MORPHO-ANATOMICAL AND MOLECULAR CHARACTERISATION OF *Dendrobium* Sw. CULTIVARS

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DECLARATION

I hereby declare that this thesis entitled **"Morpho-anatomical and molecular characterisation of *Dendrobium* Sw. cultivars"** 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 to me 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 **"Morpho-anatomical and molecular characterisation of *Dendrobium* Sw. cultivars"** is a record of research work done independently by Mr. N. Padmanaba Pillai (99-22-10) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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N. PADMANABA PILLAI

DEDICATED TO

MY FAMILY

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LIST OF ABBREVIATIONS

AFLP	–	Amplified Fragment Length Polymorphism
bp	–	base pair
DNA	–	deoxy ribonucleic acid
dNTPs	–	deoxy nucleotides
EDTA	–	Ethylene diamino tetra acetic acid disodium salt
GA	–	Genetic Advance
GCV	–	Genotypic Coefficient of Variation
H^2	–	Heritability
M	–	molar
$Mg\ Cl_2$	–	Magnesium chloride
ml	–	milliliter
mm	–	millimeter
NaCl	–	Sodium chloride
ng	–	nanogram
OD	–	Optical Density
PCR	–	Polymerase Chain Reaction
PCV	–	Phenotypic Coefficient of Variation
pM	–	picomolar
PVP	–	Polyvinyl pyrrollidone
RAPD	–	Random Amplified Polymorphic DNA
RFLP	–	Restriction Fragment Length Polymorphism
SDS	–	Sodium dedecyl sulphate
TAE	–	Tris acetic acid EDTA
Tris-HCL	–	Tris (hydroxy methyl) aminomethane hydrochloride
µl	–	microlitre
µm	–	micromolar

INTRODUCTION

1. INTRODUCTION

Orchids are among the most beautiful flowering plants in the world. They belong to the family Orchidaceae. It is the largest family among the flowering plants with about 35000 species and 800 genera with innumerable hybrids and inexhaustible varieties. Among the genus *Dendrobium* is one of the largest with over 1210 species, showing, diverse vegetative and floral characteristics. *Dendrobium* belongs to the sub tribe Dendrobiinae of sub family *Epidendroideae*. It is distributed throughout the world with a greater concentration in South East Asia. Burma, Australia, New Zealand, China and Japan. In India it is represented by about 150 species occurring mainly in Eastern Himalayas and Western ghats (Bose and Bhattacharjee, 1980). *Dendrobium* is widely used for cut flower production with much contribution to the agribusiness activity in Kerala. Majority of the commercial orchids grown today are hybrids derived from *Arachnis, Vanda, Renanthera, Ascocentrum, Cymbidium, Cattleya, Dendrobium, Oncidium, Phalaenopsis* and *Paphiopedilum* (Mercy and Dale, 1997). Most of the hybrids grown in Kerala are exotic.

Adaptation to different environments has resulted in extreme modifications of organs, particularly those of the vegetative body. The epiphytic nature of most of the orchid genera is the main reason for their stability and wider adaptation. The large number of species and diversified gross morphology are the reasons for the complexity of *Dendrobium* taxonomy and to establish its intrageneric and intrasubtribal relationships. Hence the validity of various orchid classifications stands debated. To overcome these, diagnostic characters other than gross morphology are strongly needed. Several morpho-anatomical characters have been used widely in taxonomic and phylogenetic studies because they can provide valuable information. The importance of some of the

modern taxonomic parameters is being increasingly realized but sparingly used for characterization.

Morpho-anatomical characterization has certain limitations as they could be influenced by environmental and developmental factors. But molecular markers have greater dependability utility than morphological markers.

Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) have been extensively used for characterization studies. Their important applications include cultivar identification, variability studies, hybridity testing, genome mapping etc. The orchid flora of India can be characterized for using breeding programmes by these techniques.

Keeping all these points in view an experiment was conducted with the following objectives.

1. Evaluation of growth and inflorescence production in *Dendrobium* varieties.
2. Morpho- anatomical characterization of *Dendrobium* varieties.
3. Molecular characterization of *Dendrobium* varieties using Random amplified Polymorphic DNA (RAPD) technique.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Orchids exhibit wide variations in nature with regard to specific morphological and floral characters. This may be due to their high cross pollination nature as well as the evolutionary response of germplasm to macro and micro climatic conditions within a locality. Therefore knowledge about their pattern of genetic variation enables the breeder to know the magnitude of genetic variation available within them for selection and the breeding procedure to be adopted for crop improvement. Morphological, anatomical and molecular characterizations are possible using a wide range of characters and molecular markers in orchids. In this chapter, attempts are made to review the work on morphological and anatomical variability in orchids and use of molecular markers in ornamental crops.

2.1 MORPHOLOGICAL VARIABILITY

The success of any breeding programme mainly depends upon the extent of genetic variability available in the population. Genotypic coefficient of variation indicates the relative magnitude of genetic diversity present within the population and helps to compare the pattern of genetic similarity or dissimilarity present for various characters.

The genus *Dendrobium* comprises of 1210 species making it one of the largest of Orchidaceae. The constituent species exhibit diverse vegetative and floral characteristics and have been grouped into 41 sections (Schlecter *et al.*, 1982; Shekar and Vij, 1986). Many *Dendrobium* cultivars grown for cut flowers have been derived from intersectional crosses involving the *Phalaenathe* and *Ceratobium* (spatulata) sections. *Phalaenathe* species generally have large full flowers while *Ceratobium* species generally have small individual flowers.

Intersectional hybrids have many intermediate flowers with desirable cut flower characteristics.

Barthlott (1981) and Atwood and Williams (1979) have studied morphological features of orchids as potent indicators of taxonomic relationships.

Arditti *et al.* (1979, 1980) reported that certain morphological differences such as size and colour of seed confirmed taxonomic relationships of orchids.

Balfour and Linder (1990) studied intraspecific variation of individuals from seven groups of populations of *Disa uniflora* based on 16 floral and vegetative morphological features.

Anthecology and vegetative reproductive structures especially the pollinarium studies in numerous species of five genera (*Catasetum*, *Cyenoches*, *Mormodes*, *Clowesia* and *Dressleria*) in subtribe *Catasetinae* (*Orchidaceae*, *Cymbidieae*) revealed that the subtribe is remarkably uniform in pseudobulb and leaf morphology and anatomy, with the possible exception of *Dressleria* (Romero, 1990).

Amore and Kamemoto (1992) compared the yield and morphology of eleven named diploid *Dendrobium* hybrids and their corresponding colchicine – induced amphidiploids from intersectional combinations of *Ceratobium* x *Eleutheroglossum*, *Phalaenanthe* x *Ceratobium* and *Phalaenanthe* x *Eleutheroglossum*. The amphidiploids were found to be less floriferous and produced fewer and shorter racemes than their corresponding diploids. The amphidiploids often had greater flower length and width and wider dorsal sepals, longer and wider petals.

Kores *et al.* (1993) evaluated three species of *Cyrtostylis* (*C. oblonga*, *C.reniformis* and *C.robusta*) using morphometric methods, specifically thin plante spline analysis of leaf perimeter landmarks and eigen shape analysis of leaf outlines and found that *C. oblonga* and

C. reniformis are well delimited, but *C. robusta* is morphologically indistinguishable from *C. reniformis*.

Rajeevan and Sobhana (1993) evaluated 11 orchid species on flower characters like inflorescence length and the number, size, colour, fragrance and flowering period to identify the species suitable for cut flower and pot plants.

Albert (1994) studied the cladistic relationships of the slipper orchids (*Cypripedioideae, Orchidaceae*) based on morphological and molecular characters and inferences on slipper orchid phylogeny and biogeography were made. The analysis supported the monophyly of *Cypripedium*, *Paphiopedilum* and *Phragmipedium*.

Freudenstein and Doyle (1994) studied the variation between species of *Corallorhiza* (*C.maculata, C.mertensiana* and new *Corallorhiza* species) based on plastid DNA and morphological features. The phylogenetic pattern based on plastomes revealed that, *C.mertensiana* and the new *Corallorhiza* species were derived from ancestors much like *C.maculata* or from *C.maculata* itself though the three species had unique morphological character combinations.

Pun *et al.* (1994) studied natural variation in *Vanda tessellata* in the Chandaka forest of Orissa and identified seven distinct variants. Of the variants studied variant No:2 was found to be most potent with desirable cut flower qualities.

Caputo *et al.* (1997) studied the hybrid status of a natural hybrid (*Orchis alata*) between *Orchis laxiflora* and *O.morio* based on morphological and molecular characterization. The morphological characters of the natural hybrid showed intermediacy between those of the parent species and restriction fragment length polymorphism (RFLP) analysis confirmed its hybrid origin.

Prasad *et al.* (1997) conducted environmental correlation studies in 20 orchid cultivars on characters such as plant height, number of leaves

per plant, leaf length, leaf diameter etc. and found significant negative relationship between length of leaf and diameter of flower in both seasons.

The genetic divergence within and among early flowering *Gymnadenia conopsea* subsp *conospea* and late flowering *G.conopsea* subsp. *densiflora* was studied (Solivia and Widmer, 1999) using allozyme and morphological variations. Alloenzyme variation indicated significant variation between the subspecies. Floral characters were variable within the subspecies but could not consistently separate the early from the late flowering populations. A weak separation between subspecies was found in vegetative characters.

Goldman and Orzell (2000) distinguished *Calopogon multiflorus* from *Calopogon barbatus* using several taxonomic characters and some phenological and ecological differences.

2.2 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.

2.2.1 Root

In general, in transection, the roots of *Dendrobium* are circular in outline. The velamen is 5-6 cell layers thick. The cortex is differentiated into three layers namely the exodermis, cortex proper and the endodermis; the outer and inner regions comprising of 1-2 layers each of small cells and middle region of larger ones.

Garay (1972) reported that the presence of velamen is a characteristic feature of epiphytes, though it is present in some terrestrial orchids too.

The roots of *Sobralia macrantha* was reported to be equipped with a multilayered velamen, underlaid by a distinct cortical exodermis. The velamen thickness varied between three or four cell layers (Benzing *et al.*, 1982)

Pridgeon (1982) investigated the vegetative anatomy of 200 species in 22 genera of subtribe *Pleurothallidinae*. The epidermis of all species consisted of a uniseriate, biseriate or multiseriate velamen layer usually consisted of relatively small, elliptical to polygonal cells while the inner layer was made up of elliptical to rectangular or polygonal shaped cells. The number of velamen layers varied with the genera. There was little variation in cortex among the species studied except for *Pleurothallis* species, which consisted of relatively large cells.

Benzing *et al.* (1983) studied the anatomy of shootless, semi-shootless and leafy types of epiphytic orchids. Shootless species had a more elaborate aeration apparatus at the velamen-cortex interface. Velamen thickness varied greatly among the species studied.

Liang and Zheng (1984) reported that the number of vascular bundles could be used as a morphological criterion to study evolutionary sequences in species *Anocetochilus formosanus, Goodyera velutina* and *Goodyera schlechtendaliana*.

Khasim and Rao (1986) studied the nature of velamen tissue of some epiphytic orchid roots and their habitat tolerance.

Rao and Khasim (1987) studied the vegetative anatomy of five species of *Coelogyne*, two of *Pleione*, *Otochilus alba* and *Pholidota imbricata*. The epidermis was multiseriate with two to five layers of velamen in all the taxa studied. The cortex was four or five cells thick

with one or two layers of small cells in the outer or inner layers and large cells in the middle layers.

In *Dendrobium jenkinsii*, the velamen thickness was 5-6 cell layers. The cortex was divided into outer, middle and inner regions, with the outer and inner regions comprising 1-2 layers each of small cells and the middle region of larger ones (Isaiah and Rao, 1992).

Raju (1996) studied the vegetative anatomy of *Vanilla wightiana* and reported that the root was circular in outline, with a multiseriate epidermis. The velaman was composed of 1-2 layers of radially elongated rectangular cells with cutinised walls. Cortex was 8-13 layers thick with polygonal to oval cells. The cells of the outer 1-2 layers were relatively small.

The anatomy of leaves, stems and roots of 35 orchid species (substribe : Orchidinae) was investigated by Stern (1997).

Both the aerial and terrestrial roots in vanilla had uniseriate velamen as reported by Stern and Judd (1999).

2.2.2 Leaf

In *Dendrobium*, the leaves are usually entire, fleshy, glabrous, leathery, parallel veined, linear or lanceolate and with an outer coat of wax. The leaves are generally leathery in texture.

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

Rosso (1966) distinguished plicate leaved genera and con-duplicate leaved genera of *Cypripedioideae* partially on the basis of cuticle thickness. The leaf anatomy of *Palumbina* and *Odontoglossum* sub genera *Osmoglossum* was reported by Ayensu and Williams (1972). Banerjee and Rao (1978) attempted epidermal studies in a few species of *Coelogyne*.

Inamadar (1968) studied the stomatal ontogeny in *Habenaria marginata* Coleb.

The development and organization of stomata in 48 orchid taxa including *Calanthe*, *Coelogyne*, *Cymbidium*, *Dendrobium* etc. was investigated (Singh, 1981). Three different forms of stomatal development were observed as well as combinations of different types of stomata on one and the same leaf.

Pridegeon (1982) reported several diagnostic anatomical characters like features of trichomes, cuticle, epidermal-wall thickness, stomatal apparatus, number of vein series and hypodermis of 200 species in 22 genera of sub tribe *Pleurothallidinae.*

Rao and Khasim (1987) investigated the anatomical details of leaf epidermis, petiole, pseudobulb and root in five species of *Coelogyne,* one of *Otochilus*, one of *Pholidota* and two of *Pleione.*

Four sub families *viz.*. Anomocyticeae, Diacyticeae, Cydocyticeae and Paracyticeae were identified in 53 species, based on stomatal feactures (Kaushik, 1983)

The utility of the dermal (foliar) features *viz.*, size, shape and margin of epidermal cells, the structure of trichomes and structural organization, distribution, frequency and index of stomata in understanding the phylogenetic and ecological significance of 43 species of ground growing and epiphytic orchids was reported by Vij *et al.* (1991).

Isaiah and Rao (1992) studied the vegetative anatomy of *Dendrobium jenkinsii.* The leaves are hypostomatic with paracytic stomata. The anatomical features of *Dendrobium* and *Bulbophyllum* were compared and their respective placement in the subtribes *Dendrobiinae* and *Bulbophyllinae* was supported.

Yukawa *et al.* (1992) divided 153 species of the genus *Dendrobium* into three groups *viz.*, species group I, species group II and section *Grastidium* on the basis of the shape and size of the outer stomatal ledge.

Leaf anatomy of 25 species in 15 genera of *Caladeniinae* was investigated by Pridgeon (1994) for cladistic analysis and to assess the interspecific relationship of the subtribe.

The anatomical characters of the thick leaved *Dendrobium* section *Rhizobium* was studied by Stern *et al.* (1994). The anatomical data supported the monophyly of section *Rhizobium* and the unifacial leaved species constituted a distinctive clade within the section.

Peak and Jun (1995) reported that in 33 orchids the stomata were mainly confined to the abaxial side of leaves except in *Bletilla striata*. Similarly in the five economically important species of the genus *Paphiopedilum*, the stomata occurred only on the lower epidermis and all the species had a stomatal frequency ranging from 13.7 to 46.8 stomata per mm^2 (Handique and Handique, 1996).

Carlsward *et al* (1997) compared the leaf anatomy and systematics in *Dendrobium* sections *Aporum* and *Rhizobium*. A cladistic analysis performed with various anatomical characters of the leaf confirmed that both groups are monophyletic.

2.3 GENETIC ANALYSIS AND VARIABILITY STUDIES

Rehman *et al.* (1993) conducted the genetic analysis of *Dendrobium aggregatum* and certain other species grown in the plains of West Bengal. High degree of genetic variance was recorded for length of inflorescence, number of flowers per inflorescence and flower size. Heritability and genetic advance estimates were also high for these characters indicating selection based on these characters would be successful.

Genetic analysis in *Dendrobium* hybrids was conducted by Sobhana (2000). High genetic variability was observed for number of flowers per spike, days for opening of florets and number of shoots per plant. Heritability was of moderate to high magnitude for most of the characters. Flower size exhibited the highest heritability. Length of inflorescence

exhibited high positive correlation with height of shoots and number of leaves.

Lekharani (2002) studied intra and interspecific hybridization in *Dendrobium* spp. High GCV and PCV were recorded for the number of nodes per cane, leaf area per cane, length of inflorescence, length of scape and number of flowers per inflorescence. High heritability estimates were recorded for floral characters. High positive correlation at genotypic and phenotypic levels were observed for most of the vegetative and floral characters studied.

2.4 CYTOLOGY STUDIES

The chromosome constitutes the integral part of any genetic system. Evaluation is primarily based on the changes in the chromosome number and structure. So cytological studies have an important bearing on the variation studies. The first chromosome number counts in *Dendrobium* sp were reported by Hoffman (1929). In *Dendrobium*, polyploidy and aneuploidy along with chromosome changes have played an important role in evolution.

The basic chromosome number of the *Dendrobium* species varied from n=19 to n=22. The genus *Dendrobium* has in majority of the species a basic set of 19 chromosomes (Sharma and Chatterjee, 1966).

Kashyap and Mehar (1983) conducted cytological investigations on 12 species of Orchidaceae belonging to eight genera. Studies on karyotype analysis and chromosome behavior of *Coelogyne, Pholidota, Dendrobium, Aerides, Sarcochilus, Hexisea, Cleistostoma, Rhynchostylis, Stauropsis* and *Vanda* were reported by Sau and Sharma (1983).

Chung *et al.* (1990) examined the chromosome of *Cymbidium goerinigii* samples. The chromosome number was 2n=40 with 13 pairs of metacentrics, six pairs of sub metacentrics and a pair of subtelocentrics.

Chromosomal relationships and evolution of some Sarcanthine orchids were reported by Chatterji (1990).

Lee (1991) studied the genomic constitutions and flower characteristics of selected *Aranda* orchid cultivars. The size of flower as well as of sepals and petals increased with increase in ploidy level. The diploid cultivars tend to have less compact sprays with flowers more distantly spaced out.

Bhattarai and Malla (1993) conducted karyotypic analysis of five diploid plants of *Cymbidium cyperifolium*, one cytotype had a chromosome number of 2n=36 and the other 2n=40.

2.5 MOLECULAR MARKERS

Traditionally various morphological, anatomical, cytological and physiological methods have been used to distinguish plant species and varieties. The limitations of these methods have prompted the identification and use of more reliable methods. During the last decade, the use of molecular markers has significantly contributed to our understanding of the species at the genetic level. Protein markers and DNA markers have the widest applications among these. The important applications include cultivar identification, variability studies, hybridity testing, genome mapping etc.

2.5.1 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.

Enzyme polymorphisms have been used successfully to study the variability in *Cymbidium* (Park *et al.*, 1990; Okeyo *et al.*, 1998), *Phalaenopsis* (Hsieh *et al.*, 1992) and *Epipactis* (Hollingsworth and Dickson, 1997; Ehlers and Pedersen, 2000). Characterisation of natural hybrids between *Pterostylis alveata* Garnet and *P. ophioglossa* R.B was reported by Sharma and Jones (1999).

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). Taxonomic studies on *Calanthe* (Ryuk *et al.*, 1999) and *Lilium longiflorum* Thunb. (Wen and Hsiano, 1999) using enzyme polymorphism were also reported.

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

2.5.2 DNA Markers

In contrast to the morphological and protein markers, DNA markers directly assess the genetic differences between species at the DNA level.

2.5.2.1 Restriction fragment length polymorphism (RFLP)

In this method, the genomic DNA's 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.

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

Wolff *et al.* (1994) developed RFLP probes and primers in chrysanthemum. In carnation, the locus controlling flower type (Double / semi double) was identified using RAPD and RFLP markers (Scovel *et al.*, 1998).

In petunia, RFLP mapping has been carried out for certain identified genes namely the actin gene families and chalcone synthase genes (Koes *et al.*, 1987 and McLean *et al.*, 1990).

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.

2.5.2.2 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 oligonucleotide 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 analyzed on agarose gels. Since this technique is being adopted in the present study much of the literature reviewed pertains to this technique in ornamental crops.

2.5.2.2.1 RAPDs in orchids

Fu *et al.* (1994) reported that RAPD technique was used to identify wild *Phalaenopsis* species and to study their relationship.

DNA polymorphism within the genes *Cattleya* was studied using RAPD (Benner *et al.*, 1995). A high level of molecular variability was detected among the eight species, with each of them exhibiting an unique DNA finger print with 9 out of 10 arbitrary primers used in single primer RAPD reactions.

The genetic variability in the taxa of the *Ophrys bertolonii* aggregate was studied using RAPD (Grunanger *et al.*, 1998). A high genetic variability was observed within the same populations of *Ophrys bertolonii* aggregate.

Okeyo and Kako (1998) reported that RAPD technique was used to study genetic diversity and to identify *Cymbidium* cultivars. A total of 132 RAPD markers, 78 per cent of which were polymorphic were

produced from 15, 10-mer arbitrary primers. All cultivars were distinguishable when a number of primers were considered.

Lim *et al.* (1999) studied the genetic closeness of various species of *Vanda* using RAPD markers. Strap leaved *Vanda* species (including *Vanda sanderiana*) and *Ascocentrum miniatum* were more closely related to each other than to the terete leaved *Vanda* species studied. RAPD analysis supported the suggestion that terete leaved *Vanda teres* and *Vanda hookeriana* be classified in the separate genus *Papillionanthe* and that *Vanda sanderiana* should remain in the genus *Vanda*.

2.5.2.2.2 RAPDs in other ornamentals

2.5.2.2.2.1 RAPD's for cultivar identification

Torres *et al.*, (1993) differentiated rose cultivars Cardinal, Sonia, Carta Blanca, Laser and Cartade Oro using eight primers. All cultivars were distinguished by comparing differences in DNA banding patterns. RFLP and RAPD patterns were compared and 20 of the 22 cultivars investigated were characterized (Ballard *et al.*, 1996).

RAPD markers were used to study cultivar identification in Chrysanthemum (Wolff *et al.*, 1995).

Jau-Yueh *et al.* (1999) used RAPD markers to identify and to assess genetic diversity among 15 *Anthurium* cultivars. Twenty-four DNA fragments derived from eight primers were polymorphic and were used to distinguish the cultivars.

RAPD markers were also reported to be used to identify cultivars of Callalily (Hamada and Hagimori, 1996), *Poinsettia* (Jing Tian *et al.*, 1977), *Azalea* (Kobayashi *et al.*, 1995), *Pelargonium* (Lesur *et al.*, 2000) and *Alstroemeria* (Beneditti *et al.*, 2000).

2.5.2.2.2.2 RAPD's for variability studies

Aloisi *et al.* (1996) studied the molecular polymorphism in botanical and ancient roses via RAPD analysis using 10-mer operon primers and observed large variations.

RAPD techniques have been reported to be used to study the genetic variation in Chrysanthemum (*Dendranthema grandiflora*). The variation between cultivars was high and the cultivars were distinguished from each other by using only two different primers (Wolff and Rijn, 1993).

Scott *et al.* (1996) examined twenty-one cultivars of chrysanthemum that belonged to several species using RAPD technique. A few polymorphic characters were uniquely identified in closely related cultivars within each of the species. In contrast many DNA polymorphisms were observed between members of the different families.

RAPD technique was used to study the genetic diversity and relationship between different species of *Jasminum* (Mukundan, 2000) and *Heliconia* (Goh *et al.*, 1995).

2.5.2.2.2.3 Taxonomic studies

Millan *et al.* (1996) analysed nineteen species of rose using RAPD markers. Each 10 base long arbitrary primer produced a specific banding pattern that grouped plants belonging to the same species in 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).

RAPD markers were used to assess the relationship among species, cultivars and hybrids of *Lilac* (Marsolais *et al.*, 1993) in the classification of *Lilium* (Jongsuk *et al.*, 1994; Haesun *et al.*, 1999) the taxonomic positioning of Kalanchoe (Gehrig *et al.*, 1997) and in the phylogenetic relationships between *Hibiscus syriacus* (Malvaceae) and 26 allied species (Jong-Hwa *et al.*, 1999)

2.5.2.2.2.4 Genetic map

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. Of these, 278 could be located on the 14 linkage groups of the two maps covering a total map length of 326 and 370 cM respectively.

Peltier *et al.* (1994) established a linkage map for *Petunia hybrida* based on the RAPD and phenotypic markers. The map consisted of 35 RAPD loci, which covered 262.9 cM with a mean distance of 8.2 cM, dispersed over 7 linkage groups.

2.5.2.2.2.5 Parentage analysis

Debener *et al.* (1997) reported that molecular markers were used for the identification of inter-specific roses hybrids in three crosses *viz.*, *Rosa acicularis* x *R. majalis, R. obtusifolia x R sherardii* and *R. stylosa* x *R. gallica*. All putative F_1 hybrids were unambiguously identified as true hybrids using 24 – 42 RAPD markers.

RAPD markers were used to verify inter-specific hybridization in *Alstroemeria* (Benedetti *et al.*, 2000). Five putative inter-specific hybrids and their parents were analysed by four RAPD primers. The putative parentage was confirmed in four hybrids and was excluded in one.

2.5.2.2.2.6 Identification of chimera, sports and mutants

Debener *et al.* (2000) used RAPD and AFLP markers to study the genetic difference between sports from two cut flower rose varieties as well as a garden rose variety. There was no polymorphism between the sports of the cut rose variety and the original cultivar, whereas five polymorphism were detected between the garden rose variety and its sports.

RAPD markers have been reported to be used to study sporting and chimerism in chrysanthemum (Shibata *et al.*, 1998).

Pathania and Misra (2001) confirmed the mutability of gladiolus cv Eurovision mutants. Out of 7 random primers used for PCR amplification one primer OPX-02 produced polymorphic banding pattern. A monomorphic band of 1 Kb was present in both the parent cultivar and its mutant. However a single band 0.8 Kb difference between five mutants ERM 3, ERM 4, ERM 5, ERM 6 and ERM 7 was obtained.

RAPD is a fast and sensitive method, requiring only small amounts of DNA and involving no radioactivity hazards. However RAPD's have certain quality problems like confinement to dominant gene expression and occasional low reproducibility of results.

2.5.2.3 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.

AFLP markers were used to identify rose varieties (Zhang *et al.*, 2000). With twelve pre-screened primer combinations 322 AFLP markers were generated in thirteen modern rose varieties and their polymorphism was studied. The intra variety variability was also evaluated.

Lesur *et al.* (2000) compared molecular methods namely RFLP, RAPD, SCAR (Sequence characterized amplified region) STMS (PCR amplification of micro satellite motives), Oligo finger printing Intersimple sequence repeat PCR and AFLP inorder 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 reproducibility.

AFLP proved to be much more powerful and reliable tool capable of producing a large number of genomic loci and discriminate genetic difference between phenotypically similar individuals with high reproducibility.

2.5.2.4 Minisatellites (Variable Number of Tandem Repeats (VNTR))

Jeffreys *et al.* (1985b) was the first to report on the presence of minisatellite hyper variable sequences in the human genome that could be used for DNA finger printing. Minisatellites are repeat sequences having repeat units ranging from 11 to 60 bp in length. The repeat sequences comprise upto greater than 90 percent of total DNA in certain plant genomes. The conserved sequence flanking minisatellites can be amplified using a suitable primer to reveal the polymorphism. The polymorphisms are attributed to the variation in the length of minisatellites.

Cafasso *et al.* (2001) reported the occurance of a tandem repeat in the chloroplast genome of the marsh orchid *Orchis palustris*. The repeat unit is an AT rich, 16 bp sequence located in the chloroplast tRNALEU intron. The 16 bp repeat unit was found to be present in all *O.palustris* accessions studied, as well as in closely related *O.laxiflora*.

Vainstein and Ben-Meir (1994) reported that DNA fingerprinting using mini and microsatellite probes was useful for cultivar identification in roses.

In contrast to RAPD the banding pattern yielded by minisatellites probes is highly reproducible but it involves high cost and complexity.

2.5.2.5 Microsatellites

The term microsatellites was introduced by Litt and Lutty (1989). Microsatellites are tandem repeats of DNA sequence of only a few base pairs (1-6) bp in length. The most abundant being the dinucleotide repeats. They show comparatively low degree of repetition and dispersed

distribution over many genomic loci. The microsatellites can be amplified using a suitable primer to reveal polymorphism.

Solivia *et al.* (2000) isolated and characterized a microsatellite loci from *Ophrys araneola* to study the influence of pollinating system on population genetic structure and on gene flow between similar co-flowering *Ophrys* species.

Micro satellites were used to study the genetic diversity in natural populations of *Dianthus* species and for the identification of carnation varieties. (Smulders *et al.*, 2000)

The microsatellites are highly polymorphic and thus highly informative. Being shorter in length they are easy to be cloned, sequenced and amplified through PCR. But the identification of informative microsatellite loci and consequence of suitable primer sequence is more cumbersome and expensive.

MATERIALS AND METHODS

3.MATERIALS AND METHODS

The present investigation on morpho-anatomical and molecular characterization on *Dendrobium* cultivars was carried out in the Department of Pomology and Floriculture, College of Agriculture, Vellayani during 2000-2001. The experimental site was located at an altitude of 29 m above MSL and at latitude 8°N and 76°E longitude. The materials used and the methods followed are described below.

3.1 EXPERIMENT 1

3.1.1 Materials

The following 15 varieties of near flowering size tissue cultured *Dendrobium* plants were used as the experimental material.

Varieties

1. *D.* Sonia-28
2. *D.* Rinappa-3
3. *D.* Jacquelin Concert x *D.* Mme. Udomsiri
4. *D.* Brook Shields
5. *D.* Lady Pink
6. *D.* Snow White
7. *D.* Candy Stripe x Tomie Drake
8. *D.* Kasem White
9. *D.* Tayswee Keng x Lady Charm
10. *D.* Sakura Pink
11. *D.* Midnight Velvet Lipstick
12. *D.* Jacquelin Concert x Lady Charm
13. *D.* Candy Stripe hybrid
14. *D.* Caesar Candy
15. *D.* Thailand White

3.1.2 Methods

The selected varieties were established in charcoal medium in orchid pots and experiment 1 was laid out in completely randomized design with three replications during November 2000 and continued up to October 2001. The plants were placed on raised GI frames under black UV stabilized high density polyethylene shade nets calibrated to provide 50 per cent shade.

The plants were mist irrigated with micro sprinklers once or twice a day depending on temperature conditions. A nutrient mixture containing NPK 13:27:27@225 mg/plant was given as foliar spray at fortnightly intervals. As a supplement, NPK mixture (17: 17:17) @ two per cent was also sprayed at monthly interval. Supernatant liquid drawn from a fermented slurry prepared with a mixture of cowdung @ 20g/p and neem cake @ 5g/p was diluted 5 to 6 times and applied at monthly intervals.

The plants were drenched with Bavistin 50 percent W.P @ 2 g/litre immediately after planting. Thereafter prophylactic applications of insecticides and fungicides were given once a month.

3.1.2.1 Biometric observations

The observations on the following characters were recorded from all the plants used in the experiment.

3.1.2.1.1 Growth parameters

3.1.2.1.1.1 Rate of shoot elongation (cm/month)

The length of the shoots was measured at monthly interval and the mean rate of elongation per month was recorded.

3.1.2.1.1.2 Rate of increase in shoot girth (cm/month)

The maximum girth of all the shoots was measured at monthly intervals and the mean rate of increase in girth per month was recorded.

3.1.2.1.1.3 Number of basal shoots produced

The number of basal shoots produced after planting was recorded for each plant and their mean values were calculated.

3.1.2.1.1.4 Days taken for completion of leaf unfurling

The number of days taken by the shoots from initiation to complete unfurling were recorded and the mean values calculated.

3.1.2.1.1.5 Rate of leaf elongation (cm/week)

The length of the leaves was measured in weekly intervals and the mean rate of increase in leaf length per week was recorded.

3.1.2.1.1.6 Increase in leaf area per shoot (cm^2/month)

The length and the maximum width of all the leaves in each shoot were recorded at monthly intervals. The leaf area was calculated using the formula,

$$y = k \times lw$$

Where, y - is the leaf area

l - length of the leaf

w- width of the leaf and

k- a constant

The value of the constant was found to be 0.7160 for *Dendrobium* (Thekkayam, 1996). The mean rate of increase in leaf area per shoot per month was calculated.

3.1.2.1.1.7 Leaf area at completion of leaf unfurling (cm^2)

The leaf area of the shoots after complete leaf unfurling was measured and their mean values recorded.

3.1.2.1.1.8 Leaf area at inflorescence emergence (cm^2)

The total leaf area of the plant was measured when the inflorescence was one cm length and the mean values recorded.

3.1.2.1.1.9 Leaf area at first flower opening (cm^2)

The total leaf area of the plant was calculated when the first flower of the inflorescence opened and the mean values were recorded.

3.1.2.1.1.10 Days taken from inflorescence emergence to full bloom

The number of days taken by the inflorescence from emergence to complete opening of all the flowers were counted and the mean values were recorded.

3.1.2.1.1.11 Days taken from inflorescence emergence to first flower opening

The number of days from the inflorescence emergence to first flower opening in a spike were counted and the mean recorded.

3.1.2.1.1.12 Rate of inflorescence elongation (cm/week)

The increase in the length of the inflorescence from emergence to full bloom was measured at weekly interval and the mean rate of elongation per week was recorded.

3.1.2.1.2 Morphological characters

3.1.2.1.2.1 Shoot length (cm)

The length of the shoots from the base to its tip was measured and recorded.

3.1.2.1.2.2 Shoot girth (cm)

The maximum girth of the shoots was measured and recorded.

3.1.2.1.2.3 Internodal length of the shoots (cm)

The maximum internodal length of the shoots was measured and recorded.

3.1.2.1.2.4 Number of laminate leaves per shoot

The number of leaves present in the shoots were counted and recorded.

3.1.2.1.2.5 Number of nodes per shoot

The number of nodes in the shoots was recorded

3.1.2.1.2.6 Length of the leaves (cm)

The length of the five randomly selected fully expanded leaves was measured and recorded.

3.1.2.1.2.7 Width of the leaves

The maximum width of the leaves selected for the above observation was measured and recorded.

3.1.2.1.2.8 Orientation of the leaves

The angle of inclination of the leaf tip with that of the shoot was measured using a protractor for five randomly selected leaves and the values recorded in degrees.

3.1.2.1.3 Inflorescence characters

3.1.2.1.3.1 Length of the inflorescence (cm)

The length of the inflorescence was measured from the base to the tip and recorded.

3.1.2.1.3.2 Thickness of the inflorescence stalk (cm)

The thickness of the inflorescence stalk was measured in cm from the base and the value recorded.

3.1.2.1.3.3 Internodal length of the stalk (cm)

The maximum internodal length of the stalk was measured and recorded.

3.1.2.1.3.4 Number of flowers

The number of flowers in the inflorescence were counted and recorded.

3.1.2.1.3.5 Length of the flowers (cm)

The linear spread between the tip of the dorsal sepal and the lip was measured in all the flowers in a spike and recorded.

3.1.2.1.3.6 Width of the flowers (cm)

The spread between the tip of the two lateral petals was measured for all the flowers in a spike and recorded.

3.1.2.1.4 Anatomical characters

3.1.2.1.4.1 Root thickness (mm)

The diameter of the cross section of three randomly selected roots was measured under microscope using a caliberated occular micrometer. The value of one division of the occular micrometer was standardized using a stage micrometer.

3.1.2.1.4.2 Thickness of the cortex (mm)

The thickness of the cortex of root sections was measured using a caliberated occular micrometer under 100x magnification and recorded.

3.1.2.1.4.3 Number of layers in the cortex

The number of layers present in the cortex region was recorded.

3.1.2.1.4.4 Number of layers of the velamen tissue

The number of layers present in the velamen tissue was observed and recorded.

3.1.2.1.4.5 Leaf thickness (mm)

Free hand cross-sections of three randomly selected leaves were observed under microscope and the thickness was measured using occular micrometer and rendered.

3.1.2.1.4.6 Number of stomata in the adaxial surface of the leaf (per cm^2)

A thin film of quick fix was applied over three randomly selected leaves. The film was removed after few minutes and the number of

stomatal impressions were counted using a microscope (100x magnification). The area of the microscopic field was calculated using a stage micrometer and the number of stomata per unit area was calculated and recorded.

3.1.2.1.4.7 Number of stomata in the abaxial surface of leaf (cm^2)

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

3.1.2.1.4.8 Petal thickness (mm)

Free hand sections of the petals were taken and the thickness was measured under the microscope using an occular micrometer and recorded.

3.1.2.1.4.9 Number of pigmented layers

The number of pigmented layers present in the petals of coloured varieties was observed and recorded.

3.1.2.1.5 Qualitative characters

The following qualitative characters were recorded visually.

3.1.2.1.5.1 Shape of the leaves

The shape of the leaves was recorded.

3.1.2.1.5.2 Leaf sheath colour

The leaf sheath was observed for presence or absence of pigmented streaks and was recorded.

3.1.2.1.5.3 Hairiness of the leaf sheath

The leaf sheath was observed for the presence or absence of hairs and observations recorded.

3.1.2.1.5.4 Degree and direction of torsion of the flowers

The angle described by the flower buds during resupination, relative to the initial and final position of their lip at flower opening and the direction of their movement was recorded.

3.1.2.1.5.5 Flower colour

The photographs of the flower were taken and the colour group to which they belonged was determined by comparing it with the colour chart of "The Royal Horticultural Society", London, available in the Indian Institute of Horticultural Research, Bangalore

3.1.2.1.5.6 Shape of the perianth lobes

The shape of the perianth lobes was observed and recorded.

3.1.2.1.6 2n chromosome count of the cultivars

The technique suggested by Tanaka and Kamemoto (1984) was followed to count the 2n chromosome number of the varieties. The slides were observed under 1000 X magnification. However, the greater number and relatively smaller size of the chromosomes precluded the taking of exact counts accurately.

3.1.3 Statistical analysis

The data collected were subjected to the following statistical analysis.

Mean, variance, standard error and co-efficient of variation were estimated. Analysis of Variance (ANOVA) technique was used to test the significance of genotypic differences. The character associations were estimated through correlation coefficients using Analysis of Covariance (ANACOVA) technique (Panse and Sukhatme, 1967).

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

3.1.3.1 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

3.1.3.2 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	$\sigma_{gxy} = \frac{G_{xy} - E_{xy}}{r}$	$\sigma_{exy} = E_{xy}$	$\sigma_{pxy} = \sigma_{gxy} + \sigma_{exy}$

3.1.3.3Coefficient 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

3.1.3.4 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 a broad sense was estimated as the proportion or heritable components of variation (Jain, 1982).

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

$$\text{Genetic advance as percentage of mean (GA)} = \frac{KH^2\sigma px}{\bar{x}} \times 100$$

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

3.1.3.6 Mahalanobis (D^2) analysis

Mahalanobis D^2 (1936) technique was applied to cluster the 14 Dendrobium varieties for the i^{th} and j^{th} genotypes, the D^2 value is computed as

$$D^2 = \sum_{i=1}^{k} (X1) \text{ where k is the number of characters}$$

The genotypes were grouped into several clusters based on these D^2 values by Tocher's method of clustering (Rao, 1952). One of genotype was excluded for clustering in order to have more clusters instead of one.

3.1.4 Molecular Characterisation

The investigations were carried out at the plant Molecular Biology and Bio-Technology Centre, College of Agriculture, Vellayani and the College of Horticulture, Thrissur. The materials employed and methodology followed are described below.

3.1.4.1 Materials and procedure

The 15 varieties of *Dendrobium* used in the Experiment I were studied for molecular characterization. Leaf samples were collected from young new leaves of *Dendrobium* plants

3.1.4.2 Method

The extraction protocol was modified from that of Mondal *et al.* (2000) with out the use of CTAB. Briefly 0.5 g of leaf material was pulverized in liquid nitrogen with 20.0 µl β-mercaptaethanol in pre-cooled mortar by rapid grinding to a fine powder. Then 7.5 ml of hot (65^0c) extraction buffer (100mM Tris-HCl ,20 mM EDTA ,2M NaCl, 2 percent (w/v) SDS, pH=8) and a pinch of polyvinyl pyrrollidone (PVP) were added. The fine slurry of grounded plant material was transferred to a 50 ml conical flask and incubated in water bath at 65^0C for 20 minutes with occasional gentle shaking. The lysate was then squeezed through four layers of sterile muslin cloth, on to a sterile 50 ml centrifuge tube. After that an equal volume of chloroform: Isoamyl alcohol (24:1) was added and thoroughly mixed. The mixture was centrifuged at 1000 rpm for 10 minutes at 20^0C. The supernatant was transferred to another sterile centrifuge tube with a wide bore sterile pipette tip. To this again equal volume of chloroform : Isoamyl alcohol (24:1) was added and centrifuged as in the previous step after thorough mixing. After that, to the supernatant, $1/10^{th}$ volume of 3.0 M sodium acetate followed by double volume of chilled absolute alcohol were added. It was mixed gently and then centrifuged at 10000 rpm for 10 minutes at 4^0C to pellet the DNA. The supernatant was discarded and the pellet was washed in 70 percent ethanol. The pellet was air dried and then dissolved in 0.5 ml of 1xTris EDTA buffer (10 mM Tris HCl, 1mM EDTA; pH=8) and stored at 4^0C.

3.1.4.3Quantification of DNA

The quantification of DNA is necessary before it is subjected to amplification. The quantification of DNA was carried out with the help of uv-vis 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 280nm. The concentration of the DNA was found out using the formula:

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

Where A_{260}- absorbance at 260 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 1.8 and 2.0 indicates best quality of DNA, where A_{280} is the absorbance at 280 nm.

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 percent for visualising the genomic DNA and 1.4 per cent for visualizing the amplified products) and melted in 1xTAE buffer (0.04M Tris acetate, 0.001 M EDTA, pH 8.0) by boiling. After cooling to about 50^0C ethidium bromide was added to a final concentration of 0.5 µgml^{-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 (6.0 X loading dye *viz.*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 of the length of the gel. The gel was visualized using an ultraviolet visible (UV-Vis) transilluminator.

3.1.4.5Random Amplified Polymorphic DNA (RAPD)

DNA amplification was done using forty 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 ml 10 X buffer (10 mM Tris HCI pH 9.0, 1.50 mM Mg Cl_2, 50 mM KCI and 0.01 per cent gelatin), 10 pM primer, 250 µM each of deoxynucleotides (dNTPs), 0.6 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 95^0C for 1.0 minute, followed by 45 cycles of denaturatioin at 95^0C for 1.0 minute, annealing at 35^0C for two minutes and extension at 72^0C for 2.0 minutes. A final extension at 72^0C for 10 minutes was included after the last cycle. Finally the products of amplification were cooled to 4.0^0C. 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 and recorded. The PCR was repeated at least twice in order to confirm the reproducibility. The amplified products of four primers alone which could produce amplification for most of the clones were used for further analysis.

3.1.4.6 Data analysis

The reproducable bands were scored for their presence (+) or absence (-) for all the varieties studied. A genetic similarity matrix was constructed using the Jaccard's coefficient method (Jaccard, 1908)

(formula)

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

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

b = number of bands present in the first variety 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 distance between the varieties was computed with the help of a software package SYSTAT (version 9). Using these values of distance between the varieties, a dendogram was constructed by following the nearest neighbour (single link) method (Krzanowski, 1988). Association between the various varieties was assessed from the dendogram.

3.2 EXPERIMENT II – MORPHO-ANATOMICAL CHARACTERIZATION OF NINE *DENDROBIUM* VARIETIES

3.2.1 Materials

The following 9 varieties of *Dendrobiums* available at the college of Agriculture were used. These plants were of more than 3 years age and already well established in pots

Varieties

1. *D.* Uniwai Pink
2. *D.* Madam Pompadour
3. *D.* Nave Blue
4. *D.* Purple-1
5. *D.* Purple-2
6. *D.* Yupee Deewan
7. *D.* Oshin
8. *D.* Nagoya Pink
9. *D.* Waipahu Pink

3.2.3 Methods

The experiment was laid out in completely randomized design with three replications. The experiment was carried out from November 2000 to October 2001. The growing medium consisted of charcoal brick and coconut husks. The plants were grown under fifty percent shade.

The cultural operations were carried out as in the Experiment I

3.2.3.1 Observations

The observations on morphological, floral and anatomical characters were carried out as described in the Experiment I.

3.2.4 Statistical analysis

Analysis of variance (ANOVA) technique was applied to test the significance of genotypic differences.

RESULTS

4. RESULTS

The results of the present study on evaluation of growth morpho-anatomical and molecular characterization of 15 *Dendrobium* varieties (Experiment I) and the morpho-anatomical characterization of another nine *Dendrobium* varieties (Experiment 2) are presented below.

4.1 EXPERIMENT I

4.1.1 Analysis of variance of growth characters

Among the 12 traits studied the analysis of variance (Table 1) revealed significant differences between varieties for characters such as increase in shoot girth, increase in leaf area per shoot, leaf area at completion of leaf unfurling, leaf area at inflorescence emergence, leaf area at first flower opening and days taken from inflorescence emergence to full bloom. The mean performances of all the varieties for the growth characters are presented in Table 2.

In the rate of increase in shoot girth *D.* Caesar Candy was on par with *D.* Jacquelin Concert x *D.* Mme Udomsiri and *D.* Midnight Velvet x Lipstick while *D.* Candy Stripe x Tomie Drake was on par with the rest of the varieties with *D.* Candy Stripe recording the maximum (1.300 cm) and *D.* Candy Stripe x Tomie Drake (0.673 cm) recording the minimum values.

The increase in leaf area per shoot per month was highest (68.617 cm^2) in *D.* Caesar Candy. *D.* Snow White, *D.* Tayswee Keng x Lady Charm and *D.* Candy Stripe hybrid were on par with *D.* Caesar Candy. *D.* Lady Pink recorded the lowest value of 19.880 cm^2. Leaf area at the completion of leaf unfurling was greater (181.990 cm^2) in *D.* Caesar Candy and *D.* Jacquelin Concert x *D.* MME Udomsiri was on par with it and it was lowest (59.343 cm^2) in *D.* Jacquelin Concert x *D.* Lady Charm. The leaf area at inflorescence emergence showed significant variation among the varieties. The low value was recorded by *D.* Kasem White

Table 1. Analysis of variance of growth characters in 15 *Dendrobium* varieties

Sl. No.	Characters	Mean square	
		Varieties Df=14	Error Df=30
1.	Rate of shoot elongation (cm / month)	3.976 ^{NS}	2.835
2.	Rate of increase in shoot girth (cm / month)	0.091**	0.034
3.	Number of basal shoots produced	0.061^{NS}	0.956
4.	Days taken for completion of leaf unfurling	426.062 ^{NS}	293.270
5.	Rate of leaf elongation (cm / week)	0.145^{NS}	0.101
6.	Increase in leaf area per shoot (cm^2/month)	633.324**	154.712
7.	Leaf area at completion of leaf unfurling (cm^2)	4580.844**	1432.038
8.	Leaf area of inflorescence emergence (cm^2)	4167.263**	1027.460
9.	Leaf area of first flower opening (cm^2)	4031.174**	1156.621
10.	Days taken from inflorescence emergence to first flower opening	44.443*	18.583
11.	Days taken from inflorescence emergence to full bloom	37.449**	12.957
12.	Rate of inflorescence elongation (cm / week)	1.103^{NS}	0.932

** Significant at 1 per cent level * Significant at 5 per cent level DF- Degrees of freedom

Table 2. Differences in growth parameters among fifteen *Dendrobium* varieties

Varieties	Rate of shoot elongation (cm/month)	Rate of increase in shoot girth (cm/month)	Number of basal shoots produced	Days taken for completion of leaf unfurling	Rate of leaf elongation (cm/week)	Increase in leaf area per shoot (cm^2/week)	Leaf area at completion of leaf unfurling (cm^2)	Leaf area at inflorescence emergence (cm^2)	Leaf area of first flower opening (cm^2)	Days taken from infl. emergence to first flower opening	Days taken from infl. emergence to full bloom	Rate of infl. elongation (cm/week)
D. Sonia-28	6.267	0.983	2.333	107.223	1.783	19.880	78.520	114.233	108.213	44.667	48.333	5.973
D. Rinnappa-3	5.047	0.697	1.667	138.667	1.503	46.640	139.823	176.217	158.583	43.000	46.667	4.713
D. Jacquelin Concert x D.Mme Udomsiri	5.010	1.100	1.667	124.667	1.510	68.420	180.523	180.713	176.863	32.833	38.167	4.540
D. Brook Shields	5.143	0.737	2.000	129.000	1.867	45.032	126.58	173.983	133.820	39.000	44.000	5.840
D. Lady Pink	2.650	0.737	2.000	127.223	1.470	31.033	87.897	91.247	111.337	44.333	48.000	4.943
D. Snow White	4.240	0.780	1.667	131.333	1.650	49.853	129.533	124.440	113.103	38.667	42.333	5.397
D. Candy Stripe x Tomie Drake	3.923	0.673	1.667	124.217	1.163	24.043	67.07	92.993	83.237	40.333	44.000	5.077
D. Kasem White	3.417	0.690	1.333	133.667	1.310	31.323	63.253	65.197	50.347	33.000	37.667	4.800
D. Tayswee Keng x Lady Charm	5.887	0.947	2.000	115.167	1.540	48.473	109.093	139.167	146.860	35.333	40.000	4.593
D. Sakura Pink	3.867	0.917	3.000	133.500	1.753	43.297	84.993	142.513	119.350	36.333	40.833	5.317
D. Midnight Velvet Lipstick	4.073	0.997	1.667	104.000	1.670	36.843	125.180	165.32	145.760	43.000	46.667	5.243
D. Jacquelin Concert x Lady Charm	4.267	0.933	1.667	118.833	1.343	27.797	59.343	105.887	79.510	42.000	47.333	5.267
D. Candy Stripe hybrid	3.887	0.827	1.333	110.000	1.227	49.147	120.680	106.210	97.067	41.667	45.333	5.833
D. Caesar Candy	6.753	1.300	2.667	101.050	1.837	68.617	181.99	177.960	182.727	38.500	42.583	6.793
D. Thailand White	3.300	0.830	2.000	111.443	1.447	32.453	84.72	106.973	122.28	41.833	46.833	5.143
F 14,44	1.402^{NS}	2.622**	0.671^{NS}	1.453^{NS}	1.437^{NS}	4.094**	3.199**	4.056**	3.485**	2.392**	2.890**	1.183^{NS}
Sem	0.972	0.108	0.564	9.887	0.183	7.181	21.848	18.506	19.635	2.489	2.078	0.557
CD	2.807	0.311	1.630	28.553	0.529	20.738	63.094	53.443	56.703	7.187	6.002	1.610

(65.197 cm^2) and *D.* Lady Pink, *D.* Candy Stripe X Tomie Drake, *D.* Jacquelin Concert x *D.* Lady Charm, *D.* Candy Stripe hybrid, *D.* Thailand White and *D.* Sonia 28 were on par with it. *D.* Jacquelin Concert x *D.* MME. Udomsiri had the maximum leaf area at inflorescence emergence (180.713 cm^2) and *D.* Caesar Candy, *D.* Rinappa –3, *D.* Brook Shields, *D.* Midnight Velvet Lipstick, *D.* Sakura Pink and *D.* Tayswee Keng x D. Lady Charm were on par it.

The variety D. Caesar Candy recorded maximum leaf area at first flower opening (182.727 cm^2). It was on par with *D.* Jacquelin Concert x *D.* MME Udomsiri, *D.* Rinappa –3, *D.* Tayswee Keng x *D.* Lady Charm, *D.* Midnight Velvet Lipstick and *D.* Brook Shields. The variety *D.* Kasem White had the minimum leaf area at first flower opening (50.347 cm^2). *D.* Jacquelin Concert x *D.* Lady Charm, *D.* Candy Stripe x *D.* Tomie Drake and *D.* Candy Stripe hybrid were on par with *D.* Kasem White for this character.

The days taken from inflorescence emergence to first flower opening was minimum for *D.* Jacquelin Concert x *D.* MME Udomsiri (32.833) and maximum for *D.* Sonia – 28 (44.667). *D.* Kasem White, *D.* Tayswee Keng x *D.* Lady Charm, *D.* Sakura Pink, *D.* Caesar Candy, *D.* Snow White and *D.* Brook Shields were on par with *D.* Jacquelin Concert x *D.* MME. Udomsiri. For days taken from inflorescence emergence to full bloom, D. Kasem White recorded the minimum value of 37.667 days and it was on par with *D.* Jacquelin Concert x *D.* MME. Udomsiri, *D.* Tayswee Keng x Lady Charm, *D.* Sakura Pink, *D.* Snow White and *D.* Caesar Candy.

4.1.2 Analysis of variance of morphological inflorescence and anatomical characters (Table 3)

The varieties showed significant differences in the characters studied such as length, girth, internodal length and number of nodes per shoot, length and width of the leaves, internodal length of the

Table 3. Analysis of variance of morphological, inflorescence and anatomical characters in 15 *Dendrobium* varieties

Sl. No.	Characters	Mean square	
		Varieties	Error
		Df=14	Df=30
1.	Shoot length (cm)	58.783**	10.663
2.	Shoot girth (cm)	0.750*	0.103
3.	Internodal length of shoots (cm)	1.117**	0.279
4.	Number of nodes per shoot	5.499**	1.385
5.	Number of laminate leaves per shoot	1.032^{NS}	0.644
6.	Length of the leaves (cm)	7.351**	1.184
7.	Width of the leaves (cm)	0.883**	0.138
8.	Orientation of the leaves (degrees)	151.031^{NS}	138.333
9.	Length of the inflorescence (cm)	28.786^{NS}	16.586
10.	Thickness of the inflorescence stalk (cm)	0.001^{NS}	0.001
11.	Internodal length of the stalk (cm)	3.828**	1.401
12.	Number of flowers	4.983**	0.685
13.	Length of the flower (cm)	2.796**	0.095
14.	Width of the flower (cm)	2.496**	0.066
15.	Root thickness (mm)	0.171**	0.017
16.	Thickness of the cortex (mm)	0.004**	0.001
17.	Number of layers in the cortex	0.832^{NS}	0.511
18.	Number of layers in the velamen	3.222**	1.756
19.	Leaf thickness (cm)	0.147**	0.014
20.	Number of stomato in the adaxial surface of the leaf (per cm^2)	67571.430**	7898.134
21.	Number of stomata in the abaxial surface of the leaf (per cm^2)	1828878**	83350.40
22.	Petal thickness	0.112**	0.003
		Mean square	
		Varieties Df=11	Error Df=24
23.	Thickness of pigmented layers (only for 12 varieties)	0.030**	0.0004

** Significant at 1 per cent level * Significant at 5 per cent level DF- Degrees of freedom

inflorescence stalk, number, length and width of the flowers, thickness of the root, leaf and petal, number of layers in the velamen and number of stomata in the adaxial and abaxial surface of leaf.

4.1.3 Mean performance of varieties

4.1.3.1 Morphological characters

The mean performance of 15 varieties with respect to the morphological characters studied is presented in Table 4.

The length of the shoots ranged from 11.100 cm to 26.083 cm. *D.* Jacquelin Concert x *D.* Mme. Udomsiri recorded the maximum shoot length of 26.083 cm. *D.* Caesar Candy was on par with it. *D.* Candy Stripe x Tomie Drake recorded the minimum shoot length (11.100 cm). The same trend was observed for shoot girth with maximum of 4.533 cm being recorded for *D.* Jacquelin Concert x *D.* Mme. Udomsiri followed by *D.* Caesar Candy (4.500 cm). The values ranged from 3.000 to 4.500 cm.

The internodal length of shoots was maximum (4.677 cm) in *D.* Snow White followed by *D.* Caesar Candy (4.580 cm). The minimum value (2.637 cm) was recorded in *D.* Candy Stripe x Tomie Drake. *D.* Thailand white, *D.* Candy Stripe hybrid, *D.* Kasem White, *D.* Sakura Pink, *D.* Tayswee Keng x Lady Charm and *D.* Lady Pink were on par with *D.* Candy Stripe x Tomie Drake. There was significant variation in the number of nodes per shoot. It ranged from 4.443 (*D.* Candy Stripe x Tomie Drake) to 9.330 (*D.* Caesar Candy). *D.* Caesar Candy was on par with *D.* Jacquelin Concert x *D.* Mme. Udomsiri (8.697).

The length of the leaves ranged from 10.970 cm (*D.* Candy Stripe hybrid) to 15.307 cm (*D.* Snow White). *D.* Caesar Candy, *D.* Brook Shields, *D.* Thailand White and *D.* Midnight Velvet Lipstick were on par with *D.* Candy Stripe hybrid. *D.* Caesar Candy recorded the maximum leaf width (4.653 cm) and it was significantly different from all other varieties. *D.* Kasem White had the minimum leaf width (2.427 cm) and it

Table 4. Differences in shoot and leaf morphology among fifteen Dendrobium varieties

Varieties	Shoot length (cm)	Shoot girth (cm)	Internodal length of shoots (cm)	Number of nodes per shoot	Number of laminate leaves per shoot	Length of the leaves(cm)	Width of the leaves(cm)	Orientation of the leaves
D. Sonia-28	16.203	3.433	3.810	5.490	4.223	12.253	3.623	80.000
D. Rinappa-3	18.007	3.433	3.890	6.170	4.110	12.000	3.123	86.670
D. Jacquelin Concert x D.Mme Udomsiri	26.083	4.533	4.067	8.697	5.833	15.207	3.873	100.000
D. Brook Shields	19.180	3.267	4.020	6.367	4.833	15.077	3.143	80.000
D. Lady Pink	12.203	3.133	3.340	5.097	3.890	14.697	2.953	90.000
D. Snow White	19.553	3.267	4.677	7.110	4.667	15.307	2.767	73.333
D. Candy Stripe x Tomie Drake	11.100	3.167	2.637	4.443	4.000	12.313	3.180	81.667
D. Kasem White	12.010	3.067	3.050	5.780	4.333	11.417	2.427	86.667
D. Tayswee Keng x Lady Charm	18.253	3.433	3.320	6.640	5.167	14.330	2.887	96.667
D. Sakura Pink	13.997	3.667	3.193	5.980	5.233	12.033	3.017	86.667
D. Midnight Velvet Lipstick	13.017	3.733	3.543	5.000	5.000	14.043	3.073	86.667
D. Jacquelin Concert x Lady Charm	13.203	3.600	3.670	5.083	3.833	12.090	2.587	83.333
D. Candy Stripe hybrid	12.993	2.833	2.933	5.277	4.500	10.970	3.140	90.000
D. Caesar Candy	23.513	4.500	4.580	9.330	5.167	15.203	4.653	90.000
D. Thailand White	14.84	2.900	2.833	6.033	5.000	14.110	3.127	76.667
F 14,44	5.513**	7.260**	4.005**	3.972**	1.604^{NS}	6.211**	6.387**	1.092^{NS}
SEm	1.885	0.186	0.305	0.679	0.463	0.628	0.215	6.791
CD	5.444	0.536	0.880	1.962	1.337	1.814	0.620	19.610

was on par with *D.* Jacquelin Concert x Lady Charm, *D.* Snow White, *D.* Tayswee Keng x Lady Charm, *D.* Lady Pink and *D.* Sakura Pink.

4.1.3.2 Inflorescence characters (Table 5)

There was no significant difference between varieties in the length and thickness of the inflorescence. Among varieties, internodal length of the stalk ranged from 4.000 – 9.500 cm. *D.* Jacquelin Concert x Lady Charm recorded the highest value (9.333 cm) and was on par with *D.* Snow White, *D.* Midnight Velvet : Lipstick, *D.* Caesar Candy, *D.* Sonia – 28, *D.* Lady Pink and *D.* Sakura Pink. The lowest value was recorded by *D.* Candy Stripe x Tomie Drake (4.033 cm).

The number of flowers in an inflorescence also differed significantly among the varieties. It was higher (7.000) in case of *D.* Tayswee Keng x Lady Charm followed by *D.* Candy Stripe hybrid (6.667) and *D.* Caesar Candy (6.417) and they were on par with each other. *D.* Snow White recorded the lowest value (3.000).

The length of the flowers ranged between 4.017 cm (*D.* Caesar Candy) to 7.127 cm (*D.* Brook Shields). *D.* Caesar Candy was significantly different from all the varieties. The length was higher in case of *D.* Sonia-28, *D.* Rinappa-3 and *D.* Lady Pink also. The width of the flower and maximum (8.120 cm) for *D.* Brook Shields and minimum (4.740 cm) for *D.* Caesar Candy. *D.* Brook Shields and *D.* Kasem White were on par with each other.

4.1.3.3 Anatomical characters (Table 6)

The thickness of the root varied between 1.552 mm (*D.* Caesar Candy) to 2.555 mm (*D.* Rinappa-3). None of the varieties had higher root thickness comparable to that of *D.* Rinappa – 3. The thickness was lower in *D.* Thailand White, *D.* Kasem White and *D.* Candy Stripe hybrid. Similarly the thickness of the cortex was maximum (0.327 mm) in *D.* Rinappa-3. *D.* Snow White (0.310 mm) was on par with it. *D.* Kasem White recorded the minimum value (0.204 mm). *D.* Rinappa – 3 again had

Table 5. Differences in inflorescence characters among fifteen *Dendrobium* varieties

Varieties	Length of the inflorescence(cm)	Thickness of the inflorescence stalk (cm)	Basal internodal length of the stalk (cm)	Number of flowers	Length of the flowers (cm)	Width of the flowers (cm)
D. Sonia-28	25.333	0.300	7.617	4.667	6.990	7.040
D. Rinnappa-3	24.767	0.273	7.267	3.333	6.957	7.330
D. Jacquelin Concert x D.Mme Udomsiri	21.583	0.283	6.650	5.333	5.023	6.907
D. Brook Shields	29.200	0.307	6.567	3.667	7.127	8.120
D. Lady Pink	21.467	0.243	7.533	3.667	6.600	6.573
D. Snow White	24.437	0.307	8.333	3.000	5.833	6.450
D. Candy Stripe x Tomie Drake	23.033	0.283	4.033	4.667	6.127	6.843
D. Kasem White	20.970	0.287	7.233	3.667	5.350	7.737
D. Tayswee Keng x Lady Charm	22.437	0.307	7.267	7.000	5.227	5.943
D. Sakura Pink	19.650	0.280	7.433	3.667	6.573	6.320
D. Midnight Velvet Lipstick	23.710	0.300	8.133	3.667	5.813	5.860
D. Jacquelin Concert x Lady Charm	24.433	0.317	9.333	3.667	6.403	7.060
D. Candy Stripe hybrid	25.833	0.293	7.167	6.667	4.757	5.050
D. Caeser Candy	31.180	0.313	7.667	6.417	4.017	4.740
D. Thailand White	26.703	0.283	7.233	4.164	4.603	6.400
F 14,44	1.736^{NS}	1.034^{NS}	2.731**	7.277**	29.308**	38.029**
SEm	2.351	0.018	0.683	0.478	0.178	0.148
CD	6.790	0.053	1.974	1.380	0.515	0.427

Table 6. Differences in anatomical character among fifteen *Dendrobium* varieties

Varieties	Root thickness (cm)	Cortex thickness (mm)	Number of layers in the cortex	Number of layers in velamen	Leaf thickness (mm)	Number of stomata in the adaxial surface of leaf (per cm2)	Number of stomata in the abaxial surface of leaf (per cm2)	Petal thickness (mm)	Thickness of pigmented layers (mm)
D. Sonia-28	1.791	0.252	5.667	6.667	1.248	666.000	2822.000	0.647	0.298
D. Rinnappa-3	2.555	0.327	6.000	9.333	1.064	666.333	3517.333	0.615	0.204
D. Jacquelin Concert x D.Mme Udomsiri	1.956	0.259	6.000	8.667	1.488	410.667	3035.000	0.589	0.223
D. Brook Shields	1.833	0.213	5.000	8.000	1.474	638.000	3588.333	0.556	0.294
D. Lady Pink	1.869	0.265	5.333	7.667	1.635	737.000	3006.667	0.640	0.297
**D.* Snow White	2.095	0.310	6.333	7.000	1.646	410.667	2907.667	0.760	-
D. Candy Stripe x Tomie Drake	1.963	0.307	6.000	5.667	1.407	751.333	3035.000	0.217	0.041
**D.* Kasem White	1.678	0.204	5.000	7.667	1.539	737.000	3432.000	0.433	-
D. Tayswee Keng x Lady Charm	1.992	0.252	5.333	7.000	1.614	472.667	3262.000	0.219	0.219
D. Sakura Pink	1.895	0.210	5.667	7.667	1.280	595.000	2992.333	0.440	0.146
D. Midnight Velvet Lipstick	1.908	0.223	4.667	7.333	1.672	382.333	2269.000	0.414	0.159
D. Jacquelin Concert x Lady Charm	1.775	0.259	5.667	7.000	1.546	822.000	2793.667	0.550	0.253
D. Candy Stripe hybrid	1.736	0.256	5.000	7.000	1.099	396.667	2666.333	0.197	0.035
D. Caeser Candy	1.552	0.213	5.000	6.333	1.002	694.333	5711.667	0.139	0.046
**D.* Thailand White	1.594	0.237	4.667	5.333	1.439	495.667	2708.667	.592	-
F 14,44	9.804**	4.985**	1.627^{NS}	2.788**	10.905**	8.555**	21.942**	41.114**	(F 11,35) 81.986**
SEm	0.076	0.017	0.413	0.621	0.067	51.310	166.683	0.030	0.011
CD	0.220	0.049	1.192	1.792	0.194	148.174	481.353	0.087	0.033

*Varieties with white flowers

more number of layers (9.333) in the velamen. *D.* Jacquelin Concert x *D.* MME. Udomsiri, *D.* Brook Shields, *D.* Lady Pink, *D.* Kasem White and *D.* Sakura Pink also had more layers in the velamen and was on par with *D.* Rinappa – 3. The number of layers in the velamen recorded lowest (5.333) in *D.* Thailand White. *D.* Candy Stripe x Tomie Drake, *D.* Caesar Candy, *D.* Sonia-28, *D.* Candy Stripe hybrid, *D.* Jacquelin Concert x Lady Charm, *D.* Snow White and *D.* Tayswee Keng x Lady Charm were on par with *D.* Thailand White.

The leaf thickness was minimum (1.002 mm) in *D.* Caesar Candy. *D.* Rinappa – 3 and *D.* Candy Stripe hybrid were on par with *D.* Caesar Candy. The thickness was maximum (1.672 mm) in *D.* Midnight Velvet Lipstick. The number of stomata on the adaxial surface of leaf ranged from 400 – 800 per cm^2. The highest number (822.000 cm^2) was recorded by *D.* Jacquelin Concert x Lady Charm and the lowest (382.333 cm^2) by Midnight Velvet Lipstick. D. Jacquelin Concert x Lady Charm was on par with *D.* Candy Stripe x Tomie Drake, *D.* Lady Pink, *D.* Kasem White and *D.* Caesar Candy while *D.* Midnight Velvet Lipstick was on par with *D.* Candy Stripe hybrid, *D.* Jacquelin Concert x D. Mme Udomsiri, *D.* Snow White, *D.* Tayswee Keng x Lady Charm and *D.* Thailand white. So also *D.* Midnight Velvet Lipstick recorded the lowest number (2269.000 per cm^2) of stomata in the abaxial surface. The highest number (5711.670 per cm^2) was recorded by *D.* Caesar Candy and it was remarkably different from all the varieties.

The thickness of the petals ranged from 0.139 (*D.* Caesar Candy) to 0.760 mm (*D.* Snow White). None of the varieties had greater petal thickness similar to *D.* Snow white. *D.* Caesar Candy was on par with *D.* Candy Stripe hybrid, *D.* Candy Stripe x Tomie Drake and *D.* Tayswee Keng x Lady Charm. The thickness of pigmented layer was higher (0.298 mm) in *D.* Sonia – 28 and it was on par with *D.* Lady Pink and *D.* Brook Shields. The minimum value was recorded in *D.* Candy Stripe

hybrid(0.035 mm). *D.* Candy Stripe x Tomie Drake and *D.* Caesar Candy were on par with *D.* Candy Stripe hybrid.

4.1.4 Variability studies

4.1.4.1 Morphological characters

The phenotypic, genotypic and environmental variance and the phenotypic and genotypic coefficients of variation (PCV and GCV) for the morphological characters studied are presented in Table 7 and Fig. 1.

The shoot length showed the maximum value for GCV (24.605 per cent) followed by number of nodes per shoot (18.992 per cent), width of the leaves (15.712 per cent), internodal length of shoots (14.800 per cent), shoot girth (13.404 per cent), length of the leaves (10.698 percent) and number of laminate leaves per shoot (7.736 per cent). The GCV was lowest for orientation of the leaves (2.395 per cent).

The highest PCV was observed for the shoot length (31.747 per cent) and it was lowest for length of the leaves (13.428 per cent).

The difference between phenotypic and genotypic coefficient of variation was the lowest for the length of the leaves (2.730 per cent) succeeded by shoot girth (2.896 per cent) and it was relatively high for orientation of the leaves (11.507 per cent) and number of laminate leaves per shoot (11.162 per cent).

4.1.4.2 Inflorescence characters

The genotypic and environmental components of phenotypic variance for the inflorescence characters are presented in Table 8 and Fig. 2.

Maximum variability both at the phenotypic and genotypic level was observed for the number of flowers (32.456 and 26.698 per cent respectively) while the minimum phenotypic and genotypic variability was observed for thickness of the inflorescence stalk (11.043 and 1.645 per cent respectively).

Table 7. Components of total variance for the shoot and leaf morphological characters in fifteen *Dendrobium* varieties

Sl. No.	Characters	σ_p^2	σ_g^2	σ_e^2	PCV (%)	GCV (%)
1.	Shoot length (cm)	26.703	16.040	0.663	31.747	24.605
2.	Shoot girth (cm)	0.319	0.216	0.103	16.302	13.404
3.	Internodal length of shoots (cm)	0.558	0.279	0.279	20.921	14.800
4.	Number of nodes per shoot	2.756	1.372	1.385	26.922	18.922
5.	Number of laminate leaves per shoot	0.773	0.130	0.644	18.898	7.736
6.	Length of the leaves (cm)	3.239	2.056	1.184	13.428	10.698
7.	Width of the leaves (cm)	0.387	0.248	0.138	19.605	15.712
8.	Orientation of the leaves (^{0})	142.556	4.233	138.333	13.902	2.395

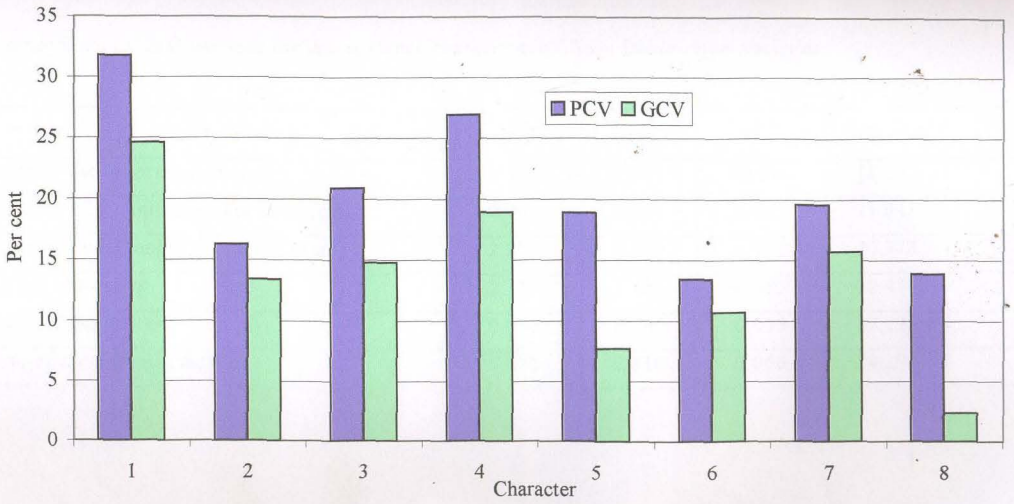


Fig. 1. GCV and PCV for the shoot and leaf morphological characters in 15 Dendrobium varieties

Table 8. Components of total variance for inflorescence characters in fifteen *Dendrobium* varieties

Sl. No.	Characters	σ_p^2	σ_g^2	σ_e^2	PCV (%)	GCV (%)
1.	Length of the inflorescence (cm)	20.653	4.067	16.586	18.690	8.293
2.	Thickness of the inflorescence stalk (cm)	0.001	0.000	0.001	11.043	1.645
3.	Basal internodal length of the stalk (cm)	2.210	0.809	1.401	20.372	12.324
4.	Number of flowers	2.117	1.433	0.685	32.456	26.698
5.	Length of the flowers	0.996	0.900	0.095	17.125	16.284
6.	Width of the flowers (cm)	0.876	0.810	0.066	14.271	13.725

1- Length of the inflorescence 2. Thickness of inflorescence stalk 3. Basal internodal length of the stalk 4. Number of flowers
5. Length of the flowers 6. Width of the flowers

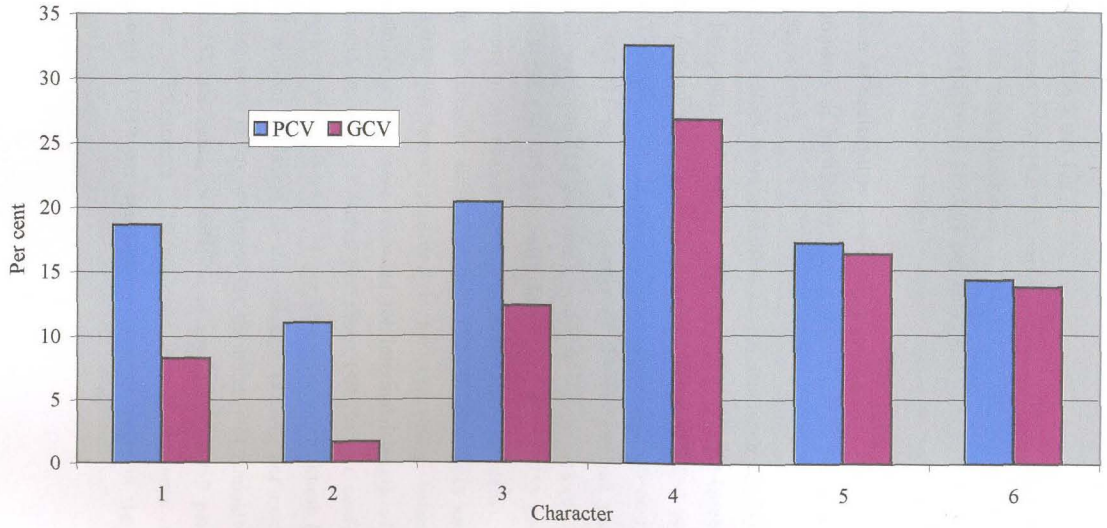


Fig. 2. GCV and PCV for the inflorescence characters in 15 Dendrobium varieties

4.1.4.3 Anatomical characters

The components of variance for the anatomical characters studied are presented in Table 9 and Fig. 3.

The maximum PCV (42.425 per cent) and GCV (40.922 per cent) were observed for petal thickness. The lowest PCV and GCV were observed for root thickness (13.951 per cent) and number of layers in the cortex (6.029 per cent) respectively.

4.1.5 Estimation of heritability and genetic advance

The estimates of heritability and genetic advance are presented in Table 10 and Fig.4 and 5.

Heritability values were classified as per Robinson (1965) as low (less than 30 per cent), medium (30 – 60 per cent) and high (greater than 60 per cent). Accordingly the characters shoot length (60.069 per cent), shoot girth (67.604 per cent), length of the leaves (63.465 per cent), width of the leaves (64.231 per cent), number of flowers (67.662 per cent), length of the flowers (90.418 per cent), width of the flowers (92.505 per cent), root thickness (74.585 per cent), leaf thickness (76.753 per cent), number of stomata in the adaxial surface of leaf (71.578 per cent), number of stomata in the abaxial surface of leaf (87.470 per cent) and petal thickness (93.042 per cent) recorded higher heritability values. The medium heritability values were recorded for internodal length of shoots (50.044 per cent), number of nodes per shoot (49.764 per cent), basal internodal length of the stalk (36.594 per cent), cortex thickness (57.051 per cent) and number of layers in the velamen (37.349 per cent). The heritability values were in lower ranges for the number of laminate leaves per shoot (16.757 per cent), orientation of the leaves (2.969 per cent), length of the inflorescence (19.690 per cent), thickness of the inflorescence stalk (1.112 per cent) and number of layers in the cortex (17.294 per cent).

Table 9. Components of total variance for anatomical characters in fifteen *Dendrobium* varieties

Sl. No.	Characters	σ_p^2	σ_g^2	σ_e^2	PCV (%)	GCV (%)
1.	Root thickness (mm)	0.069	0.051	0.018	13.951	12.048
2.	Cortex thickness (mm)	0.002	0.001	0.001	17.934	13.546
3.	Number of layers in the cortex	0.618	0.107	0.511	14.498	6.029
4.	Number of layers in the valemen	1.844	0.689	1.155	18.805	11.492
5.	Leaf thickness (mm)	0.058	0.045	0.013	17.104	14.984
6.	Number of stomata in the adaxial surface of leaf (per cm^2)	27789.230	19891.1	7898.134	28.173	23.855
7.	Number of stomata in the abaxial surface of leaf (per cm^2)	665192.100	581841.7	83350.40	25.622	23.963
8.	Petal thickness (mm)	0.039	0.037	0.002	42.425	40.922

1- Root thickness 2- Cortex thickness 3- No. of layers in the cortex 4-No. of layers in velamen 5- Leaf thickness 6- No. of stomata in adaxial sufrace of leaf 7- No.of stomata in abaxial surface of leaf 8- Petal thickness

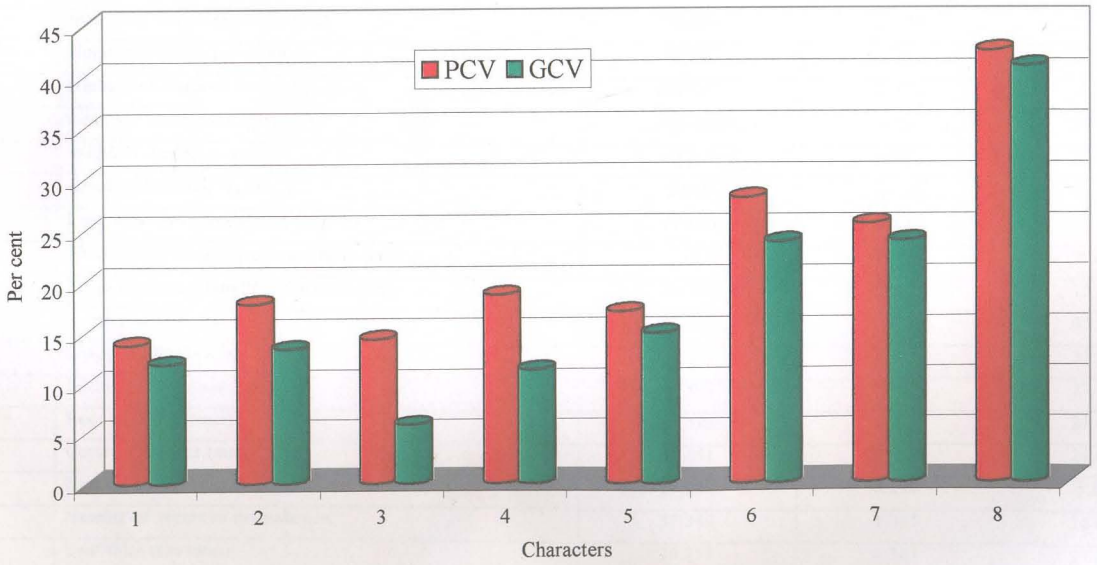


Fig. 3. GCV and PCV for the anatomical characters in fifteen Dendrobium varieties

Table 10. Heritability and Genetic advance for morpho-anatomical characters in fifteen Dendrobium varieties

Sl. No.	Characters	Heritability (%)	Genetic advance (at 5 %)	Genetic advance (as % of mean)
1.	Shoot length (cm)	60.069	6.394	39.282
2.	Shoot girth (cm)	67.604	0.787	22.705
3.	Internodal length of shoots (cm)	50.044	0.770	21.565
4.	Number of nodes per shoot	49.764	1.702	27.600
5.	Number of laminate leaves per shoot	16.757	0.304	6.523
6.	Length of the leaves (cm)	63.465	2.353	17.556
7.	Width of the leaves (cm)	64.231	0.823	25.936
8.	Orientation of the leaves (^{0})	2.969	0.730	0.850
9.	Length of the inflorescence (cm)	19.690	1.843	7.579
10.	Thickness of the inflorescence stalk (cm)	1.112	0.0007	0.253
11.	Basal internodal length of the stalk (cm)	36.594	1.120	15.356
12.	Number of flowers	67.662	2.028	45.242
13.	Length of the flowers	90.418	1.859	31.895
14.	Width of the flowers (cm)	92.505	1.783	27.194
15.	Root thickness (mm)	74.585	0.403	21.426
16.	Cortex thickness (mm)	57.051	0.053	21.028
17.	Number of layers in the cortex	17.294	0.280	5.166
18.	Number of layers in the valemen	37.349	1.045	14.468
19.	Leaf thickness (mm)	76.753	0.381	27.040
20.	Number of stomata in the adaxial surface of leaf (per cm^2)	71.578	245.803	41.541
21.	Number of stomata in the abaxial surface of leaf (per cm^2)	87.470	1469.598	46.168
22.	Petal thickness (mm)	93.042	0.0380	81.328

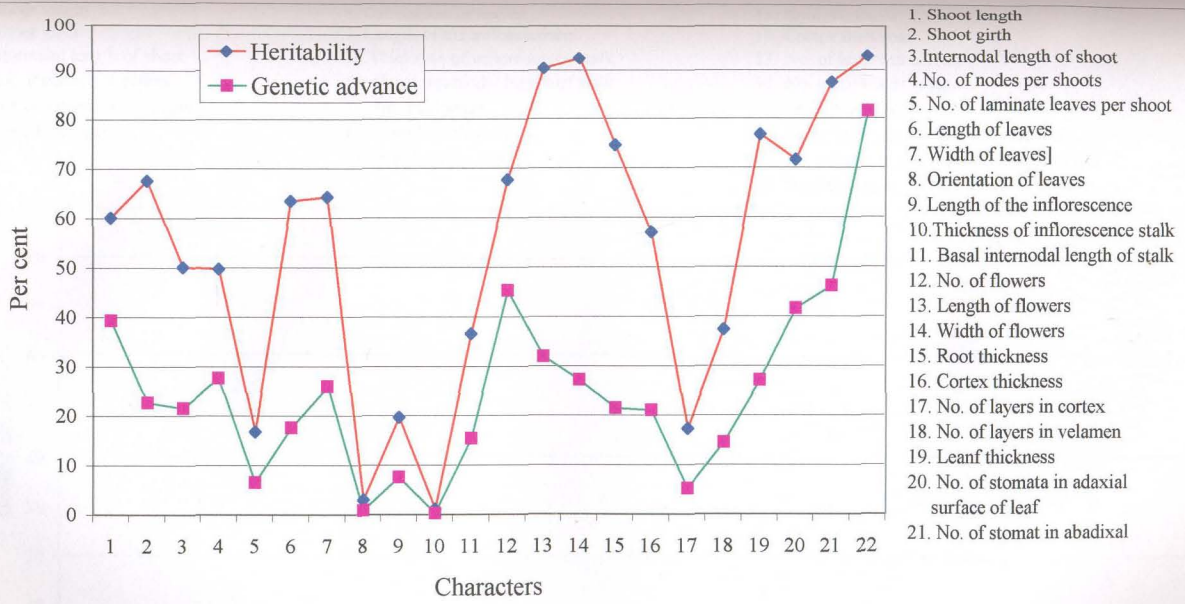


Fig. 4. Heritability and genetic advance for the morpho-anatomical characters in fifteen *Dendrobium* varieties

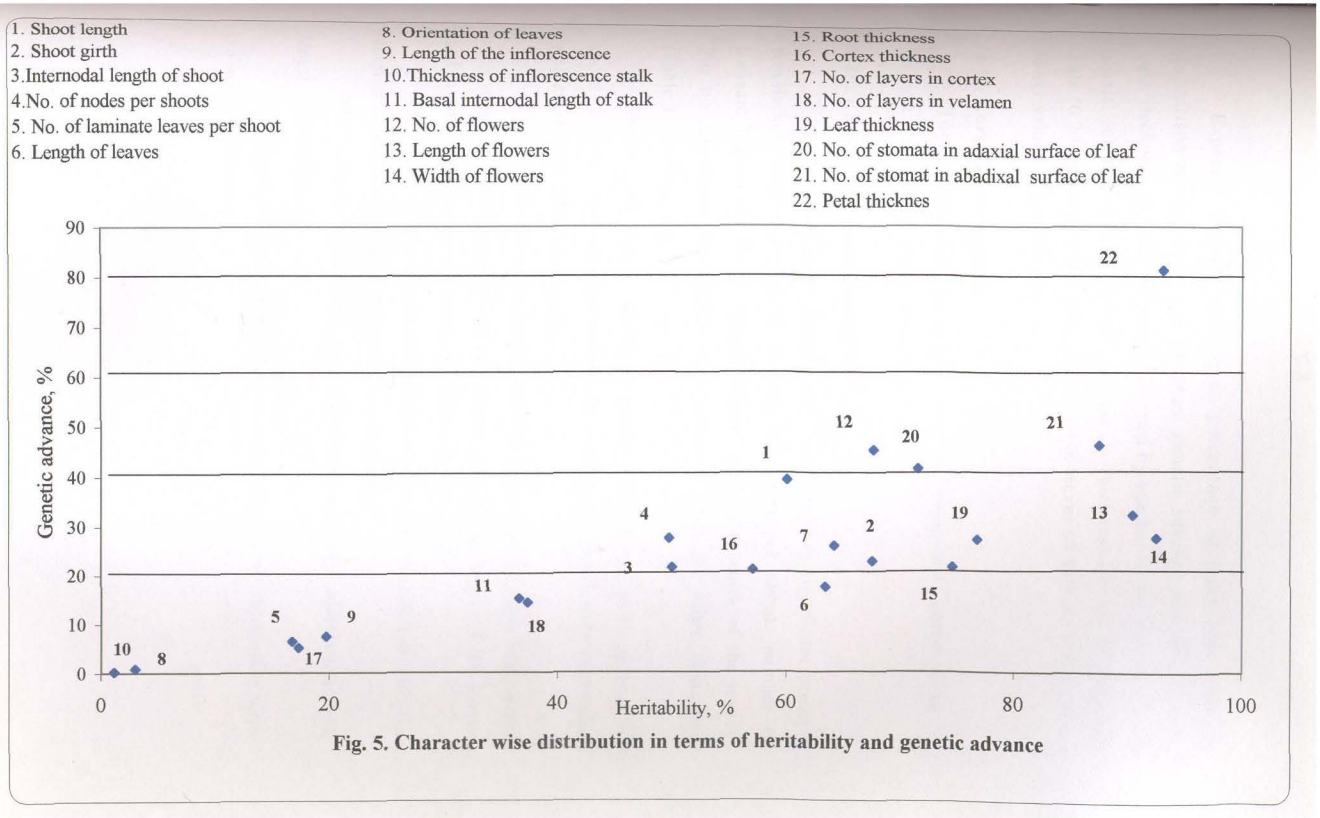


Fig. 5. Character wise distribution in terms of heritability and genetic advance

Expected genetic advance as percentage of mean was used for comparison among characters. Highest genetic advance was observed for petal thickness (81.328 per cent) followed by number of the stomata in the abaxial surface of leaf (46.168 per cent). Thickness of the inflorescence stalk (0.253 per cent) and orientation of the leaves (0.850 per cent) exhibit lower values of genetic advance.

4.1.6 Correlation analysis

The phenotypic, genotypic and environmental correlations of important characters were estimated.

4.1.6.1 Phenotypic correlations

The character shoot length was found to have significant positive correlation with shoot girth, number of nodes per shoot, number of laminate leaves per shoot, length of the leaves and width of the leaves. But no significant correlation was observed with the other characters (Table 11 and Fig. 6).

Shoot girth had a significant positive correlation with number of nodes per shoot and width of the leaves. It was not significantly correlated with any of the other characters.

The number of nodes per shoot had significant positive correlation with number of laminate leaves per shoot, length and width of the leaves and number of stomata in the abaxial surface of the leaf.

The width of the leaves had significant positive correlation with the number of stomata in the abaxial surface of the leaf.

The number of flowers had significant negative correlation with length and width of flowers.

The length of flowers had high significant positive correlation with the width of flowers.

Table 11. Phenotypic correlation coefficients among some characters in fifteen *Dendrobium* varieties

Characters		X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}
Shoot length (cm)	X_1														
Shoot girth (cm)	X_2	0.6218*													
Number of nodes per shoot	X_3	0.8237**	0.6199*												
Number of laminate leaves per shoot	X_4	0.5246*	0.3646	0.5936*											
Length of the leaves (cm)	X_5	0.6200*	0.3616	0.5220*	0.3207										
Width of the leaves (cm)	X_6	0.6441**	0.6141*	0.5850*	0.3970	0.4014									
Length of the inflorescence (cm)	X_7	0.3130	0.1597	0.2175	0.0177	0.1936	0.3747								
Number of flowers	X_8	0.1710	0.2194	0.2527	0.2484	0.0070	0.3346	0.1972							
Length of flowers (cm)	X_9	-0.2603	-0.1897	-0.3972	-0.3340	-0.1809	-0.3516	-0.1656	-0.5403*						
Width of flowers (cm)	X_{10}	-0.1187	-0.2317	-0.2090	-0.1970	-0.1236	-0.4309	-0.2006	-0.5372*	0.6620**					
Number of stomata in the adaxial surface of leaf (per cm^2)	X_{11}	-0.2247	-0.0700	-0.1432	-0.3608	-0.2459	-0.1039	0.0702	-0.2127	0.3664	0.3961				
Number of stomata in the abaxial surface of leaf (per cm^2)	X_{12}	0.4363	0.4038	0.5361*	0.1596	0.2326	0.5231*	0.4023	0.2749	-0.3241	-0.2277	0.3241			
Number of layers in the velemen	X_{13}	0.1339	0.1322	0.0757	0.0920	0.0115	-0.0832	-0.1855	-0.0581	0.3344	0.3266	0.0057	-0.0027		
Number of layers in the cortex	X_{14}	0.0898	0.0709	0.0573	0.0205	-0.0736	-0.0723	-0.4255	-0.0901	0.2823	0.2084	-0.0253	-0.0547	0.1507	

* significant at 5 per cent (r= 0.5139)
** significant at 1 per cent (r= 0.6411)

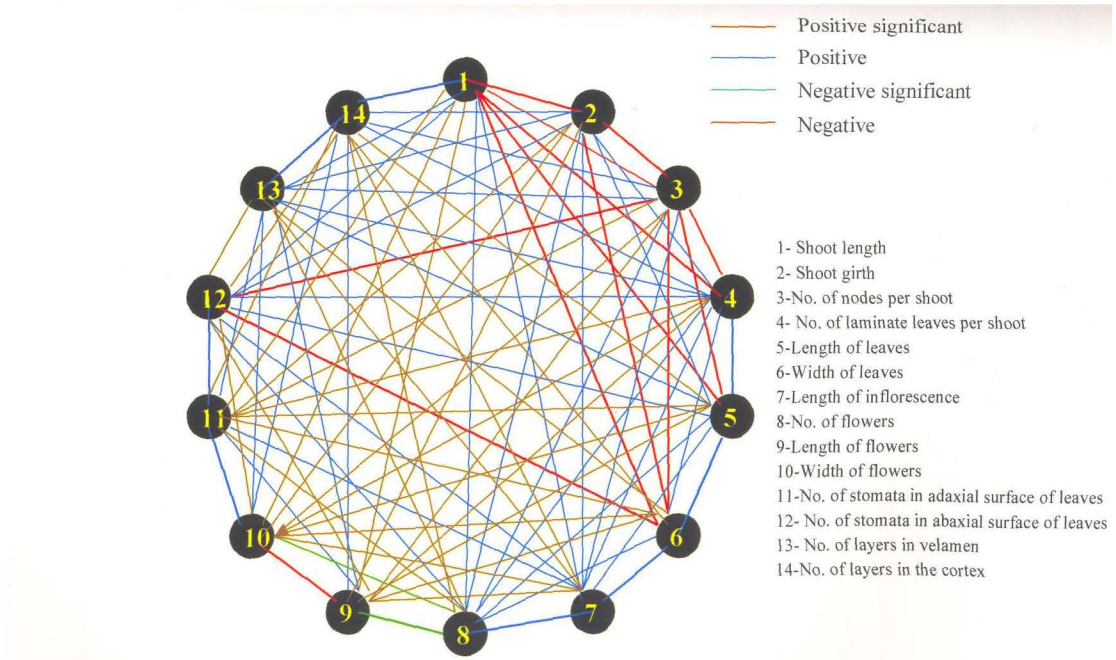


Fig. 6. Phenotypic correlation coefficients among the fourteen characters of fifteen *Dendrodium* varieties

4.1.6.2 Genotypic correlations

In general genotypic correlations coefficients were higher than phenotypic coefficients (Table 12 and Fig.7).

Shoot length had high significant positive correlation with shoot girth, number of nodes per shoot, number of laminate leaves per shoot, length of the leaves, width of the leaves and number of stomata in the abaxial surface of leaf.

High significant positive correlation was observed between shoot girth and number of nodes per shoot, number of laminate leaves per shoot, width of the leaves and number of stomata in the abaxial surface of leaf.

The number of nodes per shoot showed positive and significant correlation with number of laminate leaves per shoot, length and width of the leaves, length of the inflorescence and number of stomata in the abaxial surface of the leaf but had a significant negative correlation with the length of flowers.

The number of laminate leaves per shoot had positive significant correlation with length of the leaves, width of the leaves, number of flowers and a significant negative correlation with length of flowers, width of flowers, number of stomata in the adaxial surface of leaf and number of layers in the cortex.

Width of the leaves had significant positive correlation with length of the inflorescence, number of flowers and number of stomata in the abaxial surface of the leaf.

The length of the inflorescence registered significant positive correlation with the number of stomata in the abaxial surface of the leaf.

The number of flowers showed significant negative correlation with the length and width of the flowers.

Length of the flower had significant positive correlation with width of flower and number of layers in the cortex.

Number of layers in the velamen was significantly correlated with the number of layers in the cortex.

Table 12.Genotypic correlation coefficient among some characters in fifteen Dendrobium varieties

Characters		X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}
Shoot length (cm)	X_1														
Shoot girth (cm)	X_2	0.8002**													
Number of nodes per shoot	X_3	1.0278**	0.8290**												
Number of laminate leaves per shoot	X_4	0.9489**	0.9610**	0.9351**											
Length of the leaves (cm)	X_5	0.6583**	0.4763	0.6754**	0.8773**										
Width of the leaves (cm)	X_6	0.6723**	0.7568**	0.7591**	0.5947*	0.3679									
Length of the inflorescence (cm)	X_7	0.4843	0.0639	0.5894*	0.1282	0.4855	0.8275**								
Number of flowers	X_8	0.4086	0.2549	0.4495	0.6010*	0.0607	0.6307*	0.3206							
Length of flowers (cm)	X_9	-0.3119	-0.2699	-0.6057*	-0.8270**	-0.2293	-0.4077	-0.3640	-0.6976**						
Width of flowers (cm)	X_{10}	-0.0864	-0.2388	-0.3093	-0.5352*	-0.1101	-0.4625	-0.3109	-0.7038**	0.6800**					
Number of stomata in the adaxial surface of leaf (per cm^2)	X_{11}	-0.3779	-0.0512	0.3390	-1.1955**	-0.3571	-0.0482	-0.0013	-0.3040	0.4257	0.4301				
Number of stomata in the abaxial surface of leaf (per cm^2)	X_{12}	0.5861*	0.5521*	0.8079**	0.2462	0.3344	0.7159**	0.8167**	0.3781	-0.3424	-0.2417	0.3708			
Number of layers in the velamen	X_{13}	0.4908	0.3987	0.3065	0.0871	-0.0387	-0.1285	-0.5058	-0.4875	0.4941	0.4798	-0.0981	-0.0015		
Number of layers in the cortex	X_{14}	0.5132	0.3611	0.1839	-0.7484**	0.0158	-0.0164	-0.1355	-0.4479	0.5975*	0.4107	0.3325	-0.1560	0.5518*	

* significant at 5 per cent (r= 0.5139)
** significant at 1 per cent (r= 0.6411)

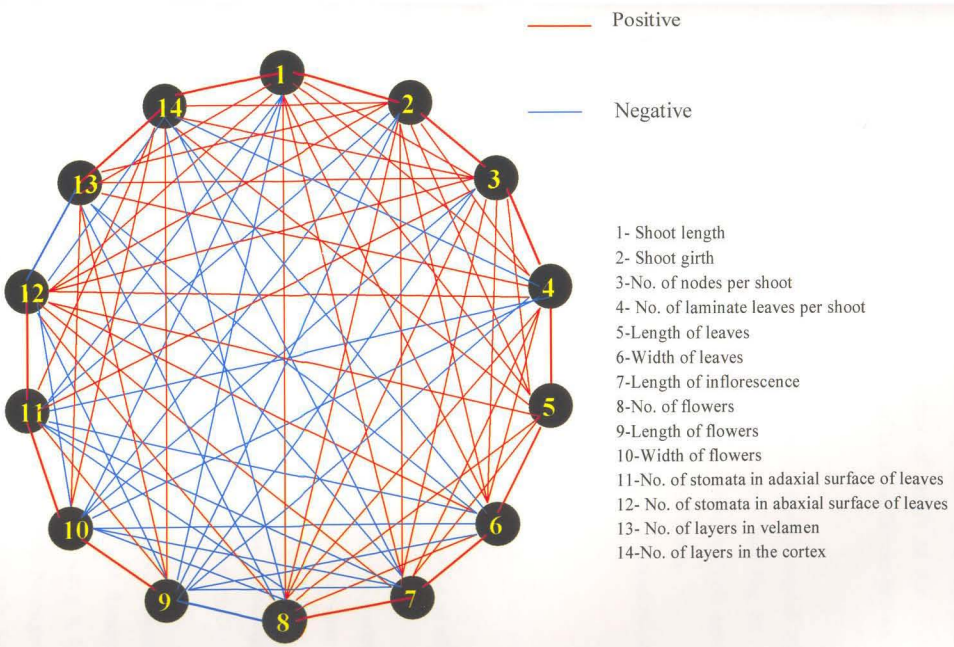


Fig. 7. Genotypic correlation coefficients among the fourteen characters of fifteen *Dendrodium* varieties

The number of layers in the root cortex showed significant positive correlation with length of flowers and number of layers in the velamen while it was negatively correlated the number of laminate leaves per shoot.

4.1.6.3 Environmental correlations

Low values of correlations coefficient due to environmental effect were obtained for most of the characters studied (Table 13 and Fig. 8).

Shoot length showed significant positive correlation with number of nodes per shoot, length of the leaves and width of the leaves.

The correlation coefficients for the other characters studied were low and insignificant.

4.1.7 Qualitative characters

The qualitative characters of the fifteen *Dendrobium* varieties in Experiment I are given in Table 14.

The shape of the leaves was found to be linear –lanceolate in all the varieties.

The leaf sheath was light green and glabrous in all the varieties. Regarding resupination, in all the varieties, it was observed that the flowers continue to resupinate to orient the flowers so that the lip was placed lower most. The direction of resupination may be clockwise or anticlock wise. In an inflorescence the flowers resupinated clockwise-anticlockwise alternately in all the varieties.

The colours of the flowers in general and of each petal in particular are described (Plate 1). The varieties *D.* Sonia-28, *D.* Rinappa-3, *D.* Jacquelin Concert x *D.* Mme. Udomsiri, *D.* Tayswee Keng x Lady Charm, *D.* Midnight Velvet Lipstick and *D.* Caesar Candy belonged to the Red Purple group. *D.* Brook Shields, *D.* Lady Pink, *D.* Candy Stripe x Tomie Drake, *D.* Sakura Pink, *D.*. Jacquelin Concert x Lady Charm and *D.* Candy Stripe hybrid belong to Purple group. *D.* Snow White, *D.* Kasem White and *D.* Thailand White were categorized under the White group.

Table 13. Environment correlation coefficients among some characters in fifteen *Dendrobium* varieties

Characters		X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}
Shoot length (cm)	X_1														
Shoot girth (cm)	X_2	0.3110													
Number of nodes per shoot	X_3	0.5844*	0.3448												
Number of laminate leaves per shoot	X_4	0.3878	0.0792	0.5003											
Length of the leaves (cm)	X_5	0.5592*	0.1442	0.3325	0.0628										
Width of the leaves (cm)	X_6	0.5993*	0.3391	0.3677	0.3699	0.4606									
Length of the inflorescence	X_7	0.2586	0.2673	0.0519	-0.0068	0.0406	0.1501								
Number of flowers	X_8	-0.2515	0.1452	-0.0201	0.0888	-0.0954	-0.2387	0.1574							
Length of flowers (cm)	X_9	-0.1557	0.1207	-0.0414	-0.0426	-0.0389	-0.2207	-0.0434	0.0303						
Width of flowers (cm)	X_{10}	-0.3136	-0.2749	0.0043	0.0548	-0.2373	-0.4548	-0.2766	0.1260	0.4731					
Number of stomata in the adaxial surface of leaf (per cm^2)	X_{11}	-0.0686	-0.1132	0.1566	0.1094	-0.0163	-0.2235	0.1480	-0.0039	0.1451	0.3162				
Number of stomata in the abaxial surface of leaf (per cm^2)	X_{12}	0.0512	-0.1032	0.0123	0.2025	-0.0774	-0.0642	0.1998	-0.0795	-0.1787	-0.1063	0.1629			
Number of layers in the velamen	X_{13}	-0.1970	-0.1511	-0.1006	0.0973	0.0634	-0.0428	-0.0682	0.4153	0.1931	0.2058	0.1337	-0.0066		
Number of layers in the cortex	X_{14}	-0.1315	-0.1016	0.0051	0.1782	-0.1433	-0.1229	-0.4914	0.1221	0.1635	0.1772	-0.2934	0.0185	0.0145	

* significant at 5 per cent (r= 0.5139)
** significant at 1 per cent (r= 0.6411)

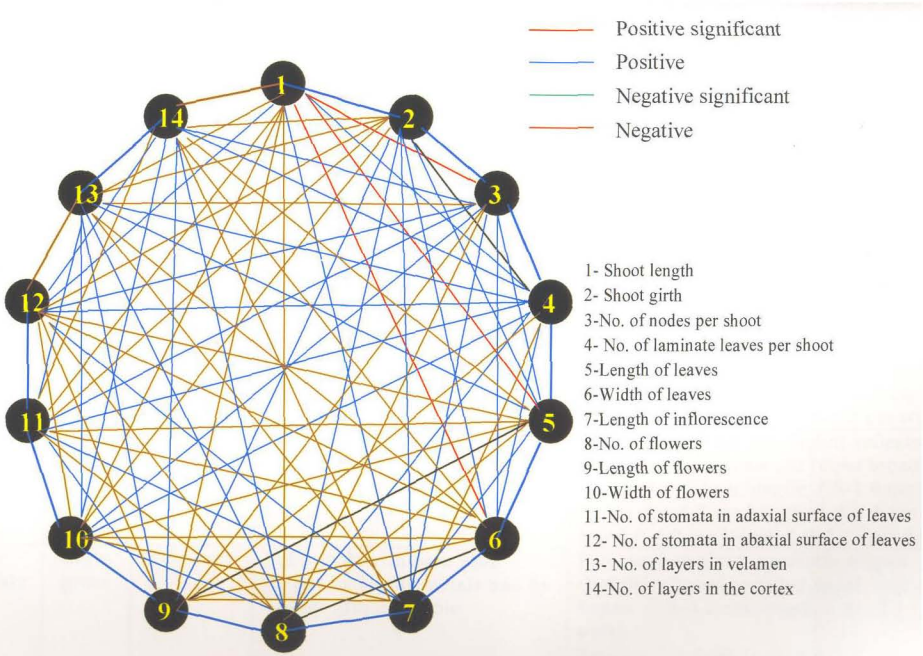


Fig. 8. Environmental correlation coefficients among the fourteen characters of fifteen *Dendrodium* varieties

Table 14. Qualitative characters of fifteen Dendrobium varieties

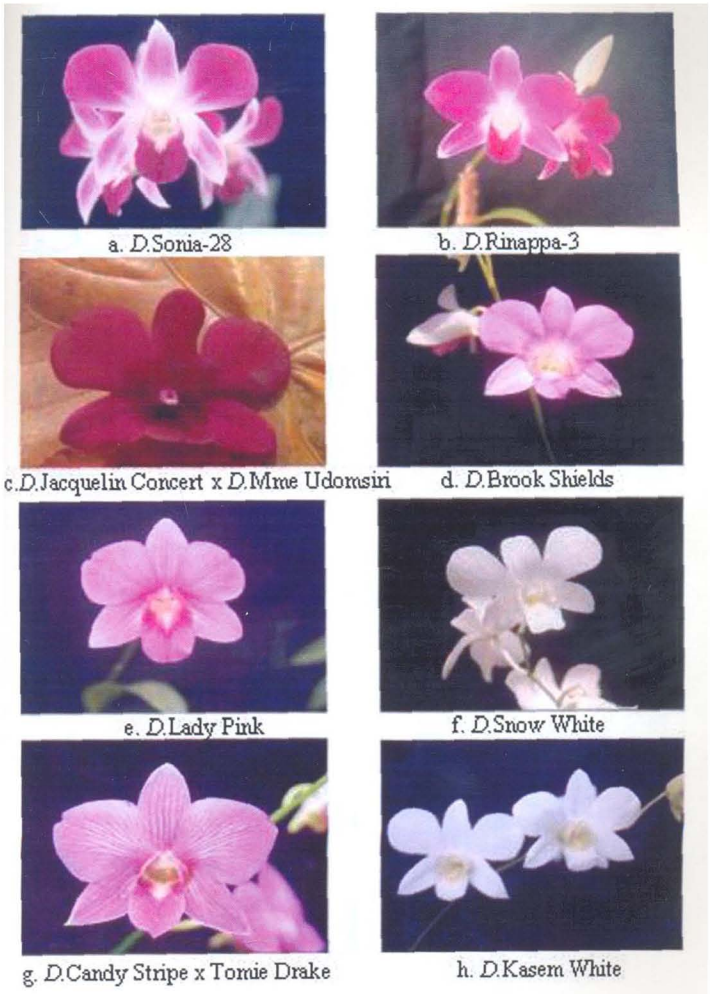
Varieties	Shape of leaves	Leaf sheath colour	Hairiness of leaf sheath	Flower colour	Size and shape of perianth lobes
D. Sonia-28	Linear-lanceolate	Light green	Glabrous	Deep purple petals with white towards centre. Sepals light purple, white towards inside. Lip deep purple Colour group -Red purple	Large flowers, petals larger than sepals and are separate, narrow and pointed sepals, rounded petals, Sepals:4.1-4.3 cm length;1.4-1.7 cm width Petals: 4.5-4.7 cm length;2.7-3.0 cm width Lip: 3.8-4 cm length;2.0-2.3 cm width
D. Rinappa-3	Linear-lanceolate	Light green	Glabrous	Petals and lip deep purple with white near the base. Sepals light purple than petals with white colouration at base and tip Colour group : Red Purple	Medium sized flower, petals broader than sepals and glossy. Sepals: 4.2-4.4 cm length;1.5-1.7cm width Petals:4.5-4.6 cm length;3.3-3.4 cm width Lip:3.5-3.7 cm length;2.0-2.3 cm width
D. Jacquelin Concert x D.Mme Udomsiri	Linear-lanceolate	Light green	Glabrous	Solid reddish purple throughout, glossy Colour group: Red Purple	Medium sized flowers, slightly reflexed, round petals Sepals :3.4-3.5 cm length; 1.7-1.8 cm width Petals:3.9-4.0 cm length; 3.0-3.1 cm width Lip: 3.1-3.3 cm length;1.6-1.8 cm width
D. Brook Shields	Linear-lanceolate	Light green	Glabrous	Light pink and white Colour group: Purple	Medium sized flowers, slightly reflexed petals, pointed sepals and round broad petals Sepals:4.0-4.1cm length; 1.6-1.8 cm width Petals:4.6-4.7 cm length; 3.4-3.5 cm width Lip:4.0-4.2 cm length;2.0-2.1 cm width
D. Lady Pink	Linear-lanceolate	Light green	Glabrous	Light pink with dark pink stripes on sepals, petals and lip Colour group: Purple	Full medium sized, perfectly shaped slightly reflexed rounded sepals and petals Sepals: 3.8-4.2 cm length; 1.8—2.1 cm width Petals:4.2-4.3 cm lenth;3.0-3.1 cm width Lip: 3.7-3.9 cm length; 2.0-2.1 cm width

Table 14. continued...

D. Snow White	Linear-lanceolate	Light green	Glabrous	White petals, petals and lip Colour group: White	Medium sized flower, sepals and petals separate, slightly reflexed, round petals Sepals:3.4-3.8 cm length; 1.6-1.8 cm width Petals :4..3-4.5 cm length; 2.7-2.9 cm width Lip: 3.1-3.3 cm length; 1.6-1.7 cm width
D. Candy Stripe x Tomie Drake	Linear-lanceolate	Light green	Glabrous	Light pink with dark pink stripes throughout, hairy thickening white Colour group: Purple	Medium sized, full. Perfectly shaped broad petals and sepals, sepals at tip Sepals:4.1-4.3 cm length; 2.1-2.3 cm width Petals :4.7-5.0 cm length; 4.7-5.0 cm width Lip:4.3-4.6 cm length;2.1-2.3 cm width
D. Kasem White	Linear-lanceolate	Light green	Glabrous	White sepals, petals and lip Colour group: White	Medium sized, full, slightly reflexed round sepals and petals Sepals:3.0-3.6 cm length; 1.5-1.7 cm width Petals:4.4-4.5 cm length; 2.8-2.9 cm width Lip:3.0-3.2 cm length; 1.6-1.7 cm width
D. Tayswee Keng x Lady Charm	Linear-lanceolate	Light green	Glabrous	Deep purple throughout, glossy Colour group:Red purple	Medium sized, slightly reflexed, round sepals and petals Sepals:3.6-3.8 cm length; 1.6-1.8 cm width Petals:3.5-3.7 cm length; 3.7-2.9 cm width Lip:3.3-3.5 cm length; 1.6-1.9 cm width
D. Sakura Pink	Linear-lanceolate	Light green	Glabrous	Pink and white sepals and petals, White towards inside Colour group: Purple	Medium sized, slightly reflexed sepals and petals of almost equal size, sepals pointed petals rounds Sepals:3.6-4.0 cm length; 1.6-1.8 cm width Petals: 3.9-4.0 cm length; 2.9-3.0 cm width Lip:3.6-4.0 cm length;1.8-2.1 cm width

Table 14. continued...

D. Midnight Velvet Lipstick	Linear-lanceolate	Light green	Glabrous	Solid purple throughout Colour group: Red Purple	Small, perfectly shaped, full and flat round sepals and petals, petals slightly broader than sepals Sepals: 3.1-3.5 cm length; 1.6-1.7 cm width Petals :3.5-3.6 cm length; 2.4-2.7 cm width Lip:2.9-3.0 cm length; 1.4-1.7 cm width
D. Jacquelin Concert x Lady Charm	Linear-lanceolate	Light green	Glabrous	Purple and white sepals and petals, white towards base and purple towards outside, margin of sepals white, lip deep purple towards outside. Colour group: Purple	Large flower, sepals and petals separate, sepals narrower and pointed at tip, petals broader and rounded Sepals: 4.0-4.2 cm length; 1.6-1.7 cm width Petals :4.5-4.8 cm length; 2.9-3.0 cm width Lip:3.4-3.7 cm length;1.6-1.9 cm width
D. Candy Stripe hybrid	Linear-lanceolate	Light green	Glabrous	Deep purple stripes on light purple flowers, sepals white and purple, petals purple towards outside, lip deep purple Colour group: Purple	Medium sized, full, slightly reflexed petals rounded, sepals slightly narrower than petals Sepals:2.7-2.8 cm length; 1.4-1.5 cm width Petals:3.0-3.2 cm length; 2.4—2.6 cm width Lip:2.6-2.8 cm length; 1.5-1.6 cm width
D. Caesar Candy	Linear-lanceolate	Light green	Glabrous	Light pink with dark pink stripes on sepals, petals and lip, hairy growth in lip reddish purple Colour group: Red purple	Small flowers, reflexed sepals, petals and lip, sepals and petals separate and of almost equal size with pointed tips. Sepals:1.9-2.0 cm length; 0.9-1.0 cm width Petals:2.0-2.1 cm length;1.1-1.8 cm width Lip:1.9-2.0 cm length; 1.2-1.3 cm width
D. Thailand White	Linear-lanceolate	Light green	Glabrous	White sepals, petals and lips, hairy labellum yellowish Colour group: White	Medium flowers, petals and sepals reflexed, round sepals and petals Sepals:3.7-3.8 cm length; 1.6-1.7 cm width Petals:4.2-4.5 cm length; 3.1-3.2 cm width Lip:3.7-3.8 cm length; 3.1-3.2 cm width



a. *D.*Sonia-28 b. *D.*Rinappa-3

c.*D.*Jacquelin Concert x *D.*Mme Udomsiri d. *D.*Brook Shields

e. *D.*Lady Pink f. *D.*Snow White

g. *D.*Candy Stripe x Tomie Drake h. *D.*Kasem White

Plate 1. *Dendrobium* **varieties used in the Experiment I**

i. *D.*Tayswee Keng x Lady Charm

j. *D.*Sakura Pink

k. *D.*Midnight Velvet Lipstick

l. *D.*Jacquelin Concert x Lady Charm

m. *D.*Candy Stripe Hybrid

n. *D.*Caesar Candy

o. *D.*Thailand White

Plate 1. *Dendrobium* **varieties used in the Experiment I (continued...)**

4.1.8 Cluster analysis through Mahalanobis D^2 statistics

The 14 varieties of *Dendrobium* were subjected to Mahalanobis D^2 statistical analysis based on the fourteen characters viz., shoot length, shoot girth, number of nodes per shoot, number of laminate leaves per shoot, length of the leaves, width of the leaves, length of the inflorescence, number of flowers, length of the flowers, width of flowers, number of stomata in the abaxial and adaxial surface of the leaf, number of layers in the velamen and number of layers in the cortex. One hundred and ninety six C-2 D^2 values were estimated. The range of these D^2 values being 1362.47 (between *D.* Lady Pink and *D.* Candy Stripe x Tomie Drake) to 2419647 (between *D.* Brook Shields and *D.* Midnight Velvet Lipstick).

Tocher's method was applied to cluster the variety based on their pair wise D^2 values and as such three clusters were formed. The different clusters with the varieties are given in Table 15.

The average intra and inter cluster distance D^2 values are presented in Tables 16 and 17.

The intracluster D^2 values was lowest (87158.998) for C-2 (four varieties) and highest (218462.10) C-3 (two varieties). The intercluster D^2 value was highest between C-2 and C-3 (1450126.013) and lowest between C-1 and C-2 (432549.243).

The cluster means of the fourteen characters are presented in Table 18 and Fig. 9. Cluster-1 showed the highest cluster mean for shoot length, shoot girth, number of nodes per shoot, length of the leaves, width of the leaves and number of stomata in the adaxial surface of the leaf. The highest cluster mean for length and width of the flower, number of stomata in the abaxial surface of leaf, number of layers in the velamen and number of layers in the cortex was registered by C-2. C-3 recorded highest cluster mean for number of laminate leaves per shoot, length of the inflorescence and number of flowers.

Table 15. Group constellation of three clusters

Cluster	No. of genotypes	Genotypes
C-1	8	*D.*Sonia-28, *D.* Jacquelin Concert x *D.* MME Udomsiri, *D.* Lady Pink, *D.* Snow White, *D.* Candy Stripe x Tomie Drake, *D.* Sakura Pink, *D.* Jacquelin, Concert x Lady Charm and *D.* Thailand White
C-2	4	*D.* Rinappa-3, *D.* Brook Shields, *D.* Kasem White and *D.* Tayswee Keng x Lady Charm
C-3	2	*D.* Midnight Velvet Lipstick and *D.* Candy Stripe hybrid

Table 16. Average intra and inter cluster distance (D^2)

Cluster name

C-1	C-2	C-3
123520.165	485395.673	432549.243
	87158.998	1450126.013
		218462.10

Bold figures in diagonals are the intra cluster distance

Table 17. Average intra and inter cluster distance ($\sqrt{D^2}$)

C-1	C-2	C-3
355.575	696.703	657.685
	295.228	1204.212
		467.399

Bold figures in diagonals are the intracluster distance

Table 18. Cluster means of the 14 characters for 14 *Dendrobium* varieties

Sl. No.	Characters	C-1	C-2	C-3	Cluster CV %
1.	Shoot length (cm)	16.982	16.863	13.005	14.488
2.	Shoot girth (cm)	3.662	3.300	3.283	6.298
3.	No. of nodes/shoot	6.404	6.239	5.139	11.602
4.	No.of laminate leaves	4.606	4.611	4.750	1.756
5.	Length of the leaves (cm)	13.638	13.206	12.507	71.147
6.	Width of the leaves (cm)	3.332	2.895	3.107	7.024
7.	Length of the inflorescence	23.890	24.344	24.772	1.812
8.	No.of flowers	4.386	4.417	5.167	9.497
9.	Length of the flowers (cm)	5.946	6.165	5.285	7.901
10.	Width of the flowers (cm)	6.492	7.283	5.455	14.302
11.	Number of stomata in the adaxial surface of leaf (per cm^2)	635.875	628.500	389.500	25.425
12.	No.of stomata in the abaxial surface of the (per cm^2)	3288.00	3449.917	2467.667	17.162
13.	No.of layers in the velamen	7.084	8.000	7.167	6.830
14.	No.of layers in the cortex	5.708	7.111	4.834	19.521

1- Shoot length 2- Shoot girth 3-No. of nodes per shoot 4- No. of laminate leaves per shoot 5-Length of leaves
6-Width of leaves 7-Length of inflorescence 8-No. of flowers 9-Length of flowers 10-Width of flowers
11-No. of stomata in adaxial surface of leaves 12- No. of stomata in abaxial surface of leaves
13- No. of layers in velamen 14-No. of layers in the cortex

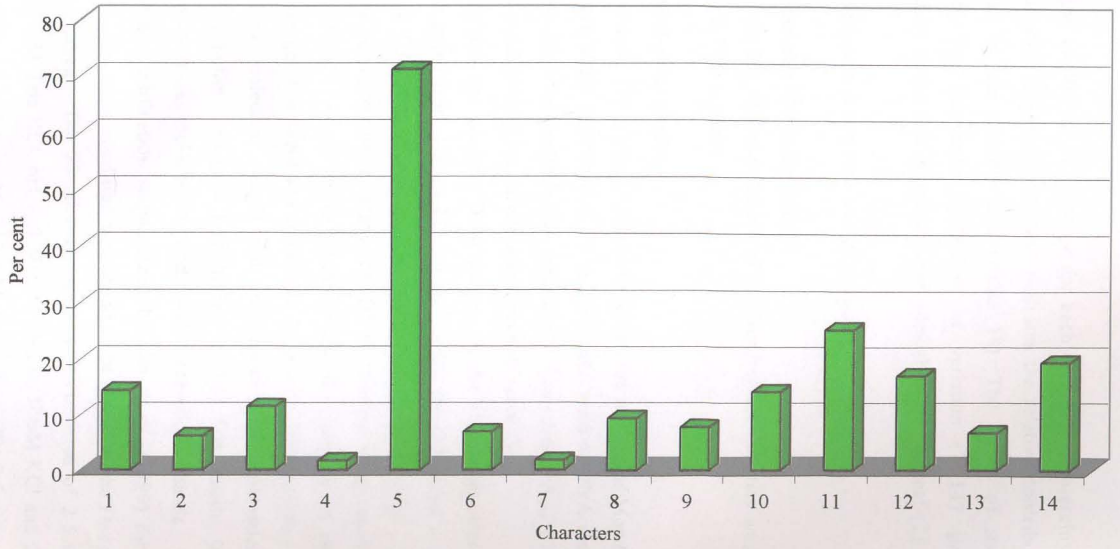


Fig. 9. Pattern of variation in terms of CV. at intercluster level

The coefficient of variation for each character at intercluster level was estimated and this coefficient indicates the relative contributions of each trait at the cluster level (Table 18). The length of the leaves registered the maximum coefficient of variation (71.147 per cent) followed by number of stomata in the adaxial surface of leaf (25.425 per cent).

Maximum divergence was observed for C-2 with C-3.

4.1.8 Molecular characterization

Molecular characterization of 15 *Dendrobium* varieties was carried out using RAPD markers.

4.1.8.1 Molecular analysis

Genomic DNA was extracted based on the method of Mondal *et al.* (2000) with slight modification. The purity and yield of DNA were good when fresh unfurled tender leaves were used. The ratio of A_{260} / A_{280} and the DNA yield of different varieties are given in Table 19.

The average genomic DNA content of the 15 varieties was 169.33 μg ml^{-1} (ranged from 120 to 225 μg ml^{-1}) while the $O.D_{260/280}$ ratios was an average 1.74 (ranged from 1.53 to 1.89).

The electrophoretic assay of DNA samples using agarose gel electrophoresis (1.4 per cent) revealed that the integrity of the DNA sample was good, without any smearing. In fact the DNA samples were in general high molecular weight and undegraded 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 bands.

DNA amplification standardized by Lim *et al.* (1999) for *Vanda* was used. The same condition was found to be good for the *Dendrobium* varieties studied. The 25 ml reaction mixture consisted of 2.5 ml 10 x buffer (10 mM Tris HCl, pH 9, 1.50 mM $MgCl_2$, 50mM KCl and 0.01 per cent gelatin), 10 pM primers, 250 mM each of dNTP's 0.6 units of Taq DNA polymerase and 20 ng of DNA gave good amplification. The

Table 19. Quality and yield of DNA from 15 *Dendrobium* varieties

Sl. No.	Variety	A260	A280	A260/ A280	DNA yield $\mu g\ ml^{-1}$
1.	*D.* Sonia-28	0.027	0.015	1.80	135
2.	*D.* Rinappa-3	0.043	0.023	1.87	215
3.	*D.* Jacquelin Concert x D.MME Udomsiri	0.031	0.019	1.63	155
4.	*D.* Brook Shields	0.037	0.022	1.68	185
5.	*D.* Lady Pink	0.045	0.025	1.80	225
6.	*D.* Snow White	0.029	0.019	1.53	145
7.	*D.* Candy Stripe x Tomie Drake	0.035	0.021	1.67	175
8.	*D.* Kasem White	0.043	0.026	1.65	215
9.	*D.* Tayswee Keng x Lady Charm	0.040	0.022	1.82	200
10.	*D.* Sakura Pink	0.027	0.017	1.59	135
11.	*D.* Midnight Velvet Lipstick	0.036	0.019	1.89	180
12.	*D.* Jacquelin Concert x Lady Charm	0.024	0.014	1.72	120
13.	*D.* Candy Stripe hybrid	0.035	0.020	1.75	175
14.	*D.* Caesar Candy	0.024	0.013	1.85	120
15.	*D.* Thailand White	0.032	0.018	1.78	160

programme consisted of an initial denaturation at 95^{0}C for one minute followed by 45 cycles of denaturation at 95^{0}C for one minute annealing at 35^{0}C for two minutes and extension at 72^{0}C for ten minutes. The amplification products were cooled to 4^{0}C after the reaction.

Of the forty primers used in this study only 35 showed amplification with the DNA from *D.* Rinappa-3 (Table 20 and Fig.10). There was no amplification with the primers OPA 06, OPA 12, OPA 20, OPD 09 and OPB 19. The total number of bands, number of intense and fair bands produced by the primers are given in Table 20. These primers amplified 109 RAPD marker bands (average of 2.73 bands per primer). Of these 102 (93.58 per cent) were polymorphic and seven were monomorphic. The primers OPA 05, OPA 08, OPA 14, OPA 16, OPA 17, OPA 18 and OPB 13 produced monomorphic bands.

The highest number of RAPD's was produced by the primers OPB 04 (10) followed by OPA 19 and OPB 02 (6 each). OPB 06 produced five bands with three faint and two intense bands. The highest number of faint bands was produced by OPB 07 (4 bands) followed by OPA 03 and OPB 06 (3 each). OPA 04, OPA 19, OPB 04 and OPB 10 produced highest number of intense bands. OPA 03, OPA 05, OPA 10, OPA 14, OPA 17, OPA 18, OPB 08, OPB 12, OPB 13,OPB 14, OPB 15, OPB 16 and OPB 20 gave one intense band each.

The four primers produce the highest number of bands as well as the highest number of intense bands was selected for DNA amplification from the 15 *Dendrobium* varieties. The PCR reaction was repeated at least twice in order to confirm the reproducibility. The data obtained from four primers that gave reproducible bands were used for statistical analysis. They are OPA 19, OPB 02, OPB 4 and OPB 10. These primers amplified 44 scorable RAPD marker bands with an average of 11 bands per primer. Of these 39 bands (88.64 per cent) were polymorphic and five were monomorphic. The number of bands resolved per amplification was primer dependent and varied from a minimum of eight to a maximum of thirteen.

Table 20. Primer associated banding patterns with the DNA of Rinappa-3 using 40 primers belonging to kit A and kit B of operon on Inc, CA, USA

Sl. No.	Primers	No.of faint bands	No.of intense bands	Total no. of bands
1.	OPA 01	2	2	4
2.	OPA02	-	2	2
3.	OPA03	3	1	4
4.	OPA04	0	4	4
5.	OPA05	0	1	1
6.	OPA06	0	0	0
7.	OPA07	1	2	3
8.	OPA08	1	0	1
9.	OPA09	0	2	2
10.	OPA10	1	1	2
11.	OPA11	0	3	3
12.	OPA12	0	0	0
13.	OPA13	0	2	2
14.	OPA14	0	1	1
15.	OPA15	2	2	4
16.	OPA16	1	0	1
17.	OPA17	0	1	1
18.	OPA18	0	1	1
19.	OPA19	5	1	6
20	OPA20	0	0	0
21.	OPB01	1	3	4
22.	OPB02	4	2	6
23	OPB03	2	2	4
24.	OPB04	10	-	10
25.	OPB05	1	3	4
26.	OPB06	3	2	5
27.	OPB07	4	0	4
28.	OPB08	2	1	3
29.	OPB09	0	0	0
30	OPB10	2	2	4
31.	OPB11	2	2	4
32.	OPB12	2	1	3
33.	OPB13	0	1	1
34.	OPB14	1	1	2
35.	OPB15	2	1	3
36.	OPB16	1	1	2
37.	OPB17	0	3	3
38.	OPB18	0	2	2
39.	OPB19	0	0	0
40.	OPB20	2	1	3

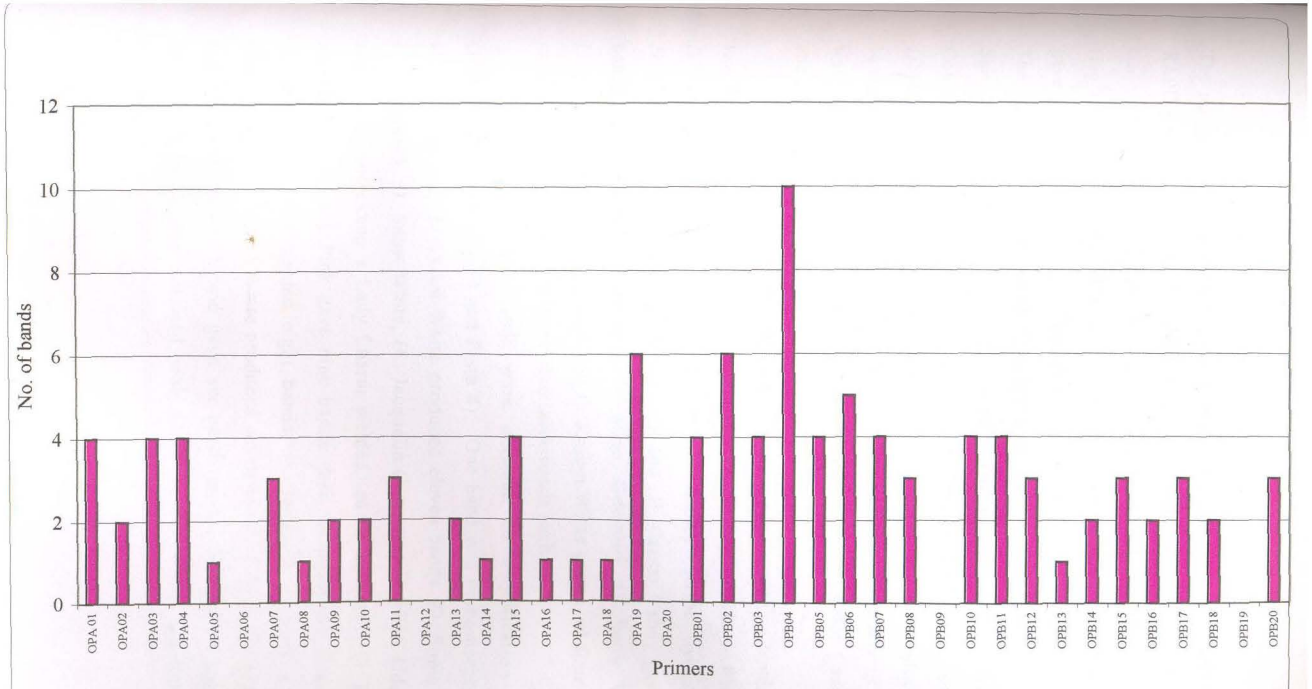


Fig. 10. Amplification profile (total bands) of the DNA of *D.* Rinappa-3 using forty primers of Kit A and Kit B

The nucleotide sequence of these primers and the number of informative RAPD markers amplified by each primer are given in Table 21 and Fig. 11.

A total of 12 scorable bands were obtained by the primer OPA 19 (Fig.12 and Plate 2). The variety *D.* Tayswee Keng X *D.* Lady Charm gave the highest number of bands (9) followed by *D.* Sakura Pink (8). The varieties *D.* Jacquelin Concert x *D.* Mme.. Udomsiri, *D.* Brook Shields, *D.* Lady Pink, *D.* Candy Stripe x Tomie Drake and *D.* Jacquelin Concert x Lady Charm produced seven bands each. *D.* Sonia-28, *D.* Rinappa, *D.* Kasem White and *D.*Candy Stripe hybrid, produced six bands each. *D.* Snow White yielded five bands. Lower number of bands was produced by *D.* Midnight Velvet Lipstick (3), *D.* Caesar Candy (2) and *D.* Thailand White (2).

The primer OPB 02 produced a total of eight scorable bands with two monomorphic bands (Fig.13 and Plate 3). *D.* Sonia - 28, *D.* Rinappa, *D.* Jacqulin Concert x Lady Charm, *D.* Candy Stripe hybrid and *D.* Thailand White yielded six bands each and *D.* Caesar Candy gave five bands. *D.* Jacquelin Concert x *D.* Mme. Udomsiri, *D.* Snow White, *D.* Candy Stripe x Tomie Drake and *D.* Kasem White produced four bands each. The rest of the varieties yielded three bands each.

Thirteen scorable bands were obtained on amplification when OPB 04 was used (Fig.14 and Plate 4). One band was monomorphic for all the varieties. D.Kasem White produced eleven bands. *D.* Sonia - 28, *D.* Rinappa-3, *D.* Snow White, *D.* Jacquelin Concert x *D.* Mme. Udomsiri and *D.* Taysureekeng x Lady Charm yielded ten bands each. *D.* Brook Shields and *D.*Lady Pink gave nine bands each. *D.* Jacquelin Concert x *D.* Mme. Udomsiri yielded eight bands. *D.* Jacquelin Concert x Lady Charm and *D.* Thailand White produced seven bands each. *D.* Sakura Pink and *D.* Candy Stripe hybrid gave six bands each. Minimum number of four bands each was obtained with *D.* Midnight Velvet Lipstick and *D.* Caesar Candy.

Table 21. Nucleotide sequence of primers and total number of informative RAPD markers amplified by them in the 15 *Dendrobium* varieties

Primer	Nucleotide sequence	Number of informative RAPD markers
OPA 19	CAAACGTCGG	12
OPB 02	TGATCCCTGG	8
OPB 04	GGACTGGAGT	13
OPB 10	CTGCTGGGAC	11

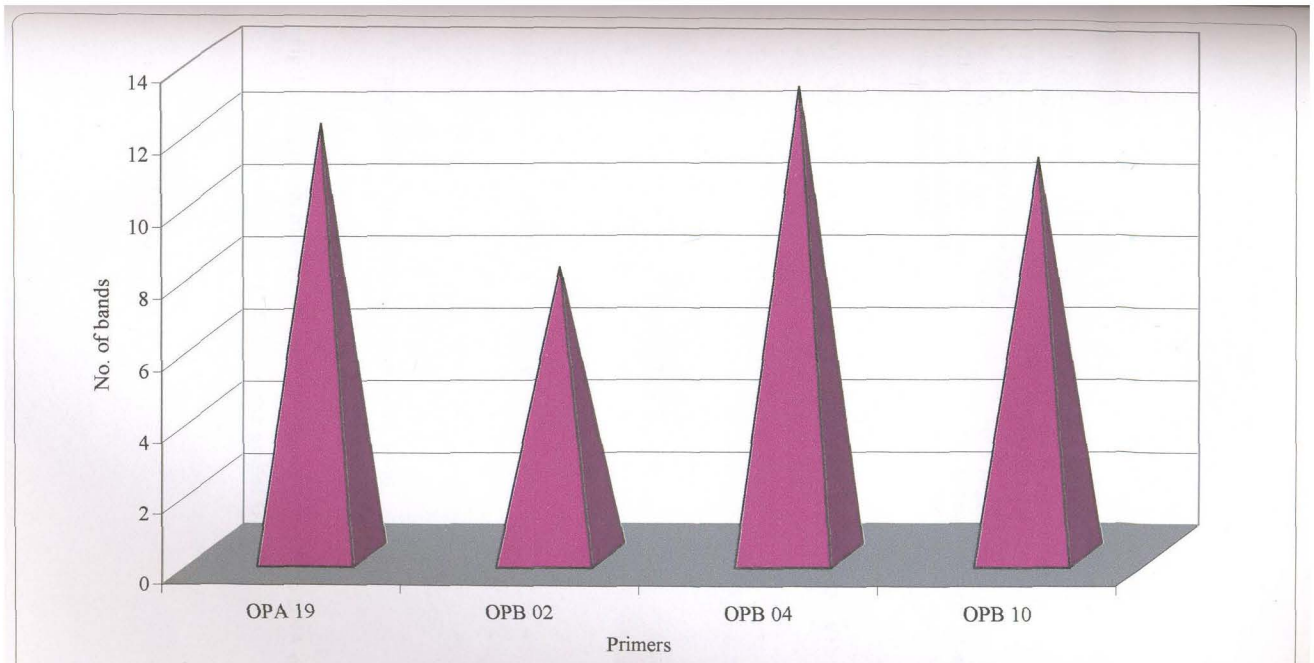


Fig. 11. Total number of informative RAPD markers amplified with the four primers in fifteen *Dendrobium* varieties

V1 -*D.* Sonia-28
V2-*D.* Rinappa-3
V3-*D.* Jacquelin Concert x D.MME Udomsiri
V4 -*D.* Brook Shields
V5 -*D.* Lady Pink
V6- *D.* Snow White
V7- *D.* Candy Stripe x Tomie Drake
V8- *D.* Kasem White
V9- *D.* Tayswee Keng x Lady Charm
V10- *D.* Sakura Pink
V11- *D.* Midnight Velvet Lipstick
V12- *D.* Jacquelin Concert x Lady Charm
V13- *D.* Candy Stripe hybrid
V14- *D.* Caesar Candy
V15- *D.* Thailand White

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15
+	+	+	+	+	+	+	-	+	-	+	+	-	-	+
+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
-	+	-	-	-	-	-	-	-	+	+	-	-	-	-
+	+	+	-	+	-	+	+	-	-	-	+	+	-	-
-	-	+	-	-	-	-	+	+	-	-	+	+	-	-
+	-	-	+	-	-	+	+	+	+	-	-	-	-	-
-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
-	+	+	+	-	-	-	-	+	+	-	-	-	-	-
-	-	-	+	+	+	+	+	+	+	-	+	+	+	-
+	-	-	+	-	+	-	-	+	+	-	+	+	-	-
-	+	+	-	+	-	+	-	+	+	-	-	-	-	-
+	-	+	+	+	+	+	+	+	+	-	+	+	-	-

+ = presence of band - = absence of band

Fig.12. Representation of the amplification profile of the DNA of fifteen *Dendrobium* varieties using the primer OPA 19

Lane 1	→	*D.* Sonia-28
Lane 2	→	*D.* Rinappa-3
Lane 3	→	*D.* Jacquelin Concert x *D.* Mme. Udomsiri
Lane 4	→	*D.* Brook Shields
Lane 5	→	*D.* Lady Pink
Lane 6	→	*D.* Snow White
Lane 7	→	*D.* Candy Stripe x Tomie Drake
Lane 8	→	*D.* Kasem White
Lane 9	→	*D.* Tayswee Keng x Lady Charm
Lane 10	→	*D.* Sakura Pink
Lane 11	→	*D.* Midnight Velvet Lipstick
Lane 12	→	*D.* Jacquelin Concert x Lady Charm
Lane 13	→	*D.* Candy Stripe hybrid
Lane 14	→	*D.* Caesar Candy
Lane 15	→	*D.* Thailand White
Lane B	→	Blank
Lane L	→	PCR Molecular marker (U.S. Biochemicals)

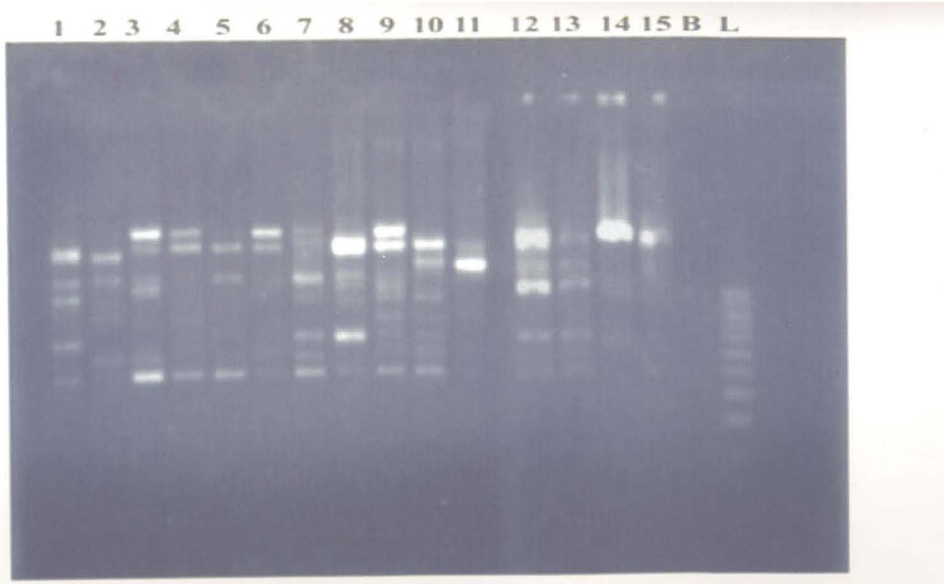


Plate 2. Amplification profiles of the DNA of fifteen *Dendrobium* varieties using the primer OPA 19

V1 -*D.* Sonia-28
V2-*D.* Rinappa-3
V3-*D.* Jacquelin Concert x D.MME Udomsiri
V4 -*D.* Brook Shields
V5 -*D.* Lady Pink
V6- *D.* Snow White
V7- *D.* Candy Stripe x Tomie Drake
V8- *D.* Kasem White
V9- *D.* Tayswee Keng x Lady Charm
V10- *D.* Sakura Pink
V11- *D.* Midnight Velvet Lipstick
V12- *D.* Jacquelin Concert x Lady Charm
V13- *D.* Candy Stripe hybrid
V14- *D.* Caesar Candy
V15- *D.* Thailand White

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15
-	-	-	-	-	-	-	--	-	-	+	-	-	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	-	-	-	-	-	-	-	-	-	+	+	-	-
+	+	+	+	+	+	+	+	+	-	-	+	+	+	+
+	+	-	-	-	-	-	+	-	+	-	+	+	-	+
-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	-	-	+	+	-	-	-	-	+	+	-	+

+ = presence of band - = absence of band

Fig. 13. Representation of the amplification profile of the DNA of fifteen *Dendrobium* varieties using the primer OPB 02

Lane 1	→	*D.* Sonia-28
Lane 2	→	*D.* Rinappa-3
Lane 3	→	*D.* Jacquelin Concert x *D.* Mme. Udomsiri
Lane 4	→	*D.* Brook Shields
Lane 5	→	*D.* Lady Pink
Lane 6	→	*D.* Snow White
Lane 7	→	*D.* Candy Stripe x Tomie Drake
Lane 8	→	*D.* Kasem White
Lane 9	→	*D.* Tayswee Keng x Lady Charm
Lane 10	→	*D.* Sakura Pink
Lane 11	→	*D.* Midnight Velvet Lipstick
Lane 12	→	*D.* Jacquelin Concert x Lady Charm
Lane 13	→	*D.* Candy Stripe hybrid
Lane 14	→	*D.* Caesar Candy
Lane 15	→	*D.* Thailand White
Lane B	→	Blank
Lane L	→	PCR Molecular marker (U.S. Biochemicals)

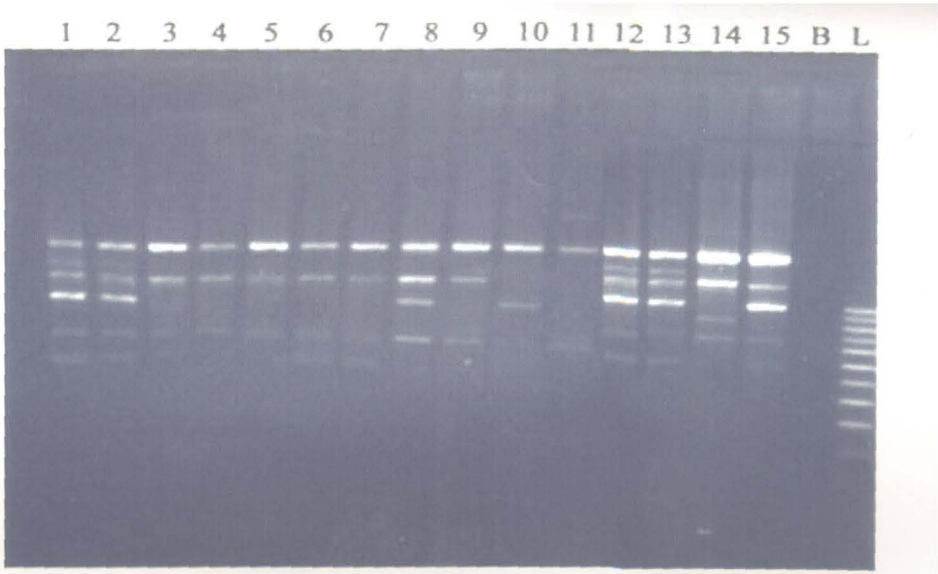


Plate 3. Amplification profiles of the DNA of fifteen *Dendrobium* varieties using the primer OPB 2

V1 -*D.* Sonia-28
V2-*D.* Rinappa-3
V3-*D.* Jacquelin Concert x D.MME Udomsiri
V4 -*D.* Brook Shields
V5 -*D.* Lady Pink
V6- *D.* Snow White
V7- *D.* Candy Stripe x Tomie Drake
V8- *D.* Kasem White
V9- *D.* Tayswee Keng x Lady Charm
V10- *D.* Sakura Pink
V11- *D.* Midnight Velvet Lipstick
V12- *D.* Jacquelin Concert x Lady Charm
V13- *D.* Candy Stripe hybrid
V14- *D.* Caesar Candy
V15- *D.* Thailand White

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15
-	-	-	-	-	+	+	+	+	+	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	-	+	+	+	+	-	-	-	+	+	+	+
+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
+	+	+	+	+	-	+	+	+	-	-	+	+	-	+
+	+	+	+	-	+	-	+	+	+	+	+	+	-	+
+	+	+	+	+	+	+	+	+	-	+	+	-	-	+
+	+	-	+	+	+	+	+	+	+	+	+	+	-	+
-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	+	+	+	+	-	+	+	-	-	-	-	-
+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
+	+	+	+	+	+	+	+	+	-	-	-	-	-	-

+ = presence of band - = absence of band

Fig. 14. Representation of the amplification profile of the DNA of fifteen *Dendrobium* varieties using the primer OPB 04

Lane 1	→	*D.* Sonia-28
Lane 2	→	*D.* Rinappa-3
Lane 3	→	*D.* Jacquelin Concert x *D.* Mme. Udomsiri
Lane 4	→	*D.* Brook Shields
Lane 5	→	*D.* Lady Pink
Lane 6	→	*D.* Snow White
Lane 7	→	*D.* Candy Stripe x Tomie Drake
Lane 8	→	*D.* Kasem White
Lane 9	→	*D.* Tayswee Keng x Lady Charm
Lane 10	→	*D.* Sakura Pink
Lane 11	→	*D.* Midnight Velvet Lipstick
Lane 12	→	*D.* Jacquelin Concert x Lady Charm
Lane 13	→	*D.* Candy Stripe hybrid
Lane 14	→	*D.* Caesar Candy
Lane 15	→	*D.* Thailand White
Lane B	→	Blank
Lane L	→	PCR Molecular marker (U.S. Biochemicals)

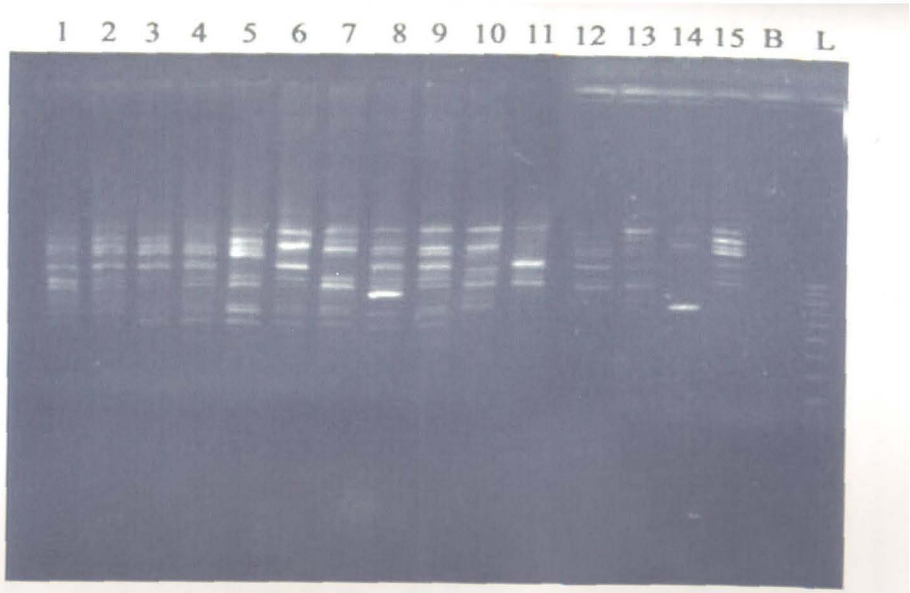


Plate 4. Amplification profiles of the DNA of fifteen *Dendrobium* varieties using the primer OPB 4

When OPB 10 was used for amplification eleven scorable RAPD bands were obtained of which two were monomorphic for all the varieties (Fig.15 and Plate 5). Maximum number of bands (8) was obtained with *D.* Snow White followed by *D.* Brook Shields and *D.* Tayswee Keng X *D.* Lady Charm (7 each). *D.* Lady Pink and *D.* Kasem White produced six bands each. Five bands each were obtained with *D* Sonia-28, *D.* Jacquelin Concert x *D.* Mme. Udomsiri, *D.* Candy Stripe x Tomie Drake, *D.* Sakura Pink, *D.* Midnight Velvet Lipstick, *D.* Caesar Candy and *D.* Thailand White. The varieties *D.* Rinappa-3 and *D.* Jacqelin Concert x Lady Charm yielded four band each. Minimum amplification was obtained with *D.* Candy Stripe hybrid (3 bands).

4.1.8.2 Data analysis

The banding pattern from RAPD analysis for each primer was scored by visual observation. The presence of an amplification product in each position was recorded as positive for presence and negative for absence. Based on the presence or absence data a genetic similarity matrix was constructed using the Jaccard's coefficient method (Table 22). The coefficient value ranged between 0.2667 and 0.8824. The lowest similarity coefficient value were those of *D.* Sakura Pink with *D.* Caesar Candy (0.2667) while the highest value for similarity index was obtained for the *D.* Sonia - 28 and *D.* Snow White pair (0.8824).

The distances between the varieties were computed using SYSTAT (Version 9) software package. The distance between the varieties and number of varieties that are grouped together are given in Table 23. The distance was lowest between *D.* Sonia-28 and *D.* Snow White (0.1176) followed by *D.* Candy Stripe hybrid – *D.* Jacqelin Concert x Lady Charm (0.1250). The distance between D. Lady Pink – *D.* Candy Stripe x Tomie Drake pair and *D.* Sonia-28 – *D.* Jacquelin Concert x Lady Charm pair were similar (0.2414). The greatest distance was between *D.* Caesar Candy – *D.* Midnight Velvet Lipstick (0.5806).

V1 -*D.* Sonia-28
V2-*D.* Rinappa-3
V3-*D.* Jacquelin Concert x D.MME Udomsiri
V4 -*D.* Brook Shields
V5 -*D.* Lady Pink
V6- *D.* Snow White
V7- *D.* Candy Stripe x Tomie Drake
V8- *D.* Kasem White
V9- *D.* Tayswee Keng x Lady Charm
V10- *D.* Sakura Pink
V11- *D.* Midnight Velvet Lipstick
V12- *D.* Jacquelin Concert x Lady Charm
V13- *D.* Candy Stripe hybrid
V14- *D.* Caesar Candy
V15- *D.* Thailand White

V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15
-	-	+	+	+	+	-	-	-	+	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	-	-	+	-	+	-	+	-	-	+	-
-	-	-	+	-	+	-	-	+	-	+	-	-	-	+
+	+	+	+	+	+	+	+	+	-	-	+	-	+	+
+	-	-	+	+	-	-	+	-	-	-	-	-	-	-
-	+	-	-	-	+	-	+	-	-	-	-	-	-	-
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
+	-	+	+	+	+	+	+	+	+	+	+	+	-	+
-	-	-	-	-	+	-	-	-	-	-	-	-	+	-

+ = presence of band - = absence of band

Fig. 15. Representation of the amplification profile of the DNA of fifteen *Dendrobium* varieties with the primer OPB 10

Lane 1	→	*D.* Sonia-28
Lane 2	→	*D.* Rinappa-3
Lane 3	→	*D.* Jacquelin Concert x *D.* Mme. Udomsiri
Lane 4	→	*D.* Brook Shields
Lane 5	→	*D.* Lady Pink
Lane 6	→	*D.* Snow White
Lane 7	→	*D.* Candy Stripe x Tomie Drake
Lane 8	→	*D.* Kasem White
Lane 9	→	*D.* Tayswee Keng x Lady Charm
Lane 10	→	*D.* Sakura Pink
Lane 11	→	*D.* Midnight Velvet Lipstick
Lane 12	→	*D.* Jacquelin Concert x Lady Charm
Lane 13	→	*D.* Candy Stripe hybrid
Lane 14	→	*D.* Caesar Candy
Lane 15	→	*D.* Thailand White
Lane B	→	Blank
Lane L	→	PCR Molecular marker (U.S. Biochemicals)

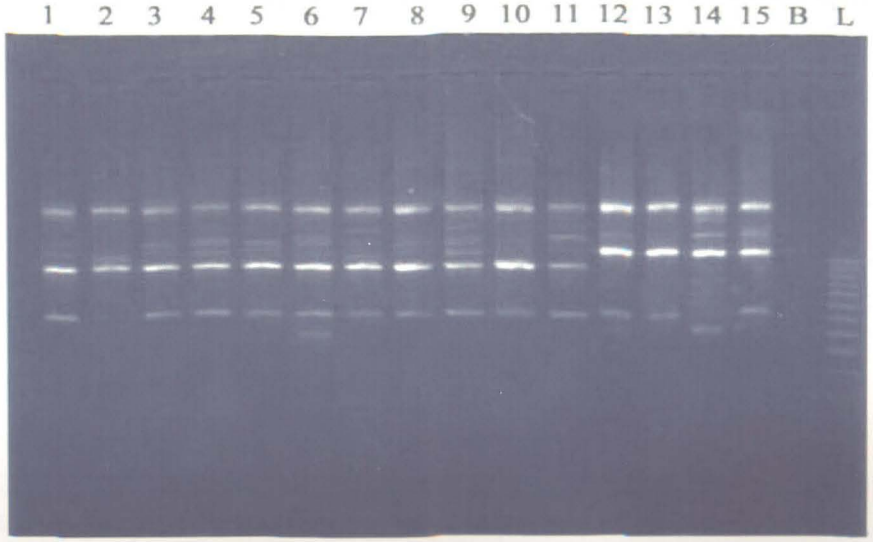


Plate 5. Amplification profiles of the DNA of fifteen *Dendrobium* varieties using the primer OPB 10

Table 22. Similarity matrix of 15 *Dendrobium* varieties based on the Jaccards Similarity Index

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1.*D.* Sonia-28	1.00														
2.*D.* Rinappa-3	0.6563	1.00													
3.*D.* Jacquelin Concert x D.MME Udomsiri	0.6452	0.6129	1.00												
4.*D.* Brook Shields	0.6364	0.4444	0.6129	1.00											
5.*D.* Lady Pink	0.6250	0.5455	0.6897	0.7000	1.00										
6.*D.* Snow White	0.8824	0.4722	0.5938	0.7097	0.6250	1.00									
7.*D.* Candy Stipe x Tomie Drake	0.6563	0.5294	0.6667	0.6250	0.7586	0.6563	1.00								
8.*D.* Kasem White	.6875	0.5143	0.5938	0.6061	0.6250	0.6667	0.6563	1.00							
9.*D.* Tayswee Keng x Lady Charm	0.5556	0.4474	0.6061	0.7742	0.5882	0.7333	0.7188	0.60000	1.00						
10.*D.* Sakura Pink	0.4000	0.3333	0.3939	0.5484	0.4242	0.4848	0.4545	0.4412	0.5938	1.00					
11.*D.* Midnight Velvet Lipstick	0.3667	0.3226	0.3448	0.4138	0.3333	0.4000	0.3667	0.3125	0.4194	0.3704	1.00				
12.*D.* Jacquelin Concert x Lady Charm	0.7586	0.5625	0.6552	0.5625	0.5806	0.5938	0.6129	0.6452	0.5588	0.4375	0.3929	1.00			
13.*D.* Candy Stripe hybrid	0.6552	0.4688	0.5517	0.4688	0.4839	0.5000	0.5161	0.6000	0.4706	0.4838	0.3333	0.8750	1.00		
14.*D.* Caesar Candy	0.6438	0.3125	0.3793	0.3548	0.4138	0.4333	0.4483	0.3871	0.3636	0.2667	0.3478	0.3793	0.3704	1.00	
15.*D.* Thailand White	0.6207	0.5333	0.5714	0.5161	0.5000	0.5667	0.5333	0.5161	0.4848	0.3548	0.5909	0.6923	0.57690	0.4400	1.00

Table 23. Genetic distance between the genotypes and the number of genotypes grouped

Genotype pairs	Genetic distance	Number of genotypes
D. Sonia-28 – *D.* Snow White	0.1176	2
D. Candy Stripe hybrid – *D.* Jacquelin Concert x Lady Charm	0.1250	2
D. Brook Shields – *D.* Tayswee Keng x Lady Charm	0.2258	2
D. Lady Pink – *D.* Candy Stripe x Tomie Drake	0.2414	2
D. Sonia-28 – *D.* Jacquelin Concert x Lady Charm	0.2414	4
D. Sonia-28 – *D.* Tayswee Keng x Lady Charm	0.2667	6
*D.*Sonia-28 - *D.* Candy Stripe x Tomie Drake	0.2812	8
*D.*Sonia-28 – *D.* Thailand White	0.3077	9
D. Thailand White – *D.* Jacquelin Concert x *D.* Mme. Udomsiri	0.3103	10
D. Jaquelin Concert x *D.* Mme. Udomsiri – *D.* Kasem White	0.3125	11
D. Kasem White – *D.*Rinappa-3	0.3437	12
D. Rinappa-3 – *D.* Sakura Pink	0.4062	13
D. Sakura Pink – *D.* Caesar Candy	0.5517	14
D. Caesar Candy – *D.* Midnight Velvet Lipstick	0.5806	15

On drawing a vertical line in the dendrogram (Fig. 16) along the point corresponding to a distance of 0.425, all the 15 varieties got divided into six clusters. Varieties D.Sonia-28, *D.* Snow White, *D.* Candy Stripe hybrid, *D.* Jacqelin Concert x Lady Charm, D. Brook Shields, *D.* Tayswee Keng X *D.* Lady Charm, *D.* Lady Pink and *D.* Candy Stripe x Tomie Drake formed a single cluster. *D.* Thailand White, *D.* Jacquelin Concert x *D.* Mme. Udomsiri, *D.* Kasem White, *D.* Rinappa and *D.* Sakura Pink formed separate cluster each. *D.* Caesar Candy and *D.* Midnight Velvet Lipstick together formed another cluster.

4.2. EXPERIMENT II

The results of the investigations on the morpho-anatomical characterization of nine *Dendrobium* varieties are presented below.

4.2.1 Analysis of variance of Morphological, Inflorescence and Anatomical Characters

The analysis of variance of 23 characters in nine *Dendrobium* varieties is presented in Table 24. Among the 23 characters studied, 18 characters showed significant difference among the varieties while five characters *viz.*, orientation of the leaves, length of the inflorescence, thickness of inflorescence stalk, basal internodal length of stalk and thickness of cortex were not significant.

4.2.2 Mean performance of the Varieties

4.2.2.1 Morphological Characters

The mean values of the varieties for the eight morphological characters studied are presented in Table 25.

The length of the shoot varied from 30 – 112 cm. *D.* Purple-1 recorded the maximum shoot length (112.00 cm) and it was significantly different from the other varieties, which were on par. Similarly shoot girth was also maximum (5.667 cm) for *D.*Purple-1. *D.* Oschin recorded

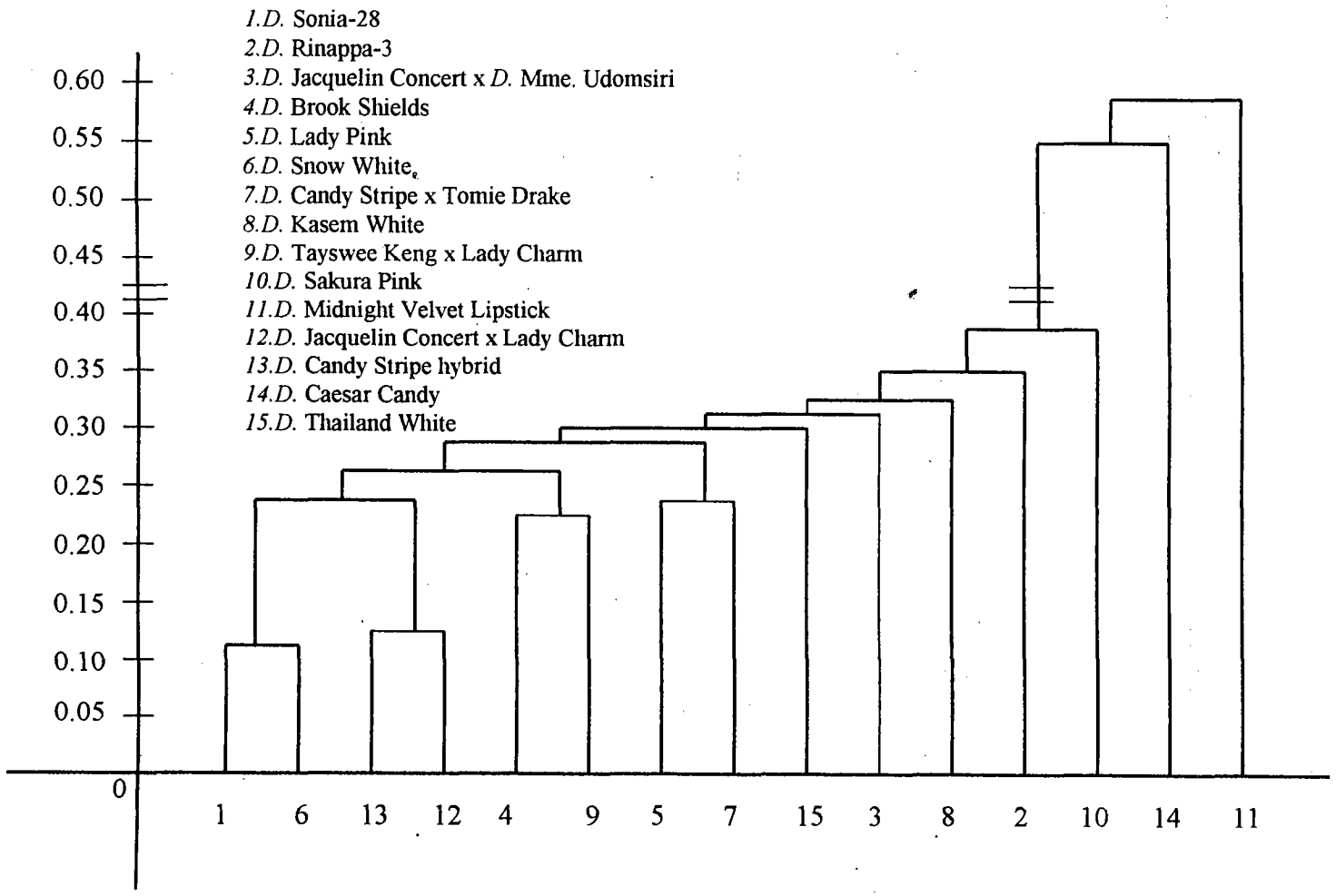


Fig. 16. Dendrogram obtained from RAPD analysis using nearest neighbour (single link) method

Table 24. Analysis of variance of characters in nine *Dendrobium* varieties

Sl. No.	Characters	Mean square	
		Genotypes 8	Error 18
1.	Shoot length (cm)	2980.093*	887.513
2.	Shoot girth (cm)	1.990**	0.207
3.	Internodal length of shoots (cm)	2.064**	0.524
4.	Number of nodes per plant	223.816**	60.499
5.	Number of laminate leaves per plant	46.925**	8.188
6.	Length of the leaves (cm)	6.191*	2.349
7.	Width of the leaves (cm)	1.392*	0.521
8.	Orientation of the leaves (^{0})	418.342^{NS}	282.000
9.	Length of the inflorescence (cm)	53.736^{NS}	72.419
10.	Thickness of the inflorescence stalk (cm)	0.005^{NS}	0.002
11.	Basal internodal length of the stalk (cm)	2.492^{NS}	1.264
12.	Number of flowers	30.897**	7.591
13.	Length of the flowers (cm)	2.942**	0.170
14.	Width of the flowers (cm)	2.032**	0.285
15	Root thickness (mm)	0.188**	0.026
16	Thickness of the cortex (mm)	0.004^{NS}	0.002
17	Number of layers in the cortex	1.583**	0.444
18	Number of layers in the valamen	5.583**	0.741
19	Leaf thickness (mm)	0.059**	0.007
20	Number of stomata in the adaxial surface of the leaf (per cm^2)	19871.25*	8513.44
21	Number of stomata in the abaxial surface of the leaf (per cm^2)	2149098**	69297.78
22	Petal thickness (mm)	0.029**	0.002
23	Thickness of pigmented layers	0.0009**	0.0002

** Significant at 1 per cent level * Significant at 5 per cent level DF- Degrees of freedom

Table 25. Differences in shoot and leaf morphology among nine *Dendrobium* varieties

Varieties	Shoot length (cm)	Shoot girth (cm)	Internodal length of shoots (cm)	Number of nodes per plant	Number of laminate leaves per shoot	Length of the leaves (cm)	Width of the leaves (cm)	Orientation of the leaves (Degrees)
D. Uniwai Pink	34.547	3.333	5.267	11.470	6.530	13.533	4.433	76.667
D. Madam Pompadour	33.750	3.433	4.667	11.667	6.833	13.747	2.830	95.000
D. Nave Blue	54.803	4.233	4.693	17.133	9.537	12.470	4.010	100.000
D. Purple –1	112.000	5.667	6.100	33.833	16.677	11.967	4.200	90.000
D. Purple – 2	81.000	4.167	6.000	23.000	11.890	11.790	4.550	111.667
D. Yupee Deewan	78.760	4.533	5.217	23.557	11.500	10.057	3.780	83.333
D. Oschin	17.003	3.033	3.610	7.610	4.223	10.377	2.900	74.333
D. Nagoya Pink	31.067	3.533	4.330	10.000	6.333	13.000	2.867	96.667
D. Waipahu Pink	30.000	3.467	4.170	10.670	5.670	14.113	3.600	86.667
F, 8, 26	3.358*	9.629**	3.939**	3.699**	5.731**	2.636*	2.671*	1.483^{NS}
SEm	17.200	0.262	0.418	4.491	1.652	0.885	0.417	9.695
CD	51.105	0.780	1.242	13.343	4.909	2.629	1.238	28.807

the lowest shoot girth (3.033 cm) and *D.*Uniwai Pink, *D.* Madam Pompadour. *D.* Waipahu Pink and *D.* Nagoya Pink were on par with it.

The maximum internodal length (6.100 cm) was recorded in *D.*Purple-1 and it was on par with *D.*Purple-2, *D.* Uniwai Pink and *D.* Yupee Deewan. The internodal length was minimum (3.610 cm) in *D.* Oschin.

Higher number of nodes per shoot (33.833) was recorded by *D.* Purple-1 followed by *D.* Yupee Deewan (23.557) and *D.*Purple-2 (23.000). *D.* Purple 1, *D.* Yupee Deewan and *D.* Purple -2 were on par with each other. The number of nodes per shoot was lower in *D.* Oschin (7.610). The varieties *D.*Uniwai Pink, *D.* Madam Pompadour, *D.* Nave Blue, *D.* Nagoya Pink and *D.* Waipahu Pink also had lower number of nodes per shoot.

The number of leaves per shoot ranged from 5 – 16 and *D.* Purple – 1 (16.667) was on par with *D.* Purple – 2 (11.890). *D.* Oschin recorded the lowest number of leaves per shoot (4.223). *D.* Waipahu pink (5.670), *D.* Nagoya pink (6.333), *D.* Uniwai pink (6.530) and Madam Pompadour (6.833) were on par with *D.* Oschin. The length of the leaf was maximum (14.113 cm) in *D.* Waipahu pink and *D.* Madam Pompadour, *D.* Uniwai pink, *D.* Nagoya pink, *D.* Purple-1 and *D.* Purple-2 were on par with it. *D.* Yupee Deewan recorded the minimum length (10.057 cm). The width of the leaf was maximum in *D.* Purple- 2 (4.550 cm) and it was on par with *D.* Uniwai Pink, *D.* Purple-1, *D.* Nave Blue, *D.* Yupee Deewan and *D.* Waipahu Pink. *D.* Madam Pompadour recorded the minimum width (2.830 cm).

4.2.2.2 Inflorescence characters

The mean performance of nine varieties for the inflorescence is presented in Table 26.

Table 26. Differences in inflorescence characters among nine *Dendrobium* varieties

Varieties	Length of the inflorescence (cm)	Thickness of the inflorescence stalk (cm)	Basal internodal length of the stalk (cm)	Number of flowers	Length of the flowers (cm)	Width of the flowers (cm)
D. Uniwai Pink	39.100	0.307	7.320	9.780	3.963	5.750
D. Madam Pompadour	31.633	0.287	7.683	7.000	5.437	6.367
D. Nave Blue	29.950	0.263	5.503	10.193	4.147	4.653
D. Purple –1	29.067	0.400	4.833	14.867	3.167	4.540
D. Purple – 2	32.667	0.317	7.167	11.000	4.190	5.283
D. Yupee Deewan	29.693	0.293	6.247	8.057	3.440	5.280
D. Oschin	25.110	0.260	6.617	3.443	5.450	6.220
D. Nagoya Pink	25.730	0.300	6.833	7.000	4.333	5.667
D. Waipahu Pink	33.500	0.290	6.967	7.333	6.123	7.090
F, 8, 26	0.742^{NS}	2.172^{NS}	1.971^{NS}	4.070**	17.348**	7.129**
SEm	4.913	0.028	0.649	1.591	0.238	0.308
CD	14.598	0.082	1.929	4.726	0.706	0.916

There was no significant difference among varieties in length of the inflorescence, thickness of the inflorescence stalk and basal internodal length of stalk.

Regarding number of flowers *D.* Purple-1 recorded maximum (14.867). *D.* Purple-2 (11.000) and *D.* Nave Blue (10.193) were on par with *D.* Purple-1. The number of flowers recorded was lowest (3.443) in *D.* Oschin. The length of the flower was maximum (6.123 cm) for *D.* Waipahu pink. *D.* Oschin (5.450 cm) and *D.* Madam Pompadour (5.437 cm) were on par with it. *D.* Purple-1 recorded the lowest value (3.167 cm). The width of the flower was also maximum (7.090 cm) for *D.* Waipahu Pink and minimum lowest (4.540 cm) for *D.* Purple-1. *D.* Purple-1,*D.* Nave Blue, *D.* Yupee Deewan and *D.*Purple-2 were on par with each other.

4.2.2.3 Anatomical Characters

The mean performance of nine varieties for the nine anatomical characters studied is presented in Table 27.

The thickness of the root ranged between 1.6 – 2.3 mm. *D.* Yupee Deewan recorded the greatest thickness (2.328 mm). *D.* Uniwai Pink (2.124 mm) and *D.*Purple-2 (2.086) were on par with *D.* Yupee Deewan. The lowest thickness (1.633 mm) was recorded by *D.* Nave blue. The number of layers in the cortex varied between 4.667 (*D.* Nave Blue) to 7.000 (*D.* Purple-2). Regarding number of layers in the velamen *D.* Purple-2 (10.333) and *D.* Uniwai Pink (9.333) recorded the highest values, were on par. The lowest number (5.667) was recorded by *D.* Nave blue.

Leaf thickness varied significantly among the varieties. The thickness was higher (1.568 mm) in *D.* Uniwai Pink. *D.* Uniwai Pink was on par with *D.* Nave Blue (1.536 mm), *D.* Purple-1 (1.533 mm) and *D.* Waipahu Pink (1.526 mm). The leaf thickness was lower (1.125 mm) in *D.* Madam Pompadour.

The number of stomata per cm^2 on the adaxial surface of leaf showed wide variation among the varieties. The higher number (694.333

Table 27. Differences in anatomical characters among nine *Dendrobium* varieties

Varieties	Root thickness (mm)	Cortex thickness (mm)	Number of layers in the cortex	Number of layers in velamen	Leaf thickness (mm)	Number of stomata in the adaxial surface of leaf (per cm^2)	Number of stomata in the abaxial surface of leaf (per cm^2)	Petal thickness (mm)	Thickness of pigmented layers (mm)
D. Uniwai Pink	2.124	0.291	5.667	9.333	1.568	595.000	2524.333	0.385	0.107
D. Madam Pompadour	1.666	0.285	6.000	6.667	1.125	637.667	2042.333	0.475	0.149
D. Nave Blue	1.633	0.230	4.667	5.667	1.536	510.000	2992.333	0.553	0.139
D. Purple –1	1.636	0.210	6.000	7.667	1.533	680.000	4807.667	0.459	0.155
D. Purple – 2	2.086	0.278	7.000	10.333	1.316	581.333	3914.333	0.446	0.165
D. Yupee Deewan	2.328	0.330	6.667	8.000	1.407	453.333	3701.667	0.647	0.126
D. Oschin	2.047	0.265	6.333	8.333	1.407	609.333	2765.960	0.297	0.155
D. Nagoya Pink	1.859	0.288	5.333	8.333	1.420	694.333	2822.330	0.492	0.136
D. Waipahu Pink	1.778	0.233	5.333	7.670	1.526	510.640	2707.229	0.452	0.136
F, 8, 26	7.157**	2.250^{NS}	3.563**	7.537**	8.848**	2.334*	31.013**	13.896**	3.975**
SEm	0.094	0.025	0.385	0.497	0.047	53.271	151.984	0.026	0.009
CD	0.278	0.074	1.144	1.476	0.140	158.283	451.586	0.078	0.026

per cm^2) was recorded the *D.* Nagoya Pink and the lower (453.333 per cm^2) in *D.* Yupee Deewan. *D.* Waipahu Pink (510.640 per cm^2) and *D.* Nave Blue (510.000 per cm^2) were on par with *D.* Yupee Deewan. *D.* Purple-1 recorded a significantly higher number of stomata (4807.667 per cm^2) in the abaxial surface than the other varieties. *D.* Madam Pompadour recorded the lower number (2042.33 3 per cm^2). Petal thickness varied between 0.297 mm (*D.* Oschin) to 0.647 mm (*D.* Yupee Deewan) among the varieties. The thickness of the pigmented layers also varied significantly among the varieties. Minimum thickness (0.107 mm) was recorded in *D.* Uniwai Pink and maximum (0.165 mm) in *D.* Purple-2. *D.* Oschin (0.155 mm), *D.* Purple-1 (0.155 mm), *D.* Madam Pompadour (0.149 mm) and *D.* Nave Blue (0.139 mm) were on par with *D.* Purple-2. *D.* Yupee Deewan (0.126 mm) was on par with *D.* Uniwai Pink.

4.2.3 Qualitative characters

The qualitative characters of the nine *Dendrobium* varieties studied in Experiment II are given in Table 28.

The shape of the leaves was linear-lanceolate in most of the varieties. In Purple-1 and Purple-2 the leaf shape was lanceolate. The leaf sheath was light green in all the varieties. In all the varieties the flowers resupinate to orient the flowers so that the lip was placed lowermost. In an inflorescence during opening the flowers resupinated clockwise and anticlockwise alternately. This was observed in all the nine varieties. The colour of the flower and the size and shape of perianth lobes are shown in Plate 6. The varieties *D.* Uniwai Pink, *D.* Madam Pompadour, *D.* Nave Blue, *D.* Nagoya Pink, *D.* Waipahu Pink were categorized under the Purple group. *D.* Purple-1, *D.* Purple-2 and *D.* Oschin belonged to the Red purple, while *D.* Yupee Deewan alone belonged to the Violet group. Most of the varieties had medium size flowers except *D.* Oschin which had large flowers.

Table 28. Qualitative characters of nine Dendrobium varieties

Varieties	Shape of leaves	Leaf sheath colour	Hairiness of leaf sheath	Flower colour	Size and shape of perianth lobes
D. Uniwai Pink	Linear-lanceolate	Light green	Glabrous	Light pink and white lip deep pink Colour group: Purple	Medium sized flowers reflexed sepals and petals Sepals: 2.9-3.2 cm length; 1.0-1.3 cm width Petals:3.3-3.5 cm length; 1.3-1.4 cm width Lip:2.9-3.0 cm length; 1.2-1.3 cm width
D. Madam Pampodour	Linear-lanceolate	Light green	Glabrous	Deep purple throughout sepals, petals and lip Colour group: Purple	Full medium sized flowers, slightly reflexed rounded sepals and petals, petals slightly broader than sepals Sepals:2.6-2.8 cm length; 1.2-1.4 cm width Petals:2.5-2.9 cm length; 2.6-2.7 cm width Lip:2.6-2.7 cm length; 1.2-1.3 cm width
D. Nave Blue	Linear-lanceolate	Light green	Glabrous	Light pink and White sepals pink and white petals and lip Colour group: Purple	Medium sized flowers, narrow sepals and petals Sepals:2.2-2.7 cm length; 1..0-1.2 cm width Petals:2.6-2.7 cm length;1.4-1.5 cm width Lip:2.5-2.8 cm length; 1.1-1.3 cm width
D. Purple-1	Lanceolate	Light green	Glabrous	Deep reddish purple throughout Colour group: Red Purple	Small flowers, sepals, petals and lip narrow and highly reflexed Sepals:2.7-2.9 cm length; 1.0-1.1 cm width Petals:3.3-3.4 cm length;1.6-1.7 cm width Lip:3.1-3.2 cm length;1.2-1.4 cm width
D .Purple-2	Lanceolate	Light green	Glabrous	Purple throughout sepals, Petals and lip Colour group: Red Purple	Small flowers, sepals, petals and lip narrow and slightly reflexed Sepals: 2.7-3.0 cm length; 1.0-1.1 cm width Petals:3.2-3.4 cm length; 1.6-1.7 cm width Lip: 3.2-3.4 cm length; 1.1-1.3 cm width
D. Yupee Deewan	Linear-lanceolate	Light green	Glabrous	Sepals and petals white lip violet with violet stripes	Reflexed sepals, petals and lip, narrow sepals and petals, lip margin reflexed Sepals:2.7-3.0 cm length; 1.0-1.2 cm width

Table 28. Continued...

				throughout Colour group: Violet	Petals:3.7-3.8 cm length; 1.2-1.3 cm width Lip:3.3-3.4 cm length; 1.3-1.5 cm width
D. Oschin	Linear-lanceolate	Light green	Glabrous	Purple and white sepals, petals and lip purple with white shade towards inner side Colour group: Red purple	Large flowers, petals round, very broader and cover of 3/4thof the sepals Sepals:3.0-3.4 cm length; 2.0-2.1 cm width Petals:3.5-3.6 cm length; 4.0-4.2 cm width Lip:3.7-3.4 cm length; 2.0-2.1 cm width
*D.*Nagoya Pink	Linear-lanceolate	Light green	Glabrous	Pink and white sepals petals and lip, white towards inside and pink towards the outside Colour group: Purple	Full medium sized, perfectly shaped flower slightly reflexed and petals. Sepals 2.6-2.8 cm length; 1.3-1.5 cm width Petals:2.9-3.1 cm length; 2.5-2.6 cm width Lip:2.4-2.6 cm length; 1.4-1.5 cm length
D. Waipahu Pink.	Linear lanceolate	Light green	Glabrous	Light pink sepals, petals and lip Colour group: Purple	Medium sized petals larger than sepals, petals and sepals separate, slightly reflexed Sepals:3.1-3.6 cm length; 1.4-1.5 cm width Petals:3.9-4.0 cm length; 2.6-2.9 cm width Lip:3.2-3.6 cm length; 1.2-1.4 cm width

a. *D.*Uniwai Pink

b. *D.*Madam Pampodour

c. *D.*Nave Blue

d. *D.*Purple-1

e. *D.*Purple-2

Plate 6. *Dendrobium* **varieties used in the Experiment II**

f. *D.* Yupee Deewan

g. *D.*Oschin

h. *D.*Nagoya Pink

i. *D.*Waipahu Pink

Plate 6. *Dendrobium* **varieties used in the Experiment II (Continued…)**

DISCUSSION

5. DISCUSSION

Orchids are very fascinating and it are one of the top ten cut flowers of the world. They exhibit a lot of variation in nature with regard to their growth and floral characters.

The present study was carried out to assess the morpho-anatomical and molecular variation among selected *Dendrobium* varieties. Any crop improvement is mainly based on the variability among the individuals of a population, which can be used in future breeding programmes. The present study was conducted as two experiments. In Experiment I the morpho anatomical variability and molecular characters of 15 *Dendrobium* varieties and in Experiment II the morpho-anatomical variability alone among nine *Dendrobium* varieties were studied.

5.1 GROWTH PARAMETERS

Analysis of variance for different growth parameters revealed significant difference among the varieties for several characters *viz.*, shoot girth, leaf area per shoot, leaf area at completion of leaf unfurling, leaf area at inflorescence emergence, leaf area at first flower opening, days taken from inflorescence emergence to first flower opening and days taken from inflorescence emergence to full bloom. Shekhar and Vij (1986) also reported diverse vegetative and floral characters in *Dendrobium*.

D. Caesar Candy recorded maximum rate of increase in shoot girth *viz.*, 1.3 cm / month and the minimum (0.673 cm/month) was recorded by *D.* Candy Stripe x Tomie Drake.

The increase in leaf area per shoot per month recorded maximum *viz.*, 68.617 cm^2 / month for *D.* Caesar Candy. *D.* Sonia-28 recorded the minimum (19.880 cm^2 /month). The variation may be due to the difference in growth habit among the varieties.

D. Caesar Candy had the maximum leaf area at completion of leaf unfurling (181.99 cm^2) and *D.* Jacquelin Concert x Lady Charm had the minimum (59.343 cm^2).

Maximum leaf area at inflorescence emergence (180.713 cm^2) was recorded for *D.* Jacquelin Concert x *D.* Mme. Udomsiri and minimum (65.197 cm^2) for *D.* Kasem white.

D. Caesar Candy recorded maximum leaf area at first flower opening (182.727 cm^2) and it was minimum for *D.* Kasem white (50.347 cm^2).

*D.*Sonia-28 took maximum number of days (44.667) from inflorescence emergence to first flower opening and it was minimum (32.833) for *D.* Jacquelin Concert x *D.* Mme Udomsiri. Similarly Shobhana (2000) reported the days taken from inflorescence emergence to first flower opening varied between 31.33 to 40 days among the ten *Dendrobium* hybrids studied. The days taken from inflorescence emergence to full bloom was maximum (48.333) for *D.* Sonia-28 and minimum (37.667) for *D.* Kasem White.

5.2 MORPHO-ANATOMICAL CHARACTERS

Most of the morpho-anatomical characters exhibitd significant variation among the 15 *Dendrobium* varieties.

The shoot length was recorded maximum for *D.* Jacquelin Concert x *D.* Mme.Udomsiri (26.083 cm) followed by *D.* Caesar Candy (23.513 cm) and it was minimum for *D.* Candy Stripe x Tomie Drake (11.100 cm). This is in similarity with the findings of Sobhana *et al.* (2000). She reported variation in plant height among ten *Dendrobium* hybrids studied. Pun et al. (1994) reported significant variation in length of stem among seven different types of *Vanda tesellata.*

Maximum shoot girth was recorded for *D.* Jacquelin Concert x *D.* Mme Udomsiri (4.533 cm) and *D.* Candy stripe hybrid recorded the minimum (2.833-cm). Pun *et al.* (1994) in a similar variability study in

seven *Vanda teselleta* types has also reported marked variation in the diameter of the stem.

D. Snow white had the maximum inter-nodal length of shoots (4.677 cm) and *D.* Candy Stripe x Tomie Drake had the minimum inter-nodal length of shoots (2.637 cm).

The number of nodes per shoot showed remarkable variation, with mean values ranging from 4.443 (*D.* Candy Stripe x Tomie Drake) to 9.330 (D. Caesar Candy). This is in accordance with the findings of Lekharani (2002). She reported a wide range of variation in the number of nodes per cane, among the parents (5.8–5.7) and hybrids (6.8–12.6) of *Dendrobium*. Generally, lengthier the shoots, higher will be the numbers of nodes.

Maximum leaf length was recorded for *D.* Snow White (15.307 cm) and minimum for *D.* Candy Stripe hybrid (10.970 cm). *D.* Caesar Candy had the maximum breadth of leaf (4.653 cm) and it was minimum for *D.* Kasem White (2.427 cm). In a similar study with ten *Dendrobium* hybrids, Sobhana (2000) reported the length of the leaf varied from 12.90 to 15.70 cm and the breadth from 3.07 to 4.80 cm. The length and width of the leaves varied from 11.5 to 17.92cm and 2.79 to 6.15 cm respectively in some *Dendrobium* hybrids evaluated by Lekharani (2002).

High variability was observed for some inflorescence characters *viz.*, basal inter-nodal length of stalk, number of flowers and the length and width of flowers.

The number of flowers per spike, length and width of the flowers are important floral characters in *Dendrobium* breeding programme as reported by many workers. In the present study, *D.* Tayswee Keng x Lady Charm had the maximum number of flowers per spike (7.00) and *D.* Snow White recorded the minimum (3.000). Sobhana (2000) and Lekharani (2002) reported high variability for the number of flowers/inflorescence among *Dendrobium* genotypes. Bobisud and Kamemoto (1982) concluded

that flower production in *Dendrobium* hybrids was primarily influenced by parental genotypes. Heritability of this character was reported by several scientists and hence this can be used for further improvement programmes.

The length and width of the flower is an important character of display value. Among the fifteen genotypes, the length and the width of the flower was recorded maximum for *D.* Brook Shields (7.127 and 8.120 cm respectively) and minimum for *D.* Candy Stripe hybrid (4.757 and 5.050 cm respectively). Amore and Kamemoto (1992) reported high variability in flower length and width among diploids and their corresponding amphidiploids in *Dendrobium*.

The larger number of species and wide diversity in *Dendrobium* makes it difficult to study the variability based on morphological characters alone. Hence anatomical factors were also used to differentiate species.

In the present study several characters showed variation. *D.* Rinappa-3 had the maximum root thickness (2.555 mm) and it was minimum (1.552 mm) for *D.* Caesar Candy. Pun *et al.* (1994) reported variation in root thickness among the seven *Vanda* types studied. The cortex thickness was maximum for *D.* Rinappa -3(0.327 mm) and minimum for *D.* Kasem White (0.204 mm).

The number of layers in the velamen recorded maximum for *D.* Rinappa-3 (9.333) and minimum for *D.* Thailand White (5.333). Rao and Khasim (1987) in *Coelogyninae* and Raju (1996) reported variation in the number of velamen layers in *Vanilla wightiana*. The variation may be attributed to variation in the thickness of the root among the genotypes.

Ayensu and Williams (1972) correlated leaf anatomy within the *Oncidiinae* as an aid in taxonomic study. In the present study the leaf thickness was maximum for *D.* Midnight Velvet Lipstick (1.672 mm) and minimum for *D.* Caesar Candy (1.002 mm).

In the present study the number of stomata in the adaxial and abaxial surface of leaf was maximum for *D.* Jacquelin Concert x Lady Charm (822 per cm^2) and *D.* Caesar Candy (5711.667 per cm^2) respectively. *D.* Midnight Velvet Lipstick recorded the minimum number of stomata in both the adaxial and abaxial surface of leaf (382.333 per cm^2 and 2269.00 per cm^2 respectively).

Vij *et al.* (1991) reported that the stomatal frequency in 43 species of epiphytic orchids ranged between 17 and 116 per mm^2. According to Goh *et al.* (1977) the stomatal frequency was distinctly related to the extent of succulence, more the succulence lesser the frequency of stomata. The low frequency of stomata in the adaxial surface may be considered as a phsychological adaptation to minimize internal water loss.

The petal thickness of *D.* Snow White was recorded the maximum (0.760 mm) and it was minimum for *D.* Caesar candy (0.139 mm). Except for the varieties with white flowers viz., *D.* Snow White, *D.* Kasem White and *D.* Thailand White, pigmented layers were presented in all other varieties. The thickness of pigmented layers was maximum for *D.*Sonia-28 (0.298 mm) and minimum for *D.* Candy Stripe hybrid (0.035 mm).

5.3 VARIATION STUDIES

The magnitude of genotypic and environmental component of phenotypic variance and the coefficients of variance at genotypic and phenotypic level in the 15 *Dendrobium* varieties were studied.

Phenotypic coefficient of variation was higher than genotypic coefficient of variation for all the characters studied and are in accordance with the results of Sobhana (2000) indicating the influence of environment.

High phenotypic (42.425) and genotypic (40.922), coefficients of variation were found for petal thickness, followed by number of flowers (PCV – 32.456; GCV – 26.698). High GCV obtained for this character is

important in orchids since it determines the market value of the spike. High phenotypic coefficient of variation for this character was also reported by Sobhana (2000).

5.4 HERITABILITY AND GENETIC ADVANCE

The magnitude of heritability is valuable in plant breeding programmes since it provides the basis for selection dependent on phenotypic performance. In the present study heritability is of low to high magnitude. Higher heritability values were recorded for the floral characters *viz.*, petal thickness (93.042 per cent), length and width of flowers (90.418 and 92.505 per cent respectively). The size of florets is one of the important characters and hence the high heritability for this character suggests scope for its improvement through selection. Rehman *et al.* (1993) and Sobhana (2000) also reported high heritability estimates for flower size.

Heritability along with genetic advance is more useful than heritability alone in predicting the resultant effects for selecting the best individual (Johnson *et al.*, 1955). This is due to the fact that a character may have high heritability but very less phenotypic variation and this gives rise to very low genetic gain. Moreover it gives an idea of the nature of gene action governing a particular character. In the present study petal thickness (81.328 per cent), number of stomata in the abaxial surface of leaf (46.168 per cent) and number of flowers (45.242 per cent) showed high genetic advance. High heritability along with high genetic gain was observed for petal thickness, number of stomata in the abaxial surface of leaf, number of flowers. Thus it may be inferred that selection based on petal thickness and number of flower which showed high genotypic coefficient of variation, heritability and genetic advance would be more effective. These characters are generally controlled by additive gene action and hence amenable to genetic improvement through selection (Panse, 1957).

5.5 CORRELATION STUDIES

The degree and direction of association between different characters could be better understood based on correlations. Some of the economic characters may be influenced by many plant characters through different physiochemical mechanisms. Therefore improvement of the economic characters is possible by knowing the association of various characters.

In this study, shoot length had significant positive phenotypic correlation with shoot girth, number of nodes per shoot, number of laminate leaves per shoot, length of the leaves and width of the leaves. The number of flowers showed a significant negative correlation with the length and width of flowers. The length of the flower had significant positive correlation with flower width. This is in accordance with the findings of Sobhana (2000). Balfour and Linder (1990) also reported a greater correlation coefficient between sepal length and width in population of *Disa uniflora* (Orchidaceae), endorsing this as an important criterion for cut flower selection.

The estimates of genotypic coefficient of correlation were much higher in magnitude than the corresponding estimates at phenotypic level. It indicated that though there was a strong inherent association between the various characters studied, the phenotypic expression of the correlation was lessened under the influence of environment.

A positive genotypic correlation between pairs of characters indicated that an improvement in one character will improve the other character also, thus enabling the breeder to select characters responsive to selection. The length of the inflorescence is influenced by the number of nodes per shoot as well as the width of the leaves. The selection for more number of nodes per shoot and higher width of leaves can thus result in larger inflorescences.

Number of flowers per spike is an important cut flower attribute. This character showed high positive correlation with number of laminate

leaves per shoot and width of the leaves and high negative correlation with length and width of flowers. Sobhana (2000) also reported positive correlation between flowers per spike and total number of leaves. So improving management practices may increase production of more number of leaves which in turn facilitate the production of inflorescence with more number of flowers.

Environment correlations are present only for a few pairs of characters. The shoot length showed significant positive correlation with number of nodes per shoot, length of the leaves and width of the leaves.

5.6 QUALITATIVE CHARACTERS

In Orchidacea, the shape of the leaves is found to vary with the species and varieties. In the present study the shape of the leaves was linear-lanceolete in all the cultivars. This character though having limited application may be profitably used for identifying various taxonomic categories when taken in conjunction with other epidermal features. The presence or absence of leaf sheath is one of the important taxonomic characters. The leaf sheath was present in all the varieties studied and was light green and glabrous in nature. Similarly, among the *Vanda tesellata* spp. studied. Pun *et al.* (1994) reported no variation in leaf sheath colour. Ossian (1990) reported that the species in section *Dendrobium* have smooth fleshy stems and leaf sheaths. Regarding resupination in all the varieties the flowers in an inflorescence resupinate to orient the flower so that the lip is placed lowermost irrespective of the angle of inflorescence axis. In a similar study in *Dendrobium* Tomie 'Tokyo' Nyman (1984) reported that just before or during opening, the buds turned positioning the lip below all the floral parts. Withner (1974) too reported similar findings. The degree of turning depends on the orientation of the inflorescence in relation to the ground (Soediono *et al.*, 1984). The reason for resupination may be due to the response to gravity and a special form of gravitropism. The direction of resupination may be

clockwise or anticlockwise. In an inflorescence the flowers resupinate clockwise-anticlockwise alternately in all the variety. This may be due to the alternate positioning on the inflorescence axis. Regarding colour of the flower, six varieties came under the Red Purple group, six varieties under the Red group and three varieties under the White group.

In the Experiment II, the varieties showed significant differences for most of the characters. The shoot length, shoot girth, internodal length of shoots, number of nodes per shoot and number of laminate leaves per shoot were recorded maximum for *D.* Purple-1 (112.000 cm, 5.667 cm, 6.100 cm, 33.833 and 16.677 cm respectively) and minimum for *D.* Oschin (17.003 cm, 3.033 cm, 3.610 cm, 7.610 cm and 4.233 cm respectively). *D.* Waipahu Pink had the maximum length of leaf (14.113 cm) and it was minimum for *D.* Yupee Deewan (10.057). Sobhana (2000) reported that the length of the leaves varied between 12.90 to 15.17 cm among the ten *Dendrobium* varieties studied. *D.* Purple-2 had the maximum width of the leaf (4.550 cm) and it was minimum for *D.* Oschin (2.900 cm).

The number of flowers per spike and the length and width of the flowers are important cut flower attributes. In the present study *D.* Purple-1 had the maximum number of flowers per spike (14.867) and it was minimum for *D.* Oschin (3.443 cm). The length and width of flowers were maximum for *D.*Waipahu Pink (6.123 and 7.090 cm respectively) and they were minimum for *D.*Purple-1 (3.167 and 4.540 cm respectively). The lesser length and width recorded with *D.* Purple-1 may be due to the reflexed nature of petals.

D. Yupee Deewan recorded the maximum root thickness (2.328 mm) and *D.* Nave Blue recorded the minimum (1.633 mm). The number of layers in the cortex and velamen were maximum for *D.* Purple-2 (7.000 and 10.333 respectively) and minimum for *D.* Nave Blue (4.667 and 5.667 respectively). This is in similarity with the findings of Rao and Khasim (1987) in *Coelogyninae*. Similarly Dycus and Knudson (1957) reported one to at least 18 cell layers in the velamen of orchid roots. In the

present study, *D.* Uniwai Pink had the maximum leaf thickness (1.568 mm) and *D.* Madam Pompadour recorded the minimum (1.125 mm).

The number of stomata in the adaxial surface of leaf varied between 453.333 per cm^2 (*D.* Yupee Deewan) to 694.333 (*D.* Nagoya Pink). The number of stomata in the abaxial surface of leaf was maximum for *D.* Purple-1 (4807.667 per cm^2) and minimum for *D.* Madam Pompadour (2042.333 cm^2).

The petal thickness was maximum for *D.* Yupee Deewan (0.647 mm) and minimum for *D.* Oschin (0.297 mm).

The thickness of pigmented layers were recorded the maximum for *D.* Purple- 2 (0.165 mm) and minimum for *D.* Uniwai Pink (0.107 mm). The thickness of pigmented layers depend on the degree of pigmentation of the petals. Datta (1993) studied the somatic mutations in flower colour in seventeen gamma ray induced somatic flower colour mutants of roses and the respective twelve controls based on qualitative and or quantitative changes of pigemented cells.

In most of the varieties the leaves were linear-lanceolate except in *D.* Purple-1 and *D.* Purple-2, both with lanceclate leaves. This may be due to primitive characteristics/origin of the varieties which have antelope type flowers. Glabrous, light green sheath was present in all the varieties. In all the varieties the flowers resupinate to orient the lip at the lower most after complete opening. Soedino *et al.* (1984) reported that resupination was a geotropic response mechanism and fully opened flowers will not continue to resupinate. The direction of resupination of the flowers in an inflorescence was clockwise and anticlockwise alternately in all varieties. In most of the varieties the flower colour was either Purple or Red Purple group except *D.* Yupee Deewan which was in the Violet colour group.

In both the experiments, the varieties showed significant differences in the characters such as length of shoot, girth of shoot, internodal length of shoot and number of nodes per shoot, length of width of leaves, number of flowers, length and width of flowers, thickness of

root, number of layers in velamen, leaf thickness, number of stomata in adaxial and abaxial surface of leaves, petal thickness and thickness of pigmented layers. This showed the varietal differences may not be attributed by the age of the plants. The characters orientation of leaves, length of inflorescence and thickness of inflorescence stalk were not significant in both the experiments.

5.7 D^2 ANALYSIS

The genetic diversity among the varieties was studied using Mahalanobis D^2 analysis. The fourteen varieties were clustered into three groups. The greater the distance between the two clusters the greater is the divergence between varieties belonging to the two clusters and vice versa. Within a cluster, the varieties are less divergent than those which are in different clusters. Length of the leaves registered maximum coefficient of variation followed by number of stomata in the adaxial surface of leaf. Thus length of the leaves was the potential contributor of differentiation at intercluster level followed by number of stomata in the adaxial surface of leaf. The low coefficient of variation recorded for length and width of the flower showed these characters are not functionally important in differentiating the clusters. Similar findings were reported earlier by Balfour and Linder (1990).

The selection of parents for hybridization programmes may be based on the relative contribution of each character to the total divergence. The parents may be selected from the clusters with maximum genetic distance to obtain maximum diversity among the hybrids. The inter-cluster distance was maximum between C-2 and C-3. So selection of parents from these divergent clusters will be very effective in hybridization. The length of the inflorescence and number of flowers were found to be high in C-3 (D. Midnight Velvet Lipstick and *D.* Candy Stripe hybrid). The length and width of the flower were found to be high in C-2.

5.8 RAPD ANALYSIS

RAPD is one of the reliable methods of identifying varieties at the genotypic level and can therefore helped to overcome the complications arising from morpho-anatomical characterization. In this study, RAPD technique was used to distinguish *Dendrobium* varieties. Moreover it is fast and comparatively less tedious.

Isolation of genomic DNA was carried out using the method of Mondal *et al.* (2000) modified to suit the plant material. Fresh unfurled tender leaves were found to yield good quality DNA. This may be due to the easy disruption of the leaves during grinding using liquid nitrogen. Young tender leaves were also reported to be used for the isolation of genomic DNA in *Chrysanthemum* (Scot *et al.*, 1996), roses (Vainstein and Ben-Meir, 1994) and Gladiolus (Pathania and Mishra, 2001).

The yield and quality of DNA varied with the varieties. The DNA yield varied from 120 ng ml^{-1} (*D.* Caesar Candy and *D.* Jacquelin Concert x Lady Charm) to 225 ng ml^{-1} (*D.* Lady Pink). The OD-260/280 ratios varied between 1.53 (*D.* Snow White) to 1.89 (*D.* Midnight Velvet Lipstick). This may be due to the varietal differences in chemical reactions taking place during extraction of DNA. All extracted DNA was of good quality with very little fragmentation.

RAPD analysis was carried out based on the method followed by Lim *et al.* (1999) in *Vanda*. Forty decamer primers (Operon Inc. CA) of kit A and Kit B was used for PCR amplification with the DNA of *D.* Rinappa-3. Thirty-five primers out of forty yielded good amplification products. There was no amplification with the primers OPA-06, OPA-12, OPA-20, OPB-09 and OPB-19. The remaining 35 primers produced a total of 109 RAPD bands ranging from 1 to 13 with an average of 3.11 bands per primer. Out of that 102 (93.58 %) were polymorphic and 7 were monomorphic. Based on the band resolution 4 primers were selected for further evaluation and repeated in at least two replications. Only bands that consistently amplified were scored. Forty-four RAPD markers were

scored from the primers and 39 of them were polymorphic. The primer OPA – 19 produced polymorphic bands in all cultivars.

In a similar study to find the genetic closeness of various *Vanda* spp. Lim *et al.* (1999) obtained a total of 158 RAPDs from eight different primers. The difference in the amplification products may be due to the difference in the primer sequence, extraction protocol, DNA quality, $MgCl_2$, ion concentration etc. The Mg^{2+} ion is known to affect the primer template interaction (Welsh and McClelland, 1990), polymerase activity and the melting temperature of double stranded DNA.

The similarity coefficient value ranged from 0.2667 and 0.8824. The genetic distance between the varieties ranged from 0.1176 (between *D.* Sonia and *D.* Snow White) to 0.5806 (between *D.* Candy Stripe and *D.* Thailand White). The genetic distance revealed the genetic variability that exists between the varieties.

The dendrogram constructed by using nearest neighbour (Single-link) Method revealed the extent of genetic relationship among the 15 *Dendrobium* varieties studied. On clustering, the 15 varieties got divided into seven clusters. The varieties *D.* Sonia-28, *D.* Brook Shields, *D.* Lady Pink, *D.* Snow White, *D.* Candy Stripe x Tomie Drake, *D.* Tayswee Keng x Lady charm, *D.* Jacquelin Concert x Lady Charm and *D.* Candy Stripe hybrid together formed the largest cluster. The varieties *D.* Thailand White, *D.* Jacquelin Concert x *D.* MME Udomsiri, *D.* Kasem White, *D.* Rinappa-3 and *D.* Sakura Pink formed a second cluster. *D.* Caesar Candy and *D.* Midnight Velvet Lipstick together formed another cluster.

The varieties *D.* Sonia-28, *D.* Lady Pink, *D.* Snow White, *D.* Candy Stripe x Tomie Drake and *D.* Jacquelin Concert x Lady Charm were clustered together in both the Mahanolobis D^2 analysis and RAPD technique.

SUMMARY

6.SUMMARY

Morpho-anatomical and molecular characterization of *Dendrobium* varieties was conducted in the Department of Pomology and Floriculture, College of Agriculture, Vellayani. The results of the studies are summarized below.

1. Evaluation of growth, morpho-anatomical and molecular characterization of 15 *Dendrobium* varieties (near flowering size plants) were studied. Besides, morpho-anatomical characterization of nine *Dendrobium* varieties (three years old) were also studied.

2. Analysis of variance revealed significant differences among the fifteen *Dendrobium* varieties for rate of increase in shoot girth, increase in leaf area per shoot, leaf area at completion of leaf unfurling, leaf area at inflorescence emergence, leaf area at first flower opening, days take from inflorescence emergence to first flower opening and days taken from inflorescence emergence to full bloom.

3. Significant varietal differences were observed between fifteen varieties for shoot length, shoot girth, internodal length of shoots, number of nodes per shoot, length and width of leaves, basal internodal length of stalk, number of flowers, length and width of flowers, root thickness, cortex thickness, number of layers in velamen, leaf thickness, number of stomata in the adaxial and abaxial surface of leaf, petal thickness and thickness of pigmented layers.

4. Significant varietal differences were observed among nine *Dendrobium* varieties studied separately, for shoot length, shoot girth, internodal length of shoots, number of nodes per shoot,

length and width of leaves, number of flowers, length and width of flowers, root thickness, number of layers in cortex and velamen, leaf thickness, number of stomata in adaxial and abaxial surface of leaf petal thickness and thickness of pigmented layers. Besides, qualitative characters of the leaves and flowers were analysed.

5. In both the experiments, the varieties showed significant differences in the characters such as length of shoot, girth of shoot, internodal length of shoot and number of nodes per shoot, length of width of leaves, number of flowers, length and width of flowers, thickness of root, number of layers in velamen, leaf thickness, number of stomata in adaxial and abaxial surface of leaves, petal thickness and thickness of pigmented layers. This showed the varietal differences may not be attributed by the age of the plants. The characters orientation of leaves, length of inflorescence and thickness of inflorescence stalk were not significant in both the experiments.

6. Variability studies indicated high GCV and PCV for the character petal thickness followed by number of flowers.

7. High heritability was observed for petal thickness and length and width of flowers.

8. High heritability along with genetic advance was found for petal thickness, number of stomata in the abaxial surface of leaf and number of flowers. Selection of parents based on these characters may result in a significant improvement in the next generation.

9. The shoot length showed significant positive phenotypic correlation with shoot girth, number of nodes per shoot, number of laminate leaves per shoot, length and width of leaves. The length of the flower had significant positive correlation with width of flower.

10. High genotypic correlation was observed between the length of inflorescence and number of nodes per shoot as well as the width of leaves.

11. Environment correlation was observed to be low in comparison with genotypic and phenotypic correlation. The shoot length showed significant positive correlation with number of nodes per shoot, length of leaves and width of leaves.

12. The qualitative characters of leaf and flower were analysed for all the varieties.

13. The genetic diversity among the fourteen *Dendrobium* varieties was studied using Mahalanobis D^2 analysis. The fourteen varieties were grouped into three clusters.

14. The cluster C-2 was found to have maximum intercluster distance with two of the three clusters formed. This was followed by C-3

15. Selection of parents from the divergent clusters may be effectively used for selection of parents in future hybridization programmes.

16. Molecular characterization of 15 *Dendrobium* varieties were carried out using RAPD technique.

17. The DNA yield varied from 120 ng ml^{-1} to 225 ng m^{-1}.

18. The primers OPA-19, OPB-02, OPB-04 and OPB-10 yielded good resolution bands out of forty decamer primers tested.

19. A total of 44 RAPD markers were amplified by the primers OPA-19, OPB-02, OPB-04 and OPB-10 of which 39 were polymorphic and five were monomorphic

20. The similarity coefficient value of the varieties assessed ranged from 0.2667 to 0.8824.

21. The genetic distance between the varieties ranged from 0.1176 to 0.5806

22. The 15 varieties got divided into six clusters on drawing a vertical line in the dendrogram at a distance of 0.425.

23. A detailed descriptive blank of 24 *Dendrobium* varieties have been prepared (Appendix I)

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*Originals not seen

APPENDICES

APPENDIX I

Descriptive blank of the twenty four *Dendrobium* varieties used in Experiments

Varieties	Shoot length (cm)	Shoot girth (cm)	Length of the leaves (cm)	Width of the leaves (cm)	Length of the inflorescence (cm)	Thickness of the inflorescence stalk (cm)	Basal internodal length of the stalk (cm)	Length of the flower (cm)	Width of the flowers (cm)
D. Sonia-28	7.80-28.20	2.60-4.00	11.23-13.00	3.13-4.37	22.00-32.50	0.27-0.30	1.90-8.60	6.50-7.50	6.30-8.00
D. Rinappa-3	7.50-25.0	2.50-3.70	10.97-13.95	2.72-3.37	24.50-26.70	0.25-0.30	2.30-8.00	6.80-7.10	7.10-7.50
D. Jacquelin Concert x D.Mme Udomsiri	19.00-34.50	3.80-4.93	14.75-15.65	3.80-3.98	20.50-22.00	0.25-0.30	1.50-8.30	4.50-5.50	6.50-7.20
D. Brook Shields	7.60-25.30	2.60-3.70	13.73-16.33	2.10-3.43	25.00-38.10	0.28-0.34	2.50-7.80	7.00-7.60	7.40-8.30
D. Lady Pink	6.00-18.50	2.50-3.40	13.85-16.17	2.73-3.53	19.50-20.60	0.20-0.27	2.00-8.50	6.40-7.00	6.40-6.70
D. Snow White	14.50-25.00	2.60-3.40	14.85-15.72	2.60-2.85	21.80-23.20	0.30-0.32	1.70-9.70	4.70-6.30	5.40-7.20
D. Candy Stipe x Tomme Drake	6.00-18.60	2.40-3.40	11.83-12.78	3.07-3.30	19.50-23.20	0.24-0.34	1.80-5.20	5.90-6.30	6.70-6.90
D. Kasem White	8.00-16.20	2.70-3.40	10.50-11.90	2.23-2.55	18.20-19.50	0.25-0.31	1.60-8.30	5.00-5.70	7.50-8.00
D. Tayswee Keng x Lady Charm	7.60-28.00	2.80-3.70	13.53-15.23	2.53-3.08	15.40-17.80	0.30-0.32	2.30-8.10	5.00-5.40	5.60-6.20
D. Sakura Pink	6.50-20.50	2.60-3.80	11.57-12.88	2.90-3.08	15.20-24.70	0.25-0.30	1.65-8.50	6.30-7.00	5.90-6.70
D. Midnight Velvet Lipstick	7.00-18.50	2.90-3.90	13.13-15.17	3.00-3.17	21.00-23.90	0.25-0.30	1.70-9.70	5.30-6.00	5.50-6.00
D. Jacquelin Concert x Lady Charm	7.00-19.70	2.80-3.80	11.30-12.63	2.53-2.67	21.50-26.00	0.25-0.40	1.40-10.60	4.50-7.10	5.60-7.50
D. Candy Stripe hybrid	7.50-17.50	2.40-3.10	10.23-11.90	2.70-3.55	28.20-32.00	0.28-0.30	1.30-8.00	4.50-5.00	4.80-5.20
D. Caeser Candy	10.50-39.00	4.00-5.10	13.76-17.35	4.00-5.48	25.00-44.50	0.30-0.32	1.55-8.00	3.50-4.30	3.70-5.30
D. Thailand White	8.00-27.00	2.40-3.20	12.38-15.25	2.60-3.60	27.50-30.20	0.25-0.30	2.45-8.80	3.70-4.50	3.70-4.50
D. Uniwai Pink	9.80-63.40	2.70-3.60	9.00-17.00	2.10-6.20	21.00-51.40	0.15-0.20	1.00-8.30	3.70-4.20	4.60-6.30
D. Madam Pompadour	16.00-54.00	3.00-3.60	8.30-18.00	1.60-4.00	21.80-40.00	0.15-0.20	1.50-9.10	4.80-5.80	5.90-6.90
D. Nave Blue	35.70-75.40	3.60-4.50	6.00-16.20	2.20-5.20	19.00-45.00	0.20-0.30	0.60-7.50	3.00-5.00	3.60-5.60
D. Purple –1	41.00-184.00	5.00-5.80	10.50-13.40	3.50-5.60	18.00-44.00	0.20-0.30	0.70-8.00	2.70-3.70	3.70-5.50
D. Purple – 2	43.20-128.40	3.50-4.30	9.00-17.50	3.50-6.10	29.00-42.00	0.25-0.32	0.30-0.80	3.70-4.40	4.70-6.00
D. Yupee Deewan	28.20-144.00	4.00-5.00	5.60-13.20	2.00-5.00	14.00-47.50	0.21-0.29	0.50-0.80	2.90-4.00	4.10-6.00
D. Oschin	13.00-21.00	2.60-3.40	7.10-12.00	2.40-3.50	20.30-30.50	0.23-0.29	1.00-9.30	5.10-6.10	5.40-7.00
D. Nagoya Pink	16.00-35.50	3.10-3.80	11.50-14.00	2.20-3.30.	25.00-26.70	0.19-0.27	0.90-7.50	4.10-4.50	5.60-5.70
D. Waipahu Pink	19.00-3250	3.00-3.70	11.20-17.30	3.00-4.20	18.00-49.00	0.23-0.26	1.20-8.50	5.50-6.70	6.70-7.30

Appendix I. continued...

Varieties	Shape of leaves	Leaf sheath colour	Hairiness of leaf sheath	Flower colour	Size and shape of perianth lobes
D. Sonia-28	Linear-lanceolate	Light green	Glabrous	Deep purple petals with white towards centre. Sepals light purple, white towards inside. Lip deep purple Colour group -Red purple	Large flowers, petals larger than sepals and are separate, narrow and pointed sepals, rounded petals, Sepals:4.1-4.3 cm length;1.4-1.7 cm width Petals: 4.5-4.7 cm length;2.7-3.0 cm width Lip: 3.8-4 cm length;2.0-2.3 cm width
D. Rinappa-3	Linear-lanceolate	Light green	Glabrous	Petals and lip deep purple with white near the base. Sepals light purple than petals with white colouration at base and tip Colour group : Red Purple	Medium sized flower, petals broader than sepals and glossy. Sepals: 4.2-4.4 cm length;1.5-1.7cm width Petals:4.5-4.6 cm length;3.3-3.4 cm width Lip:3.5-3.7 cm length;2.0-2.3 cm width
D. Jacquelin Concert x D.Mme Udomsiri	Linear-lanceolate	Light green	Glabrous	Solid reddish purple throughout, glossy Colour group: Red Purple	Medium sized flowers, slightly reflexed, round petals Sepals :3.4-3.5 cm length; 1.7-1.8 cm width Petals:3.9-4.0 cm length; 3.0-3.1 cm width Lip: 3.1-3.3 cm length;1.6-1.8 cm width
D. Brook Shields	Linear-lanceolate	Light green	Glabrous	Light pink and white Colour group: Purple	Medium sized flowers, slightly reflexed petals, pointed sepals and round broad petals Sepals:4.0-4.1cm length; 1.6-1.8 cm width Petals:4.6-4.7 cm length; 3.4-3.5 cm width Lip:4.0-4.2 cm length;2.0-2.1 cm width
D. Lady Pink	Linear-lanceolate	Light green	Glabrous	Light pink with dark pink stripes on sepals, petals and lip Colour group: Purple	Full medium sized, perfectly shaped slightly reflexed rounded sepals and petals Sepals: 3.8-4.2 cm length; 1.8—2.1 cm width Petals:4.2-4.3 cm lenth;3.0-3.1 cm width Lip: 3.7-3.9 cm length; 2.0-2.1 cm width

Appendix I. continued...

D. Snow White	Linear-lanceolate	Light green	Glabrous	White petals, petals and lip Colour group: White	Medium sized flower, sepals and petals separate, slightly reflexed, round petals Sepals:3.4-3.8 cm length; 1.6-1.8 cm width Petals :4..3-4.5 cm length; 2.7-2.9 cm width Lip: 3.1-3.3 cm length; 1.6-1.7 cm width
D. Candy Stripe x Tomie Drake	Linear-lanceolate	Light green	Glabrous	Light pink with dark pink stripes throughout, hairy thickening white Colour group: Purple	Medium sized, full. Perfectly shaped broad petals and sepals, sepals at tip Sepals:4.1-4.3 cm length; 2.1-2.3 cm width Petals :4.7-5.0 cm length; 4.7-5.0 cm width Lip:4.3-4.6 cm length;2.1-2.3 cm width
D. Kasem White	Linear-lanceolate	Light green	Glabrous	White sepals, petals and lip Colour group: White	Medium sized, full, slightly reflexed round sepals and petals Sepals:3.0-3.6 cm length; 1.5-1.7 cm width Petals:4.4-4.5 cm length; 2.8-2.9 cm width Lip:3.0-3.2 cm length; 1.6-1.7 cm width
D. Tayswee Keng x Lady Charm	Linear-lanceolate	Light green	Glabrous	Deep purple throughout, glossy Colour group:Red purple	Medium sized, slightly reflexed, round sepals and petals Sepals:3.6-3.8 cm length; 1.6-1.8 cm width Petals:3.5-3.7 cm length; 3.7-2.9 cm width Lip:3.3-3.5 cm length; 1.6-1.9 cm width
D. Sakura Pink	Linear-lanceolate	Light green	Glabrous	Pink and white sepals and petals, White towards inside Colour group: Purple	Medium sized, slightly reflexed sepals and petals of almost equal size, sepals pointed petals rounds Sepals:3.6-4.0 cm length; 1.6-1.8 cm width Petals: 3.9-4.0 cm length; 2.9-3.0 cm width Lip:3.6-4.0 cm length;1.8-2.1 cm width

Appendix I. continued...

D. Midnight Velvet Lipstick	Linear-lanceolate	Light green	Glabrous	Solid purple throughout Colour group: Red Purple	Small, perfectly shaped, full and flat round sepals and petals, petals slightly broader than sepals Sepals: 3.1-3.5 cm length; 1.6-1.7 cm width Petals :3.5-3.6 cm length; 2.4-2.7 cm width Lip:2.9-3.0 cm length; 1.4-1.7 cm width
D. Jacquelin Concert x Lady Charm	Linear-lanceolate	Light green	Glabrous	Purple and white sepals and petals, white towards base and purple towards outside, margin of sepals white, lip deep purple towards outside. Colour group: Purple	Large flower, sepals and petals separate, sepals narrower and pointed at tip, petals broader and rounded Sepals: 4.0-4.2 cm length; 1.6-1.7 cm width Petals :4.5-4.8 cm length; 2.9-3.0 cm width Lip:3.4-3.7 cm length;1.6-1.9 cm width
D. Candy Stripe hybrid	Linear-lanceolate	Light green	Glabrous	Deep purple stripes on light purple flowers, sepals white and purple, petals purple towards outside, lip deep purple Colour group: Purple	Medium sized, full, slightly reflexed petals rounded, sepals slightly narrower than petals Sepals:2.7-2.8 cm length; 1.4-1.5 cm width Petals:3.0-3.2 cm length; 2.4—2.6 cm width Lip:2.6-2.8 cm length; 1.5-1.6 cm width
D. Caesar Candy	Linear-lanceolate	Light green	Glabrous	Light pink with dark pink stripes on sepals, petals and lip, hairy growth in lip reddish purple Colour group: Red purple	Small flowers, reflexed sepals, petals and lip, sepals and petals separate and of almost equal size with pointed tips. Sepals:1.9-2.0 cm length; 0.9-1.0 cm width Petals:2.0-2.1 cm length;1.1-1.8 cm width Lip:1.9-2.0 cm length; 1.2-1.3 cm width
D. Thailand White	Linear-lanceolate	Light green	Glabrous	White sepals, petals and lips, hairy labellum yellowish Colour group: White	Medium flowers, petals and sepals reflexed, round sepals and petals Sepals:3.7-3.8 cm length; 1.6-1.7 cm width Petals:4.2-4.5 cm length; 3.1-3.2 cm width Lip:3.7-3.8 cm length; 3.1-3.2 cm width

Appendix I. continued...

D. Uniwai Pink	Linear-lanceolate	Light green	Glabrous	Light pink and white lip deep pink Colour group: Purple	Medium sized flowers reflexed sepals and petals Sepals: 2.9-3.2 cm length; 1.0-1.3 cm width Petals:3.3-3.5 cm length; 1.3-1.4 cm width Lip:2.9-3.0 cm length; 1.2-1.3 cm width
D. Madam Pampodour	Linear-lanceolate	Light green	Glabrous	Deep purple throughout sepals, petals and lip Colour group: Purple	Full medium sized flowers, slightly reflexed rounded sepals and petals, petals slightly broader than sepals Sepals:2.6-2.8 cm length; 1.2-1.4 cm width Petals:2.5-2.9 cm length; 2.6-2.7 cm width Lip:2.6-2.7 cm length; 1.2-1.3 cm width
D. Nave Blue	Linear-lanceolate	Light green	Glabrous	Light pink and White sepals pink and white petals and lip Colour group: Purple	Medium sized flowers, narrow sepals and petals Sepals:2.2-2.7 cm length; 1..0-1.2 cm width Petals:2.6-2.7 cm length;1.4-1.5 cm width Lip:2.5-2.8 cm length; 1.1-1.3 cm width
D. Purple-1	Lanceolate	Light green	Glabrous	Deep reddish purple throughout Colour group: Red Purple	Small flowers, sepals, petals and lip narrow and highly reflexed Sepals:2.7-2.9 cm length; 1.0-1.1 cm width Petals:3.3-3.4 cm length;1.6-1.7 cm width Lip:3.1-3.2 cm length;1.2-1.4 cm width
D .Purple-2	Lanceolate	Light green	Glabrous	Purple throughout sepals, Petals and lip Colour group: Red Purple	Small flowers, sepals, petals and lip narrow and slightly reflexed Sepals: 2.7-3.0 cm length; 1.0-1.1 cm width Petals:3.2-3.4 cm length; 1.6-1.7 cm width Lip: 3.2-3.4 cm length; 1.1-1.3 cm width
D. Yupee Deewan	Linear-lanceolate	Light green	Glabrous	Sepals and petals white lip violet with violet stripes throughout Colour group: Violet	Reflexed sepals, petals and lip, narrow sepals and petals, lip margin reflexed Sepals:2.7-3.0 cm length; 1.0-1.2 cm width Petals:3.7-3.8 cm length; 1.2-1.3 cm width Lip:3.3-3.4 cm length; 1.3-1.5 cm width

Appendix I. continued...

D. Oschin	Linear-lanceolate	Light green	Glabrous	Purple and white sepals, petals and lip purple with white shade towards inner side Colour group: Red purple	Large flowers, petals round, very broader and cover of 3/4thof the sepals Sepals:3.0-3.4 cm length; 2.0-2.1 cm width Petals:3.5-3.6 cm length; 4.0-4.2 cm width Lip:3.7-3.4 cm length; 2.0-2.1 cm width
*D.*Nagoya Pink	Linear-lanceolate	Light green	Glabrous	Pink and white sepals petals and lip, white towards inside and pink towards the outside Colour group: Purple	Full medium sized, perfectly shaped flower slightly reflexed and petals. Sepals 2.6-2.8 cm length; 1.3-1.5 cm width Petals:2.9-3.1 cm length; 2.5-2.6 cm width Lip:2.4-2.6 cm length; 1.4-1.5 cm length
D. Waipahu Pink.	Linear lanceolate	Light green	Glabrous	Light pink sepals, petals and lip Colour group: Purple	Medium sized petals larger than sepals, petals and sepals separate, slightly reflexed Sepals:3.1-3.6 cm length; 1.4-1.5 cm width Petals:3.9-4.0 cm length; 2.6-2.9 cm width Lip:3.2-3.6 cm length; 1.2-1.4 cm width

APPENDIX II

Modified Mondal, Singh and Ahuja's Method

Leaves were ground to fine powder using liquid nitrogen

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Hot (65^0C) extraction buffer and polyvinyl pyrollidone (PVP) was added to it

↓

Slurry of grounded plant material was transferred to extraction buffer kept in water bath at 65^0C for 20 minutes

↓

Lysate was squeezed through four layers of sterile muslin cloth

↓

Chloroform : Isoamyl Alcohol (24:1) extraction was done

↓

To the supernatent, two volume of cold absolute ethanol and $1/10^{th}$ volume of 3.0 M sodium acetate were added

↓

DNA was precipitated

↓

70 per cent ethanol wash was given

↓

DNA was stored in Tris Hcl-EDTA buffer

MORPHO-ANATOMICAL AND MOLECULAR CHARACTERISATION OF *Dendrobium* Sw. CULTIVARS

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Abstract of the
thesis submitted in partial fulfillment of the requirement
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ABSTRACT

An investigation on morpho-anatomical and molecular characterization of *Dendrobium* varieties was conducted in the Department of Pomology and Floriculture, College of Agriculture, Vellayani during 1999-2001.

Fifteen *Dendrobium* varieties of near flowering size plants were evaluated for their growth morpho-anatomical and molecular characterization and another nine *Dendrobium* varieties of three year old plants were evaluated for the morpho-anatomical characters.

The fifteen *Dendrobium* varieties differed significantly for the growth parameters *viz*., rate of increase in shoot girth, increase of leaf area of the shoot, leaf area at completion of leaf unfurling, leaf area at inflorescence emergence, leaf area at first flower opening, days taken from inflorescence emergence to first flower opening and days taken from inflorescence emergence to full bloom. Significant varietal difference were observed among the fifteen varieties for shoot length, shoot girth, internodal length of shoots, number of nodes per shoot, length and width of leaves, basal internodal length of stalk, number of flowers, length and width of flowers, root thickness, cortex thickness, number of layers in velaman, leaf thickness, number of stomata in the adaxial and abaxial surface of leaf, petal thickness and thickness of pigmented layers.

The nine *Dendrobium* varieties (three year old plants) evaluated separately showed significant difference for shoot length, shoot girth, internodal length of shoots, number of nodes per shoot, length and width of leaves, number of flowers, length and width of flowers, root thickness, number of layers in cortex and velaman, leaf thickness, number of stomata in adaxial and abaxial surface of leaf, petal thickness and thickness of pigmeted layers.

High GCV and PCV were observed for petal thickness followed by number of flowers, while high heritability was observed for petal

thickness and length and width of flowers. Petal thickness, number of stomata in the abaxial surface of leaves and number of flowers exhibit high heritability along with genetic advance.

The shoot length showed significant positive genotypic correlation with shoot girth, number of nodes per shoot, number of laminate leaves per shoot, length and width of the leaves. The number of flowers showed positive correlation with length and width of flowers. High genotypic correlation was observed between the length of inflorescence and number of nodes per shoot as well as the width of leaves. Significant positive environment correlation was observed between shoot length and number of nodes per shoot, length of leaves, width of leaves.

The genetic diversity among the fifteen *Dendrobium* varieties was studied using Mahalanobis D^2 analysis. The fifteen varieties were grouped into three clusters.

The qualitative characters of leaf and flowers were analysed for all the 24 *Dendrobium* varieties used in the investigation.

Molecular characterization of fifteen *Dendrobium* varieties evaluated in the experiment I was carried out using RAPD technique. The DNA yield varied from 120 to 225 ng/ ml. The primers OPA-19, OPB-02, OPB-04 and OPB-10 yielded good resolution bands out of 40 decamer primers tested. These primers amplify 44 RAPD markers of which 39 were polymorphic and five were monomorphic. The Similarity Coefficients value of the varieties ranged from 0.2667 to 0.8824. The genetic distance ranged from 0.1176 to 0.5806. The fifteen varieties got divided into six clusters on drawing a vertical line in the Dendrogram at a distance of 0.425.

A detailed descriptive blank of 24 *Dendrobium* varieties evaluated in the investigation was prepared.