leaves and the tender portion of the stem are used as an antidote for poisoning.
- The oil obtained from male inflorescence is administered for headache and rheumatism
- The anthers of male inflorescence are used against earache and headache.
- The juice obtained from the whole inflorescence is said to be useful in rheumatic arthritis in animals.
- Decoctions of the stilt root is effective in the treatment of venereal diseases.
- The ashes of wood are said to promote the healing of wounds
- The oily *Pandanus* seeds are said to be effective in strengthening of heart and liver
- There is a believe in the rural areas that keeping male inflorescence under the pillow will induce better and peaceful sleep.
- The leaves are used as a green manure for coconut
- The leaves along with calcium carbonate is effective in controlling gray blight disease of coconut
- The dried male inflorescence especially andrecious fescicles are kept in wordrobes and boxes to protect the stored garments from insect damage and also to lend a long lasting fragrance to them.
- The cured leaf strips of *Pandanus* is good for rheumatism, heat of body and blood pressure.
- A fleshy cabbage like growing meristem surrounded by whorls of tender leaves is an excellent food and it is good for heart diseases.
- Special hair adornments prepared from the male inflorescence of *Pandanus* finds place in wedding ceremonies.
- Fully ripe *Pandanus* fruits are used as a pest repellent as against houseflies cockroaches and rats. The pests are usually attracted by the alcoholic odour emitted by the ripe fruit.

APPENDIX - II

DNA isolation method

(modified Murray and Thompson method)

Grind 500 mg tender leaf material in liquid nitrogen

↓

Transfer the powder into 15 ml centrifuge tube

↓

Add 5 ml of pre warmed CTAB extraction buffer and 1ml of 1 percent PVP solution

↓

Incubate at 60°C for 30 minutes

↓

Keep at Room temperature for 10 minutes

↓

Add equal volume of phenol chloroform isoamylalcohol mix (25:24:1) and mix by inversion

↓

Spin at 15,000 rpm for 10 min at 5°C

↓

Remove aqueous phase and extract again with phenol: chloroform isoamyl alcohol followed by chloroform isoamyl alcohol

↓

Take aqueous phase and add 2/3 volume of isopropanol +0.1 volume of 3M sodium acetate and mix by gentle inversion

↓

Pellet the DNA by centrifugation at 15,000 rpm for 5 min at 5°C

↓

Decant the supernatant and wash the pellet with 70% cold ethanol

↓

Air dry the pellet and dissolve in 50-100 µl of TE buffer

APPENDIX - III

Composition of Murashige and Skoog (1962) medium

	Quantity	Volume made up	Volume pipetted
Solution A			
Ammonium nitrate	16.5 g	1 litre	100 ml
Potassium nitrate	19.0 g		
Magnesium sulphate	3.7 g		
Potassium dihydrogen Phosphate	1.7 g		
Solution B			
Calcium chloride	4.4 g	500 ml	50 ml
Solution C			
Boric acid	0.62 g	100 ml	1 ml
Manganese sulphate	2.23 g		
Zinc sulphate	0.86 g		
Potassium iodide	0.083 g		
Sodium molybdate	0.025 g		
Solution D			
Ferrous sulphate	2.78 g	500 ml	5 ml
Sodium EDTA	3.73 g		
Solution E			
Cobalt chloride	0.025 g	1 litre	1 ml
Copper Sulphate	0.025 g		
Solution F			
Nicotinic acid	50 mg	100 ml	1 ml
Pyridoxine HCl	50 mg		
Thiamine HCl	10 mg		
Glycine HCl	200 mg		
Sucrose	30.00 g		
Inositol	100.00 mg		
Agar	7.00 g		

APPENDIX - IV

Scorecard for quality evaluation of *Pandanus* mats

Product : Mat Tested by :

Date Age

1. Colour
   - white : 5
   - Half white / light yellow : 4
   - Yellowish brown : 3
   - Brown : 2
   - Dark brown : 1
2. Texture
   - very soft : 5
   - soft : 4
   - moderately soft : 3
   - neither hard nor soft : 2
   - hard : 1
3. Eye appeal
   - excellent : 5
   - good : 4
   - fair : 3
   - poor : 2
   - very poor : 1
4. Durability of the mat
   - highly durable : 5
   - durable : 4
   - moderately durable : 3
   - less durable : 2
   - not durable : 1
5. Over all acceptability
   - highly acceptable : 5
   - acceptable : 4
   - moderately acceptable : 3
   - slightly acceptable : 2
   - not acceptable : 1

MORPHOMOLECULAR CHARACTERIZATION AND EVALUATION OF *Pandanus* spp.

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Abstract of the
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ABSTRACT

Investigation on "Morphomolecular characterization and evaluation of *Pandanus* spp." was undertaken at College of Agriculture, Vellayani during 2002-2004 with the view of estimating the genetic diversity among 30 genotypes of *Pandanus* selected from different locations of Kerala and Tamil Nadu based on morphological, anatomical and molecular markers. Variability and divergence studies between 22 male genotypes of *P. fasicularis,* standardization of different propagation techniques and leaf curing technology were also undertaken.

Morphological characters *viz.*, stem, leaf and spine growth characters and leaf anatomical characters such as leaf thickness and number of stomata revealed significant variability among the 30 genotypes of *Pandanus*.

In RAPD analysis, the four primers such as OPB-11, OPB-12, OPB-18 and OPB-20 produced the highest number of intense and polymorphic bands. A total of 41 scorable RAPD markers were amplified by the four primers and out of these, 35 were polymorphic and six were monomorphic. The UPGMA based dendrogram generated through RAPD analysis grouped the 30 genotypes into eight clusters and each cluster consisted of similar morphotypes.

In variability and divergence studies, the genotypic and phenotypic coefficient of variation (GCV and PCV), heritability and genetic advance showed highest values for most of the characters. Inflorescence yield per plant had high significant positive phenotypic and genotypic correlation with leaf length, width, weight, inflorescence length, girth and inflorescence weight. It had significant negative correlation with spine length.

D^2 analysis grouped the 22 male genotypes of *Pandanus* into six clusters. The major characters contributed towards divergence were inflorescence yield and number of inflorescence per plant.

The selection of best genotypes for leaf purpose based on the leaf characters *viz.*, number of leaves, leaf length, width, weight and thickness indicated that, the genotype PF 21, PF 22 (grayish green leaf type) and PF 25 (long dark green and short spiny type) identified from Chavara and Karunagapally areas of Kollam respectively are the superior ones. Selection based on the inflorescence yield and related characters revealed that, the genotypes PF 10, PF 19 and PF 25 are the best. The selection for oil content and its fragrance showed that long spiny yellow inflorescence types are good. Genotype PF 18 had the highest oil content of 0.21 per cent.

The effect of different physical and chemical seed treatments on the germination and growth performance of *Pandanus* seedlings indicated that, the treatment T_6 (soaking the seeds in diluted cow urine), T_7 (cow urine + cowdung slurry) and T_8 (cow urine + GA_3 100 ppm) were the superior while considering the germination and other growth parameters.

Different vegetative propagation materials *viz.*, terminal cuttings with crown (T_1), stem cuttings without crown (T_2), aerial suckers (T_3) and ground suckers (T_4) were evaluated for their growth characters such as establishment percentage, number of days taken for the establishment, plant height, girth, number of leaves, length of leaf, width of leaf, leaf yield, number of suckers and number of prop roots. Terminal cuttings with crown showed significant influence on all growth characters invariably at all stages of growth and it was followed by ground suckers.

In micro propagation for the initial establishment of shoot tip cultures, the treatment combination involving BA 1 mg l^{-1} and NAA 0.4 mg l^{-1} (T_7) was found to be the best. For the induction of axillary buds the treatment combination with BA 4 mg l^{-1} and NAA 1 mg l^{-1} (T_9) was

considered as the best. The treatment combination involving 0.6 per cent activated charcoal along with the growth hormones *viz.*, NAA 2 mg l^{-1} and IBA 2 mg l^{-1} (T_6) produced the highest number of roots per shoot (11.0). The *in vitro* raised plantlets showed 70 per cent survival in the field.

The effect of different leaf curing treatments on the quality attributes of the *Pandanus* mats were assessed in terms of colour, texture, eye appeal, durability and overall acceptance. Treatment T_9 (boiling and sun drying of leaves + colouring with synthetic dyes) and T_3 (boiling and sun drying of leaves) obtained highest score values for almost all the quality attributes except texture. With regard to texture treatment T_5 (soaking in water for 48 hours + sun drying) excelled the other treatments.