EXPERIMENTAL TAXONOMY OF *PAPHIOPEDILUM* Pfitz. OF NORTH EASTERN REGION OF INDIA

ABSTRACT

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The North-Eastern region of India is one of the richest geographical zone of India for orchid with its diverse topography: altitude, climate and many other phytogeographical factors. This region has got immense treasure of orchid taxa which grow in profusion and also at the zenith with regard to their ornamental importance (Joy et al., 1994). However, various destructive activities and ruthless exploitation by the inhabitants disturb the delicately balanced natural habitat of orchids. Several orchid species have become extinct, many are at the verge of extinction and some are endangered.

Orchids are of tremendous horticultural interest, very promissing as well as challenging for various botanical investigations because of their characteristic and unique mode of life and reproduction. The genus Paphiopedilum is commercially most immportant group of orchids, with marvellous, beautiful, long lasting and large flowers.

In India, all the species of Paphiopedilum except P. druryi are found in North-Eastern region between 60-2220 m altitude. These are terrestrial or occassionally epiphytic, sympodial herbs with horizontally spreading thick roots. Leaves are conduplicate, oblong, elliptic, distichous, two to seveal, coriaceous, green or green mottled with light green or purple markings. Inflorescence terminal, terete. Flowers one or two per inflorescence, waxy in apprearance. Dorsal sepal erect, large, lateral sepals, united to form a synsepalum. Petals spreading, horizontal or pendent. Lip is slipper shaped, side lobes incurved. Column horizontal short with fleshy staminode at

apex infront of two fertile, ventral anther; pollinia two, glutinous. Stigma large, ventral, fleshy, short stalked, more or less hidden by side lobes of lip.

North-Eastern region of India is a trijunction - a meeting place of (a) Himalayan element, high altitude, (b) South, South-East Asian and far-east flora and (c) Peninsular India. Out of the seven existing species, six are confined to North-East, whereas P. druryi is only found in the Travancore hills of South India. This shows a discontinuous distribution of the species. Various ecological and adaphic factors could be the possible reasons for the distribution pattern of these species.

There are eight species reported from this region, viz., P. charlesworthii, P. fairrieanum, P. hirsutisimum, P. insigne, P. spicerianum, P. venustum, P. villosumand P. wardii. Out of these species, P. charlesworthii and P. wardii are supposed to be extinct from the nature. Every species has got distinct characteristics for identification, such as size and colour of leaf, leaf apex, lip and dorsal sepal. There are many variations and varieties among each species of Paphiopedilum of this region. Species diversity can be defined with satisfaction, the same may not be true in case of ecosystem diversity. Still, the ecosystem diversity/regional diversity can be identified on the basis of the increase or decrease in the number of species. Among all the species of Paphiopedilum, P. venustum showed the maximum number of variations, especially within the populations of Meghalaya. Hence, it may be suggested that Meghalaya could be considered as the centre of origin and dispersion of P. venustum.

The incessant sequence of biological diversity and gene pool should be efficiently utilized for the conservation of these species. The increasing importance of *in vitro* multiplication and perpetuation of species requires pure/natural species (biodiversity) for basis breeding stock.

Phenological observations are pre-requisite for sceintific multiplication and hybridization. The concept and significance of phenological investigations have been discussed in detail by Lieth (1970), Lieth and Radford (1971). Most of the characteristics are found species specific. The leaf apex may be considered as a very distinguishing character of species, for the identification even in vegetative stage. Various genetical as well as ecological factors influenced the phenology of **Paphiopedilum** species of this region. Out of six species studied, five species except **P. hirsutissimum** has got the flowering season from October-March i.e., from autumn to the end of winter. The floral longivity was minimum in **P. hirsutissimum** (blooming, March-June). This observation indicate that temperature, atomospheric humidity, intensity of light etc., have the direct impact on the flowering and longivity. Less relative humidity, high temperatue, high intensity of light have a negative impact on the floral longivity of **Paphiopedilum** species. The periodicity of different phenophases reflects seasonal distribution of specific kind of resources such as flower, pollen, fruit, seeds etc. Regular seasonal pattern observed in all the species may be due to the conducive climate of the

region. This kind of informations would be a great help in implementing sceintific multiplication/developmental programmes, proper utilization and management of resources in the orchid industry as well as for the conservation of the species. Since, the **Paphiopedilum** species are facing threat to their survival in nature, propagation and conservation of the species are the need of the time.

Epidermal and cuticular characters of the leaf have remarkable value in the field of palaeobotany, palaeoecology, pharmacognosy and taxonomy (Stace, 1966; Hardin, 1979; Wurdack, 1986; Singh and Dube, 1991). **Paphiopedilum** species under present investigation has got specific characteristics of leaf surface morphology. The cuticular sculpturing was different in all the species. The sculpturing of cuticle may be corelated with the light intensity of the environment. Absence of epidermal hairs observed in all the species is a general feature of Orchidaceae. Hyperstomatic chamber as well as lack of stomates on upper surface of the leave observed in the present study may function as water reservoir. The structural pattern of stoma present in all the species of **Paphiopedilum** supporting the premitive nature of the group. The overall similarity observed in the structure and only one type of stomata (elliptical) suggest that the group represent one phyletic line and might have evolved under a common environmental factors. Cuticular sculpturing, presence/absence of glandular hairs or trichomes, distribution, size , shape and structure of stomata, stomatal opening etc., are much significant in elucidating the phylogeny of the species. Leaf topology could

be used as a secondary or supporting characteristic in tracing out the taxonomic position/phylogeny of **Paphiopedilum** species along with the available information from other aspects of botany.

Orchid seeds are smallest among the seeds produced by the flowering plants and **Paphiopedilum** seeds are very small devoid of endosperm. Usually the number of seeds per capsule is ranging from 1,300 to 10,00,000. In the case of **Paphiopedilum** species this number is in the range of 42,150 to 1,03,250. Seeds of all the species studied are of floating nature.

The viability test is usually conducted by germinating the seeds. In the present study, a standradised staining method with Triphenyl Tetra Chloride and Malachite green has been described for the viability test of the **Paphiopedilum** species. With this method, the red stained embryos with green reticulations are considered as viable; whereas the wholly green stained ones are considered to be sterile.

Seeds of **Paphiopedilum** species were viable even after 4-6 months of bursting the capsule. This may be due to the appropriate maintenance of optimum temperature (4 C) required for the viability of seeds. Seeds are variously adapted with their structure to perform the function of propagation in nature. However, the rate of germination in nature is very meagre, because of non-availability of the specific fungal requirements and suitable atmospheric conditions. Singh (1992) reported that only 0.2%-0.3% of the seeds germinate in nature. Hadley (1970),

Harvais (1974), Arditti et al., (1981), Fast (1982) reported that the terrestrial orchids are very difficult to germinate.

The in vivo germination of **Paphiopedilum** species in the present study has only been partially successful with a very low percentage of germination. This may be due to the lack of proper endomycorrhizal association (an essential requirement) or various other factors like soil and atmospheric conditions. Other possible reasons may be the presence of germination inhibitors, onset of dormancy and the light intensity. These aspects are need to be further investigated to have a better conclusion. However, propagation through in vivo seed germination if followed properly will be of great advantage.

Cytology of **Paphiopedilum** species was very fascinating but a difficult task. Chromosomes of different species of **Paphiopedilum** showed a striking resemblance. The genus has got a basic chromosome number, n = 13. **P. fairrieanum, P. hirsutissimum, P. insigne** and **P. villosum** are represented by 2n = 26; whereas **P. spicerianum** has got 15 pairs of chromosomes (2n = 30) and **P. venustum** has 21 pairs of chromosomes (2n = 42). The karyotype of each species showed very distinct characteristics in the shape as well as position of centromeres. Chromosomes of all the species are large and distinct. However, **P. hirsutissimum** and **P. villosum** have quite large and distinct chromosomes out of all the six species studied.

There are lot of variations and varieties among each species of **Paphiopedilum** of North-Eastern region and have many natural

hybrids. This incessant series of biological diversity and gene pool should be properly utilized in various multiplication, propagation and conservation programmes. The knowledge of germ plasm, distribution, phenology, leaf topology, seed viability and germination, and cytologyetc., are the remarkable requirements for the scientific approach to the conservation of the species. The present study on Paphiopedilum species of North-Eastern region of India is a modest attempt to contribute some relevant informations towards the academic as well as applied venture.

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CERTIFICATE

I certify that the thesis entitled "EXPERIMENTAL TAXONOMY OF PAPHIOPEDILUM Pfitz. OF NORTH EASTERN REGION OF INDIA", submitted by Mr. JOY. K.A., in fulfilment of the requirement of the degree of DOCTOR OF PHILOSOPHY of the North-Eastern Hill University embodies the record of the original investigation carried out by him under my supervision. He has been duly registered, and the thesis presented is worthy of being considered for the award of the Ph.D. Degree. This work has not been submitted for any degree of any other University.

Shillong,
Dated: 28th June, 96

(DR. YOGENDRA KUMAR)
Supervisor of Research

DEDICATED TO
THE ETERNAL SOUL
OF
MY FATHER

CONTENTS

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(JOY K.A.)

CHAPTER – I

GENERAL INTRODUCTION

Plant Taxonomy is the basis to other fields of plant sciences and at the same time dependent on them. It has to depend for its improvement and existence on the information from other fields. Currently there is an emphasis on the importance of taxonomy in the light of recent biodiversity convention. Now there is renewed stress on the kind of information like exact identity, intraspecific variations, size of population, economic factors, hybrids, variations, distribution, habitat preference, cytology, ultrastructure, palynology etc., needed for basic biology and applied aspects (Khoshoo, 1995).

Northeastern region of India is considered as one of the richest biodiversity centre of the Indian subcontinent. A wide range of physiography and ecoclimatic conditions have contributed to a rich gene pool of both wild and cutivated plant species. The Northeastern India with a geographical area of about 2,55,050 sq.km. embraces the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland and Tripura. Phytogeographically, the region has been divided into two distinct regions,i.e., Assam and Eastern Himalayas (Clarke, 1898; Puri et al., 1990). The two distinct phytogeographical regions, viz., Eastern Himalaya and North-East India are identified based on the knowledge of vegetation and floristic elements. The later includes the plain regions of Arunachal Pradesh and all the other six sister states.

The northeast India lying between 22° - 30° N latitude and 89° - 97° E longitude forming the richest botanical zone of the country. It is claimed to be a `cradle of flowering plants' and is a sanctuary of primitive angiosperms. The climate, which determines the vegetation pattern of an area is quite varied. The region is characterised by high rainfall and humidity. The annual rainfall is usually above 2,000 mm, although, there are rain shadow belts also. The relative humidity is usually 80% during the morning hours and more than 90% during the evening hours. The altitude also varied from near sea level to 5,500 meters. The climate ranges from that of tropical plains to temperate and alpine hills. Due to these factors, the region represents one of the richest regions in the Indian subcontinent (Hooker and Thompson, 1855; Clarke, 1898; Hooker, 1906; Chatterjee, 1962; Rao, 1968, 1974; Joseph, 1982) and has been considered as a paradise for botanists. Hooker made a huge collection of plants from Khasi and Jaintia hills during 1851, and remarked that the vegetation of Khasi hills in Meghalaya is richest in India and probably in the whole of Asia. However, the rich genetic diversity has depleted and many plant species are facing threat in their natural habitats (Jain and Sastry, 1980; Sastry et al., 1983; Haridasan and Rao, 1985, 1987; Nayar and Sastry, 1987, 1988, 1990). Natural calamities like landslides, erosion and floods also contribute to the present conditions of the waste lands.

The Orchidaceae is one of the largest family of the flowering plants, with more than 20,000 - 25,000 wild species,

distributed world over under about 700 - 800 genera (Garay, 1960; Schultes and Pease, 1963; Dressler, 1981; Alberto and Rossi, 1988; Chadha, 1992; Peter, 1994). In India, the nearest estimate of orchids is about 1300 species belonging to 140 genera (Arora and Mukherjee, 1983; Jain and Mehrotra, 1984; Manilal and Kumar, 1986; Chadha, 1992). Orchidaceae occupies the first position amongst the ten dominant families of flowering plants in India (after Hooker, 1904). The Indian subcontinent is also considered primary/secondary centre of origin for orchids (Jain, 1986). Out of over 1000 species reported from India about 700 species flourish in this region (Sharma, 1993; Kumar et al., 1994). The orchids of northeastern region are also at the zenith with regard to their ornamental value when compared to the orchids of other parts of India (Sharma, 1993; Kumar et al., 1994).

Orchids have been well known to horticulturists for the past 400 years (Arditti, 1966; Garay, 1974). Their flowers have an irresistable appeal, and long lasting quality has been responsible for the development of a profitable industry but elsewhere. Several countries viz., Japan, Korea, Malaysia, Singapore, Taiwan, Thailand etc., are now running multimillion dollar business in orchids. However, commercial orchid growing in India is yet to be properly organized.

The orchids are not only well known for their beauty but also for their other utility. Besides their immense

floricultural values, they find effective utilization in indigenous systems of medicines. In the Ayurvedic system of medicine, a group of eight drugs known as ashtavarga, is employed in the preparation of a number of rejuvenating formulations and tonics. Important constituents of ashtavarga are reported as follows - jivak (**Microstylis wallichii**), kakoli (**Habenaria acuminata**) and ridhi vardhi (**H. intermedia**). Other commonly used orchid drugs are salem (**Orchis latifolia**, **Eulophia latifolia**), jiwanti (**Dendrobium alpestre**) and rasna (**Acampe papillosa**) (Handa, 1986).

Several important taxon of the family Orchidaceae, deserve urgent investigation and the **Paphiopedilum** Pfitz., is one of them. The **Paphiopedilum** (common name-Lady's slipper orchid) genus which was thaught to consist of 60 species (Cribb, 1985, 1987), with new discoveries this number has now increased to about 70 species (Cribb, 1995). This genus belongs to the sub-family Cypripedioideae and is distributed throughout Asia from India eastwards to the Malay islands, the Philippines, New Guinea and the Solomon Islands (Cribb, 1985, 1987, 1995). Considerable works have been done on different aspects, viz., seed morphometry, palynology, leaf surface morphology, cytology, *in vitro* seed germination etc., of this subfamily (Stoutamire, 1967; Newton and Williams, 1978; Arditti et al., 1979,1980; Atwood, 1984; Burns-Balogh and Funk, 1986; Burns-Balogh and Hesse, 1988; Vij et al., 1992; Garg et al., 1992). The Cypripedioideae form a very distinct and a homogenously well defined group, it differs from Orchidoideae in having two fertile

stamens (Rasmussen ,1985). Dahlgren and his co-workers (c.f. Dressler, 1986) treat this group as a distinct family.

The **Paphiopedilum** flowers are resupinate, strongly zygomorphic. Flowers are in three whorls. The dorsal sepals are fused into a synsepalum. Ventral sepal is free. The lateral petals are considerably longer. The median petal (lip) is characteristicaly slipper shaped. The two lateral stamens of the inner whorls are always present and the median stamen of the outer whorls is present as a characteristic shield-like staminode. The filaments are fused with the style, forming a thick inflexed gynostemium. The anthers are subglobose and latrose, dehising by longitudinal slits. The pollen grains are free, sulcate, porate and reticulate (Joy, 1992). The ovary is unilocular with parietal placentation.

In India, nine species are reported of Paphiopedilums, of which two species **P. charlesworthii** and **P. wardii** from Mizoram and Arunachal Pradesh respectively, are supposed to be extinct from India. One of the species **P. druryi** is confined to the Travancore hills of Kerala State once extinct in nature and recently has been re-located (Menon et al., 1994). The other six species viz., **P. fairrieanum**, **P. hirsutissimum**, **P. insigne**, **P. spicerianum**, **P. venustum** and **P. villosum** are confined to Northeastern India.

Biodiversity is the totality of genes, species and ecosys-

tems in a region. In simple words it could be defined as the great variability that exists in the living organism. The whole range of variations of a species can be used for plant improvement, breeding programmes and effective and meaningful conservation (Kumar et al., 1995). There are large number of floristic works on Indian flora but we do not know much about diversity within the species.

Phenology is an important aspect concerned with the study of different phenophases such as flowering, fruiting, maturation, dehiscence etc., of a given species in different seasons. Phenological observations are pre-requisite for the scientific multiplication, plant improvement, breeding, hybridisation etc. Reports are available on the phenological data of some orchid species (Nilsson, 1988; Peakall, 1989; Borg-Karlson, 1990; Dafni and Bernhardt, 1990; Paulus and Gack, 1990 b; Robertson and Wyatt, 1990; Firmage, 1992; Stern et al., 1994). However, phenological data on the Paphiopedilum species of Northeastern region of India is lacking. Therefore, in the present study both the vegetative as well as reproductive phenophases has been studied.

Characters of the leaf are second to only those of flowers and fruits in the extend to which they are used, and in their value in taxonomical studies. Leaf surface characteristics are much significant than the floral organs, since they are generally present on the plant for a much great part of its life span.

For this reason they are valuable in making primary taxonomical decisions (Stace, 1984). Significance of leaf surface character in the present study is to investigate a phylogenetic relationships between species. For the past thirty years, the botanical science have witnessed a remarkable increase in the attention paid to leaf surface characters. The advent of the Scanning Electron Microscopy (SEM) made the investigation much easier. There are few reports of microcharacteristics on leaf surface morphology in various groups of plants (Stace, 1965, 1973, 1984; Napp-Zinn ,1966,1973,1974; Tomlinson, 1969, 1974; Paliwal, 1969; Payne, 1970, 1979; Van Cotthem, 1970, 1971; Martin and Juniper 1970; Heywood, 1971; Preece and Dickinson , 1971;Fryns-Claessens and Van Cotthem , 1973; Patel, 1978; Stewens and Martin, 1978; Wilkinson, 1979; Rasmussen, 1981 a,b). The investigations on the leaf surface morphology of orchids in general are scanty (Yukawa et al., 1992; Stern et al., 1994) and on **Paphiopedilum** species, there is no comprehensive study. Therefore, in the present study an attempt has been made to investigate the leaf surface morphology of **Paphiopedilum** species of Northeastern India by using Light Microscope as well as Scanning Electron Microscope.

Orchid seeds are unique in that they are exceedingly small and are produced in large numbers ranging from 1,300 to 10,00,000 per capsule (Garg et al., 1992). Each seed comprises an undifferentiated embryo enclosed within a transparent integument or seed coat.

Orchid seeds are usually non-endospermic and in nature they

require the association of mycorrhizal fungus for their germination. There are reports, particularly on the *in vitro* germination of orchid seeds (Knudson, 1922; Hegarty, 1955; Murashige and Skoog, 1962; Lawrence and Arditti, 1964; Arditti, 1967, 1979; Harrison and Arditti, 1970; Harvais, 1973; Mukherjee et al., 1974; Henrich et al., 1981; Lee et al., 1983; Singh and Prakash, 1985; Reddy et al., 1992). However, it is found that there is no comprehensive works on the viability of orchid seeds and their *in vivo* germination . An attempt has been made to study these aspects also in the present investigation.

Cytotaxonomical investigations are important to study the evolutionary and phylogenetic aspects of the plant species with regard to the relevant structural and quantitative features of the karyotype (Greilhuber, 1984). In addition, the chromosome number and morphology of the species can be investigated. Cytological investigations of the Himalayan orchids have been a main attraction for a number of years to the botanists. Cytological and karyological investigations on Orchidaceae have been carried out by D'emerico et al., 1993. Mehra (1983) and Sehgal and Sehgal (1989) prepared a monograph on the cytology of orchids of Khasi and Jaintia hills. In the field of cytology of Indian orchids, Biswas (1980, 1986) and Vij et al., (1986) have made significant contributions. However, no comprehensive studies have been conducted on the cytological aspects of Northeastern **Paphiopedilum** species except some scattered reports. Therefore, in the present study an attempt has been made to investigate the

cytology of **Paphiopedilum** species of Northeastern India.

Therefore, in the present work, following aspects viz., Biodiversity, Phenology, Leaf topology, Seed viability and *in vivo* germination and Cytology of **Paphiopedilum** of the North-Eastern region of India have been investigated.

CHAPTER – II

REVIEW OF LITERATURE

BIODIVERSITY

The concept of biodiversity has got multifaced significance in the study of every organism on the earth. There are various definitions put forward for the term "biodiversity", time to time. According to Brieger (1975) the extreme degree of morphological variability in orchids is attributed to genetic drift. Jain and Sastry (1980) ; Sastry et al., (1983); Nayar and Sastry (1987, 1988, 1990) reported that the rich genetic diversity of various species is facing threat in their natural habitats.

Wilcox (1984) defined the concept of biological diversity as the variety or life forms, in a given region, the ecological roles they perform and the genetic diversity they contain. Whereas, Reid and Miller (1989) provided a comprehensive definition: "biodiveristy is the variety of the world's organisms, their genetic diversity and the assemblages they form".

General account of Paphiopedilums and the world wide distribution has been dealt by Cribb (1987). Kataki (1986) provided a general account on Indian Paphiopedilums. Recently, the concept of biodiversity has been highlighted by Menon et al., (1994) and Heywood (1996). Peter Endress (1994) dealt in detail the biodiversity and evolutionary biology of tropical flowers. Koopowitz (1995) published an annotated checklist of the genus

Paphiopedilums. However, a thorough perusal of the available literature revealed that there is a need of a comprehensive account on the biodiversity of each species of Indian **Paphiopedilum**.

PHENOLOGY

Since 1942, or rather still long back the botanical science was in possession of phenological data of flora from various regions of the world (Holmes, 1942; Sagreoya, 1942; Ganapathya and Rangarajan, 1964; Kaul and Raina, 1980; Bisht et al., 1986; Navchoo and Kachroo, 1986; Beniwal, 1987). The investigators like Cooke (1903-08); Santapau (1953); Anonymous (1957); Ghate and Vartak (1987) remarked on flowering of different groups of plants in floras. Foster (1982 b); Leigh and Windsor (1982); Prasad (1983); Wada (1983); Appanah (1985) and others explained and discussed the various phenological events and other sociological impacts. According to Prasad and Hegde (1986) there are mainly nine phenological events (production of young leaves, maturation of leaves, abscission of leaves, production of young flowers, maturation of flowers, abscission of flowers, production of young fruits, maturation of fruits and ripening of fruits) in the life history of a plant.

Phenological studies on selected tree species of Northeastern India was carried out by Boojh and Ramakrishnan (1982) and Shukla and Ramakrishnan (1982, 1984). Whereas Bhat (1992) studied the phenology of tree species of tropical moist forest of Uttara Kannada district of Karnataka State. Vinaya and Kumbhojkar (1991) have discussed the phenology of deciduous ornamental trees from Western Maharashtra.

Kay (1987) studied a comparative ecology of flowering. Flowering phenology was further dealt by Totland (1993). Waser (1979) and Thompson (1980) suggested that there is a natural selection to synchronize flowering phenology.

There are certain studies which revealed the relation between pollination and reproductive phenology. Darwin (1885); Dressler (1968); Waser (1983); Javier et al., (1992) reported the existence of infrequent pollinators, in many plant groups. Pollen dispersal over longer distances is revealed by the studies of Melampy (1981) and Schmitt et al., (1987).

Flowering and any subsequent seed production require the diversion of resources from vegetative growth (Evenson, 1983). However, Werner (1975) and Gross (1981) reported that the plant size, but not the age determines the time of reproduction.

Some of the workers advocated the influence of duration of seasons on the phenophases of diffferent plants. Mosquin (1971); Pleasant (1980, 1983) and Waser (1983) reported the interspecific competition for pollinator as the most frequently cited explanation for distribition pattern of flowering times. Subsequently, Poole and Rathcke (1979); Rathcke (1983, 1984) reported that the flowering time within a community should be regularly distributed through the season. However, a positive interaction for pollination occurs if species experience a high visitation rate when flowering simultaneously than sequentially. Arroyo et al.,(1985) measured a decrease in visitation rate with

increasing altitude. Futher studies of Rathcke and Lacey (1985) and Primack (1987) revealed that the length of season may influence flowering times.

There is a small array of investigations on the phenological aspects, particularly on orchids. Though, the informations are found to be inadequate for the thorough knowledge on the phenological events of a vast family like Orchidaceae. Some of the most fascinating examples of floral adaptation among orchids are highlighted by some of the workers (Vander Pijl and Dodson , 1966; Nillson, 1988; Peakall, 1989; Borg Karlson, 1990; Dafni and Bernhardt, 1990; Paulus and Gack, 1990 b; Robertson and Wyatt, 1990; Kim et al., 1994). Hubert (1987) studied the effect of vernalization on the development of native orchids, Platanthera, Cypripedium and Calopogon. David (1992) investigated the flowering frequency and reproductive cost in Platanthera blephariglottis (Orchidaceae) in relation to micro-habitat. Benzing and Atwood (1984) pointed out that there is always a competition for pollinators in orchids.

Reproduction and development of fruits in orchids are also been dealt by some investigators. According to Ackerman (1990) there are short and long term limitations to fruit production in a tropical orchid. Ackerman (1986) reported that orchids generally produce fewer fruits than flowers. Arditti (1979) and Dressler (1981) opined that many aspects of orchid reproduction have generally been neglected. However, structural aspects of reproduction have been studied for some orchid species (Johansen, 1950; Maheswari, 1950; Wirth and Withner, 1959; Veyret, 1974).

According to these investigations, orchid fruit development is generaly characterised by long period necessay for mature embryosac to form after pollination. Taylor et al., (1982) studied the hormonal and structural aspects of fruit development in the orchid, **Epidendrum**.

A review of the available literature revealed that there is a dirth of informations on the methedology to study the phenological aspects in all groups of plants in general and Orchidaceae in particular. However, the various methodologies adopted are discussed by a few investigators (Montalvo and Ackerman, 1987; Javier et al., 1992). Though, it is found that the informations are still meagre. Montalvo and Ackerman (1987) discussed the various methodology for the general phenological studies. Methods for phenolgical observations and effects of display size was dealt in detail by Javier et al., (1992).

LEAF TOPOLOGY

Prantl (1872-81) classified the stomata for the first time into two ontogenetic types - direct and indirect. Since Prantl's work, various kinds of classifications were carried out by many investigators (Strassburger, 1866-67; Vesque, 1889;Florin, 1931-33; Metcalfe and Chalk, 1951; Metcalfe, 1961; Maroti, 1961; Pant, 1965; Stace, 1965; Guyoti, 1966; Mickel and Lersten, 1967; Van Cotthem, 1970, 1971; Probst, 1971; Fryns-claessens and Van Cotthem, 1973; Patel, 1978; Stevens and Martin, 1978; Rasmussen, 1981 a,b; Benton, 1988). Vesque in the year 1889, recognised four types of stomata among angiosperms - Ranunculaceous, Rubiaceous, Cruciferous and Caryophyllaceous. Metcalfe and Chalk, 1950 proposed the terms anomocytic, paracytic, anisocytic and diacytic respectively.

The volumes of modern terminology for stomatal types were prepared by some investigators around decades ago (Barthlott and Ehler, 1977; Wilkinson, 1979; Stevens and Martin, 1978; Wilkinson, 1979; Rasmussen, 1981; Benton, 1988).

Reviews on the stomatal classification, anatomy and morphology was published time to time by various workers (Dickinson, 1949, 1979;Meidner and Mansfield, 1968; Ellingboe, 1972; Day, 1972; Wynn,. 1976; Wilkinson, 1979; Pring, 1980; Palevitz, 1981; Willmer, 1983; Inamdar et al., 1986).

Vegetative and leaf anatomy of plants has been a main

attraction since long ago to various workers (Stahl, 1914; Haberlandt, 1914; Solereder and Meyer, 1930; Martin and Juniper, 1970; Newton, 1972; Williams, 1974; Hull and Bleckmann, 1977; Pridgeon, 1981, 1994). Haberlandt in 1914 studied the epidermal surface features in general. Similar studies were conducted by Stern and Strauss, 1983 and Clarke et al., 1991. Ogundipe (1992) studied the leaf epidermal characters in the genus **Datura** Linn. (Solanaceae). Whereas the structure of plant cuticle was described and the characters were analysed by workers like Haberlandt (1914); Stace (1965) ; Martin and Juniper (1970); Newton (1972); Stant (1973); Williams (1974); Hull and Bleckmann (1977).

Intensive studies on the ontogeny of stomata was started a century ago (Hidebrand, 1866; Oudemans, 1866; Strassburger, 1866-67; Rauter, 1870; Prantl, 1872; Benecke, 1892; Tognini, 1897; Porsch, 1905). Recently Paliwal (1969) studied in detail the ontogeny of different types of stomata.

Various kinds of anatomical studies on stomata were carried out by many investigators (Roelofsen, 1959; Stalfelt, 1966; Mazliak, 1968; Kolattukudy, 1970; Martin and Juniper, 1970; Pallas, 1971; De Michele and Sharpe, 1973; Hoch, 1975; Edwards et al., 1976; Reed, 1979; Wattendorf and Holloway, 1980; Weyer and Travis, 1981; Rogers et al., 1981; Sack and Paolillo, 1983).

Stomatal density is found to be related to various factors. Ticha (1982) and Smith et al., (1989) reported that stomatal density extremely variable with species habitat, height of

insertion and areas of leaf. Whereas Schoch et al., 1980; Yegappan et al., 1982, Ferris, 1991; Rahim and Fordham, 1991 stressed the influence of environmental factors on the stomatal density. Ticha (1982) opined that stomatal density is fixed during leaf development. According to Korner et al., (1979) and Woodward (1986), the density is increased with altitude. Zalenski (1904) reported greater stomatal density as a xeromorphic adaptation. Sharma (1990) pointed out an inverse relationship between stomatal density and pollution. General studies has also been conducted by Paoletti and Gellini (1993) and Peng and Weyers (1994). Inverse relationship between Co_2 concentration and stomatal density was reported by Maden (1973); Oberhauer et al., (1985); Woodward (1987, 1993); Woodward and Bazzaz (1988).

There are various informations on the stomatal physiology and other details particularly in the family Orchidaceae. Solereder and Meyer (1930) and Pridgeon (1981) studied the vegetative anatomy of Orchidaceae. Strassburger (1966-67) reported `aperigenous' stomata for Orchidaceae. Stomatal diurnal fluctuations of acidity in orchids was investigated by Warburg (1986); Goh et al., (1977). Withner et al., (1974) investigated the relationship between water conservation and lack of stomata on upper surface of orchids.

Fu and Hew (1982) studied CAM in orchids under water stress. Whereas Holttum (1960, 1964) and Sanford (1975) investigated the relationship between water availability and distribution of

orchids. The fixation of Co_2 by orchids via CAM was investigated by Nurerbergk (1963), Mc Milliams (1970); Neales and Hew (1975); Hew (1976) and Goh et al., (1977). Goh et al., (1984) reported CAM in young orchid leaves. Kaushik (1978) found that there is a consistency of mature stomata in different taxonomic groups within Orchidaceae and suggested a phylogenetic classification of Orchidaceae.

Various studies on stomatal morphology as well as physiology were carried out specifically in the genus **Paphiopedilum** by some workers. Nelson and Mayo (1975, 1977) and Rutter and Willmer (1979) reported achlorophyllus guard cells in **Paphiopedilum** species (**P. leanum**, **P. insigne**, **P. harisseanum**, **P. fairrieanum** and **P. venustum**). However they found that these guard cells respond to Co_2 and light as other species. Similar observations were recorded by Schnabl and Raschke (1980) and Outlaw et al., (1982). Virgin (1957) and Shaw (1958) reported the necessity of chlorophyllus guard cell for stomatal transpiration. However, Nelson and Mayo (1975) and Rutter and Willmer (1979) contradicted the above view as in the case of **Paphiopedilum** species.

Solereder and Meyer (1930) reported the presence of sunken hairs in orchids in general, but these are reported to be absent in more premitive terrestrials like **Paphiopedilum**, **Spiranthes** and **Prescottia**. A hyperstomatic chamber was observed by Haberlandt (1928) in **Cypripedium venustum** and in many other orchids. This characteristic is considered as a xerophytic adaptation (Gessner,

1956; Nuernbergk, 1963; Knauff and Arditti, 1969).

The diagnostic characteristics of stomata have been a very significant tool in solving various taxonomic problems. Many investigators in the field of botanical research have correlated the stomatal characteristics with taxonomical approach. There are many reports emphasizing the significance of characteristics of stomata in analysing and diagonising the taxonomic uncertainty (Paliwal, 1969; Tomlinson, 1969, 1974; Payne, 1970, 1979; Dilcher, 1974; Shah and Kothari, 1975; Hardin, 1969; Dehgan, 1980; Soladoye, 1982; Stace, 1984; Wurdack, 1986; Singh and Dube, 1991). Dehgan (1980) stressed the importance of epidermal morphology to taxonomic delimitations in the genus Jatropha L. (Euphorbiaceae). According to Stace (1965) the epidermal morphology is very significant and has sufficient taxonomic value. The significance of similar studies has further emphasized by many other investigators (Hagerup, 1953; Ahmed, 1963, 1974; Cuttler, 1979, 1984; Wilkinson, 1979; Ogundipe and Olatunji, 1991 a,b).

To observe and study the stomatal characteristics in detail many investigators have stressed the importance of Scanning Electron Microscope (SEM). SEM has got many advantages over Light Microscope (LM) in the study of stomatal morphology, as well as in various taxonomic studies in general. Heywood (1971) edited a volume of research works on the use of SEM in taxonomic investigations. Turner and Heichel (1977) and Shiraishi et al., (1978) investigated the stomatal morphology by

using both LM and SEM. Whereas Heath (1959) and Meidner and Mansfield (1968) emphasised the importance of SEM and high magnification for the purpose. Singh and Dube (1991) conducted SEM studies on leaves of different plants. Eveling and Mc Call (1983) ;Sargent (1983) and Eveling (1984) discussed the effect of freeze drying and critical point drying (CPD) techniques on petals and peelings for SEM. Brown and Johnson (1962) has conducted SEM studies in Grasses.

While studying the developmental pattern of stomata, Bunning and Sagromsky (1948) and Galatis and Mitrakos (1979) made use of LM and SEM. Before the wide use of SEM in the field of botanical investigations many workers have used only LM (Monzi, 1939; Went, 1944; Stalfelt, 1929, 1959; Raschke, 1970; Meidner, 1981). Fischer (1968) and Fischer and Hsiao (1968) studied the stomatal aperture by LM. However, Ayensu (1974); Ehler (1974); Rollins and Banerjee (1975); Cuttler and Brandham (1977) and Atwood and Williams (1979) opined that comparative morphology of leaf epidermis using SEM has been limited.

There are various methods and procedures for studying stomatal characteristics both by LM and SEM. A range of methodology was adopted by many investigators (Lloyd, 1908; Metcalfe, 1960; Meidner and Mansfield, 1968; Baker and Parsons, 1971; Atwood and Williams, 1979; Edwards and Meidner, 1979; Shah and Beckett, 1979; Weyers and Travis, 1981; Pridgeon, 1981, 1994; Meidner, 1981; Ziegler et al., 1983; Kramer, 1988; Passiomura,

1988; Nonaniu et al., 1990; Davies and Zhang, 1991; Tardieu and Davies, 1992; Tardieu and Davies, 1992; Meidner and Willmer, 1993). Weyers and Travis (1981) cited the wide use of epidermal strips for experimentation. Applicability of stereological methods to the quantitative evaluation of plant structure surveyed by few investigators (Briarty, 1975; Parkhurst, 1982; Toth, 1982; Kubinova, 1993, 1994). Kramer (1988), Passiomura, (1988) and Davies and Zhang (1991) discussed the effect of dehydration. Fixation in gluteraldehyde and post fixation in Osmium tetroxide was followed by Zeigler et al., (1983) and Nonaniu et al., (1990).

There are some techniques for obtaining epidermal peels for the study (Metcalfe, 1960; Meidner and Mansfield, 1968; Humble and Hsiao, 1970; Meidner, 1981; Weyers and Travis, 1981). Shah and Backett (1979) reported that the problem by dehydration can be reduced by moist environment SEM. Edwards and Meidner (1979) explained the immediate consequences of peeling. Epidermal stripping is done by dissection (Von Mohl, 1856), low pH (Squire and Mansfield, 1972), rolling and pressure (Allaway and Hsiao,) sonication (Ogawa et al., 1978). Method of isolating stomata by pH treatment was further discussed by Squire and Mansfield (1972). Stomatal study by leaf surface impression was dealt by Florell and Rufelt (1960) and Apel (1967). Kenji et al., (1983) proposed the observation of stomatal movements of intact plants by image instrumentation system. A numerical taxonoic approach to study angiosperm leaves was drafted by Robert (1980). Dolph (1978) proposed a computer data banking for gross morphology

(Hickey, 1973), cuticular characters (Dilcher, 1974) as well as for both (Blackburn, 1978 a). Humble and Hsiao (1969) prepared an incubation media for stomatal opening. Glower (1967) opined that the efficiency of replication varies with aperture. Glinka and Meidner (1968) suggested the need of direct measrement of stomata. Sampson (1961) and Weyers and Johanson (1985) reported that accurate estimation of stomatal aperture was possible from silicon rubber impressions. Various methods for the specific study of stomatal aperture was dealt by a few investigators (Lloyd, 1908; Molisch, 1912; Alvim and Harvis, 1954; Stalfelt, 1956, 1959; Sampson, 1961; Zelitch, 1963). Willmer and Mansfield (1969) done a critical examination of the uses of detached epidermis in studies of stomatal physiology. Methods to study stomatal frequency (Quirrie and Jones, 1977) and intervennial distance (Crookstin and Moss, 1974) are also available.

SEED VIABILITY AND IN VIVO GERMINATION

Roberts (1973) classified the seeds in general into two types - Orthodox and Recalcitrant, according to its water resistant capabilities. Effect of water and imbibition injury etc., on dry seeds were investigated by Tompsett (1984 b, 1987, 1988); Powell and Mathews, (1978); Ellis et al., (1982 b). Roberts and Ellis (1989) discussed in detail the effect of water and seed survival. King and Roberts (1979) and Tompsett (1983) hypothesized that seed longivity increases with increased moisture content. Similar studies were conductued by Ellis et al., (1990). Viability in storage and moisture content was investigated by Nutile, 1964; Nakamura, 1975; Ellis and Roberts, 1982; Osei-Bonsu and Roberts, 1982; Ellis et al., 1986. Arditti et al., (1979, 1980, 1981) suggested the storage of seeds at 4 C in smaller paper envelopes for retaining the viability. Abdalla and Roberts (1968, 1969); Roberts (1972 a, 1978) reported a genetic damage associated with loss of viability. Dickie et al., (1990) and Ellis and Roberts (1980 b) studied the effect of temperature on seed storage and longivity. Ellis et al., (1982); Dickie et al., (1985); Ellis (1988) suggested that the effect of temperature may not differ among species.

Arditti (1967) and Arditti and Ernst (1984) studied the seeds of orchids and reported lack of endosperm im embryo. There are other reports on the seed reserve food (Manning and Van Staden, 1987; Rasmussen, 1990; Richardson et al., 1992). Bain

and Mercer (1964), Opik (1966, 1968), Srivasthava and Paulson (1968), Simolova (1971) described protein and lipids as the reserve food in seeds. Ultrastructural studies on various seeds were conducted by Swamy (1949); Alvarez and Sagawa (1965); Ricardo and Alvarez (1971); Harrison (1977). Orchid seeds lack endosperm (Maheswari, 1950; Wirth and Withner, 1959; Veyret, 1974; Arditti, 1979; Dressler, 1981). Histochemical and physiological details regarding **Cypripedium** seeds are given by Zinger and Arnoldi, 1966.

Arditti (1967, 1979) reported the formation of protocorms during germination and defined the germination as formation of green or white protocorms (Arditti et al., 1981). Development of protocorm was also observed by Burgeff (1969); Warcup (1975); and Arditti et al., (1990). Miller and Conn (1980) reported the physiological changes during germination. The investigators like Burgeff, 1954; Arditti, 1967, 1979, 1982; Clements, 1982; Fast, 1982 tried the germination of terrestrial orchid seeds and reported that the methods appropriate for one species may not be suitable for others. Requirements for germination are varied with species (Arditti, 1967; Harley, 1969; Harvais and Pekkala, 1975; Arditti and Michaud, 1979; Arditti et al., 1979, 1980; Healey et al., 1980;Fast, 1982; Dc Paw and Remphrey, 1992). Arditti (1967) reviewed the factors affecting germination of orchid seeds.

Mostly the investigations on the orchid seed germination has been conducted *in vitro*, but not *in vivo*. A thorough, perusal of the literature revealed that there are many reports regarding the

requirements of germination in vitro. Utilization of vitamins etc., during germination has been postulated by Burgeff, 1936; Schaffstein, 1938; Noggle and Wynd, 1943; Bahme, 1949; Henrikson, 1951; Mariat, 1952; Withner, 1959; Hadley and Harvais, 1968; Hadley, 1968, 1969; Nakamura, 1982. Poddubnaya-Arnoldi (1954); Zinger (1958); Zinger and Poddubnaya-Arnoldi (1959, 1963) suggested the seeds as ideal material for studying embryonic process. Alvarez and Sagawa (1965) and Zinger and Arnoldi (1966) described the metabolism of germination. Harvais (1982) found that most important growth regulator during germination of terrestrial orchids is cytokinin. Arditti (1967, 1969) and Arditti and Ernst (1984) found that the gibberellic acid has no positive effect on germination. Comparative studies on genotype variability and seed germination were conducted by Linden (1980); Henrich et al., (1981); Arditti et al., (1982) and Oliva and Arditti, (1984).

There is a wide spectrum of informations on the mycorrhizal asociation and their functions, specificity on orchids and seed germination. Eventhough, there is different opinions on the specificity of fungal association, there is no second view on the necessity of endomycorrhiza for the germination of orchid seeds (Harley, 1959; Harvais and Hadley, 1987). According to Smith (1952) orchid parasite on fungus rather than symbiosis. Purves and Hadley (1976) described the physiology of symbiosis, existing between fungal mycelium and orchids.

Taxonomic investiations on orchid endophytic fungi, like **Rhizoctonia** have been conducted by many workers (Bernard, 1909; Burgeff, 1936; Curtis, 1939; Gaumann et al., 1960; Harvais and Hadley, 1967; Warcup and Talbot, 1967, 1971, 1980; Harvais, 1973; Nishikawa and Ui, 1976; Alexander and Hadley, 1983). Hadley (1970) reported 32 species of **Rhizoctonia** from orchid isolation. Hadley (1975-82); Clements (1988) and Paterson and Currah (1990) observed fungal association with protocorms of various species of orchids. Knudson (1922), Downie (1943), Smith (1960, 1967), Harvais (1965), Harley (1969), Hadley and Williamson (1971), Harvais and Raitsakes (1975) in their various studies questioned the growth stimulation by digestion of endophytic fungi. Harvais and Hadley (1967) found that fungal strains parasitise protocorms when carbon source is not suitably controlled. Bernard (1909) and Burgeff (1936) reported that the mycorrhizal fungi broke down complex carbohydrates in the substrates and transported simple sugars into orchid seedlings. Hollander (1936); Smith (1966, 1967) opined that fungi able to utilize cellulose. Harvais and Hadley (1967) investigated the effect of temperature on the interaction between host and fungus. Studies shown that the endophytic fungi enter to protocorm through suspensor cells, as in **Paphiopedilum hyperborea** (Burgeff, 1959; Clements, 1988).

Rasmussen (1990) found that there is an inhibitory action against fungal hyphae in **Dendrobium majalis**. According to Smith (1967); Alexander et al., (1984) and Arditti et al., (1990), there is a translocation of number of substances, sugars,

phosphates etc., by endophytic fungi. Ricardo and Alvarez (1971), Borris et al., (1971) and Hadley (1975) reported crystal containing bodies in orchid cells colonized by endophytic fungi. Hadley (1982) observed that protocorms degrades endophytic fungi to a clump and significance of callose deposition around degrading hyphae. Paterson and Currah (1990) is also highlighted, this aspect. However, Arditti et al., (1990) and Richardson et al., (1992) reported that the interaction between orchids and endophytic fungi remain a mystery.

New records and new taxa of fungi from the mycorrhizae of terrestrial orchids of Alberta was reported by Currah et al., (1987) whereas Rasmussen (1992) observed the germination and growth of mycorrhizal seedlings of **Tipularia discolor** on woody debris. Warcup (1973) reported symbiotic germination of some Australian terrestrial orchids. Arditti (1967) suggested that mycorrhizal association is a universal characteristic of germination.

Hadley (1970); Purves and Hadley (1975); Benzing (1982) suggested the possible role of mycorrhizal fungi for carbon source. According to Warcup and Talbot (1967) and Hadley (1969, 1970), the endophytic fungi are host specific. However, Campbell (1962, 1970) contradicted the above findings. Non-specificity of fungi has been stressed by Bernard (1909); Knudson (1925); Curtis (1939); Hadley (1970). Hadley and Williams (1972) described the changes after fungal infection and the findings of Heyman and Mosse (1971); Daughtridge et al., (1986) supports a

marked increase in seedling growth on mycorrhizal association. Mosse and Heymann (1971) corelated soil conditions and mycorrhizal growth. Mycorrhizal relationship of Australian orchid is demonstrated by Warcup (1981). Alexander and Hadley (1985) observed carbon movement between host and mycorrhizal endophyte during development of orchid, **Goodyera repens**. Movement of carbon, further demonstrated by Smith (1966, 1967); Hadley (1969, 1970); Purves and Hadley (1975); Hadley and Smith (1983); Alexander et al., (1984). Cochrane (1958) reported the fungal role in carbon translocation. Carbohydrate translocation in orchid mycorrhiza was demonstrated by Smith (1967). Warcup and Talbot (1967) described the perfect status of Rhizoctonias associated with orchids. Physiology and ecology of orchid mycorrhizal fungi with reference to seedling nutrition was reported by Smith (1966).

Bernard (1904, 1909), Burgeff (1936) studied the direct role of mycorrhiza in nutrition of seedlings. Hadley and Williamson (1972) explained the features of mycorrhizal infection in some Malayan orchids. Variation in symbiotic activity of **Rhizoctonia** was reported by Alexander and Hadley (1983). Blakeman et al., (1976) investigated the effect of mycorrhizal infection on respiration and activity of some oxidase enzymes of orchid protocorms. Lewis (1973) opined that orchid may be necrotrophically parasitic on fungus. Smith (1973) reported asymbiotic germination of orchid seed on carbohydrates of fungal origin. Dependence of orchids on mycorrhizal fungi for

germination was further stressed by Warcup and Talbot (1971); Warcup (1971, 1973, 1975);Stoutamire (1974).

However, reports say that the terrestrial orchids especially those from temperate region are difficult to germinate (Curtis, 1936, 1943; Downie, 1940, 1949; Knudson, 1941; Vermeulen, 1947; Liddeu, 1954; Arditti, 1967; Harvais, 1973, 1974; Fast, 1976; Clements and Ellyard, 1979; Linden, 1980). Some of the investigators have pointed out the possible reasons for poor germination in orchids. According to Harvais (1980), Van Waes and Debergh (1986 a,b); the hydrophobic nature of seed coat during development, may inhibit germination of mature terrestrial orchid seeds. Again, they suggested the use of hypochlorite solution for sterilization, which may enhance the germination by removing the suberin of mature seed coat. Fast (1974) reported presence of germination inhibitors whereas Stoutamire (1974) opined the onset of dormancy in mature seeds as the important reasons for poor germination.

Arditti (1967, 1979); Stoutamire (1974); Ernst (1982); Olvin and Arditti (1984) observed the best germination in dark for terrestrial species whereas epiphytic orchids can germinate both in light and dark. However, for the development both groups require illumination/light. Arditti et al., (1981) reported that illumination has no significance in the germination of Spiranthes, Aplectrum or Cypripedium. Effects of hydration on seed germination was highlighted by various workers (Long et al., 1981; Dasgupta and Bewley,1982; Dasgupta et al., 1982; Kermode et

al., 1985; Misra et al., 1985). Yeutur and Leopold (1976) studied the respiratory transition during seed germination. Marcus and Feiley (1964) suggested that nucliec acid synthesis is an essential part of germination. Gilles et al., (1993) studied the morphogenesis of the protocorm of **Cypripedium acaule**. Hasegawa and Esashi (1994) investigated the histochemical activities during pre-germination.

A variety of methods and procedures have been proposed to study the orchid seed morphometry, viability and their germination by many investigators. Van Waes and Debergh (1986) developed a technique to determine the percentage of seed germination. Aseptic germination techniques for many orchids was drafted long time back by Knudson (1922, 1947). Philip and Nainar (1986) described the tissue culture methods and clonal propagation.

Frosch (1982) and Hadley (1982) discussed the effect of pre-treatment for a long time on the germination, in species with resistant seed coats. Arditti (1982) and Harvais (1982) proposed the optimal temperature for terrestrials as 23 2 C. There are reports on the effect and need of chilling (3-6 C) in **Cypripedium calceolus** and **Epipactic** species, by Borris (1969), Borris and Albrecht (1969), Stoutamire (1974). However, Fast (1978) and Linden (1980) contradicted the above findings. Again, Fast (1982) reported that cold treatment may be required for breaking dormancy. Curtis (1943) has opined that cold treatment for

breaking seed dormancy is different from the same for epicotyl dormancy.

Tamanaha et al., (1979) studied various stages of germination using growth index. This idea was put forward by Spoerl in 1948 and supported by Harrison (1973) and Arditti (1979). Beardmore and Pegg (1981) developed a technique for the establishment of mycorrhizal infection in orchid tissue grown in aseptic culture. Ellis and Roberts (1980) proposed an improved equation for the prediction of seed longivity.

Lauzer et al., (1993) described the proces of in vitro germination and tetrazolium staining in mature seeds of **Cypripedium acaule**. Sosa and Luna (1994) discussed the morphometrics and character state recognition for cladistic analysis in the **Bletia reflexa** complex.

CYTOLOGY

In the year 1964, Levan et al., adopted a detailed, well organized chromosome classification. There are a quite number of researches on orchid cytology (Chardard, 1963; Sharma and Chatterji, 1966; Tara and Kamemoto, 1970; Roy and Sharma, 1972; Mehra and Vij, 1972 a,b; Vij and Mehra, 1974, 1976; Vij and Vohra 1974 a,b; Jorapur, 1976, Dressler, 1981, Vij et al., 1986; Freudenstein, 1994; Freudenstein and Doyle, 1994).

Duncan (1959) attempted to draw an evolutionary scheme in orchids, for the first time. According to many investigators, polyploidy appears to have played an important role in evolution of many plants (Lewis and John, 1963, 1964; Stebbins, 1971; De Wet, 1971; Greilhuber and Ehrendorfer, 1975; Cauwet-Marc and Balayer, 1985; Bianco et al., 1991; D'emerico et al., 1992). Merchant and Brighton (1974); Sieber et al., (1980); Lewis (1967); discussed the possible evoltution of triploid. Brandham (1982) reported the production of diploid gamete by triploid specimen.

Vij and Vohra (1974) investigated the overlapping morphological features in orchid genera. Dressler (1981) described Orchidaceae as one of the most morphologically diverse and species-rich families of flowering plants. New et al., (1975) studied the levels of genetic variation in orchids. Gill (1989) found exceedingly low level of genetic variation in Cypripedium acaule. Atwood (1984) suggested Cypripedioideae as an old lineage.

Templeton et al., (1990) studied the species level variation in **Cypripedium**. Presence of **Cypripedium** species abundantly in more open areas of transient successional stages than in mature forest was observed by Case (1987, 1994). Case (1994) further investigated the extensive variation in this species. Studies of chromosome on **Cypripedium** species was conducted by many investigators (Love and Ritchie, 1966; Love and Simon, 1968; Cox and Chase, 1994). The counting of chromosome and other details have been investigated in different orchid species like **Vanila planifolia** (Heusser, 1938, Ravindran,1979; Capmeri and Rossi, 1987); **Himantoglossum adriaticum** (Vermeulen, 1947, 1949; Del Prete, 1978; Capmeri and Rossi, 1987; Del Prete et al., 1991, 1992); **Orchis and Ophrys** (Lima de Faria, 1980). Wilfret and Kamemoto (1970) conducted genome and karyotype studies in different species. Salamuddin and Ramesh (1993) conducted detailed karyological studies in different plant species. Sheviak (1986) conducted cytotaxonomic studies on **Spiranthes**. Genetic diversity and systematic affinities of **Phragmipedium lindenii** was investigated by Mc Cook (1987).

Somatic association of chromosome and other abnormalities (mitotic) have been investigated by many workers (Kitani, 1963; Maguire, 1967; Chauhan and Abel, 1968; Stack and Brown, 1969; Sadasiviah et al., 1969; Feldman, 1969; Feldman et al., 1972, 1973; Thomas, 1973; Yoshida et al., 1974; Godin and Staik, 1976; Ferrer and Lacadena, 1977; Lavania and Sharma, 1980, 1984; Nair and Ravindran, 1994).

Harlan and De Wet (1975) investigated triploidy in **Ophrys tenthredinifera** and other species. D'emerico et al., (1993) conducted a detailed study on cytoloical and karyological aspects on Orchidaceae.

There are only few investigations, particularly on the cytology of **Paphiopedilum** species. Garay (1972) reported absolute incompatibility between four genera, **Cypripedium**, **Paphiopedilum**, **Phragmipedium** and **Salenipedium**. In the year 1974, Tanaka and Aoyama found that `insigne' complex is more premitive than `venustum'. Biswas (1986) has done some cytological studies on the genus. Mehlquist (1947); Duncan (1947); Duncan and Macheod (1948-50); Kamemoto et al., (1963); Tanaka (1964, 1965); Sasa and Torigata (1967); Tanaka and Aoyama (1974) observed highly variable chromosome number in the genus **Paphiopedilum**. Kamemoto et al., (1963) and Tanaka and Aoyama (1974) found that terminal chromosomes derrived from the median chromosomes by centric fission.

The methods for chromosomal studies was dealt in detail by Battaglia (1957 a,b) and Levan et al., (1964).

CHAPTER – III

BIODIVERSITY

INTRODUCTION

The term Diversity means to be different or unlike. A region with a large number of individual species is said to be biologically diverse. Floristic diversity refers to the group of varied plants. Either it refers to the number of types/taxa in a given region or a group. The concept of biological diversity is defined as the variety or life forms, in a given region, the ecological roles they perform and the genetic diversity they contain (Wilcox, 1984). A species with a large number of interpopulational differences, where each population gets geneticaly adapted to specific environmental conditions is said to be genetically diverse, i.e., genetic diversity of a species. The extreme degree of morphological variability in orchids is attributed to genetic drift (Brieger, 1975). Though there are a variety of definitions for the term biodiveristy, most of the definitions consider three main sub-systems of biodiveristy, viz., genetic diversity, species diversity and ecosystem diversity. Reid and Miller (1989) provided a comprehensive definition: "biodiversity is the variety of the world's organisms, their genetic diversity and the assemblages they form".

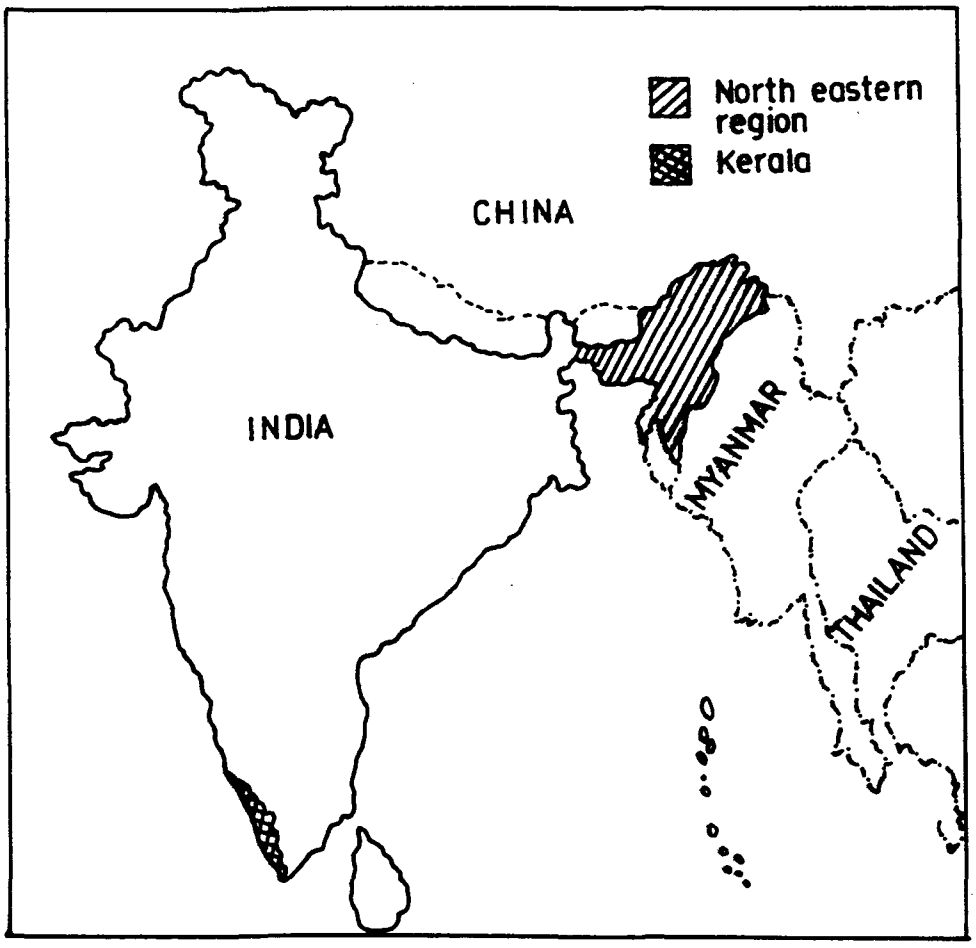
Species are the repository of genetic biodiversity. Species diversity is an important aspect of biodiversity as genetic biodiversity often evolves as a result of the interaction between different individual of same species and sometimes other sub-

system species. Species richness or number of species in an ecosystem is considered as biodiversity. Thus, species are the central object to the concept of biodiversity and the knowledge of species taxonomy is current currency of biodiversity (Heywood, 1996).

Plants differ in many ways. No two plants are exactly alike, eventhough we may limit our observation to a single species . Variations within a species are of two kinds: (a) variations due to environment, and (b) variations due to heredity. Environmental variations may be discerned by growing plants with similar heredity in different environments. Heredity variations are the result of plants possessing different genetic characters and may be simple and easily observed. Variety (cultivar) is a gorup of similar plants which by structural features and performance may be identified from other variation within the same species.

Since the concept of sustainable development became the most important objective, conservation of biodiversity is the urgent need to be tackled from all angles. The first step to start with in this direction,is the Taxonomic accounts for academic as well as for an affective and sustainable utilization of plant resource.

Fig. 1 : Phytogeographical distribution of Genus Paphiopedilum Pfitz. in India.


North eastern region
Kerala
CHINA
INDIA
MYANMAR
THAILAND

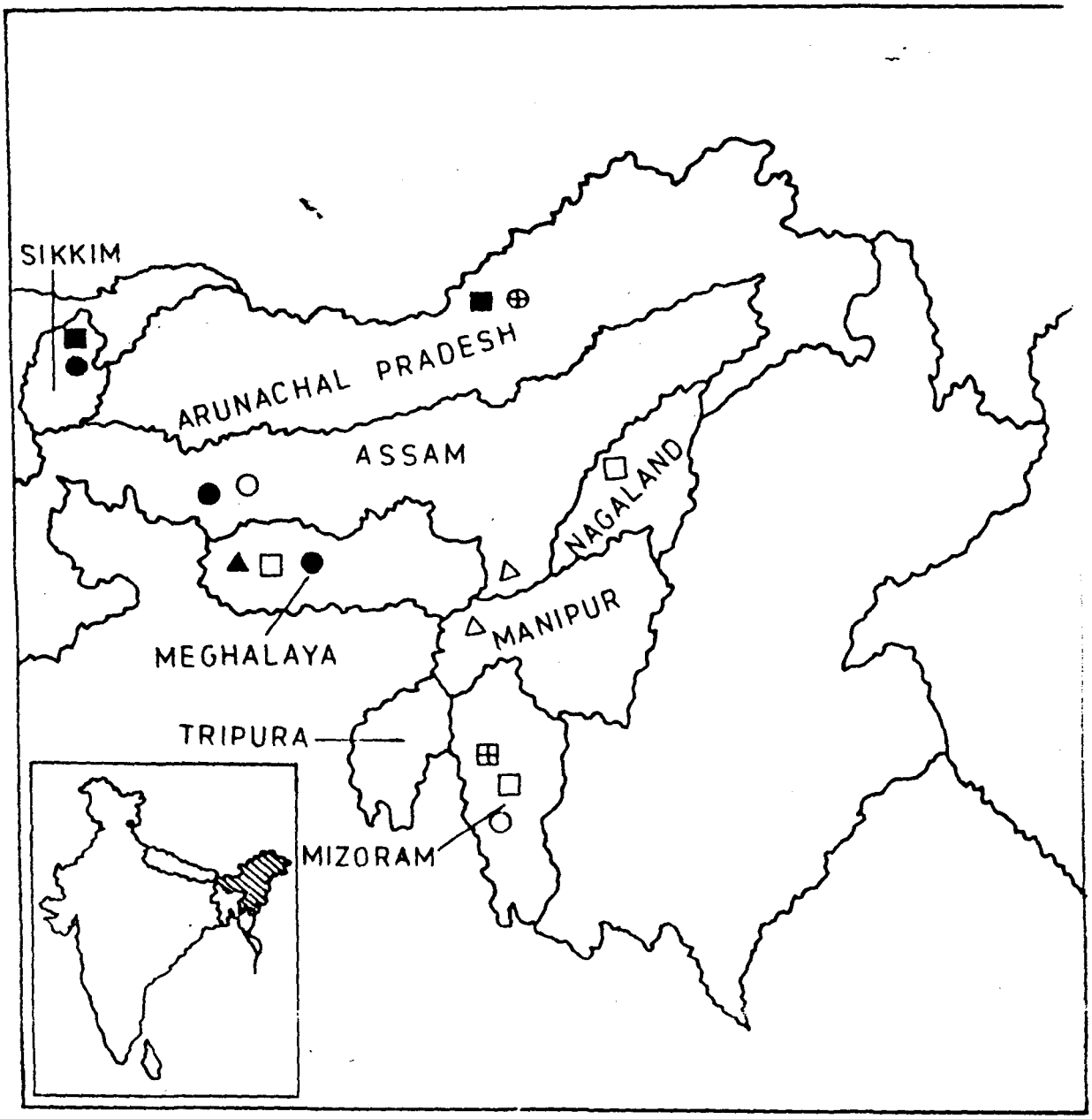


Fig.2: Distribution of Paphiopedilum species in Nort Eastern region of India.

■ P. fairrieanum (Lindl.) Stein, □ P. hirsutissimum (Lin ex Hook.) Stein, ▲ P. insigne (Lindl.) Pfitz, △ P. s cerianum (Reichb.f. ex Masters & T Moore) Pfitz. – ● P. venustum (Wall.) Pfitz., ○ P. villosum (Lindl.) Ste ⊞ P. charlesworthii (Rolfe) Pfitz., ⊕ P. wardii Summerh.

The Paphiopedilums are commercially most important group of orchids. Because of long lasting, elegant floral display, enchanting and exquisite floral variations, the cut flowers and the potted plants fetch a remarkable appreciation in the trade. A thorough knowledge on Biodiversity is necessary for the better prospects of Indian Paphiopedilums.

GENERIC DESCRIPTION:

Paphiopedilums are terrestrial or occassionally epiphytic sympodial herbs, mostly 50 cms tall with thick; horizontal spreading roots borne at the base of the plant. Leaves conduplicate, oblong, elliptic or ligulate, distichous, two to several, coriaceous, green or green mottled with light green or purple markings, acute to obtuse. Inflorescence terete, Indian species usually one flowered, occasionally two ('biflora') (Fig. 4 j), waxy in appearance. Dorsal sepal erect, large, lateral sepals united to form a synsepalum. Petals spreading, lip pouched, side lobes incurved, horizontal column, two fertile anthers, large stigma, fleshy on short stalk.

DIVERSITY OF INDIAN PAPHIOPEDILUMS

The phytogeographical distribution of **Paphiopedilum** species is illustrated by the figures 1 & 2. General account of Indian **Paphiopedilum** has been dealt by Kataki, 1986; Joy, 1992; Joy et al., 1995, however, there is a need of a comprehensive account on the biodiversity of each species. This rich genetic diversity

is facing threat in their natural habitats (Jain and Sastry, 1980; Sastry et al., 1983; Nayar and Sastry, 1987, 1988, 1990). Therefore it is attempted to provide an account on the biodiversity of Paphiopedilums of Northeatern India.

In India, nine species are reported of Paphiopedilums, of which two species P. charlesworthii and P. wardii from Mizoram and Arunachal Pradesh respectively, are supposed to be extinct from India. One of the species P. druryi is confined to the Travancore hills of Kerala State, once believed to be extinct in nature (Nayar and Sastry, 1987), recently has been re-located (Menon et al., 1994). In India the other six species viz., P. fairrieanum, P. hirsutissimum, P. insigne, P. spicerianum, P. venustum and P. villosum are confined to Northeastern region.

Key to the species of North-eastern Paphiopedilums:

1a. Leaves mottled, green and purple beneath:

2a. Lip yellow, prominently veined with green .....P. venustum

2b. Lip yellow, no prominent green veins .....P. wardii

1b. Leaves green throughout:

3a. Bracts equalling or longer than the ovary:

4a. Dorsal sepal white with lower part greenish white with purple spots or blotches; ovate-orbicular .....P. insigne

4b. Dorsal sepal greenish with lower part brown, purple; spathulate .....P. villosum

3b. Bracts shorter than the ovary:

5a. Petals horizontal: margin crisped, lower half violet, black hairs on the petal:

6a. Staminode flushed purple in apical half ..P. hirsutissimum

6b. Staminode white in colour P. charlesworthii

5b. Petals not necessarily horizontal, sometimes deflexed, margin wavy, yellowish-green with spots or veins:

7a. Dorsal sepal white, base greenish white with purple dots ....P. spicerianum

7b. Dorsal sepal greenish-white with dark purple veins ....P. fairrieanum

The diversity among the species of **Paphiopedilum** can be summarised as follows.

Paphiopedilum charlesworthii (Rolfe) Pfitzer in Engler, Bot. Jahrb., 29: 40, 1984 & in Engler, Pflanzenr. Orch. Pleon. 75, 1903; Jain & Mehrotra, Pril. Inv. Orch. Ind., 102, 1984; Cribb, The genus Paph., 142, 1987; Karthikeyan et al., Fl. Ind. Enu. Monocot., 159, 1989.

Type: Burma (East Indies) Hort. Charlesworth, Shuttleworth & Co.

Cypripedium charlesworthii Rolfe in Orchid Rev., 1: 303, 1813.

Distribution: India (Assam); BURMA, CHINA.

Fig. 3 : Floral diversity in **Paphiopedilum** species.

a. P. charlesworthii

b. P. druryi

c. P. fairrieanum

d. P. hirsutissimum

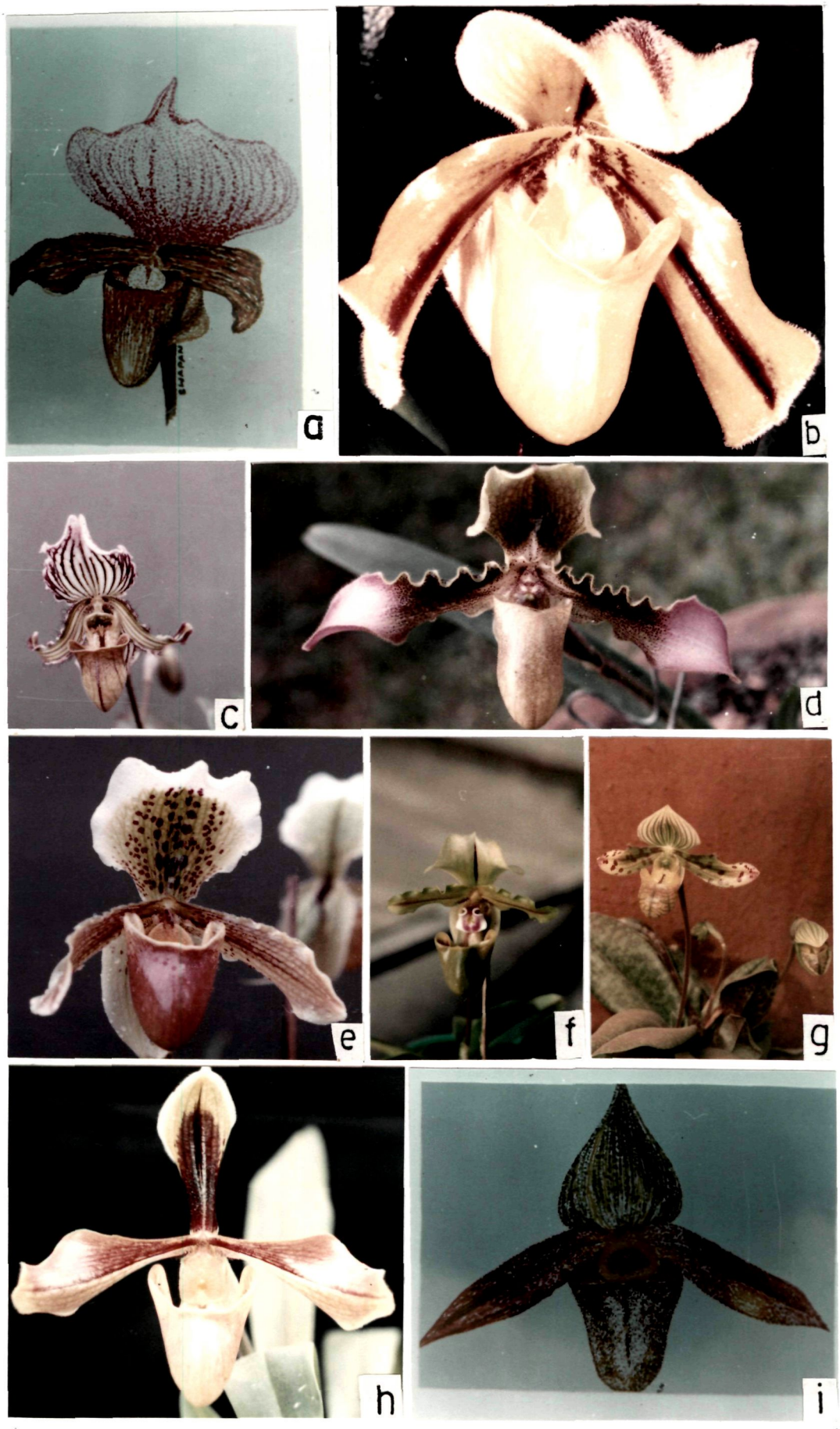
e. P. insigne

f. P. spicerianum

g. P. venustum

h. P. villosum

i. P. wardii


a
b
c
d
e
f
g
h
i

One of the most distinctive species in the genus. This species is invariably distinguished by colour and shape of dorsal sepal and the pure white staminode (Fig.3 a). It is noteworthy that inspite of a number of endeavours to locate this species from the reported habitats, the venture remained to be unsuccessful. Hence, it may be considered that this species is no more existing in wild.

P. druryi (Bedd.) Stein, Orchideenbuch 466. 1892; Cribb in Curtis' Bot. Mag. 182: 65, t. 764, f. 1. 1978; Cribb, The genus Paph., 157, 1987; Karthikeyan et al., Fl. Ind. Enu. Monocot. 159, 1989.

Cypripedium druryi Bedd., Ic. Pl. Ind. Or. 1: 23, t. 112. 1874; Hook. f. Fl. Brit. India 6: 172. 1890.

Distribition : India (Western Ghats, Kerala): Endemic.

Distinct with maroon-spotted dorsal sepal with a broad white margin. Flower is very peculiar with yellow to honey yellow lip and prominently tridentate petals (Fig.3 b).

Inflorescence with two flowers (biflora) also occurs in this species. This species has been successfully introduced to north-east and established in experimental garden of Botany department, N.E.H.U., Shillong.

Paphiopedilum fairrieanum (Lindl.) Stein., Orchideenbuch 467, 1892; Pfitzer in Engler, Pflanzenr. Orch. Pleon, 77, 1903;

Fig. 4 : Floral variations within the species- P. venustum

Identification and Nomenclature of these natural variations still to find a proper solution.


a
b
c
d
e
f
g
h
i
j
k
l

Wellesley & Rolfe in Orchid Rev. 13:11, 1904; G. Pradhan in Orchid Rev. 77: 256, 1969; U.C. Pradhan in Orchid Dig. 42: 151, 1978; Kataki, Lady's slipper Orch. India, 18, 1984; Jain & Mehrotra, Pril. Inv. Orch. in India., 102, 1984; Cribb, The Genus Paph., 157, 1987; Karthikeyan et al., Fl. Ind. Enu. Monocot. 159, 1989.

Type: cult. Fairrie.

Cypripedium fairrieanum Lindl. in Gard. Chron. 1857: 740, 1857.

Distribution : India (Arunachal Pradesh, Sikkim); BHUTAN.

An exceptionally distinct slipper orchid. This species is readily distinguished by its large white and boldly purple stripped dorsal sepal with an undulate margin and blunt apex. Petals are more or less "s" shaped with undulate margins and three toothed at the apex with prominent central one (Fig.3 c).

This species was considered as a relatively uniform species in its floral morphology. But, recently several distinctive variants have been reported. Biodiversity of the species is very well exhibited by the var. **album** (var. **bohlmannianum**) (Fig.5 a) var. **nigrescens** (small flowers, deep purple coloured); var. **giganteum** (large flowers, long lip); var. **flavuum** (albino, with yellow lip) and even forma **longisepalum** (long narrow dorsal sepal without reticulate purple veins).

Paphiopedilum hirsutissimum (Lindl. ex Hook.) Stein., Orchideenbuch, 470, 1892; Pfitzer in Engler, Bot. Jahrb. 19: 41,

1984 & in Engler, Pflanzenr. Orch. Pleon. 69, 1903; G. Pradhan in Paphiopedilum World 2: 84, 1972; U. Pradhan, Indian Orchids 1: 39, 1986; Kataki, Lady's slipper Orch. Ind. 16, 1984; Jain Mehrotra, Pril. Inv. Orch. Ind. 102, 1984; Cribb, The Genus paph., 138, 1987; Karthikeyan et al., Fl. Ind. Enu. Monocot. 159. 1989.

Distribution : India (Meghalaya, Mizoram, Nagaland); BURMA, CHINA, THAILAND.

One of the most distinctive species, recognised by hairy peduncle, long spathulate petals, undulate on the upper margin, relatively small blunt dorsal sepal and converse sub-quadrate staminode (Fig.3 d).

In view of the minute differences in hirsuteness, Cribb (1987) recognised a variety in this species, var. **esquirolei** and again he attributed a varietal rank to a group of plants, with much smaller flower as var. **chiwuanum**.

Paphiopedilum insigne (Lindl.) Pfitzer in Engler & Prantl, Nat. Pflanzenr. 2(6); 84, 1889 & in Englr, Pflanzenr. Orch. Pleon, 73, 1903; Kataki, Lady's slipper Orch. Ind., 12, 1984; Jain & Mehrotra, Pril. Inv. Orch. Ind., 102, 1984; Cribb, The Genus paph., 148, 1987; Karthikeyan et al., Fl. Ind. Enu. Monocot., 159, 1989.

Type: Sylhet cult. Liverpool B.G. ex Wallich, 7022.

Cypripedium insigne Wall. ex Lindl., Collect. Bot. t., 32, 1821 & Gen: Spec. Orch. 530, 1840; Hook. in Curtis' Bot. Mag., 62: t.3412, 1835.

Fig. 5 : Floral variations observed in different species of Paphiopedilum


a
b
c
d
e
f

Distribution : India (Meghalaya); BENGLADESH, NEPAL.

Floral size and colouration are particularly variable in this species. Dorsal sepal is very typical with many small dark brown spots on greenish background (Fig.3 e). The specific diversity is represented by a number of varieties, viz., var. **sanderianum** (bright canary yellow and white flowers, lacking purple markings) (Fig.5 b); var. **sanderae** (flowers with green veins, very few purple dots on the dorsal sepal); var. **bonhoffianum** (spots on dorsal sepal completely absent, but shaded with purplish brown); var. **chantii** (peculiar with glossy chestnut-brown lip); var. **maulei** (large flowers, petals and lip pale in colour).

Recently, a natural variation was observed in this species with two dorsal sepals and two staminodes in the same flower. However, one of the sepals and staminodes were found to be smaller in size compared to the other one (Fig.5 c).

Paphiopedilum spicerianum (Rchb.f. ex Masters & T. Moore) Pfitzer, in Pringsh. Jahrb. Wiss. Bot. 19: 164, 1889; in Engler, Bot. jahrb. 10: 11, 1894 & in Engler, Pflanzenr. Orch. Pleon., 76, 1903; Kataki, Lady's slipper Orch. Ind., 17, 1984; Jain & Mehrotra, Pril. Inv. Orch. Ind., 102, 1984; Cribb, The Genus Paph., 155, 1987; Karthikeyan et al., Fl. Ind. Enu. Monocot., 159, 1989.

Type: Assam, Cult. Veich ex Spicer.

Cypripedium spicerianum Reichb.f. ex Masters & T. Moore in Gard. Chron. n.s. 12: 505, 1879; Reichb.f. in Gard. Chron. n.s. 13: 40, 1880.

Distribution : India (Assam, Manipur, Mizoram); BURMA.

One of the most beautiful and distinct species characterised by elegant arching, large, white dorsal sepal, marked with a central maroon vein, short falcate petals. Upper margin of the petal is undulate. Dorsal sepal arches over the lip (Fig.3 f).

In some cases, the flowers are lacking any green colouration and is designated as var. **mercatellianum**.

Paphiopedilum venustum (Wall.) Pfitzer ex Stein., Orchideenbuch, 489, 1892; Pfitzer in Engler, Bot. Jahrb., 19: 41, 1894 & in Engler, Pflanzenr. Orch. Pleon. 81, 1903; G. Pradhan in Orchid Dig., 38: 195, 1974; U.C. Pradhan in Orchid Dig. 40: 92, 1976 & loc. cit., 185; Kataki, Lady's slipper Orch. Ind., 12, 1984; Jain & Mehrotra, Pril. Inv. Orch. Ind., 102, 1984; Cribb, The Genus Paph., 211, 1987; Karthikeyan et al., Fl. Ind. Enu. Monocot., 160, 1989.

Type: Sylhet, Wallich, 7023.

Distribution : India (Assam,Meghalaya,Sikkim); BENGLADESH, BHUTAN,NEPAL.

The first species in the genus to be described and introduced into cultivation. This species is easily recognised

by attractively mottled leaves, prominent veins on lip, recurved ciliated petals with large maroon raised spots on the lower half and margins.

Large number of variations are observed within this species in the intensity of marron-black spotting on petals and striations and shape of the dorsal sepal (Fig.3 g). Cribb (1987) referred the albino plants as var. **measuresianum** (Fig.4 d) lacking any maroon or purple colour on the leaves and flowers. Varieties like **rubra** (Fig.4 h), **teestaensis** (Fig.4 h) and **bhutanensis** (Fig.4 h) have also been reported, with distinct spots on the lateral petals.

Paphiopedilum villosum (Lindl.) Stein., Orchideenbuch, 490, 1892; Pfitzer in Engler, Bot. Jahrb. 19: 41, 1894 & in Engler, Pflanzenr. Orch. Pleon., 72, 1903; Van Delden in Orchid Dig., 34: 45, 1970; Stiles in Austr. Orchid Rev., 39: 124, 1974; Kataki, Lady's slipper Orch. Ind., 14, 1984; Cribb, The Genus Paph., 150, 1987; Jain & Mehrotra, Pril. Inv. Orch. Ind., 103, 1984; Karthikeyan et al., Fl. Ind. Enu. Monocot., 160, 1989.

Type: Burma, Lobb.

Cypripedium villosum (Lindl.) Rolfe in Orchid Rev., 20: 2, 1912.

Distribution : India (Assam, Mizoram); BURMA, THAILAND.

This species is very much distinguished by its shorter scape, larger bract and distinctively coloured flowers. Petals

are more spathulate (Fig.3 h).

There are varieties within this species. viz., var. **boxalli** (Fig.5 d) differs from typical species in the colouration of flowers, with a heavily spotted and acuminate dorsal sepal. On the other hand, var. **atratum**, is more boldly marked dorsal sepal and purple petals; var. **annamense**, with a dark violet-purple central streak distinguished from other varieties.

Recently, during present work, a specific characteristic was observed within a population of this species collected from Cherrapunjee (Meghalaya). The lip of the flowers were found bigger in size and there were ridges on them (Fig.5 e). This characteristic may be an additional natural contrivance for the attraction of pollinators and also attribute to the floricultural value of the species.

Paphiopedilum wardii Summerh. in Gard. Chron. Sur., 3, 92: 446, fig. 218, 1932 & Curtis Bot. Mag., 160: t. 9481, 1937; Asher in Orchid Dig., 45: 15, 1981; Karasawa & Saito in Bull., Hiroshima Bot. Gard., 5: 60, 1982; Karasawa, The genus Paph., 228, 1982; Jain & Mehrotra, Pril. Inv. Orch. Ind., 102, 1984; Cribb, The genus Paph., 209, 1987; Karthikeyan et al., Fl. Ind. Enu. Monocot., 160, 1989.
Type: N. Burma, Kingdon Ward, Cult. R.B.G. Kew.

Cypripedium wardii (Summerh.) Curtis in Orchid Rev., 41: 2, Fig.

P.g. 43, 1933 & 30, 1935, non Rolfe.

Distribution : India (Arunachal Pradesh); BURMA, CHINA.

This species can be very easily differentiated with the green coloured dorsal sepal with white veins, petals with dark-maroon spots all over and the brown spotted lip. Synsepalum is lanceolate and staminode finely pubescent, lunate (fig.3 i). There is an assertion that P. wardii is a natural hybrid between P. venustum and P. sukhakulii (Bream, 1988). However, recent examination of many flowers of P. Double Deception, the man-made hybrid between the two species, revealed no similarities between actual hybrid flowers and those of P. wardii (Koopowitz, 1995). Therefore, we suggest in support of Koopovitz (1995) that P. wardii may be considered as a legitimate species in its own right. However, this species is another victim of human being: deforestation, various developmental activities- road construction etc., and over exploitation for trade and believed to be extinct from India.

CONCLUSION

The species diversity is the most important aspect and the central object to the concept of biodiveristy. The term is often used as a synonym of species diversity or richness. The Paphiopedilum species has contributed distinctly different features in the breeding of hybrid slipper orchids.

The range of phytogeogrpahical distribution of Paphiopedilum

species could be corelated with the long distance dispersal of the light seeds. Present study indicates that there is a discontinuous phytogeographical distribution of **Paphiopedilum** species in India which supports the 'Satpura Hypothesis' put forward by Dr. Hora (1949). Out of the existing seven species of India, except **P. druryi**, all the six species are distributed in the Northeastern region, whereas, **P. druryi** is confined to the Tranvancore hills of Kerala. Various ecological and adaphic factors might also be the possible reasons for the distribution pattern of these species.

It has been observed that plants were reported from different localities other than the actual place of occurrence to confuse the competitors in horticultural trade. For example, **P. spicerianum** was over collected and the entire habitat was ransacked making its distribution in Bhutan a myth (Fowlie, 1970).

Present study indicates that species diversity can be defined with satisfaction, the same may not be true in case of ecosystem diversity. Still, the ecosystem diversity/regional diversity can be identified on the basis of the increase or decrease in the number of species.

There are enormous number of variations and clones within the **Paphiopedilum** species. It has been observed that there are a quite many number of natural variations in **P. insigne**, **P. venustum** and **P. villosum**, of which **P. venustum** showed the maximum

number of variations. However, it has not been possible to elucidate the complete picture of this incessant series except in P. venustum . It has been observed that there are a great many variations within the populations of Meghalaya. Hence, it may be suggested that Meghalaya could be considered as the centre of origin and dispersion for P. venustum.

For those species that are now threatened or extinct due to either over-collection or habitat conversion, possibilities of resolving some of the ambiguities in the taxon seems difficult. However, P. wardii, we may consider as a legitimate species which is no longer existing in wild.

The unending sequence of biological diversity and gene pool should be effectively utilized for the conservation of these species. The increasing importance of in vitro multiplication and perpetuation of species requires pure/natural species (biodiversity) for basic breeding stock.

CHAPTER – IV

PHENOLOGY

INTRODUCTION

Phenology is concerned with the study of different phenophases like new leaf growth, flowering, fruiting etc. of a given taxon in different seasons. The term "phenology" was first proposed by Charles Mooren in 1853 (c.f. Hope, 1974). Leith (1974) described phenology as "the art of observing life cycle phases or activities of plants and animals in their temporal occurrence throughout the year".

The concepts of phenological studies and its significance in understanding the functional aspects of various species have been dealt by Leith (1970) and Leith and Radford (1971). The periodicity of different phenophases reflects the seasonal distribution of specific kind of resources like pollen, nectar, fruit and seeds (Totland, 1993). The phenological events of plants are inevitable from the point of view of germplasm conservation as well as for a better understanding of the ecological adaptations and interactions of individual species (Stern and Roche, 1974, Waser, 1979; Thompson, 1980).

Phenological observations are pre-requisite for reproductive biology and proper utilization of specific resources like pollen and seeds for breeding and scientific multiplication programme. Thus, phenological calander of different orchid taxon is important for orchid industry and cut flower trade.

The phenology of about 100 horticulturally important species from Arunachal Pradesh was studied by Hegde (1980). Boojh and Ramakrishnan (1982), and Shukla and Ramakrishnan (1982, 1984) carried out the phenological studies on tree species of Northeastern India. Studies on various phenological aspects of orchid species have been conducted by a number of workers (Borg Karlson, 1990; Dafni and Bernhardt, 1990; Paulus and Gack, 1990 b; Robertson and Wyatt, 1990; David, 1992; Javier et al., 1992). However, as far as the genus **Paphiopedilum** of North eastern India is concerned, there is no comprehensive studies on phenology of this group except earlier preliminary attempts of the author.

In view of the paucity of our knowledge, a detailed investigation on the phenology of the **Paphiopedilum** species of Northeastern region of India is attempted in the present study with an emphasis on the vegetative and reproductive phenophases.

MATERIALS AND METHODS

Phenological observations were carried out, both in the experimental garden of Botany department, NEHU, Shillong as well as in a local nursery where plants are maintained almost in natural conditions. The observation were however recorded from the plants in the local nursery. The phenological observations viz., leaf length and leaf breadth were recorded of the fully grown leaves in all the six species of **Paphiopedilum**. Longivity of flower was recorded in days from the day of flower opening to the day of wilting of the flower. Flower size parameters viz., size across; length and breadth of dorsal sepal, ventral sepal,

labellum, lateral petals, staminode and their respective shapes were recorded from the fully opened flowers. Observation of shape and number of pollinia; ovary shape, length and its ornamentation; fruit initiation to maturation, and dehiscence were also observed.

Leaf:

The leaf length was measured in cm by taking mature leaf from ten different plants starting right from the base to the tip of the leaf. Breadth of the leaf was measured by taking the maximum expanded portion of the same leaf.

Flower:

Observation of flower was recorded from fully opened flower. Length of the peduncle was measured by taking the length from the base of the peduncle to the base of the pedicel. The flower size (across) was measured by taking the maximum spread out of the lateral petals. Length of sepal and petal was measured from the base to the tip respectively and their breadth were measured at maximum width. Length of the labellum(lip) was measured by taking the length from the base to the tip of the lip. The breadth at maximum of the lip was taken as the measurement of the width of the pouch. Length of the ovary was measured from the point of attachment of the pedicel to the attachment of floral leaves.

The important phenophases taken into account were the flower longivity, fruit initiation to maturation and dehiscence.

Climatic data viz., rainfall (mm), maximum and minimum temperature (C) and the relative humidity (morning and evening in %) for study site were obtained from the Seismological Department, Shillong, for 1993-94. The monthly average was calculated by taking arithmetic mean of the daily observations.

CLIMATE OF SHILLONG

Phenological observations in the present study is confined to the Shillong Plateau. The climate data are presented for the years 1993 and 1994 in the table(1-3) and figure 6. Rainfall and humidity are generally high throughout the Northeast region. Temperature in this region usually doesnot exceed beyond 35 C. The climate of the study site i.e., central part of Meghalaya (Khasi hills) and other parts of the region is conducive with heavy rainfall and high humidity for the growth and propagation of the species.

Fairly high temperature was recorded for the most part of the year i.e., from March to October with July and August being the hottest months (Table 1). In the phenological study site, Shillong (latitude 25 34'N, 91 56'E and Altitude about 1500 msl) temperature in winter season dropped to 5.85 C (January) and very rarely rises above 24 C during the hottest part of the year. During winter (December-February) 'a chilling experience' is a usual phenomena in Shillong plateau.

The rainy season commence from April and extends till

Table 1 : Monthly Mean Temperature (°C) Recorded for Shillong.

MONTHS	1993		1994		AVERAGE	
	Max.	Min.	Max.	Min.	Max.	Min.
JANUARY	13.3	4.8	16.3	6.9	14.80	5.85
FEBRUARY	17.2	8.5	15.5	6.2	16.35	7.35
MARCH	20.0	10.1	20.2	11.3	20.10	10.70
APRIL	22.4	13.2	23.5	14.2	22.95	13.70
MAY	22.5	14.9	23.8	16.0	23.15	15.45
JUNE	23.5	17.0	23.9	17.7	23.70	17.35
JULY	23.9	17.9	24.7	18.1	24.30	18.00
AUGUST	24.0	18.1	24.6	18.3	24.30	18.20
SEPTEMBER	23.2	16.7	24.2	16.6	23.70	16.65
OCTOBER	21.8	13.8	21.4	13.9	21.60	13.85
NOVEMBER	18.4	10.6	18.1	11.0	18.25	10.80
DECEMBER	16.7	7.6	15.7	6.9	16.20	7.25

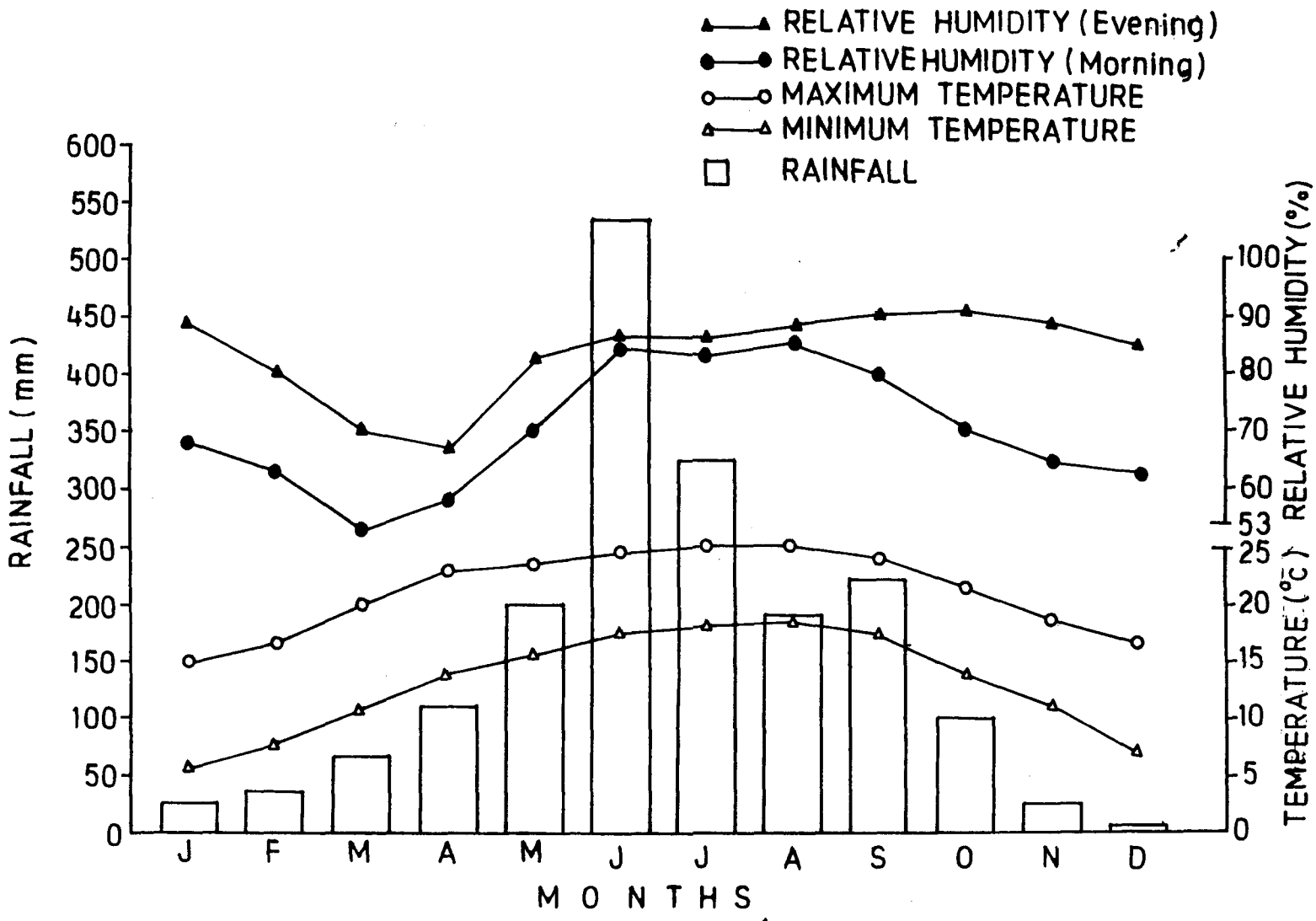
Table 2 : Monthly Rainfall (mm) Recorded for Shillong.

MONTHS	1993	1994	AVERAGE
JANUARY	35.9	12.8	24.35
FEBRUARY	19.7	44.9	32.30
MARCH	46.4	86.6	66.50
APRIL	177.5	44.9	111.20
MAY	192.7	204.5	198.60
JUNE	594.4	475.5	534.95
JULY	514.4	137.9	326.15
AUGUST	160.3	222.0	191.15
SEPTEMBER	282.7	156.5	219.60
OCTOBER	44.0	152.2	98.10
NOVEMBER	25.1	21.2	23.15
DECEMBER	0.6	5.7	3.15

Table 3 : Monthly Relative Humidity (%) Recorded in Shillong, at morning - 8.30 AM and evening - 17.30 PM.

MONTHS	1993		1994		AVERAGE	
	Morn.	Even.	Morn.	Even.	Morn.	Even.
JANUARY	83	94	53	85	68.00	89.50
FEBRUARY	64	80	62	80	63.00	80.00
MARCH	51	68	56	71	53.50	69.50
APRIL	59	68	58	66	58.50	67.00
MAY	76	86	75	80	75.50	83.00
JUNE	86	86	85	86	85.50	86.00
JULY	84	88	83	84	83.50	86.00
AUGUST	87	89	84	88	85.50	88.50
SEPTEMBER	82	91	78	90	80.00	90.50
OCTOBER	70	92	69	91	69.50	91.50
NOVEMBER	59	90	69	89	64.00	89.50
DECEMBER	72	85	55	86	63.50	85.50

Fig. 6 : Graphical representation of climatic data.


RELATIVE HUMIDITY (Evening)
RELATIVE HUMIDITY (Morning)
MAXIMUM TEMPERATURE
MINIMUM TEMPERATURE
RAINFALL
RAINFALL (mm)
600
550
500
450
400
350
300
250
200
150
100
50
0
100
90
80
70
60
53
25
20
15
10
5
0
TEMPERATURE (°C) RELATIVE HUMIDITY (%)
J F M A M J J A S O N D
MONTHS

October. Highest average rainfall was recorded in the month of June (539.95 mm) and the second highest in the month of July (326.15 mm) (Table 2). Winter was conspicously dry with an average total rainfall of 149.45 mm during November-March. The minimum average rainfall was recorded in the month of December (3.15 mm).

Average relative humidity in the morning (8.30 AM) was recorded highest in the months of June and August (85.5 %), whereas in the evening (17.30 PM). the highest average relative humidity was recorded in the month of October(91.5 %) (Table 3). Relative humidity was always maximum in the evening time.

The most fascinating climatic feature of this region is the rainfall during April-October. with an average annual rainfall of about 2093 mm. This is one of the distinguishing climatic factor which is very distinct in Shillong plateau.

RESULT/OBSERVATIONS

A detailed observation on the vegetative as well as reproductive phenophases of the **Paphiopedilum** species revealed that there are clear cut differences on the characteristics of various species. The characteristics observed are distinct and specific to each species. The phenological characteristics are evident as given in the table (4).

Table 4: Phenological observations of Paphiopedilum species.

Parameters	P. fairrieanum	P. hirsutissimum	P. insigne	P. spicerianum	P.venustum	P. villosum
VEGETATIVE						
STEM	Abbreviated	Abbreviated	Abbreviated	Abbreviated	Abbreviated	Abbreviated
LEAF						
Shape	Linear-ligulate	Linear-ligulate	Ligulate	Oblong-elliptic ligulate	Oblong-elliptic	Linear-ligulate
Length	14.78 cm	35.43 cm	36.44 cm	15.11 cm	20.03 cm	32.43 cm
Breadth	2.27 cm	3.07 cm	2.55 cm	3.45 cm	5.22 cm	3.76 cm
Length/Breadth	6.53 cm	11.70 cm	14.36 cm	4.39 cm	3.84 cm	8.53 cm
Apex	*Unequally tri-denticulate*	*Obliquely-bilobed*	*Bilobulate*	*Bi-denticulate*	*Tri-cuspidate*	*Unequally-bilobulate*
Texture	Smooth	Smooth	Smooth	Smooth	Rough	Smooth
Hairs	Absent	Absent	Absent	Absent	Absent	Absent
Margin	Serrulate towards apex	Smooth	Smooth	Undulate base	Smooth,ciliated base	Smooth, ciliated base
Colour						
A)Upper surface	Dark green	Green	Green	Gloosy dark green	Tessellated dark green	Dark green
B)Lower surface	Pale green	Slight purple base	Purple spot base	Purple spotted base	Purple spotted	Purple spotted base
REPRODUCTIVE						
INFLORESCENCE						
Position	Terminal	Terminal	Terminal	Terminal	Terminal	Terminal
No. of flowers	One(?)	One	One	One(?)	One (?)	One
Stalk	Errect	Errect	Errect	Errect-suberrect	Errect	Sub-errect
Nature	Purple hairy	Densely haired	Pubescent	Glabrous	Hairy	Purple haired
Length	17.72 cm	19.22 cm	23.68 cm	12.68 cm	10.51 cm	22.09 cm
Bract	Present	Present	Present	Present	Present	Present
Nature of bract	Pubescent-elliptic	Pubescent-subacute	Glabrous-oblong	Pubescent-elliptic	Pubescent- elli-ptic-lanceolate	Glabrous-elliptic
Length	1.63 cm	2.10 cm	4.57 cm	2.33 cm	2.10 cm	4.36 cm
Breadth	0.82 cm	0.89 cm	1.04 cm	0.77 cm	0.75 cm	3.27 cm
Length/Breadth	1.99 cm	2.36 cm	4.56 cm	3.01 cm	2.67 cm	1.33 cm
FLOWER						
Size(across)	3.49 cm	11.98 cm	8.14 cm	6.40 cm	8.51 cm	9.73 cm
Perianth lobes	Unequal	Unequal	Unequal	Unequal	Unequal	Unequal
Dorsal sepal						
Shape	Elliptic	Obovate	Ovate-elliptic	Obovate	Ovate	Obovate
Length	3.64 cm	4.06 cm	4.78 cm	3.48 cm	3.42 cm	5.56 cm
Breadth	3.38 cm	3.22 cm	3.40 cm	4.32 cm	2.51 cm	3.18 cm
Length/Breadth	1.07 cm	1.27 cm	1.41 cm	0.80 cm	1.36 cm	1.73 cm
Ventral sepal (Synsepalum)						
Shape	Ovate	Ovate	Elliptic	Ovate	Ovate	Ovate
Length	2.50 cm	3.34 cm	4.44 cm	3.12 cm	2.60 cm	4.41 cm
Breadth	1.76 cm	1.62 cm	2.20 cm	1.89 cm	1.61 cm	2.15 cm
Length/Breadth	1.43 cm	2.15 cm	2.02 cm	1.66 cm	1.62 cm	2.06 cm

Table 4 (contd....)

Parameters	P. fairrieanum	P. hirsutissimum	P. insigne	P. spicerianum	P.venustum	P. villosum
Labellum(Lip)						
Shape	Slipper shaped	Slipper shaped	Slipper shaped	Slipper shaped	Slipper shaped	Slipper shaped
Length	3.56 cm	3.87 cm	4.65 cm	3.89 cm	3.96 cm	5.03 cm
Width	1.91 cm	2.05 cm	2.88 cm	2.76 cm	3.01 cm	3.45 cm
Length/Width	1.87 cm	1.89 cm	1.61 cm	1.40 cm	1.31 cm	1.46 cm
Lateral petal						
Shape	"S" shaped/ Deflexed	Spathulate-incurved	Linear	Linear-tapering	Oblanceolate	Spathulate-incurved
Length	4.36 cm	6.07 cm	5.84 cm	3.52 cm	5.21 cm	5.34 cm
Breadth	1.21 cm	1.50 cm	1.34 cm	1.03 cm	1.25 cm	2.71 cm
Length/Breadth	3.61 cm	4.12 cm	4.50 cm	3.51 cm	4.28 cm	1.97 cm
Staminode						
Shape	Elliptic	Obovate-cordate	Obovate	Obovate-obcordate	Reniform	Obcordate
Length	0.94 cm	0.99 cm	0.99 cm	0.99 cm	0.81 cm	1.63 cm
Breadth	0.73 cm	0.80 cm	0.74 cm	0.58 cm	1.00 cm	1.25 cm
Length/Breadth	1.29 cm	1.24 cm	1.33 cm	1.72 cm	0.81 cm	1.31 cm
Longivity of flr	105 days	97 days	114 days	88 days	125 days	133days
POLLINIA						
Shape	Bilobed lump	Bilobed lump	Bilobed lump	Bilobed lump	Bilobed lump	Bilobed lump
Number/flower	Two	Two	Two	Two	Two	Two
OVARY						
Shape	Ellipsoidal	Ellipsoidal	Ellipsoidal	Ellipsoidal	Ellipsoidal	Triangular
Length	3.70 cm	5.80 cm	5.44 cm	4.57 cm	4.28 cm	4.43 cm
Ornamentation	Hairy	Hairy	Pubescent	Glabrous	Pubescent	Hairy
Placentation	Parietal	Parietal	Parietal	Parietal	Parietal	Parietal
FRUIT						
Initiation-maturation	180 days	205 days	270 days	190 days	310 days	325 days
Maturation-dehiscence	190 days	170 days	178 days	205 days	115 days	105 days

Fig. 7 : Diagramatic representation of leaf apices of *Paphiopedilum* species.

a. *P. fairrieanum*

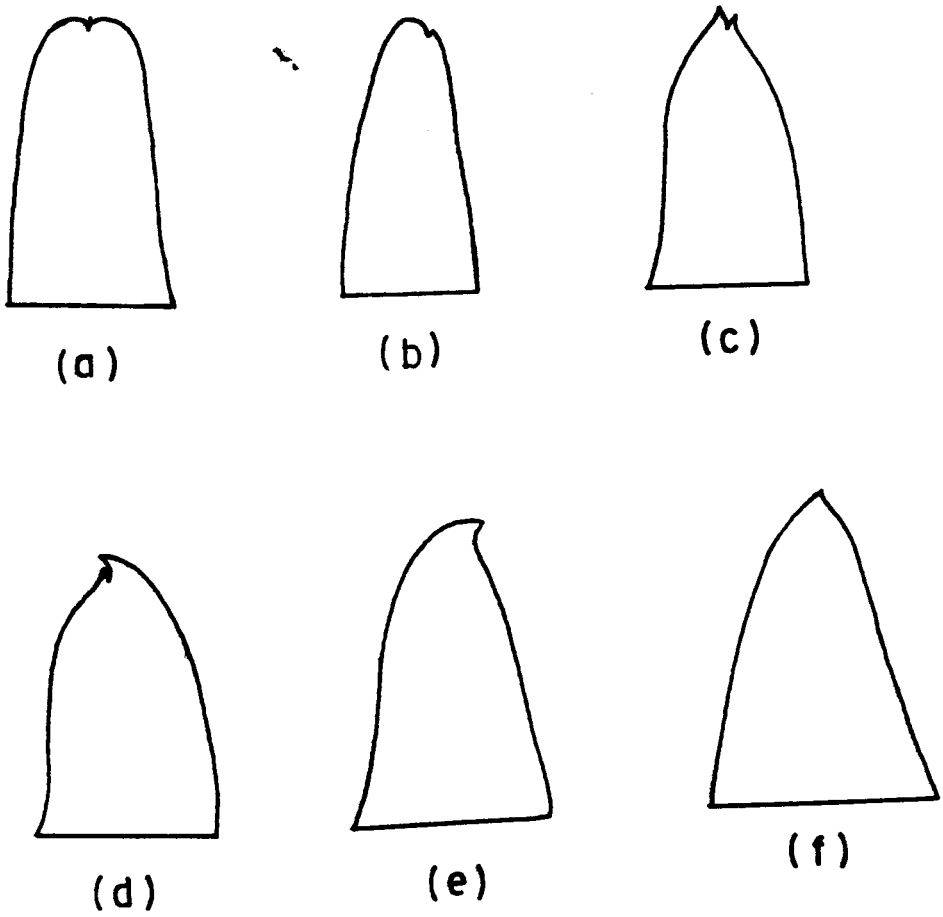
b. *P. hirsutissimum*

c. *P. insigne*

d. *P. spicerianum*

e. *P. venustum*

f. *P. villosum*


(a)
(b)
(c)
(d)
(e)
(f)

The leaf apex of each species are observed to be species specific and can be used as an criterion for the identification of species even in vegetative phase (Fig.7). The length of the leaf varied among the species. Maximum leaf length was observed in the species P. insigne (36.44 cm) followed by P. hirsutissimum (35.43 cm), P. villosum (32.43 cm), P. venustum (20.03 cm), P. spicerianum (15.11 cm) and minimum in P. fairrieanum (14.78 cm). Whereas the leaf breadth was recorded maximum in P. venustum (5.22 cm) followed by P. villosum (3.76 cm), P. spicerianum (3.45 cm), P. hirsutissimum (3.07 cm), P. insigne (2.55 cm) and the minimum in P. fairrieanum (2.27 cm). Accordingly the leaf length/breadth ratio also varied. This ratio was highest in P. insigne (14.36) followed by P. hirsutissimum (11.70), P. villosum (8.54), P. fairrieanum (6.53), P. spicerianum (4.39) and the lowest in P. venustum (3.84).

The length of the pedicel was maximum in P. insigne (23.68 cm) followed by P. villosum (22.09 cm), P. hirsutissimum (19.22 cm) P. fairrieanum (17.72 cm) P. spicerianum (12.68 cm) and the minimum in P. venustum (10.51 cm).

The flower size was also found to be varied with species. The size was measured across the flower. The maximum size was observed in P. hirsutissimum (11.98 cm), followed by P. villosum (9.73 cm), P. venustum (8.51 cm), P. insigne (8.14 cm), P. spicerianum (6.40 cm) and the minimum was observed in P. fairrieanum (3.49 cm).

The length of dorsal sepal was recorded maximum in P. villosum (5.56 cm) followed by P. insigne (4.78 cm). P. hirsutissimum (4.06 cm), P. fairrieanum (3.64 cm), P. spicerianum (3.48 cm) and the minimum in P. venustum(3.42 cm). Breadth of dorsal sepal was maximum in P. spicerianum (4.32 cm) followed by P. insigne (3.40 cm), P. fairrieanum (3.38 cm), P. hirsutissimum (3.22 cm), P. villosum (3.18 cm) and the minimum in P. venustum (2.51 cm). The length/breadth ratio was maximum in P. villosum (1.73), followed by P. insigne (1.41), P. venustum (1.36), P. hirsutisssimum(1.27), P. fairrieanum (1.07) and the minimum in P. spicerianum (0.80).

The ventral sepal (synsepalum) was also varied in size. The length of ventral sepal was recorded maximum in P. insigne(4.44 cm) followed by P. villosum (4.41 cm), P. hirsutissimum(3.34 cm), P. spicerianum (3.12 cm), P. venustum (2.60 cm) and minimum in P. fairrieanum(2.50 cm). Breadth of ventral sepal was maximum in P. insigne (2.20 cm), P. villosum (2.15 cm), P. spicerianum (1.89 cm), P. fairrieanum (1.70 cm), P. hirsutissimum (1.62 cm) and the minimum in P. venustum (1.61 cm). Length/breadth ratio found maximum in P. hirsutissimum (2.15), subsequently in P. villosum (2.06), P. insigne(2.02), P. spicerianum (1.66), P. venustum (1.62) and minimum in P. fairrieanum (1.43).

The length of the labellum (lip) was recorded maximum in P. villosum (5.03 cm) followed by P. insigne (4.65 cm), P. venustum

(3.96 cm), P. spicerianum (3.89 cm), P. hirsutissimum (3.87 cm) and the minimum in P. fairrieanum(3.56 cm). Width of the labellum was maximum in P. villosum (3.45 cm) followed by P. venustum (3.01 cm), P. insigne (2.88 cm), P. spicerianum (2.76 cm), P. hirsutissimum (2.05 cm) and the minimum in P. fairrieanum (1.91 cm). Length/breadth ratio was found maximum in P. hirsutissimum (1.89) followed by P. fairrieanum (1.87), P. insigne (1.61), P. villosum (1.46), P. spicerianum (1.40) and the minimum in P. venustum (1.31).

Length of the lateral petal was maximum in P. hirsutissimum (6.07 cm) followed by P. insigne (5.84 cm), P. villosum (5.34 cm), P. venustum (5.21 cm), P. fairrieanum (4.36 cm) and minimum in P. spicerianum (3.52 cm). Breadth of the lateral petal was maximum in P. villosum (2.71 cm) followed by P. hirsutissimum (1.50 cm), P. insigne (1.34 cm), P. venstum (1.25 cm), P. fairrieanum (1.21 cm) and the minimum in P. spicerianum (1.03 cm). The length/breadth ratio was found maximum in P. insigne (4.50), followed by P. vensutum (4.28), P. hirsutissimum (4.12), P. fairrieanum (3.61), P. spicerianum (3.51) and the minimum in P. villosum (1.97).

The length of staminode was maximum in P. villosum (1.63 cm) and the values were found equal in P. hirsutissimum , P. insigne and P. spicerianum (0.99 cm). This was followed by P. fairrieanum (0.94 cm) and the minimum was recorded in P. venustum (0.81 cm). Breadth of the staminode was found maximum in P. villosum (1.25 cm) followed by P. venustum (1.00 cm), P.

Table 5 : Important Phenological events in *Paphiopedilum* species.

Name of species	No. of plants studied	Flowering Period	Fruiting Period	Inter-phenophase duration(in days)		
					FRUIT	
				Flower Initiation-Senescence	Initiation-Maturation	Maturation-Dehiscence
P. fairrieanum	10	Oct - Feb	Mar - Nov	105	190	195
P. hirsutissimum	10	Mar - May	Jun - Apr	88	205	178
P. insigne	10	Oct - Feb	Feb - Oct	114	270	170
P. spicerianum	10	Oct - Jan	Feb - Nov	97	180	205
P. venustum	10	Nov - Mar	Mar - Dec	125	310	115
P. villosum	10	Nov - Mar	Mar - Nov	133	325	105

hirsutissimum (0.80cm), P. insigne (0.74 cm), P. fairrieanum (0.73 cm) and the minimum in P. spicerianum (0.58 cm). Length/breadth ratio was maximum in P. spicerianum (1.72) followed by P. insigne (1.33), P. villosum (1.31), P. fairrieanum (1.29), P. hirsutissimum (1.24) and the minimum in P. venstum (0.81).

The length of the ovary was recorded maximum in P. hirsutissimum (5.80 cm) followed by P. insigne (.5.44 cm), P. spicerianum (4.57 cm), P. villosum (4.43 cm), P. venustum (4.28 cm) and minimum in P. fairrieanum (3.70 cm).

Table (5) depicts the important phenophases and interphenophase duration of Paphiopedilum species.

Flowering periodicity revealed that all the species of Paphiopedilum of this region has a monocyclic pattern of flowering. There are mainly two categories among the species, according to flowering peiod. The first category comprises all the species except P. hirsutissimum (P. fairrieanum, P. insigne, P. spicerianum, P. venustum and P. villosum) which has the flowering period from October to March. Whereas P. hirsutissimum blooms during May to June, falls in the second category.

The duration of flower initiation to senescence was maximum in P. villosum (133 days), followed by P. venustum (125 days), P. insigne (114 days), P. fairrieanum (105 days), P. spicerianum (97

days) and the minimum in P. hirsutissimum (88 days).

The interphenophase duration for fruit initiation-maturation was recorded maximum in P. villosum (325 days), followed by P. venustum (310 days), P. insigne (270 days), P. hirsutissimum (205 days), P. fairrieanum (190 days) and the minimum in P. spicerianum (180 days).

The last phenophase i.e., fruit maturation - dehiscence was found maximum in P. spicerianum (205 days), followed by P. fairrieanum (195 days), P. hirsutissimum (178 days), P. insigne (170 days), P. venustum (115 days) and the minimum in P. villosum (105 days).

DISCUSSION

The measurements made on various parts of the plants (both vegetative and reproductive) showed characteristic variation with the species. This may be due to genetical features in combination with the environmental aspects.

The present study revealed that most of the characteristics are species specific. The leaf apex was found to be a very distinguishing character of species, which would be helpful in identifying the species even in vegetative stage. The vegetative growth of the Paphiopedilum species are very slow and the present findings on the vegetative phenophase also suggest this fact. The leaf longivity is perennial in the sense that it

lasts for several years.

In the present study out of six species of this region, five has got the flowering season from October to March i.e., from autumn to the end of winter. This season is useful in avoiding the physical damage to flower. Maximum blooming in the dry period prior to rain was corelated with high pollination activity in American tropical forest (Jenzen, 1977). The floral morphology and modification in orchids can be corelated to the pollination contrivances and competition for pollinators (Waser, 1983; Benzing and Atwood, 1984; Borg-Karlson, 1990; Paulus and Gack, 1990; Javier et al., 1992; Kim et al., 1994). Flowering towards the dry periods may also result in maximum fruit production during wet season.

Floral display was found to be varied with the species. Small floral display are likely to limit geitonogamy (transfer of pollen among flowers of same plant) and promoting outcrossing in self compatible plants (Wyatt, 1982; Schoen and Dubuc, 1990) and favouring pollen dispersal over longer distances (Melampy, 1987; Schmitt et al., 1987). Effects of display size further stressed by Javier et al., 1992.

The longivity of flower was found minimum in **P. hirsutissimum** (88 days). This observations is an indication that temperature, atmospheric humidity, intensity of light etc., have the direct impact on the flowering and longivity. Since, the temperature is higher and the relative humidity is lesser in

the months of March-June. P. hirsutissimum showed the minimum duration of flower longivity. Thus, we can draw a conclusion that the less relative humidity, high temperature and high intensity of light have a negative impact on the floral longivity of Paphiopedilum speices.

Regular seasonal patterns observed in all the species may be due to the conducive climate of the area. Fruit maturatin generally observed during wet period is regarded as favourable for the development. The dehiscence of capsules during windy and dry season, helps in easy and efficient dispersal of seeds by wind. The light weighted and minute seeds of orchids are specially adapted for wind dispersal.

The knowledge of orchid flowering initiation and the significance of environmental cues for the development of flowers are of utmost importance for proper utilization, and management of the resources in the orchid industry. Floral initiation in orchids is determined by the genotype and its development is in interaction with environmental conditions (Bose and Yadav, 1986). Temperature, humidity, light intensity and photoperiod etc., are some of the important environmental factors. Most of the orchids thrive well and produce flowers when the atmospheric moisture is not less than 30% at night and more than 80% during day time (Bose and Bhattacharjee, 1980).

Various ecological as well as genetical factors play major

role in the phenology of the **Paphiopedilum** species of this region. The data depicted in the present study on the various phenophases and other phenological characteristics of the species would be helpful in implementing scientific multiplication/developmental programmes as well as for the conservation of the species.

CHAPTER – V

LEAF TOPOLOGY

INTRODUCTION

The diandrous orchid sub-family, Cypripedioideae consists of four genera, Salenipedium, Cypripedium, Phragmipedium and Paphiopedilum. The former two genera are distinguished by their broad plicate leaves, distributed usually on a distinct abbreviated stem, whereas the later two genera are distinguished by their ligulate, conduplicate leaves produced in a basal distichous rosette (Atwood and Williams, 1979). Characters other than gross morphology may also be useful in determining the taxonomic position of a taxon. Leaf surface morphology, such as the presence/absence and type of trichomes, cuticular sculpturing, distribution and length/width ratios of stomata etc., may contribute in determining the phylogeny of a particular group of plants.

Epidermal and cuticular characters of leaf play an important role in palaeobotany, palaeoecology, pharmacognosy and taxonomy (Stace, 1965, 1966; Dilcher, 1974; Wilkinson, 1979; Hardin, 1979; Dehgan, 1980; Soladoye, 1982; Wurdack, 1986; Singh and Dube, 1991). Soon after the commercial arrival of Scanning Electron Microscope in 1967 it was readily taken into use by the morphologist and all types of plant organ began to be subjected to its scrutiny. Thus, SEM revolutionised the field of micromorphology/ultrastructure in the study of leaf surfaces, seeds, spores, fruits and pollen etc. Micromorphological

characters on leaf surface have been used by workers like Rasmussen, 1987; Yukawa et al., 1991, 1992.

Stomata are the essential pores through which much of the gaseous exchange involved in photosynthesis, respiration and transpiration take place (Willis and Jefferies, 1963; Waggoner and Zelitch, 1965; Zelitch, 1967; Willis and Balasubramaniam, 1968; Balasubramaniam and Willis, 1969). The stomatal control of diffusion through leaf surface depends on the number of stomata in a specific area and the dimensions of stomata. The stomatal complex in a morphological context, consists of the stoma and its neighbouring and subsidiary cells (Rasmussen, 1981). The guard cell and the pore between them constitute the stoma (Metcalfe and Chalk, 1950; Van Cotthem, 1970; Wilkinson, 1979; Rasmussen, 1981).

Stomata in plants are generally considered homologous structures. Therefore, a comparative study of stomata is meaningful. The structure of the mature stomatal complex provides a character of certain taxonomic importance.Therefore, the leaf surface study of **Paphiopedilum** species was conducted to find out if these could have any bearing on the taxonomy and phylogeny of the genus.

The present chapter deals with the leaf surface morphology of all the six species of **Paphiopedilum**, reported from the northeastern region of India. An attempt has been made to study the stomatal characteristics of all the six species with the help

of light microscope as well as scanning electron microscope.

MATERIALS AND METHODS

The voucher specimens of all the species examined are housed in the experimental garden, Botany department, NEHU, Shillong.

Scanning Electron Microscopy:

Scanning electron microscope was used to investigate stomatal morphology. Mature leaves were cut into small pieces of approx. 5 mm x 5 mm from an area between the midvein and the leaf margin. The leaf pieces measuring $5mm^2$ were fixed in 6% gluteraldehyde for 75 minutes, and post fixed with 2% osmium tetroxide for 30 minutes (Ziegler et al., 1983; Nonami et al., 1990). They were then dehydrated through an acetone series (50-100%), followed by critical point drying. The specimens were coated with gold palladium alloy and were mounted on bronze stubs with the help of adhesive, and observed under a SEM (JSM CF - 35) at an accelerated voltage of 15 KV.

Light Microscopy:

Epidermal peels were used for the light microscopic studies. The epidermis was peeled off with fine forceps after loosening the tissue with a razor blade.

Peeling Technique:

The leaf to be peeled was detached, placed on a glass plate, and cut with a razor blade or scalpel into lamina strips of manageable width (5 mm). This was found to be best done between the midrib and margin of the leaf. Lamina strips so obtained were floated on water to avoid desiccation. Then, a 'tab' was formed in the lamina strip by cutting through the upper epidermis near one end without damaging the lower epidermis. The lamina strip was then turned over, and the tab formed was gently pulled back with forceps for a few mm to seperate the tissues. The remaining epidermis was then peeled off by pulling the tab vertically away from the rest of the lamina, holding the latter in position with forceps or fingernails. This was carried out with an even, slow speed, and by taking care to be consistent in the angle of peeling. The strip was then mounted on a thin layer of water (cuticle up) on the slide, and cut into small pieces measuring 5 mm in length with a sharp blade. Adaxial epidermis could also be obtained in the same manner. Techniques for obtaining epidermal strips were followed as discussed by Metcalfe (1960); Meidner and Mansfield (1968); Weyer and Travis (1981). These peels were stained in acetocarmine, rinsed and mounted in glycerine for the temporary mounts and observed under a light microscope.

The following measurements (stomatal density, stomatal ledge length, stomatal ledge width , stomatal opening length and

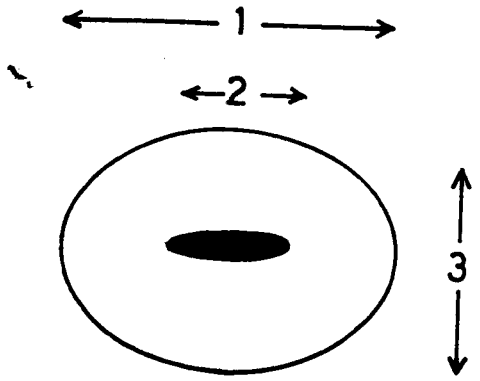


Fig. Illustration of stomatal dimensions measured

1. Length of Ledge
2. Opening length
3. Width of Ledge

Fig. 9 : Scanning Electron Micrographs of Stomata of Paphiopedilum fairrieanum.

a. Cuticular sculpturing x 4000

b. Stomatal density x 220

c. Stomata further magnified x 400

d. Stomata showing antechamber and aperture x 2000

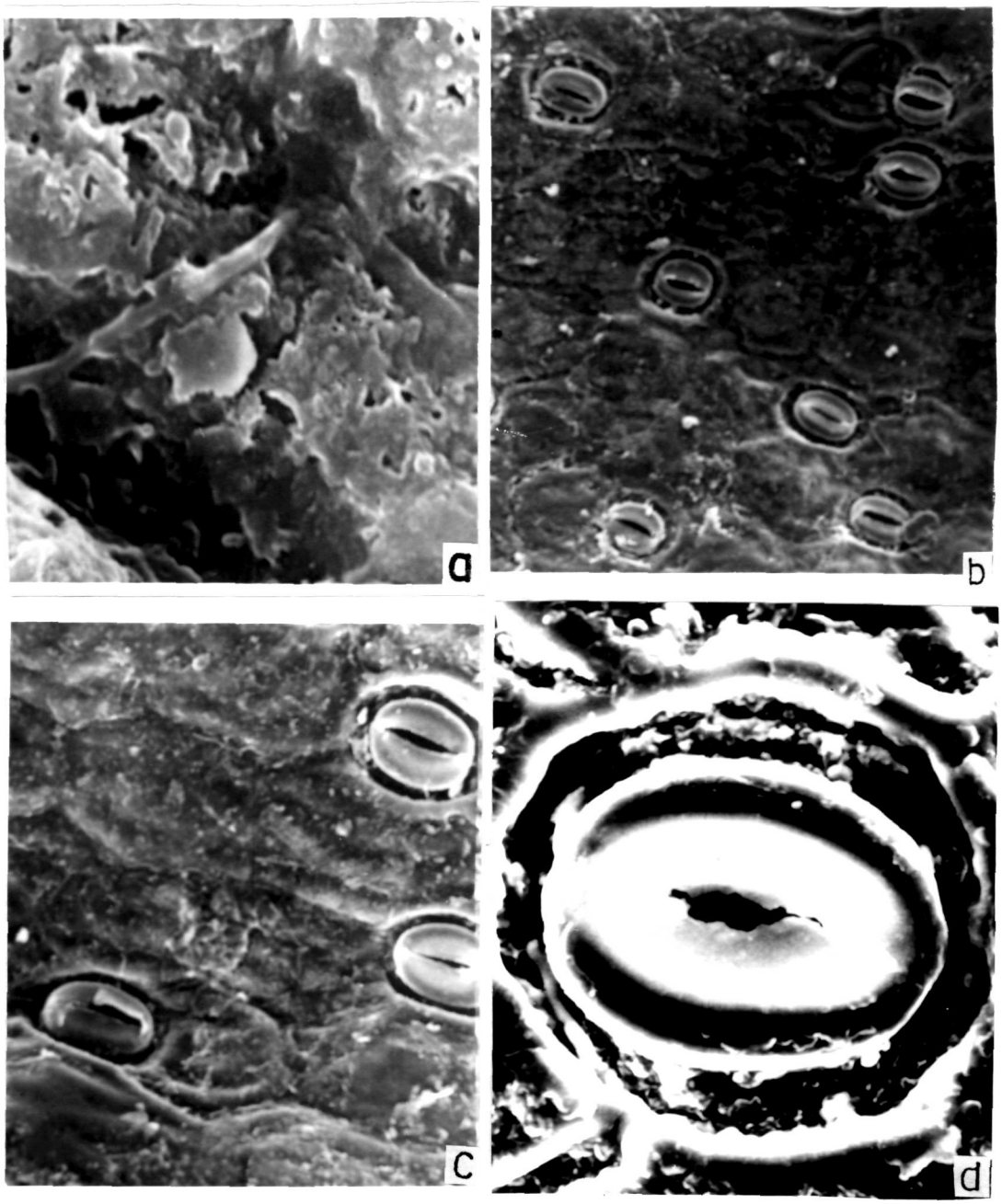

a
b
c
d

Fig. 10 : Scanning Electron Micrographs of Stomata of Paphiopedilum hirsutissimum.

a. Cuticular sculpturing x 4000

b. Stomatal density x 220

c. Stomata further magnified x 400

d. Stomata showing antechamber and aperture x 2000

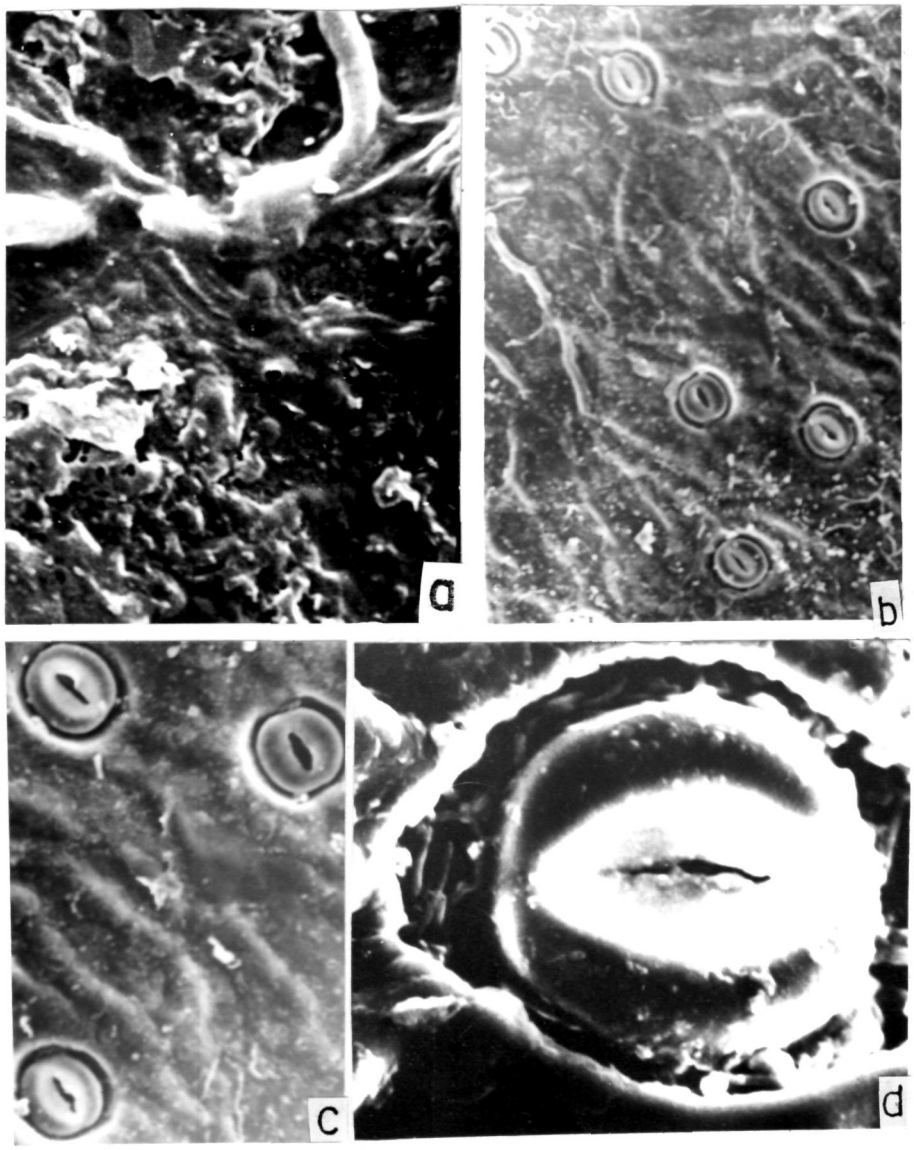

a
b
c
d

Fig. 11 : Scanning Electron Micrographs of Stomata of Paphiopedilum insigne.

a. Cuticular sculpturing x 4000

b. Stomatal density x 220

c. Stomata further magnified x 400

d. Stomata showing antechamber and aperture x 2000

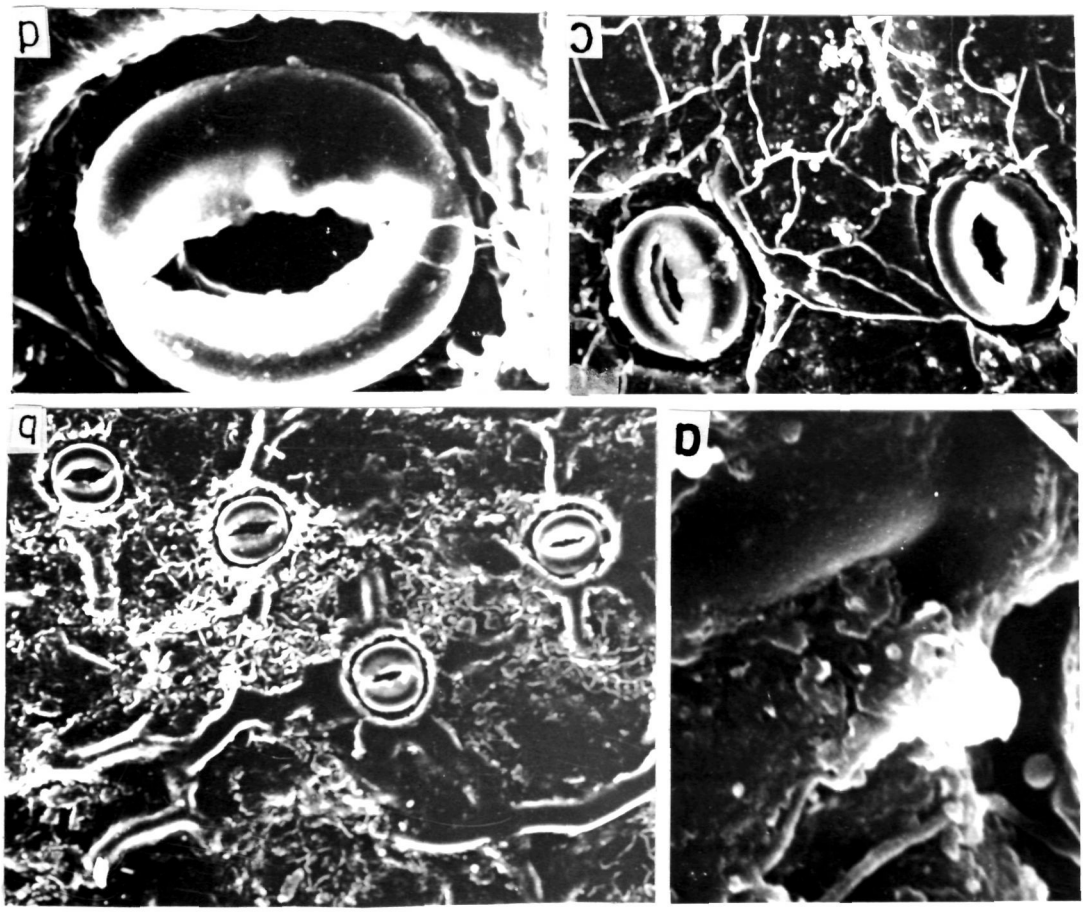

p
c
q
D

Fig. 12 : Scanning Electron Micrographs of Stomata of Paphiopedilum spicerianum.

a. Cuticular sculpturing x 4000

b. Stomatal density x 220

c. Stomata further magnified x 400

d. Stomata showing antechamber and aperture x 2000

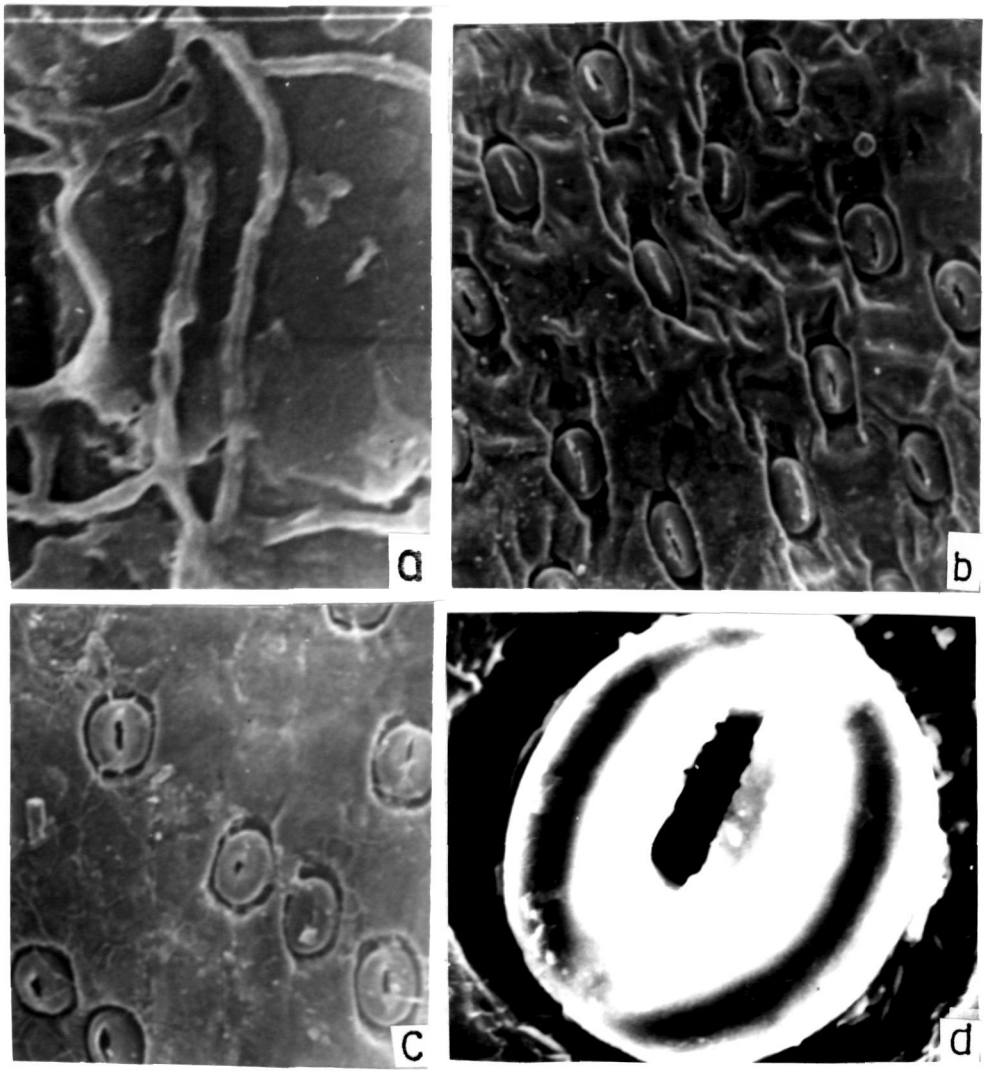

a
b
c
d

Fig. 13 : Scanning Electron Micrographs of Stomata of Paphiopedilum venustum.

a. Cuticular sculpturing x 4000

b. Stomatal density x 220

c. Stomata further magnified x 400

d. Stomata showing antechamber and aperture x 2000

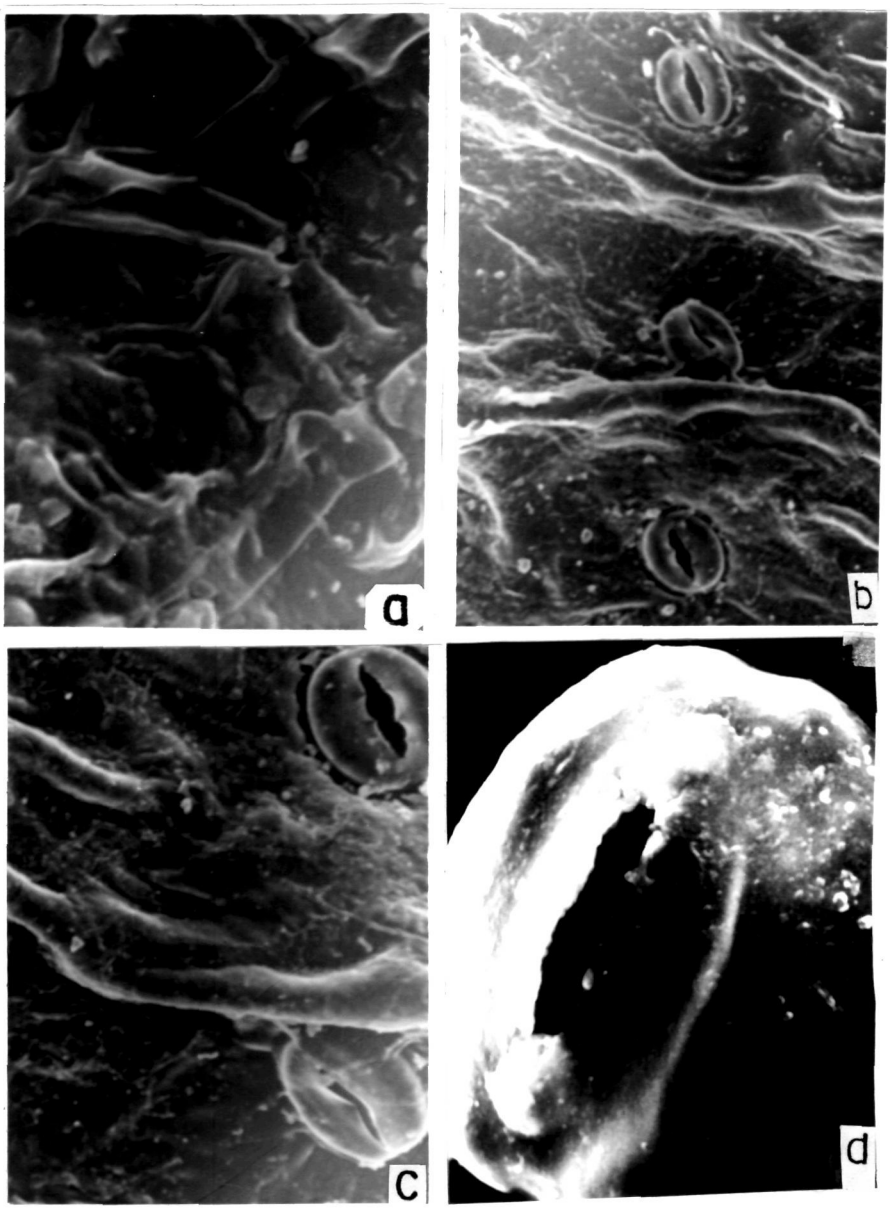

a
b
c
d

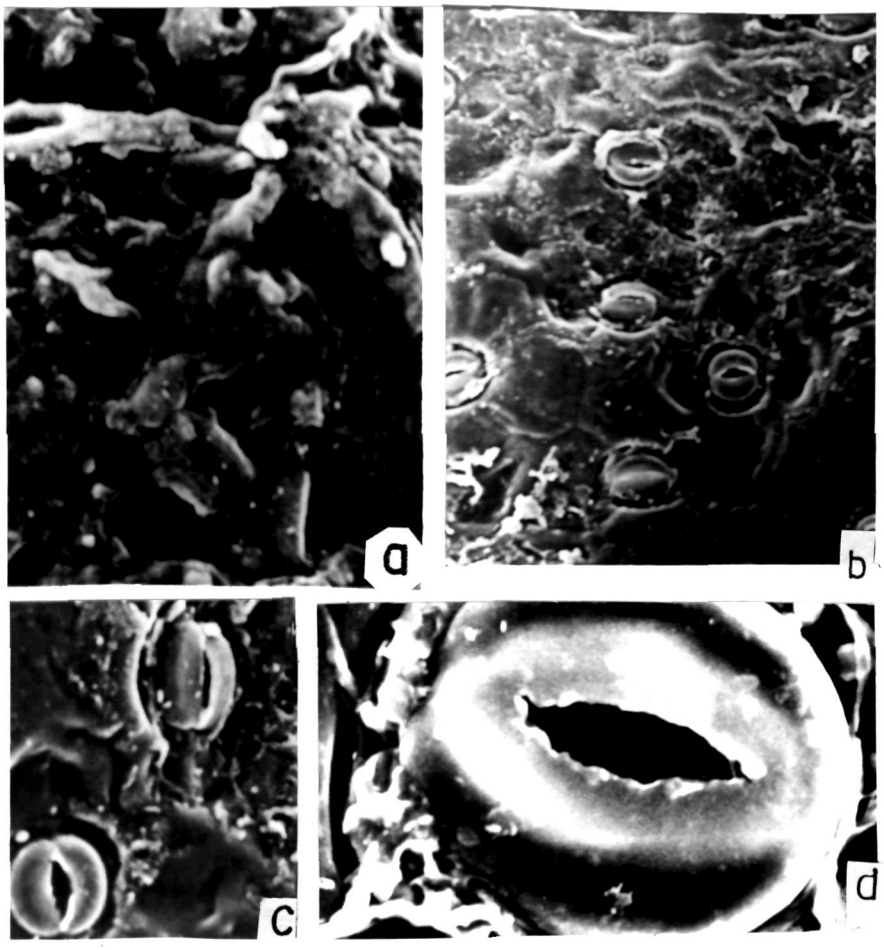
Fig. 14 : Scanning Electron Micrographs of Stomata of Paphiopedilum villosum.

a. Cuticular sculpturing x 4000

b. Stomatal density x 220

c. Stomata further magnified x 400

d. Stomata showing antechamber and aperture x 2000


a
b
c
d

number of epidermal cells) of stomata and epidermis on abaxial leaf surface were made with light microscopic micrographs.

1. Stomatal density: This was determined by counting the number of stomata in epidermal peelings of approx. 1cm x 1cm area.
2. Stomatal ledge length: (Fig.8)
3. Stomatal ledge width: (Fig.8)
4. Stomatal opening length: (Fig.8)
5. Number of epidermal cells/cm^2

The stomatal index was calculated by using the formula, $SI = S/(E+S)*100$ (Robert, 1980), where SI-stomatal index, S-number of stomates in cm^2 area, and E-number of epidermal cells/cm^2 area (Robert, 1980).

RESULTS

Stomata were completely absent on the upper surface of the leaves. Epidermal cells are tetrahedral, axially elongated with anticlinal wall. Epidermis in all the species had variously sculptured cuticle and was devoid of trichomes (Fig.9-14).

"Anomocytic" stomata i.e., stoma with only the ordinary epidermal cells, but no subsidiary cells were observed in all the species of **Paphiopedilum**(Fig.9-14). Only one type of stomata has been observed in all the six species studied, i.e., stoma with a pore, two guard cells and their outer ledge. Stoma is elliptic, pore is slitlike with a gradually slopping sides

Table 6 Measurements of stomatal characters of *Paphiopedilum* species.

Species	Epidermal cells		Stomata						
	Number of cells per cm^2	Density Stomata/cm^2	Ledge				Opening		Stomatal Index
			Width	Length	Width/length	Width x Length	Length	Opening length/ Ledge length	
P. fairrieanum	899.00	*166.88	0.0378	0.0412	0.8382	1.69	0.0188	0.4162	16.96
		±9.02	0.0023	0.0130	0.0591	1.37	0.0022	0.0451	4.29
		~2.85	0.0007	0.0041	0.0187	4.33	0.0007	0.0143	1.36
P. hirsutissimum	981.00	124.32	0.0382	0.0446	0.8586	1.65	0.0188	0.4233	12.96
		4.02	0.0018	0.0026	0.0572	1.51	0.0022	0.0586	3.89
		1.27	0.0006	0.0008	0.0181	4.77	0.0007	0.0185	1.23
P. insigne	414.00	116.16	0.0458	0.0603	0.7614	2.70	0.0333	0.5543	22.08
		4.53	0.0026	0.0032	0.0683	1.94	0.0026	0.0494	2.31
		1.43	0.0008	0.0010	0.0216	6.15	0.0008	0.0156	0.73
P. spicerianum	524.00	92.68	0.0394	0.0456	0.8731	1.74	0.0198	0.4389	15.44
		4.80	0.0018	0.0021	0.0562	1.17	0.0026	0.0582	3.00
		1.52	0.0006	0.0007	0.0178	3.71	0.0008	0.0184	0.95
P. venustum	1028.00	140.30	0.0464	0.0679	0.6843	3.08	0.0289	0.4255	13.69
		1.62	0.0025	0.0029	0.0520	1.99	0.0026	0.0319	4.77
		0.51	0.0008	0.0009	0.0164	6.23	0.0008	0.0101	1.51
P. villosum	546.00	115.12	0.0326	0.0389	0.7716	1.34	0.0291	0.6909	18.20
		10.11	0.0033	0.0115	0.0764	1.96	0.0024	0.0777	4.03
		3.19	0.0010	0.0036	0.0241	6.18	0.0008	0.0246	1.27

Fig. 15 : Graphical representation of Stomatal density of Paphiopedilum species.

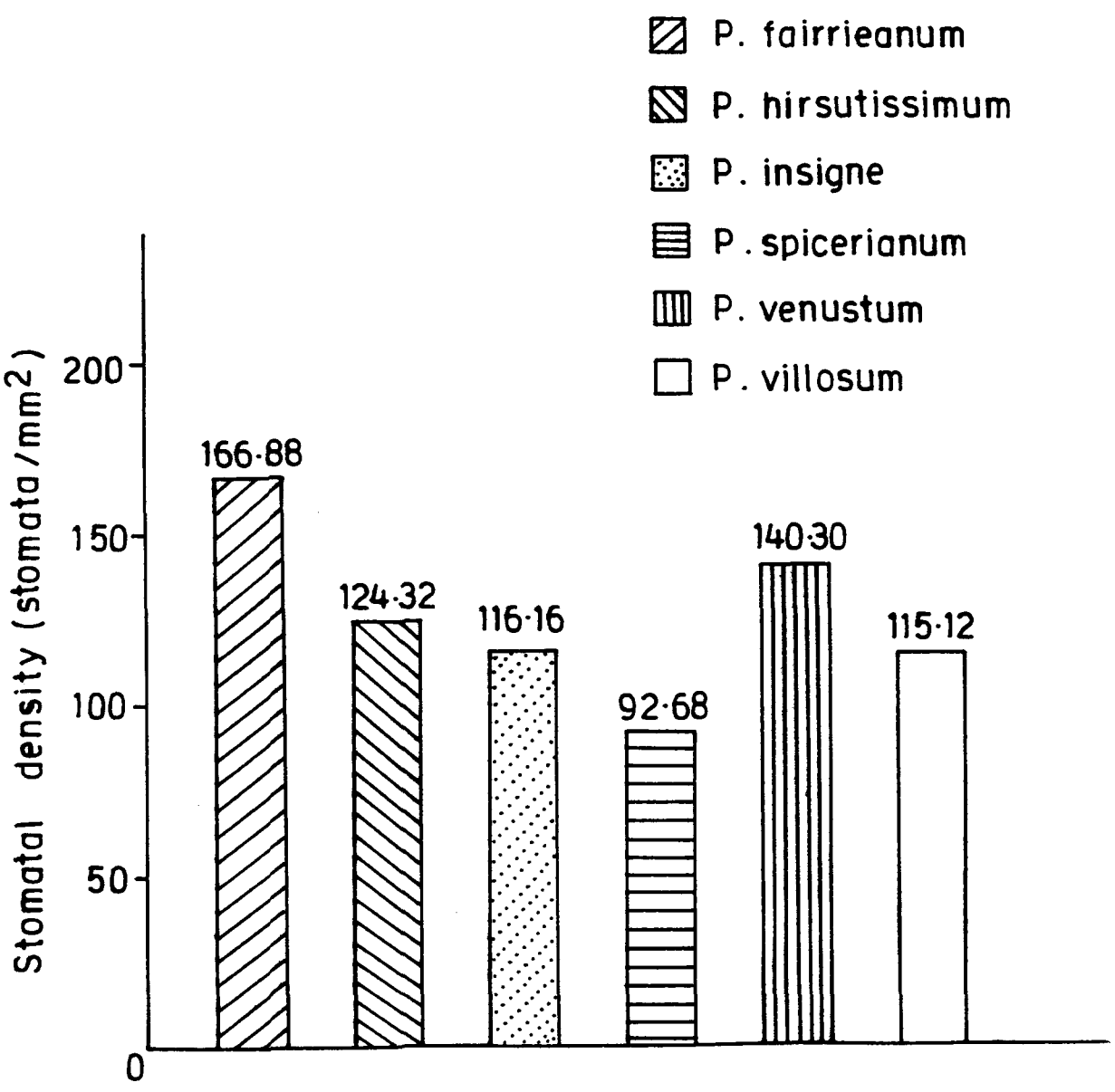

P. fairrieanum
P. hirsutissimum
P. insigne
P. spicerianum
P. venustum
P. villosum
Stomatal density (stomata/mm²)
200
150
100
50
0
166·88
124·32
116·16
92·68
140·30
115·12

Fig. 16 : Graphical representation of Stomatal ledge length of Paphiopedilum species.

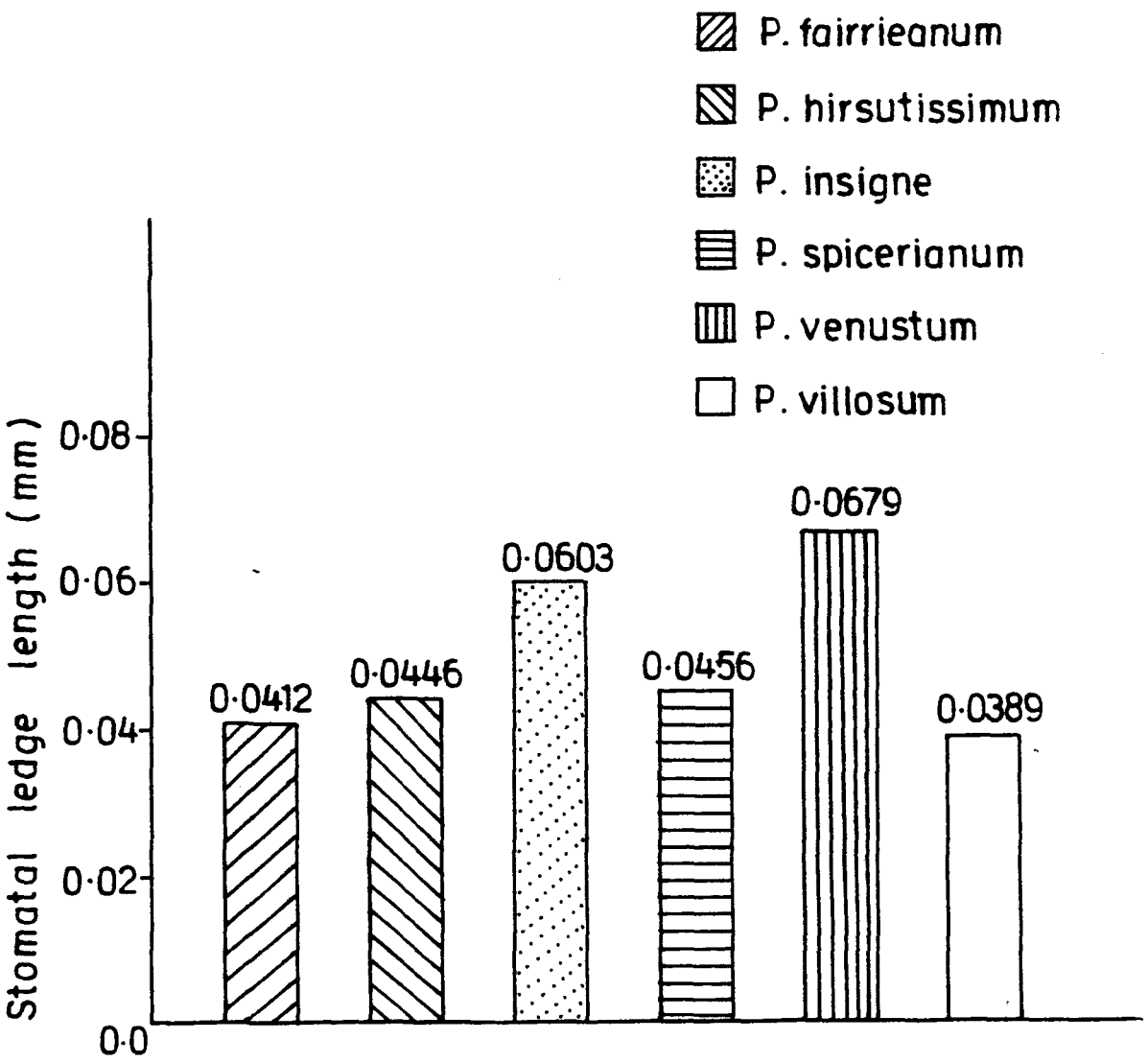

P. fairrieanum
P. hirsutissimum
P. insigne
P. spicerianum
P. venustum
P. villosum
Stomatal ledge length (mm)
0·08
0·06
0·04
0·02
0·0
0·0412
0·0446
0·0603
0·0456
0·0679
0·0389

Fig. 17 : Graphical representation of Stomatal ledge width of Paphiopedilum species.

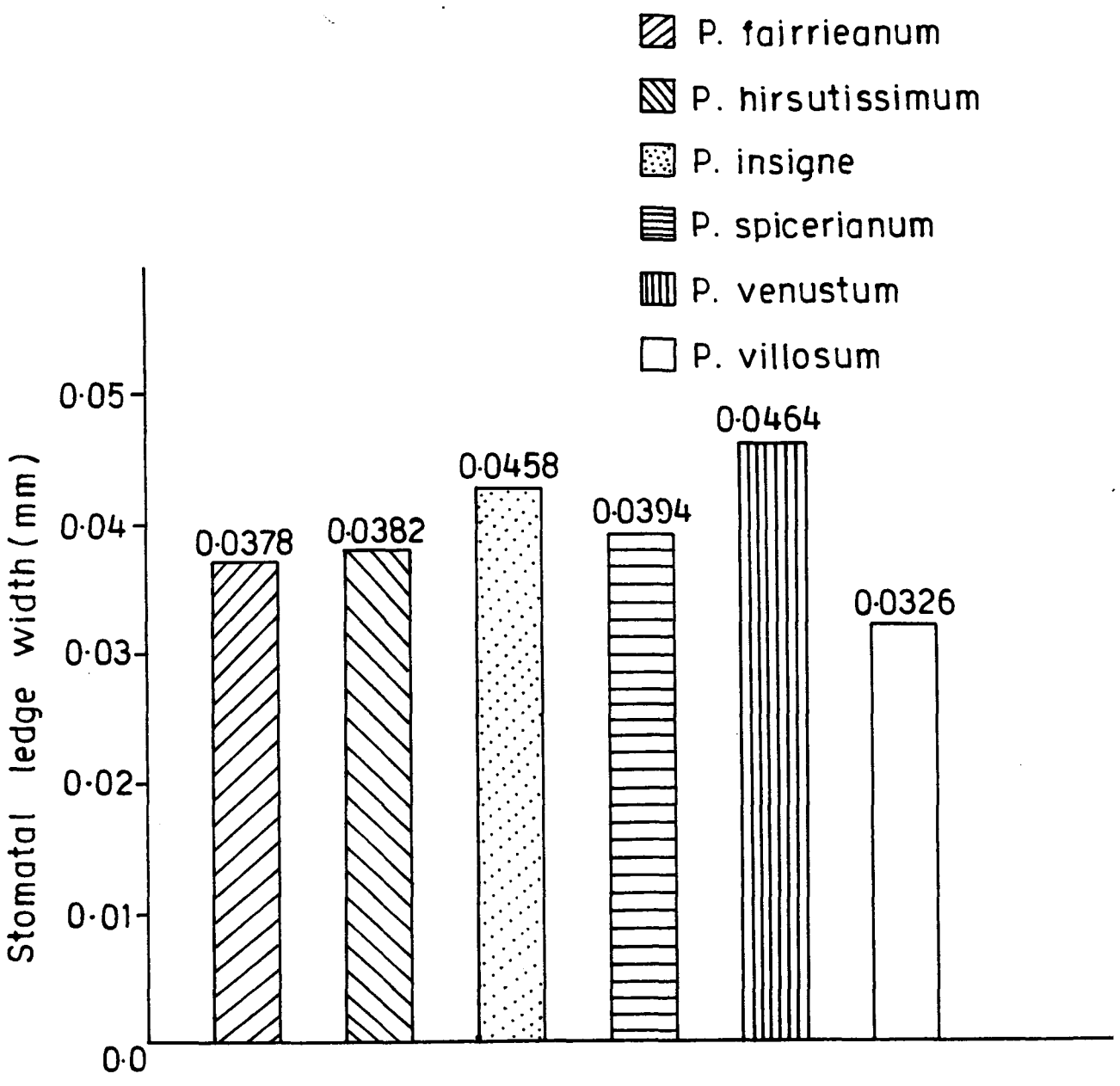

P. fairrieanum
P. hirsutissimum
P. insigne
P. spicerianum
P. venustum
P. villosum
Stomatal ledge width (mm)
0·05
0·04
0·03
0·02
0·01
0·0
0·0378
0·0382
0·0458
0·0394
0·0464
0·0326

Fig. 18 : Graphical representation of Stomatal ledge: width/length of Paphiopedilum species.

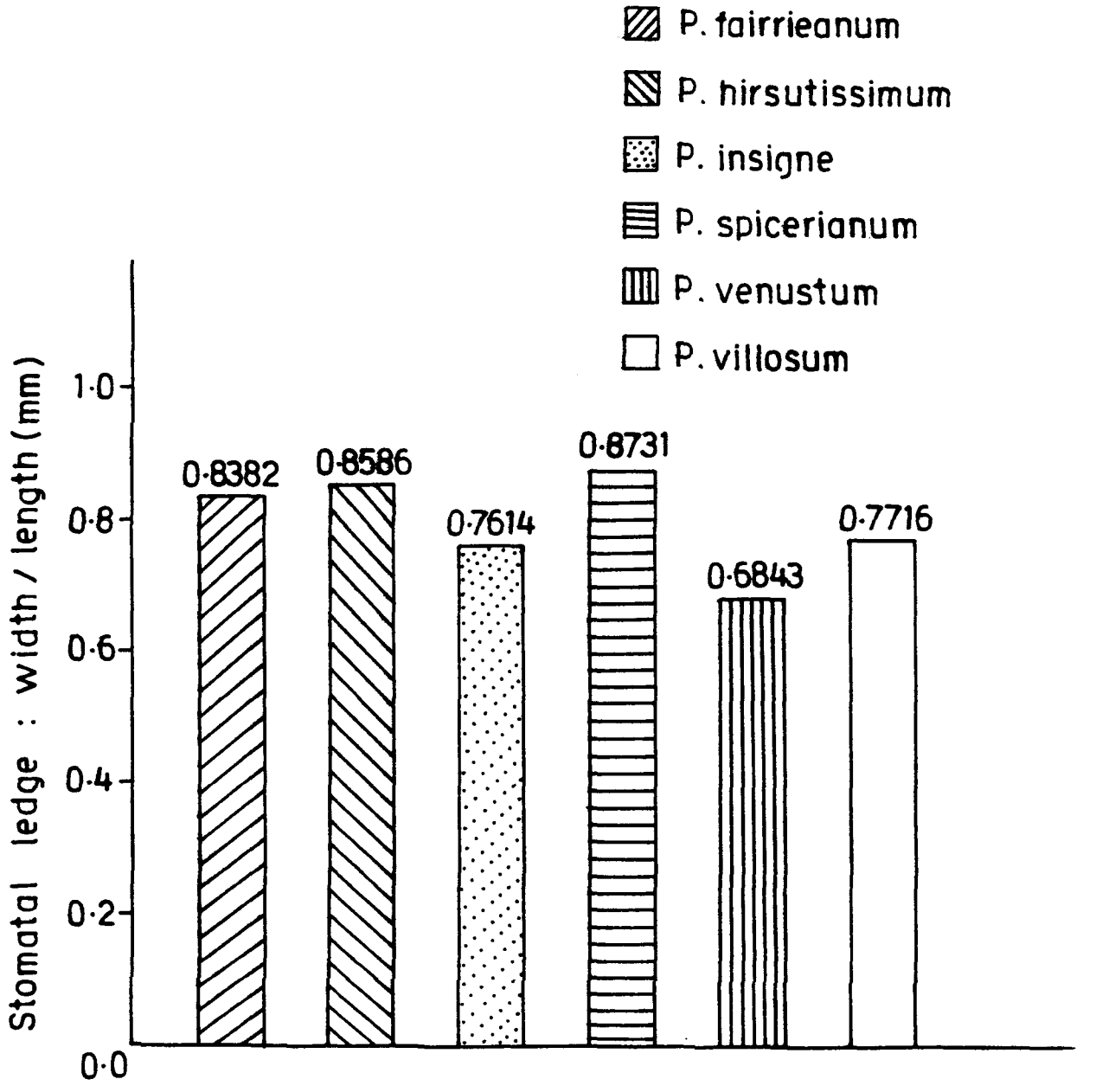

P. fairrieanum
P. hirsutissimum
P. insigne
P. spicerianum
P. venustum
P. villosum
Stomatal ledge : width / length (mm)
1·0
0·8
0·6
0·4
0·2
0·0
0·8382
0·8586
0·7614
0·8731
0·6843
0·7716

Fig. 19 : Graphical representation of Stomatal ledge: width x length of Paphiopedilum species.

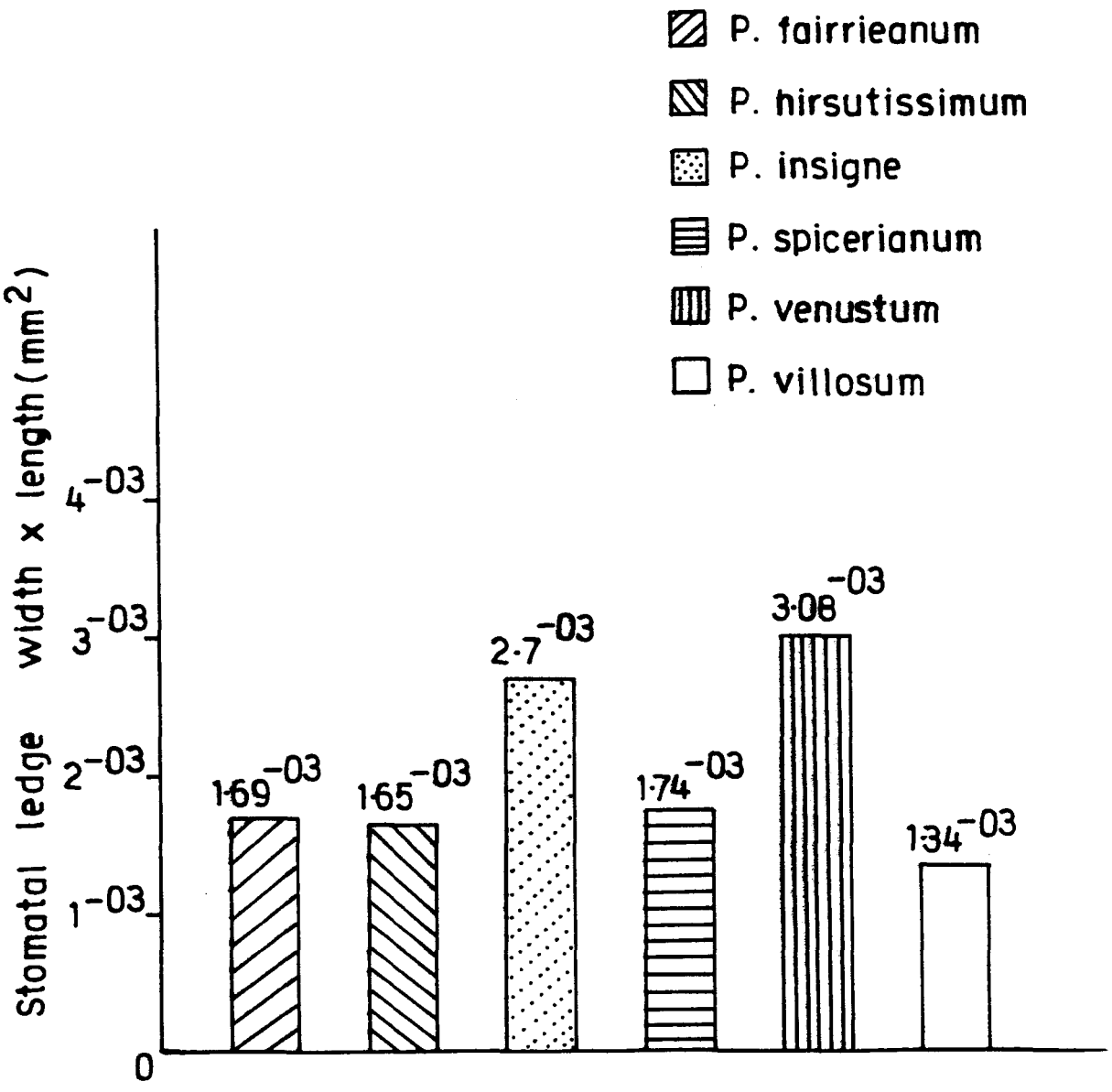

P. fairrieanum
P. hirsutissimum
P. insigne
P. spicerianum
P. venustum
P. villosum
Stomatal ledge width x length (mm²)
4⁻⁰³
3⁻⁰³
2⁻⁰³
1⁻⁰³
0
1·69⁻⁰³
1·65⁻⁰³
2·7⁻⁰³
1·74⁻⁰³
3·08⁻⁰³
1·34⁻⁰³

Fig. 20 : Graphical representation of Stomatal Opening length of Paphiopedilum species.

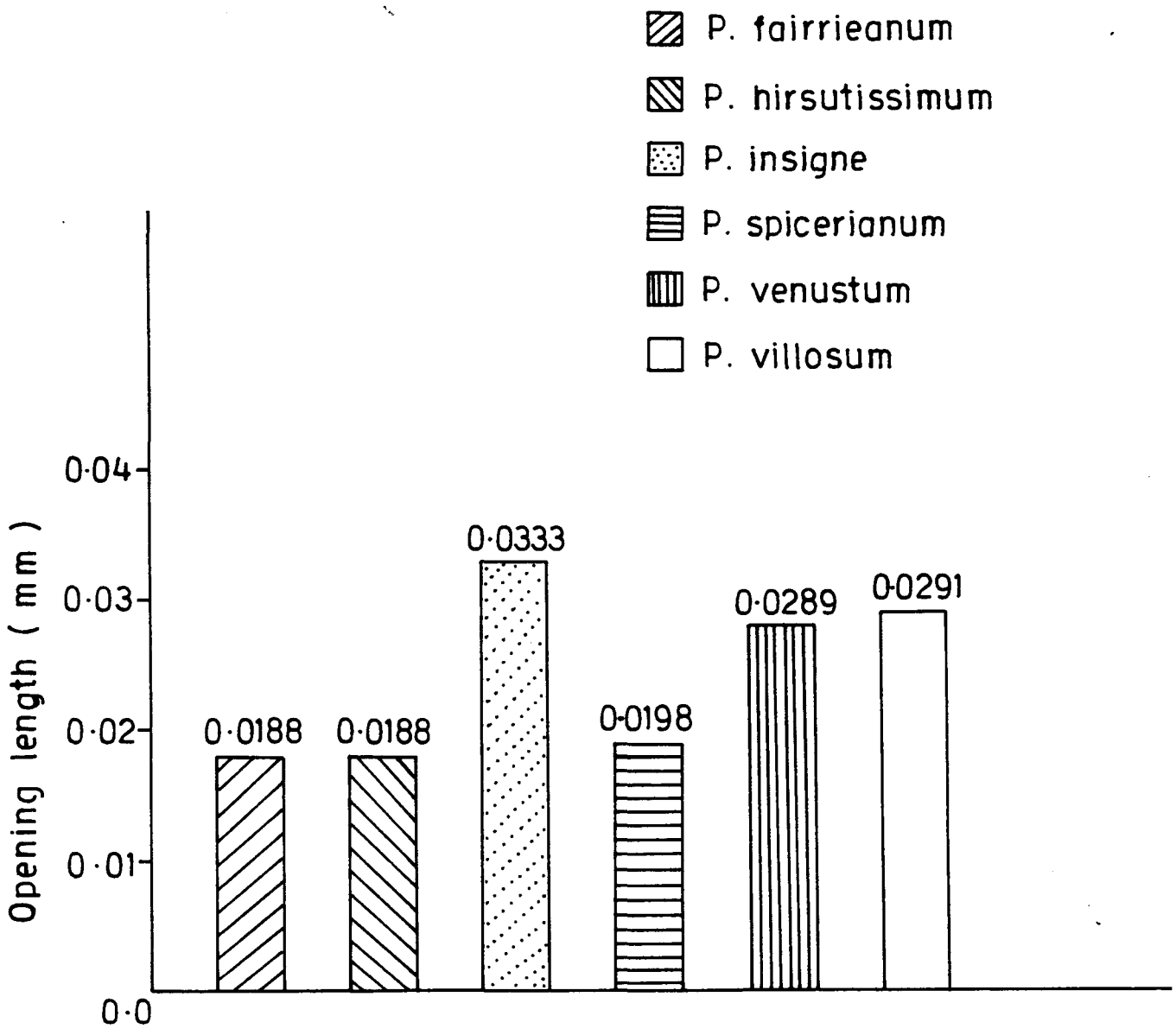

P. fairrieanum
P. hirsutissimum
P. insigne
P. spicerianum
P. venustum
P. villosum
Opening length (mm)
0·04
0·03
0·02
0·01
0·0
0·0188
0·0188
0·0333
0·0198
0·0289
0·0291

Fig. 21 : Graphical representation of Stomatal Opening length/ stomatal ledge length of Paphiopedilum species.

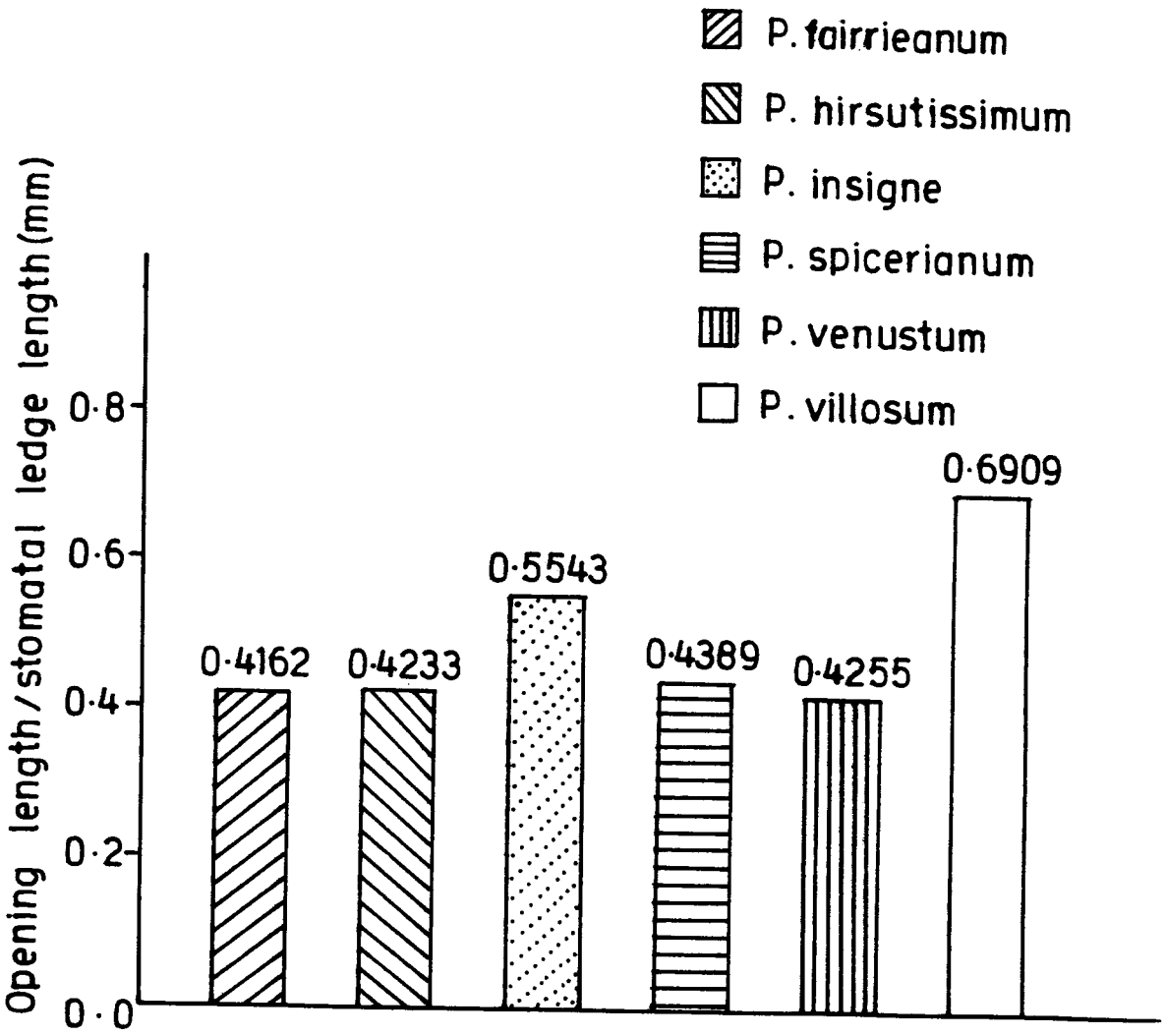
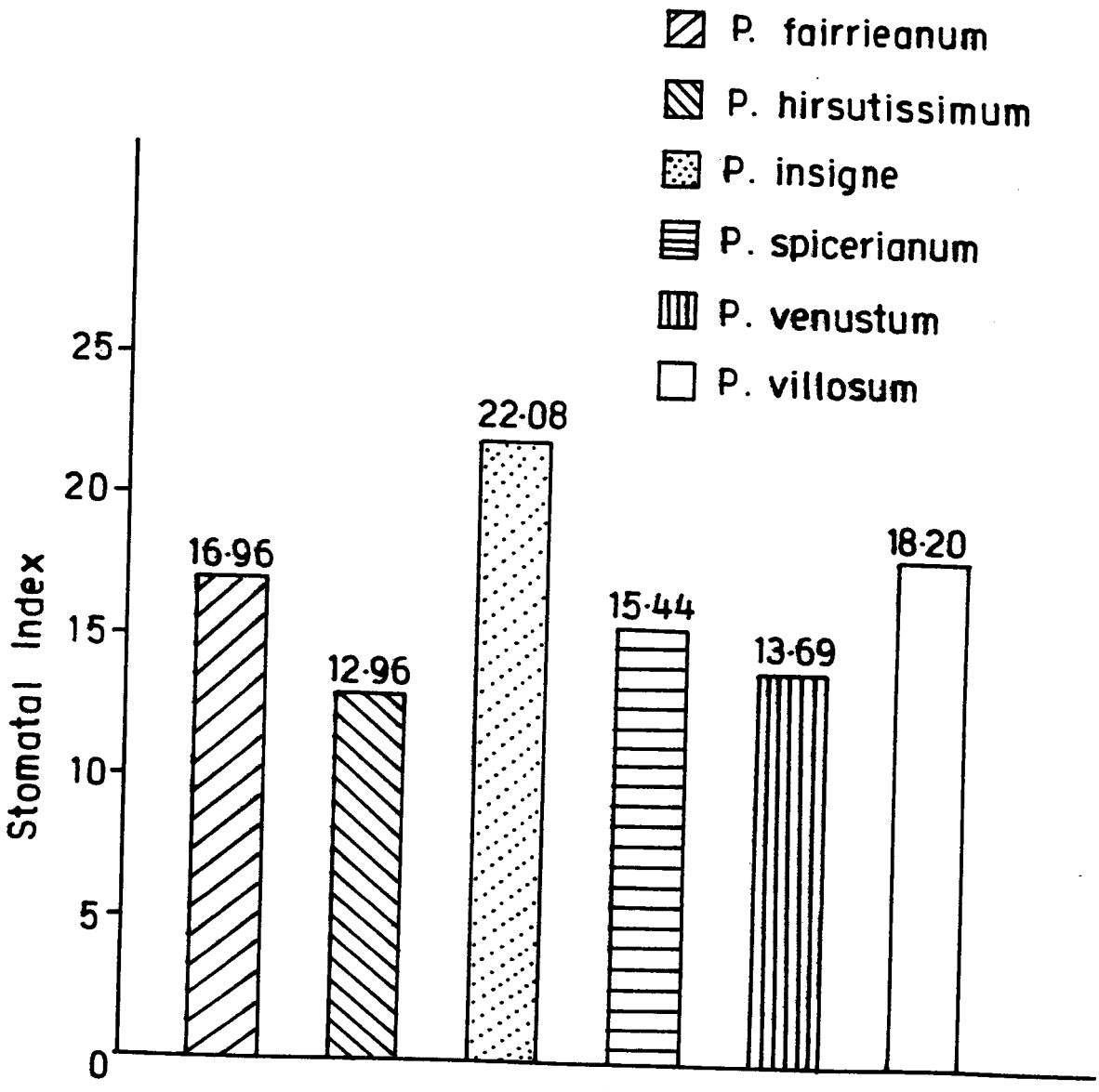

P. fairrieanum
P. hirsutissimum
P. insigne
P. spicerianum
P. venustum
P. villosum
Opening length/stomatal ledge length (mm)
0·8
0·6
0·4
0·2
0·0
0·4162
0·4233
0·5543
0·4389
0·4255
0·6909

Fig. 22 : Graphical representation of Stomatal index of Paphiopedilum species.


P. fairrieanum
P. hirsutissimum
P. insigne
P. spicerianum
P. venustum
P. villosum
Stomatal Index
0
5
10
15
20
25
16·96
12·96
22·08
15·44
13·69
18·20

(Fig.9-14). A hyperstomatic chamber was observed in all the species, covering the stomatal pore around the outer ledges (Fig.9-14).

Stomatal density:

The stomatal density was found highest in P. fairrieanum (166.88) followed by P. venustum (140.3), P. hirsutissimum (124.32), P. insigne (116.16), P. villosum (115.12) and the least in P. spicerianum (92.68) (Table 6: Fig.15-22).

Stomatal ledge width, length and ratio:

The stomatal ledge width was maximum in P. venustum (0.0464), succeeded by P. insigne (0.0458), P. spicerianum (0.0394), P. hirsutissimum (0.0382), P. fairrieanum (0.0378) and the minimum in P. villosum (0.0326) (Table 6: Fig.15-22). The values for stomatal ledge length was recorded highest in P. venustum (0.0679) and the lowest in P. villosum (0.0389). The intermediate values were found in P. insigne (0.0603), P. spicerianum (0.0456), P. hirsutissimum (0.0446) and P. fairrieanum (0.0412) (Table 6: Fig.15-22). The stomatal ledge width/length ratio was highest in P. spicerianum (0.8731), followed by P. hirsutissimum (0.8586), P. fairrieanum (0.8382), P. villosum (0.7716), P. insigne (0.7614) and the lowest value in P. venustum (0.6843) (Table 6: Fig.15-22). Accordingly, the products of stomatal ledge width and length showed marked difference among the species. P. venustum recorded the highest

value (3.08×10^{-03}) whereas P. villosum, the lowest value (1.34×10^{-03}). The second highest value was observed in P. insigne (2.7×10^{-03}), followed by P. spicerianum (1.74×10^{-03}), P. fairrieanum (1.69×10^{-03}), P. hirsutissimum (1.65×10^{-03}) and P. villosum (1.34×10^{-03}) (Table 6: Fig.15-22).

Opening length, stomatal ledge length and ratio:

The highest value for opening length was observed in P. insigne (0.0333), followed by P. villosum (0.0291), P. venustum (0.0289), P. spicerianum (0.0198) and the lowest value was recorded both in P. fairrieanum and P. hirsutissimum (0.0188) (Table 6; Fig.15-22). The ratio between the opening length and stomatal ledge length was found to be varied for species. The highest value for this ratio was recorded in P. villosum (0.6909) and the lowest in P. fairrieanum (0.4162). The intermediate values were recorded in P. insigne (0.5543), P. spicerianum (0.4389), P. venustum (0.4255) and P. hirsutissimum (0.4233) (Table 6; Fig.15-22).

Stomatal index:

Stomatal index was maximum in P. insigne (22.08) followed by P. villosum (18.2), P. fairrieanum (16.96), P. spicerianum (15.44), P. venustum (13.69) and P. hirsutissimum (12.96) (Table 6; Fig. 15-22).

DISCUSSION

The present study revealed that the epidermal cells of the **Paphiopedilum** species has different sculpturing of cuticle . This is in accordance with the findings of Atwood and Williams. 1979, in **Paphiopedilum** species. The cuticular sculpturing may be corelated with the light intensity of the environment.

Epidermal hairs were found to be absent in all the species, which is a general feature of the Orchidaceae, where the epidermal hairs are not commonly associated with vegetative structures. Swanson (1980) and Pridgeon (1981) reported similar observation in the family Orchidaceae.

Hyperstomatic chamber (antechamber) was observed in all the species studied. This may function as a water reservoir. The relation of antechamber in orchid leaf stomata to water conservation as well as prevention of excessive transpiration has been duscussed by some workers (Haberlandt, 1928; Gessner, 1956; Knauff and Arditti, 1969; Nuerbergk, 1963; Goh et al., 1977; Hew et al., 1980).

Stomata were completely absent on the upper surface of the leaves. The lack of stomata on the upper surface may further contribute to the water conservation. The absence of stomata on the upper surface of orchid leaves has also been reported by Withner et al., 1974; Nelson and Mayo, 1975; and Goh et al., 1977. The stomatal type in **Paphiopedilum** was found to be

`anomocytic' i.e., the stoma is surrounded by definite number of ordinary epidermal cells . There were no subsidiary cells surrounding the stomata. The lack of subsidiary cells in the leaf stomata of orchids has been reported by Hew et al., 1980. Thus, the structural pattern of stomata present in all the species of **Paphiopedilum** studied supporting the premitive nature of the group.

The stomatal density was found varied with the species. The density of stomata can be corelated with the rate of transpiration, which is very significant for the establishment of the plant in a specific habitat. The stomatal ledge width and length were found to be species specific and varied according to the size of the stomata. A wide range of variations in the stomatal size has been reported by Goh, 1975 in Dendrobium gonzalesii, D. jenkinsii, D. victoriae-reginae and D. pachyglossum. It is reported that epiphytic orchids have generally smaller stomata than the terrestrial ones (Solereder and Meyer, 1930). Though there is little differences in the values of stomatal ledge length and width, the ratio as well as the products of the stomatal ledge width and length determines the size and shape of the stomata in each species. The overall similarity observed in the structure of stomata and only one type of stomata, i.e., elliptical, suggest that the group studied represent one phyletic line. This is an indication that all the species have evolved under a common environmental factors. This observation also suggest that similar ecological and

physiological adpatations might have occured in all the species. Various modification of stomatal shape are known to exist within Orchidaceae, e.g., elliptical, circular, transversely elliptical and angular (Rasmussen, 1987). In the present study the shape of the stomatal opening found to be narrow slit like, whereas in Orchidaceae various shapes of stomatal openings i.e., narrow slit, circular, blunty cornered rectangle etc., has been reported by Rasmussen, 1987. In Dendrobium species two shapes of stomatal openings i.e., slit like and circular are reported (Yukawa et al., 1992).

The opening length showed no substantial difference between the species. It may be due to the similarity in the atmospheric conditions avaialble to all the species in a given area. Many workers have corelated the opening length of stomata with the atmospheric factors (Stalfelt, 1955; Mouravieff, 1958; Meidner and Heath, 1959; Kuper, 1964; Heath et al., 1965; Meidner, 1965; Nelson and Mayo, 1975; Farguhar, 1978;Outlaw et al., 1981, 1982; Lange et al., 1971; Jarvis and Morison, 1981; Meidner, 1986; Kappen et al., 1987; Losch and Tenhunen, 1981; Nonami et al., 1990; Kappen and Haeger, 1991).

The ratio between the opening length and the stomatal ledge length is an indication of the comparative efficiency of the guard cells in the process of transpiration. The stomatal idex attribute to the frequency of stomata in differnet species.

The various characteristics taken into account for the present study showed that each species of **Paphiopedilum** has got its own specificity. Leaf surface morphology such as the cuticular sculpturing, distribution,type, size and shape of stomata, the stomatal aperture etc., are significant in determining the phylogeny of each species.

There is no doubt, the leaf surface morphology could be used as secondary or supporting characteristic along with the data available from other aspects to elucidate the taxonomic position/phylogeny of the **Paphiopedilum** species. For example as discussed earlier various shapes of stomatal openings (narrow slit, circular, blunty cornered rectangle etc.) occur in Orchidaceae but in the case of species studied have one type (narrow slit) stomatal opening. It suggest that this character is specific to the group.

CHAPTER – VI

SEED VIABILITY & IN VIVO GERMINATION

INTRODUCTION

Orchid seeds are very minute and consists of a simple oval embryo without endosperm and are enclosed within a transparent, often fusiform testa (Arditti, 1967, 1969; Arditti and Ernst, 1984; Barthlott, 1976). Testa and embryos of different genera and species may vary in size, shape , colour and volumes (Patrick et al., 1980). The morphometric characteristics can be useful in elucidating various taxonomical problems. Seed morphometry of all the six species of **Paphiopedilum** has been dealt earlier in detail by Joy, 1992.

The viability of seeds can be attributed to the germination aspects of the species. The seeds are variously adapted to perform the functions of propagation in nature. However, the rate of germination in nature is very low, because of non-availability of the specific fungal requirements. Singh (1992) reported that only 0.2-0.3% of the seeds germinate in nature. The seeds which have no stocks of nutritive substances are dispersed by the wind and in order to germinate they have to be invaded by symbiotic fungi (mycorrhiza) that provide the necessary substances, especialy in the early stages of development (Alberto and Rossi, 1988) .

In the case of orchid seeds, viability was tested through germinating seeds *in vitro* which is a long process, tidious and time taking. To investigate the germination of terrestrial

orchids *in vitro*. Van Waes and Debergh (1986) developed a technique to determine the % of seeds with viable embryos. This information is a pre-requisite for studying the conditions for optimal germination. Germination % approximate the % of coloured embryos obtained with the adapted tetrazolium test (Van Waes and Debergh, 1986). Cribb (1987) categorically emphasised that the studies on orchid seed viability would be of great help.

Orchid seed germination differs from that of other seeds because of the absence of an endosperm, radicle, and leaf rudiments. Swelling of the embryo , followed by the formation of a round, top-shaped body called protocorm, is generally known as germination (Arditti et al., 1981).There are investigations on the *in vitro* germination of orchid seeds (Warcup, 1971, 1973; Warcup and Talbot, 1971: Stoutamire, 1974; Arditti et al., 1982: Van Waes and Debergh, 1986; Hadley and Pegg, 1989: De Pauw and Remphrey, 1992; Sharma, 1996).

Indian species of **Paphiopedilum** are becoming extremely rare due to the destruction of their natural habitat for many reasons. The propagation methods that has received little attention for **Paphiopedilum** (terrestrial) species is by *in vitro* seed germination (Sharma, 1996). Moreover, terrestrial orchids are generally difficult to germinate *in vitro* and have been partially successful (Downie, 1940,1949: Knudson, 1941: Stoutamire, 1965: Hadley, 1970: Harvais, 1974: Fast, 1976; Clements and Ellyard, 1979: Arditti et al., 1981: Clements, 1982; Fast, 1982).

Various facts on the mycorrhizal association and the physiological aspects of orchid seed germination have been brought out by Arditti (1967). Zeigler et al..(1967) reported that the orchid seeds are difficult to germinate for the lack of endosperm.

A detailed survey of the literature revealed that the reports on the in vivo germination of orchids are scanty (Arditti et al., 1990; Rasmussen. 1990; Richardson et al., 1992). There are reports available on the staining technique for viability test for the species of **Aerides**, **Bletilla**, **Dendrobium**, **Epidendrum** and **Spathoglottis** (Singh, 1981). However, standardised methods for testing the viability of **Papiopedilum** seeds were lacking. Further, there is no report on the **in vivo** germination of **Paphiopedilum** species of Northeast India. Therefore, in the present study an attempt has been made to observe the **in vivo** germination of all the six species of Paphiopedilums.

MATERIALS AND METHODS

Capsules were collected from different regions/locations of the Northeast india. Orchid Research and Development Centre, Tipi, Arunachal Pradesh, as well as from the experimental garden, Botany department, NEHU, Shillong. Subsequently, the capsules were stored in small Borosil specimen tubes (25 ml capacity) and kept at 4 C. Fresh seeds from the capsules of right age (Sharma, 1993) were used to study the viability, as well as in vivo seed

germination.

Counting of seeds per capsule:

The capsules were cut open with a razor and seeds were taken out with a needle. The total weight of seeds per capsule was measured with an electronic balance. Then, the seeds measured 1/100th of the total weight was separated out in ten replicates. Each replicate was counted separately, an average was taken and multiplied by 100 to get the total number of seeds (approximate) per capsule.

Staining procedure for viability test:

Seeds were spread on the filter paper (Whatman No.1), kept in the petri dishes (5 cm diameter). Distilled water was dripped along the sides of the petri dish, which assured the sufficient water for the imbibition. Seeds were soaked for around 48 hours, by keeping the petri dishes inside the incubator at 35 C. This was followed by the thorough washing with distilled water. Then seeds were kept in 1% TTC (Triphenyl Tetrazolium Chloride) solution in a test tube, in dark at 30 C for 36-48 hours. The seeds were taken out and washed with distilled water. This was followed by the malachite green staining, by keeping the seeds in a watchglass containing 0.01% aqueous solution of malachite green for 1-2 minutes. Then, seeds were mounted on a slide with a drop of glycerine, sealed with cover slip, observed under microscope.

Table 7Percentage of seed viability of *Paphiopedilum* species.

Name of species	No. of seeds tested	No. of viable seeds	Percentage of viability
P. fairrieanum	200	187	93.50
P. hirsutissimum	200	191	95.50
P. insigne	200	189	94.50
P. spicerianum	200	186	93.00
P. venustum	200	184	92.00
P. villosum	200	193	96.50

Fig. 23 : Seeds of Paphiopedilum species after viability test.

a. P. fairrieanum

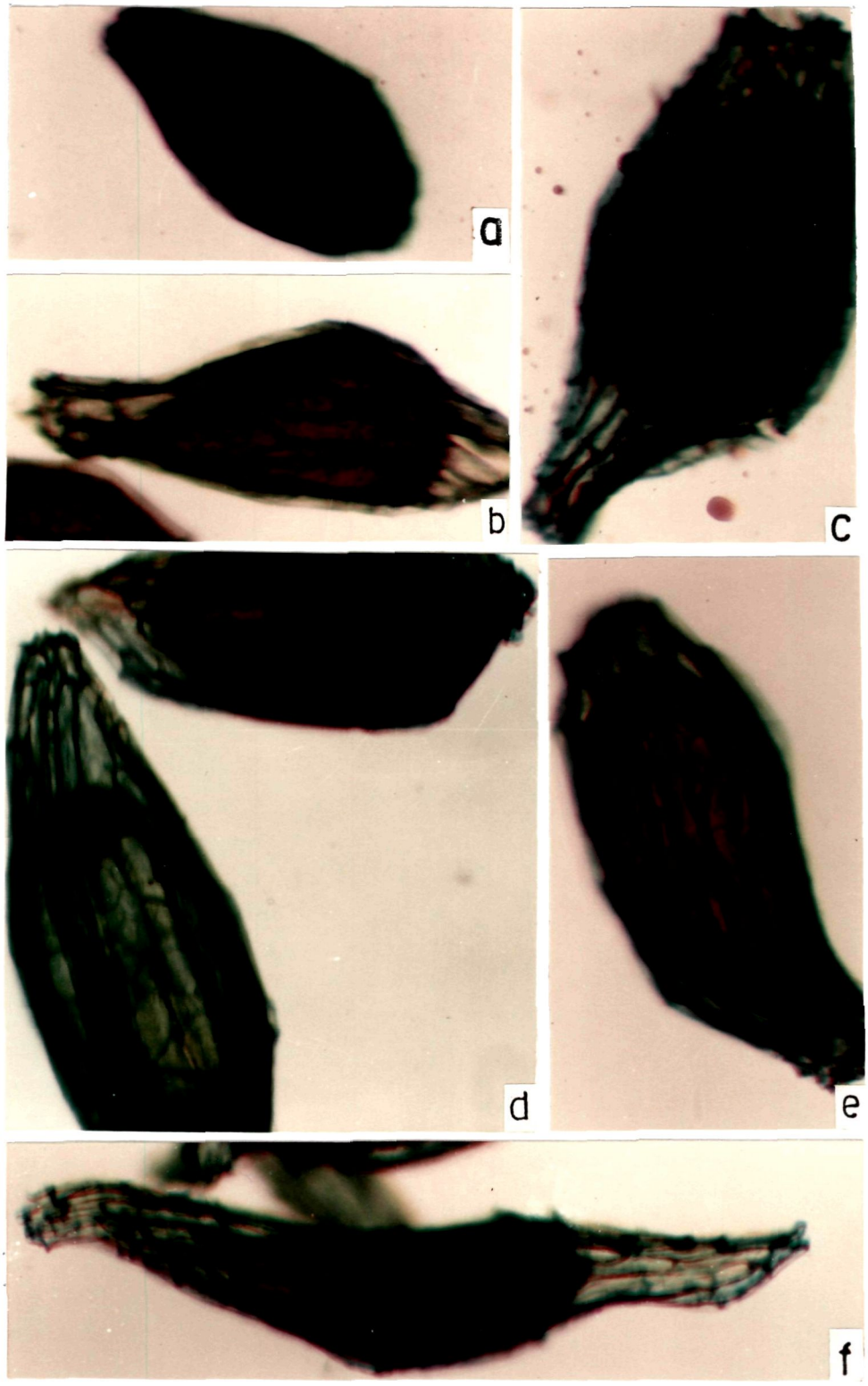
b. P. hirsutissimum

c. P. insigne

d. P. spicerianum

e. P. venustum

f. P. villosum


a
b
c
d
e
f

Micrographs were taken with the help of WILD dissecting microscope with photographic attachments using Kodak-gold (100 ASA) film, and enlarged suitably.

In vivo seed germination:

For the in vivo germination studies, seeds were soaked with distilled water for 30-35 hours for the proper imbibition of water, as a pre-requisite for germination. Then the seeds were packed in small paper envelopes , tagged, and placed in the vicinity of root system of vigorously growing mother plants. The observations for germination were carried out periodically by retrieving the seeds and observing under the microscope.

OBSERVATIONS/RESULTS

Seed Viability:

Seeds of all the species were of floating nature. Hence, soaking was effective only with the help of filter paper. After the viability test with TTC and Malachite green, it was found that in most of the seeds, embryo stains red and the testa cells green (Fig. 23).

Observations on the viability is listed in the Table (7). TTC was imbibed by the seeds as colourless solution and reduced by the enzymes present in the viable embryo to a red coloured substance "formazan". The intensity of red colour indicated the viability of embryos. Dark-red stained embryos indicate that the seeds are in good viable condition (Fig.23). Whereas, light

Fig. 24 : *In vivo* germination of *Paphiopedilum insigne*.

a. Fully imbibed seeds x 25

b. Single seeds enlarged x 100

c. Association of fungal hyphae x 40

d. Rupturing of seed coat (initiation of germination) x 100

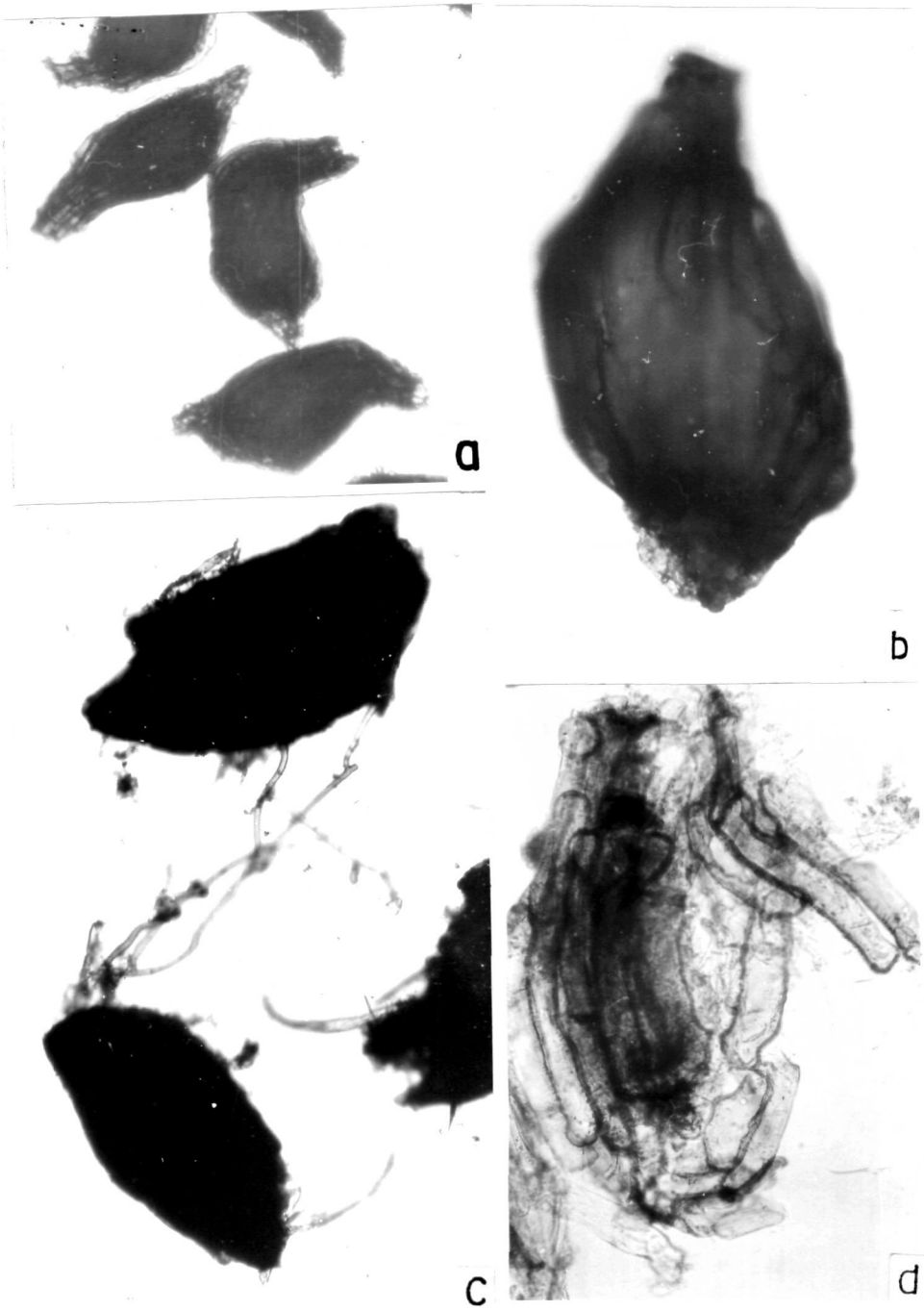

a
b
c
d

Fig. 25 : *In vivo* germination of *Paphiopedilum spicerianum*.

a. Fully imbibed seeds x 25

b. Single seeds enlarged x 100

c. Association of fungal hyphae x 40

d. Rupturing of seed coat (initiation of germination) x 100

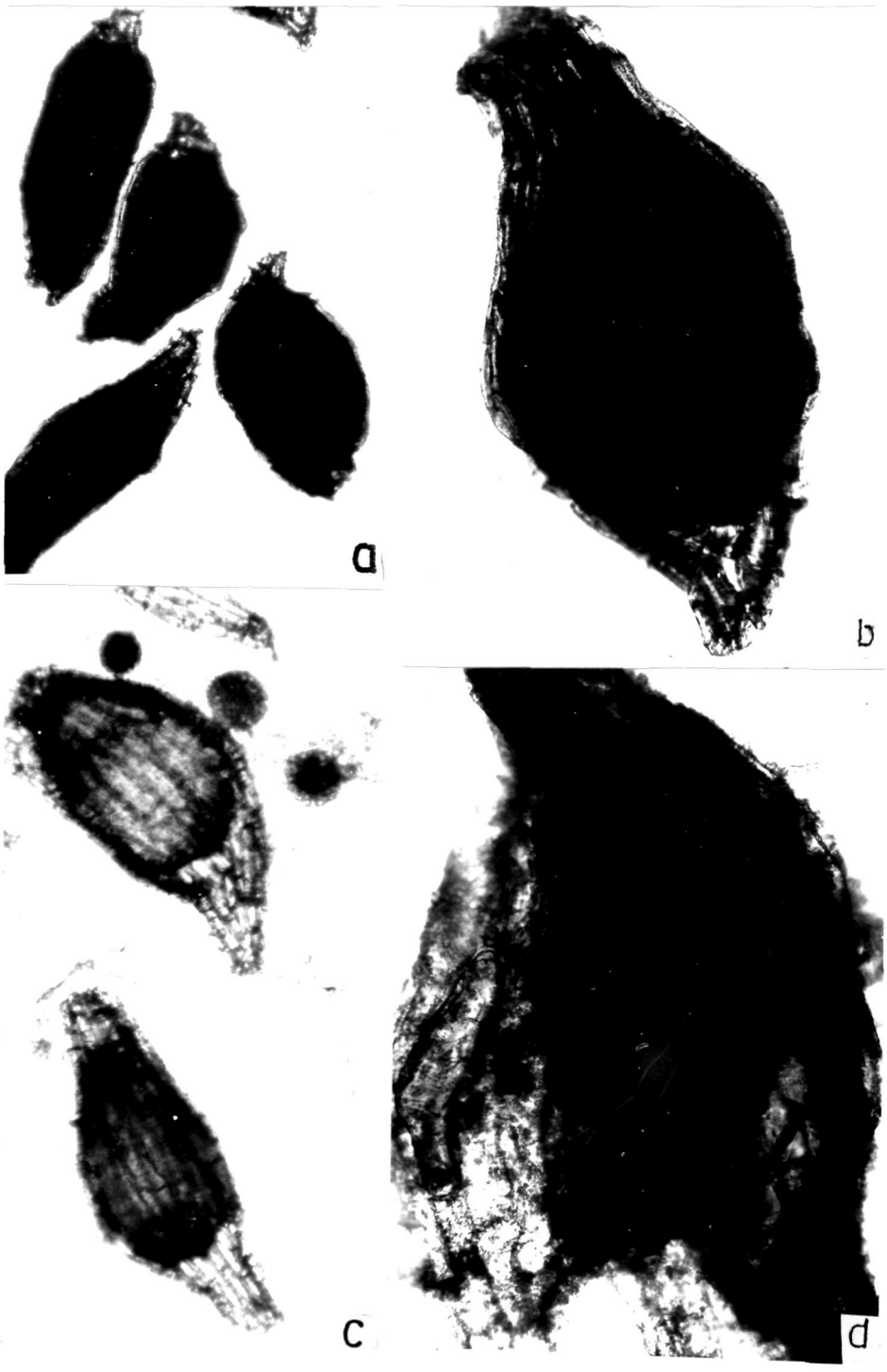

a
b
c
d

Fig. 26 : *In vivo* germination of *Paphiopedilum venustum*.

a. Fully imbibed seed x 25

b. Single seeds enlarged x 100

c. Association of fungal hyphae x 100

d. Rupturing of seed coat (initiation of germination) x 100

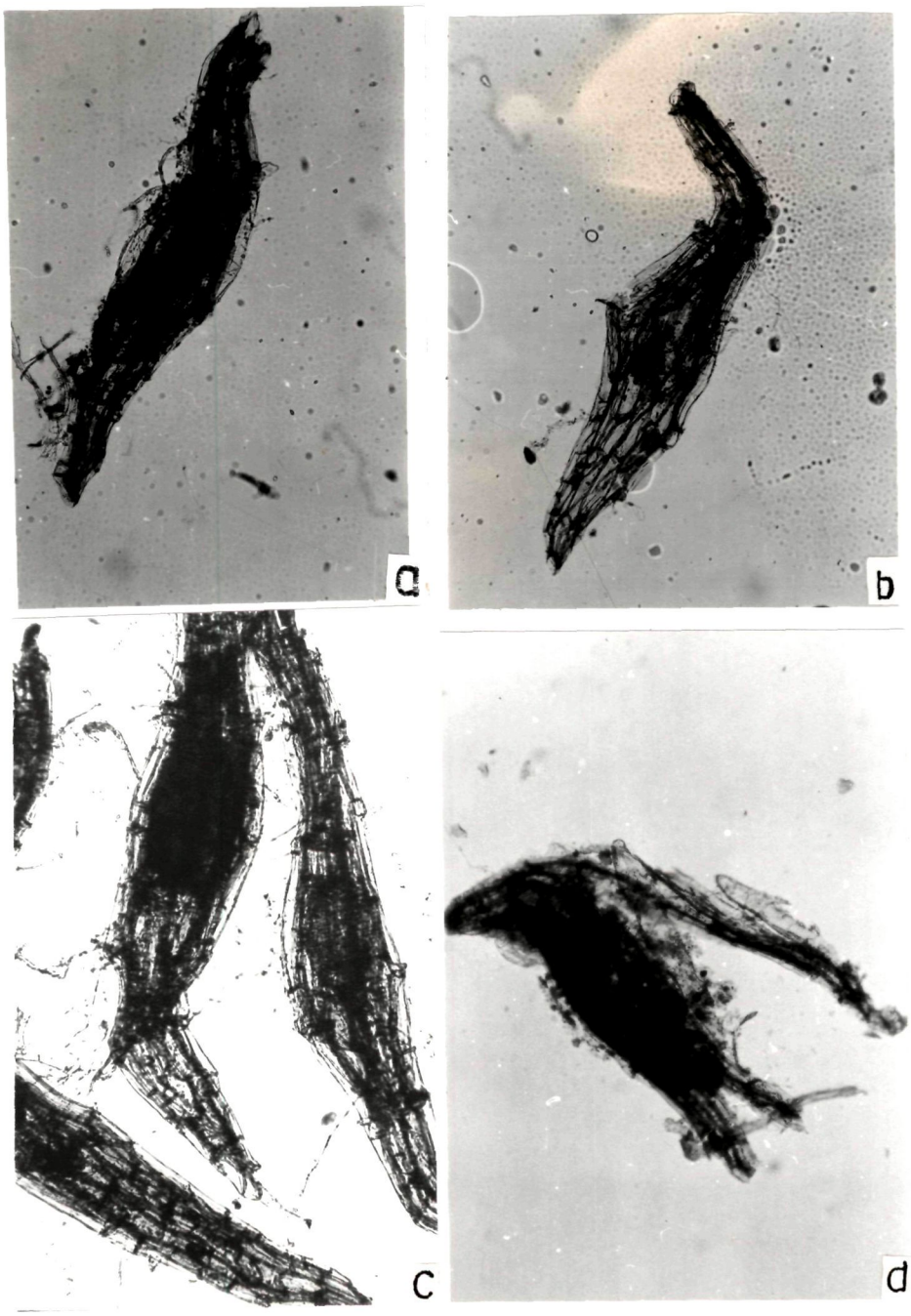

a
b
c
d

Fig. 27 : *In vivo* germination of *Paphiopedilum villosum*.

a. Fully imbibed seeds x 25

b. Single seeds enlarged x 100

c. Association of fungal hyphae x 40

d. Rupturing of seed coat (initiation of germination) x 100

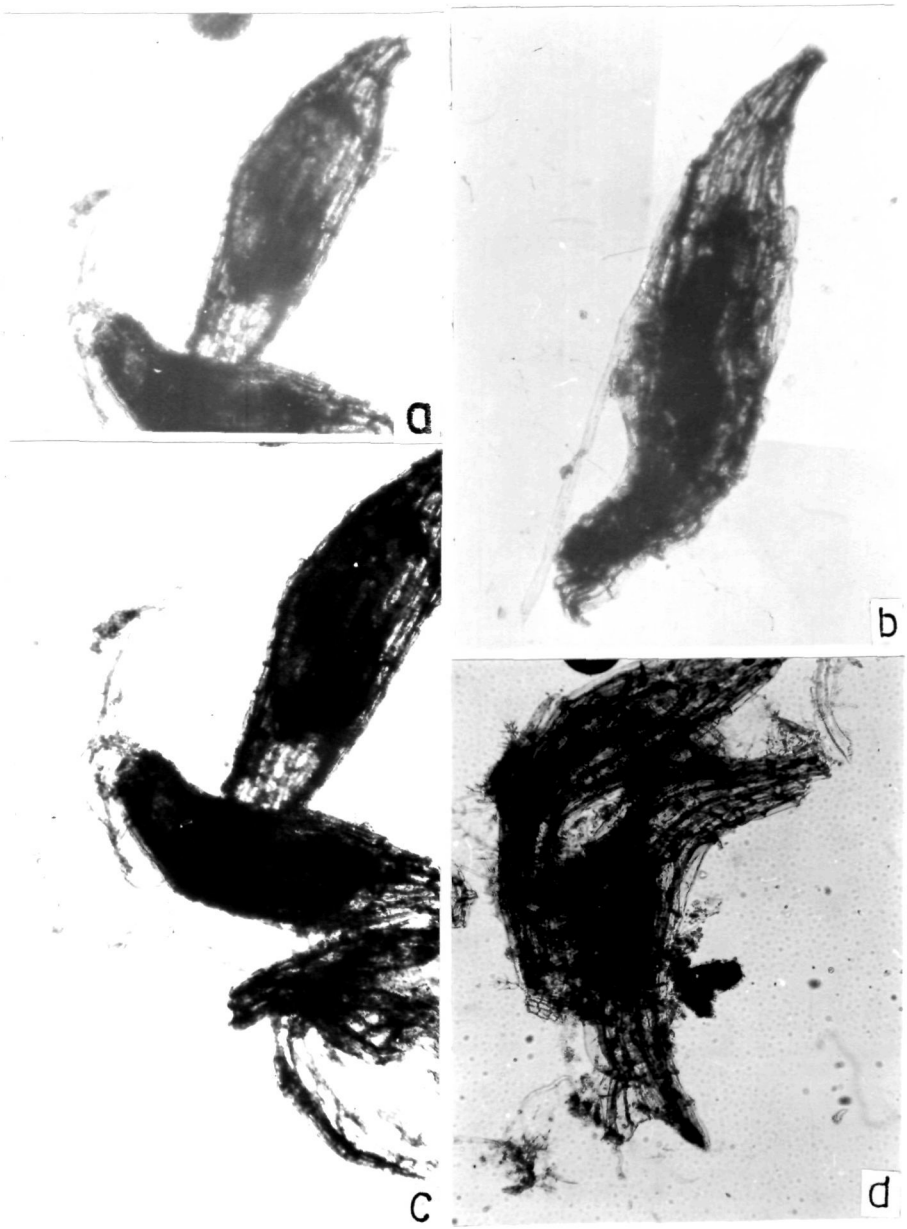

a
b
c
d

Fig. 28 : Fully developed seedlings of Paphiopedilum species

a. P. insigne

b. P. spicerianum

c. P. venustum

d. P. villosum

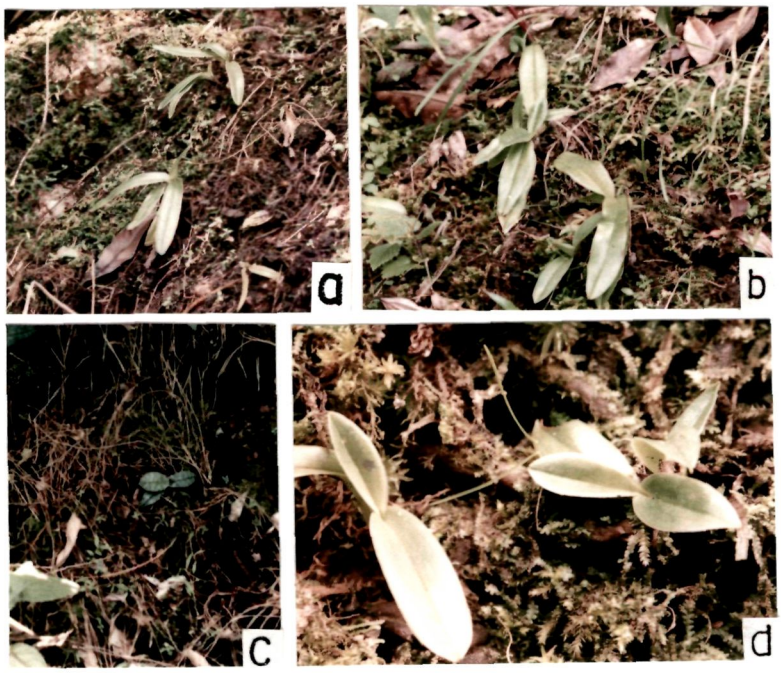

a
b
c
d

Table 8 : Number of seeds per capsule and *in vivo* germination of **Paphiopedilum** species.

Species	Total weight of seeds/capsule(gm)	Number of seeds per capsule	Number of seeds sowed	Number of seeds germinated	Percentage of germination
P. fairrieanum	0.2049	~ 90,750	200	1	0.5
P. hirsutissimum	0.2923	~ 1,03,250	200	2	1.0
P. insigne	0.2494	~ 50.350	200	2	1.0
P. spicerianum	0.2157	~ 1.02,400	200	2	1.0
P. venustum	0.2738	~ 42.150	200	1	0.5
P. villosum	0.2958	~ 78.500	200	2	1.0

colour is the indication of loosing the viability (Fig.23 c). It was observed that some seeds remain completely green after staining and those seeds were considered as sterile (Fig.23 d).

Seeds per Capsule:

The number of seeds per capsule varied among the species. The maximum number of seeds per capsule was recorded in *P. hirsutissimum* and the minimum in *P. venustum* (Table 8). The second highest number was in *P. spicerianum*, followed by *P. fairrieanum*, *P. villosum* and *P. insigne* (Table 8).

In vivo Germination:

In vivo germination of *Paphiopedilum* species required three-four weeks under appropriate atmospheric conditions. Inspite of constant attempts to get a better result, *in vivo* germination in *Paphiopedilum* species was found to be very difficult. Out of all the six species attempted for *in vivo* germination, only four species (*P. insigne*, *P. spicerianum* , *P. venustum* and *P. villosum*) were found to be successful, with the development of seedlings (Fig.24-27). The percentage of seed germination in *Paphiopedilum* species ranges from 0.5 to 1 (Table 8).

DISCUSSION

The seeds of *Paphiopedilum* species are very small devoid of endosperm. Seeds of orchids are reported to be very minute and non-endospermous (Arditti, 1967, 1969; Arditti and Ernst, 1984; Barthlott, 1976). Though more than 90% of seeds were viable in

all the species. lack of endosperm was very critical for *in vivo* germination. Moreover, the endomycorrhizal association was very essential for the *in vivo* germination.

In the case of orchid seeds usually viability is tested by germinating the seeds. But unfortunately, **Paphiopedilum** species are very difficult to germinate under experimental conditions and even if it germinates, take a long time. The staining procedure described in the present study was developed as a result of a number of trials and specifically useful to the viability test of **Paphiopedilum** species.

Seeds of **Paphiopedilum** species of North eastern region of India are very small and non-endospermous. Seeds remain viable for a long time when stored at about 4 C, usually in small paper envelopes. TTC and Malachite green staining was found to be promising for the viability test of **Paphiopedilum** species.

Germination and seedling developement in orchids are strikingly different from other flowering plants. The orchid embryo is relatively undifferentiated when mature with neither endosperm nor cotyledon. Food reserves are stored in the embryo proper.

Attempts to germinate seeds of terrestrial orchids have been only partially successful (Arditti, 1967, 1979, 1982; Burgeff, 1954; Clements, 1982; Fast, 1982; Van Waes and Debergh, 1986).

These reports are in accordance with the present observations. Though there are contradictory views regarding the specificity of fungal requirements for germination, there is no conflict regarding the necessity of endomycorrhizal association (Nakamura, 1982).

The nutritional physiology of the orchid-fungus symbiosis remains largely obscure. It is generally accepted that symbiotic fungi satisfy some nutrient requirements of the host (Hadley, 1969). The possible reason for poor in vivo germination in the **Paphiopedilum** species could be due to the lack of proper fungal association. The other reasons may be of the presence of germination inhibitors, the onset of dormancy and light factor. Fast (1974) suggested the presence of germination inhibitors and Stoutamire (1974) pointed out the onset of dormancy as the possible causes for poor germination in orchids. Darkness was an important factor for germination of **Paphiopedilum** species (Sharma, 1996). Arditti (1967, 1979) and Stoutamire (1974) reported that terrestrial orchids germinate best in the dark and/or inhibited by light. In vivo germination of **Paphiopedilum** species in the present study was found to be partially successful due to variety of reasons. However, propagation through in vivo seed germination if followed properly will be of great use. It is evident from the present study that out of one capsule 500-1000 seedlings can be obtained. Growth hormones can also be used after a certain stage of seedling development to enhance the in vivo growth rate.

CHAPTER – VII

CYTOLOGY

INTRODUCTION

The family Orchidaceae is one of the most morphologically diversed and species-rich families of flowering plants (Dressler, 1981). Despite of the family's tremendous diversity, very little is known about the level of genetic diversity within the population (Case, 1994).

Orchids, though cytologically fascinating, are not a very easy material to work with. A survey indicates that only little more than 15% of the orchidaceous species are cytologically known yet and that too are restricted to one or two individuals of a taxon, in many cases. The family exhibits a wide spectrum of numerical and structural variations of chromosomes (Chatterji, 1986). Cytological data could be well utilized scientifically as an auxilliary in the critical revision of many dubious orchid taxa and even the family as a whole.

Templeton et al., 1990 suggested a species level variation in most of the orchids. However, Case (1994) reported the chances to loss the species-level genetic variation due to large scale disturbances in the habitat.

The chromosome derrive their prominance as a tool in taxonomy from their direct relation to the genetic system of which they are an integral part (Lewis, 1957). Their behaviour during division reveals the genetic potential of organisms, whereas their comparative organisation may indicate the extent of relationships. As regards morphology, in general, orchid

chromosomes are small. Different size classes are however noticed in different groups (Chatterji, 1986). Till date, chromosome morphology has been studied in more than 175 Indian species (Sharma and Chatterji, 1966; Vij and Gupta, 1975; Kashyap and Mehra, 1983 a,b; Mehra, 1983; Sehgal and Sehgal, 1989; Salimuddin and Ramesh, 1993). Large to fairly large chromosomes are prone to morphological and structural repatterning (Vij, 1989).

None of the major taxonomic treatments of this family has yet taken chromosome as a biosystematic parameter. This may be due to the fact that cytodata is still very thin to be suitably utilized (Salimuddin and Ramesh, 1993). Duncan (1959) for the first time attempted to draw an evolutionary scheme in orchids on the basis of available chromosome records. In recent years, there is a progressive trend to emphasize the orchid cytology in researches (Chardard, 1963; Sharma and Chatterji, 1966; Tara and Kamemoto, 1970; Roy and Sharma, 1972; Mehra and Vij, 1972 ; Vij and Mehra, 1974 , 1976; Vij and Vohra, 1974 ; Vij and Gupta, 1975; Jorapur, 1976; Dressler, 1981; Kashyap and Mehra, 1983 a,b;Mehra, 1983; Biswas, 1986; Chatterji, 1986; Sehgal and Sehgal, 1989;Gill, 1989; Templeton et al., 1990; Bianco et al., 1991; D'emerico et al., 1992, 1993; Salimuddin and Ramesh, 1993; Case, 1994).

The cytological investigations on the genus **Paphiopedilum** of Northeast India have been carried out by some workers (Mehra, 1983; Biswas, 1986; Sehgal and Sehgal, 1989). However, these

reports are not consistant enough with the detailed mitotic characteristics of chromosomes for all the species of Northeast Indian Paphiopedilums. Therefore, in the present study , a thorough investigations on the mitotic behaviour/characteristics of chromsomes has been attempted . All the six species have been taken up for the study to draw a concrete conclusion as well as for the confirmation of the available reports on the cytodata of the genus Paphiopedilum.

MATERIALS AND METHODS

The following preparations were necessary for the cytotaxonomical studies.

1. Preparation of Aceto-Orcein stain:

100 ml. of 45% acetic acid was prepared by pouring 45 ml. of glacial acetic acid in 55 ml of distilled water. 1 gm of orcein was taken,added to the acetic acid (45% prepared) and heated to dissolve orcein powder. The solution was cooled, filtered and transferred to dropper bottle.

2. Preparation of Para-dichlorobenzene solution:

A saturated solution of Para-dichlorobenzene (PDB) was prepared in distilled water. Pre-treatment of root tips with PDB cause contraction and improve spreading of chromosomes.

3. Preparation of Carnoy's fluid:

Carnoy's fluid was prepared by adding 3 parts of ethyl alcohol and 1 part of glacial acetic acid. This was used as a fixative to kill the cells rapidly with minimum shrinkage,

swelling, distortions, and other artefacts.

4. Processing of root tips and preparation of cytological slides:

Actively growing root tips were collected, washed with water and placed in small vial containing PDB solution for around 3 hours at room temperature. The root tips were then washed with water and fixed in the carnoy's fluid for about 3 to 4 hours. This was followed by the hydrolysis of the root tips with 1N HCl at 60 C, for 10-15 minutes and washed thoroughly with distilled water. The root tips were then placed on a slide with a drop of 45% acetic acid. The root cap was removed and the cells were separated with a sterilized needle. The excess of acetic acid was blotted out, added 1 or 2 drops of aceto-orcein and kept aside for 5 minutes. The processed material was mounted with coverslip and tapped thoroughly, for the spreading out of the cells.The coverslip was sealed on the edges to prevent additional drying out.

5. Karyotype preparation:

The morphology of the chromosomes was observed both from the metaphase as well as anaphase stages of cell division. Microphotographs were taken with the help of Leitz microscope with photographic attachment (35 mm) and suitably enlarged. Karyotypes were made from the microphotographs for different species, by arranging the homologous chromosomes in decreasing order of length, by maintaining the centromeres in the same plane.

Table 9 : Chromosome number of *Paphiopedilum* species of North-Eastern India (earlier reports).

Taxon	Phytogeographic region	Chromosome nos. n	Chromosome nos. 2n	Author(s)
P. fairrieanum	NEH	13	--	Vij & Mehra, 1974; Shekhar, 1984.
	NEI	13	--	Mehra & Sehgal, 1980.
P. hirsutissimum	NEH	--	26	Biswas & Sharma,1979; Vij et al., 1982; Biswas & Sharma, 1979.
	NEI	13	--	Mehra & Sehgal, 1975,'76.
P. insigne	NEH	13	--	Vij & Mehra, 1974.
		--	26	Chatterji, 1865a; Vij et al., 1982; Biswas & Sharma, 1979.
	NEI	14	--	Mehra & Sehgal, 1976.
		--	21,24,27	Mehra & Sehgal, 1980.
P. spicerianum	NEH	--	26	Vij et al., 1982.
		--	26,30	Shekhar, 1984.
		--	30	Biswas & Sharma, 1979.
P. venustum	NEH	20+1B	--	Mehra & Vij, 1970.
		--	39	Shekhar, 1984.
		--	34-42,38,57	Chatterji. 1966.
		--	40+2B	Vij & Mehra, 1974.
	NEI	19	--	Mehra & Sehgal. 1980.
		--	40	Joseph, 1960 (see Panigrahi, 1960); Chatterji. 1968b; Kataki, 1963.
P. villosum	NEH	13	--	Vij & Mehra, 1974.
		--	26	Vij et al., 1982.
	NEI	13	--	Mehra & Sehgal, 1980.

NEH: North-East Himalaya
NEI: North-East India

Table 10 Chromosome counting and karyotype of **Paphiopedilum** species.

Species	Chromosome nos. n	2n	Karyotype
P. fairrieanum	13	26	14m + 8sm + 4st
P. hirsutissimum	13	26	16m + 6sm + 4st
P. insigne	13	26	16m + 4sm + 6st
P. spicerianum	15	30	18m + 8sm + 4st
P. venustum	21	42	34m + 6sm + 2st
P. villosum	13	26	10m + 14sm + 2st

m - metacentric
sm - sub metacentric
st - sub telocentric

Fig. 29 : Somatic chromosomes of **Paphiopedilum** species.

a. P. fairrieanum x 100

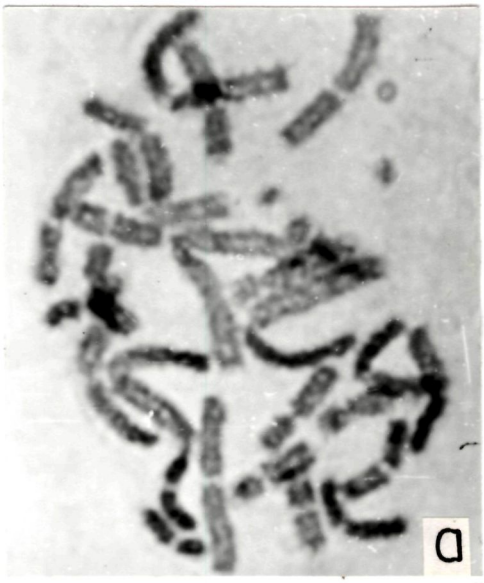
b. P. hirsutissimum x 100

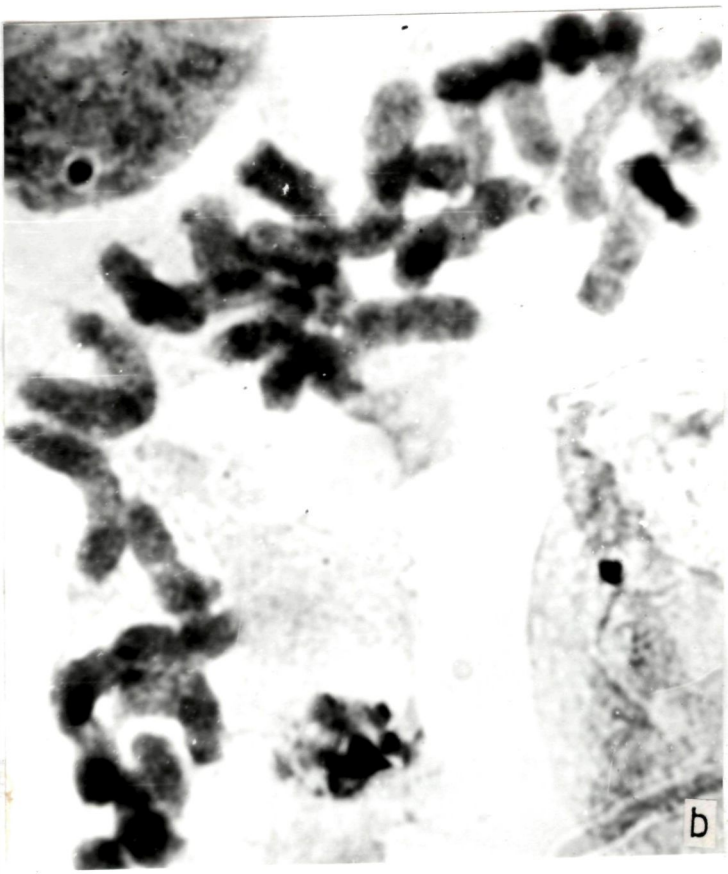
c. P. insigne x 100

d. P. spicerianum x 100

e. P. venustum x 100

f. P. villosum x 100


a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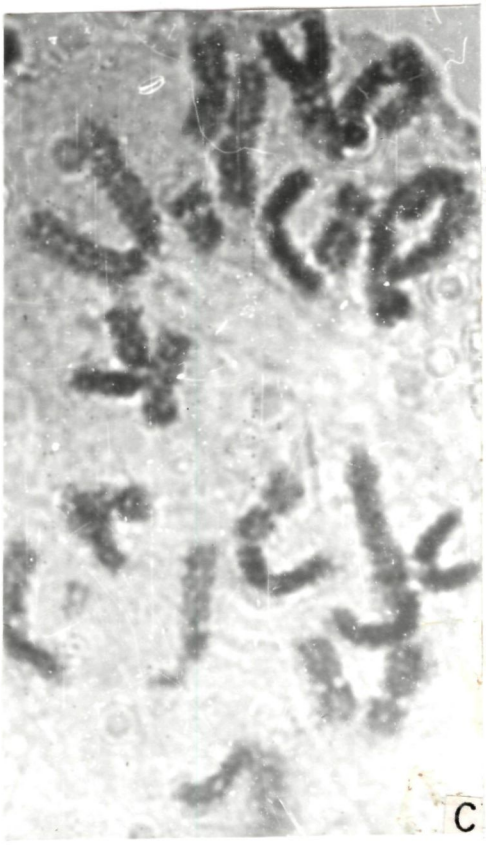
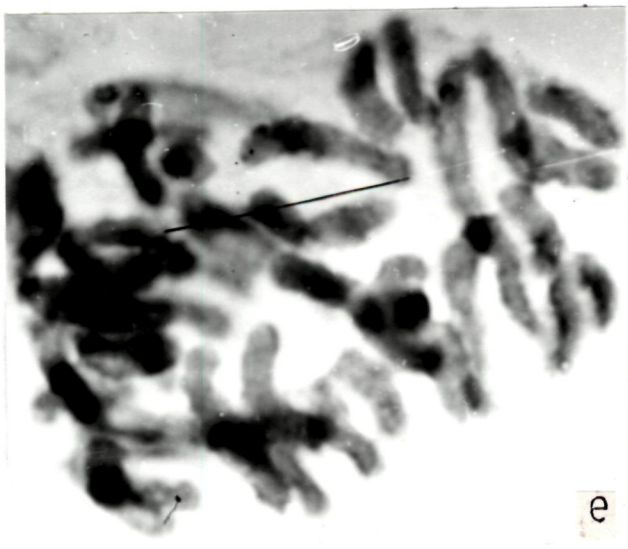

c


b


e

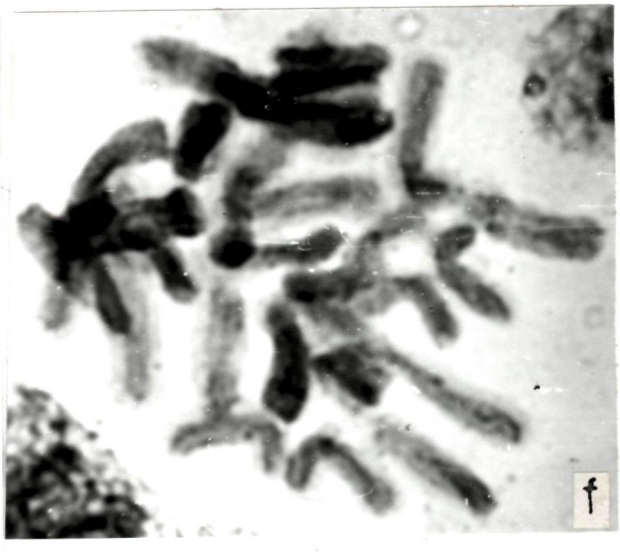

f

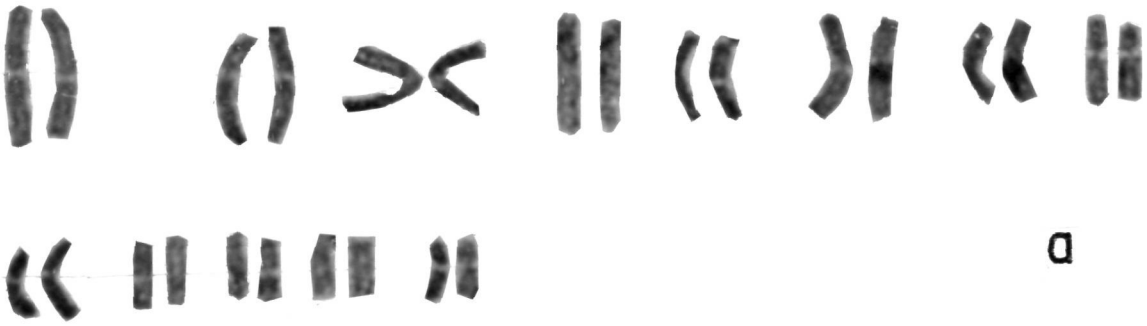
Fig. 30 : Karyotype of Paphiopedilum species

a. P. fairrieanum

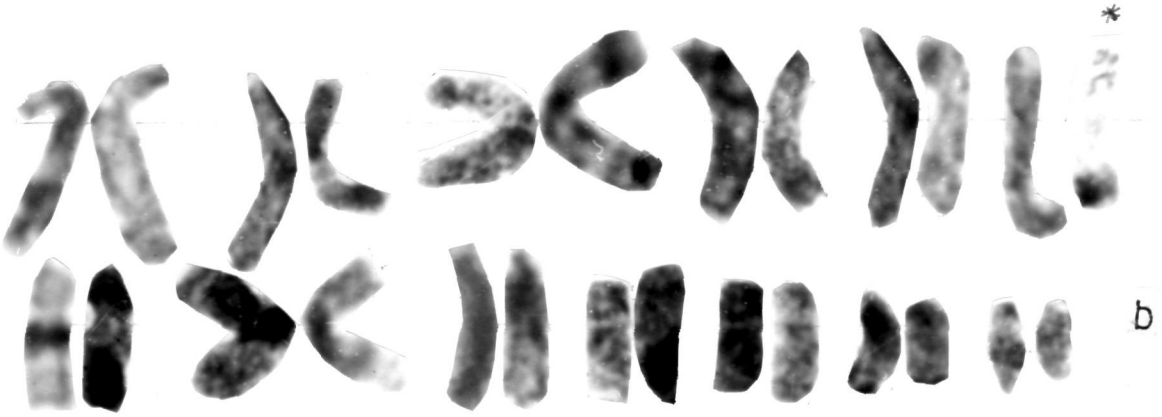
b. P. hirsutissimum

c. P. insigne

* Chromosome with secondary constriction



a



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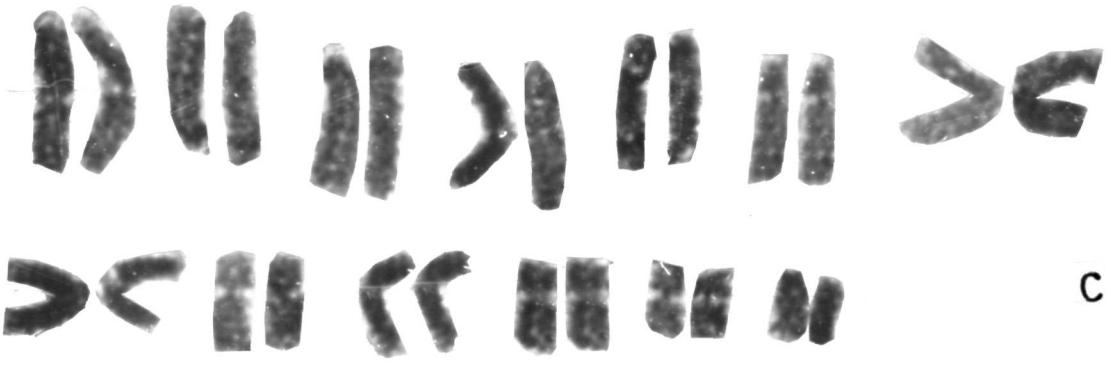

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RESULTS

The somatic chromosome count from the root tip cells of different species under investigation are illustrated by fig. 29. Out of the six species studied, four species viz., P. fairrieanum, P. hirsutissimum, P. insigne and P. villosum has 13 pairs of chromosomes and the structure was found to be apparantly uniform. Whereas in the case of P. spicerianum diploid number of chromosomes was equal to thirty (2n = 30) and in P. venustum, diploid number of chromosomes was found to be forty two (2n = 42).

The earlier reports on the chromosome number of Paphiopedilum species of North-Eastern region have been listed in the table 9.

The centromere was located in the centre of the chromosomes (metacentric) or near the centre (sub metacentric). Hence, the arms of the chromosome were almost equal in length.

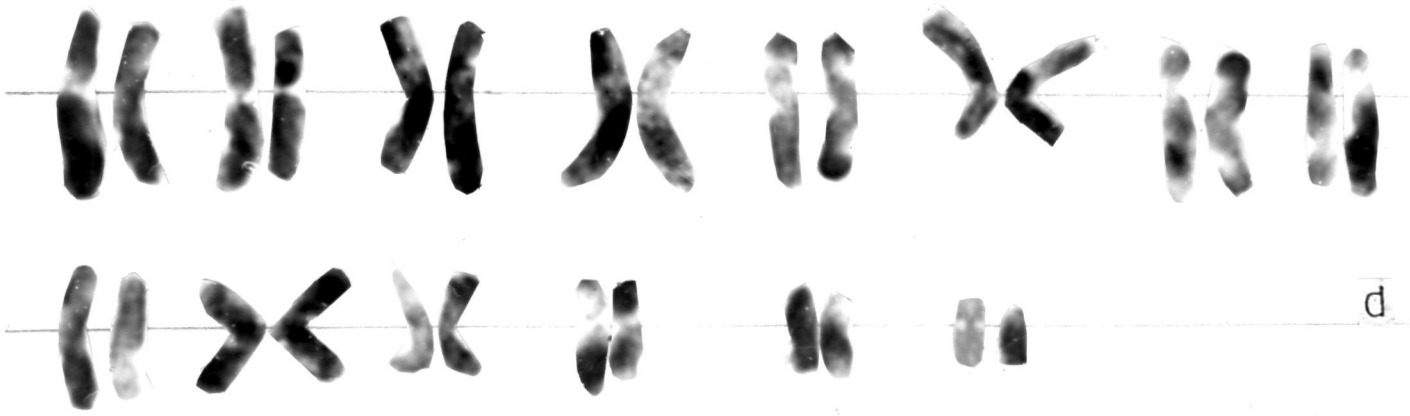
Out of 13 pairs of chromosomes in P. fairrieanum, 7 pairs were found metacentric, 4 pairs were sub metacentric, and the remaining 2 pairs were sub telocentric chromosomes. The third pair of chromosomes were observed as 'V' shaped (fig. 30 a). The karyotype formula of P. fairrieanum was 14m + 8sm + 4st (fig.30 a; table 10). In P. hirsutissimum, 8 chromosome pairs were observed to be metacentic, 3 chromosome pairs were observed to be submetacentric, and the remaining two pairs were found to be (fig. 30 b). Besides, the primary constriction i.e., the centromere, a secondary constriction was also found in the sixth pair of chromosome (fig. 30 b). During the anaphase two types of

Fig. 31 : Karyotype of **Paphiopedilum** species

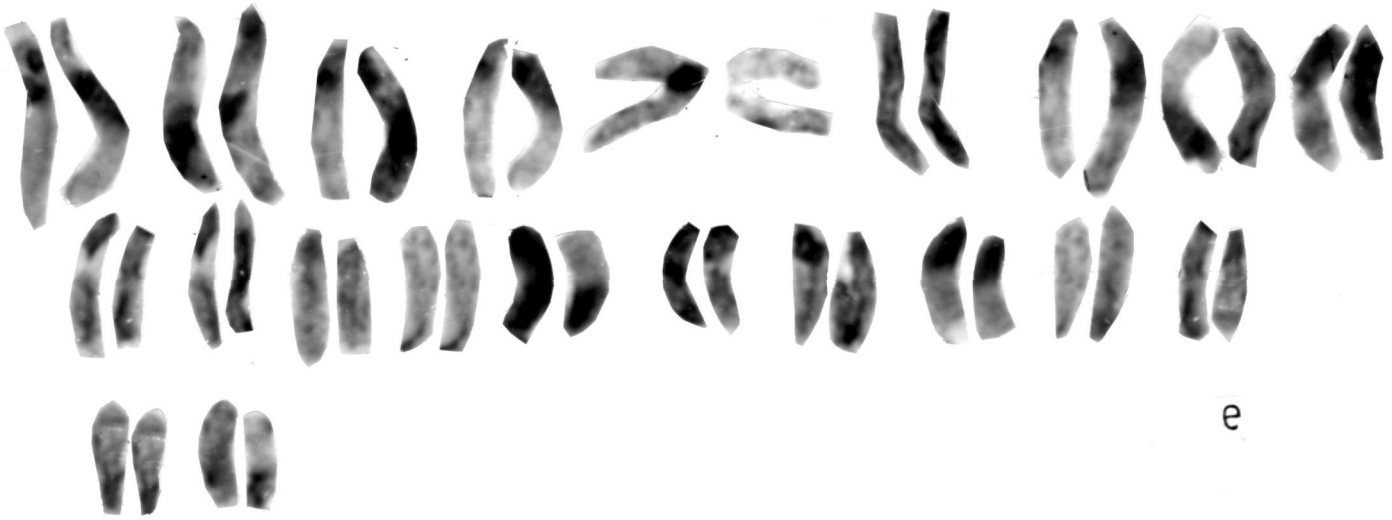
a. P. spicerianum

b. P. venustum

c. P. villosum



d



e

f

chromosome shapes were observed, viz., 'V' shaped and 'J' shaped. The number of 'V' shaped chromosomes were more when compared with the 'J' shaped chromosomes. The karyotype formula was 16m + 6sm + 4st (fig. 30 b; table 10). In the case of P. insigne, 8 pairs of metacentric, 2 pairs of sub metacentric and 3 pairs of sub telocentric chromosomes were observed. There were two pairs of 'V' shaped chromosomes. The karyotype formula of P. insigne is 16m + 4sm + 6st. In P. spicerianum, out of 15 pairs of chromosomes, 9 pairs of metacentric, 4 pairs of sub metacentric and 2 pairs of sub telocentric chromosomes were found. Two pairs of 'V' shaped and 'J' shaped chomosomes were observed. The karyotype formula of P. spicerianum was 18m + 8sm + 4st (fig. 31 a; table 10). P. venustum had altogether 21 pairs of chromosomes. Out of these chromosomes 17 pairs were metacentric, 3 pairs were sub metacentric and the remaining 1 pair were sub telocentric. There were 3 pairs of 'V' shaped and 2 pairs of 'J' shaped chromosomes. The karyotype formula of P. venustum was 34m + 6sm + 2st (fig. 31 b; table 10). In the case of P. villosum, the karyotype revealed that 5 pairs were of metacentric, 7 pairs sub metacentric and only 1 pair of sub telocentric chromosomes. 4 pairs of 'J' shaped and 1 pair of 'V' shaped chromosomes were observed in this species. The karyotype formula of P. villosum was 10m + 14sm + 2st.(fig. 31 c; table 10).

DISCUSSION

Chromosome counting of six species of Paphiopedilum of North-Eastern region of India, revealed that except two species

(P. spicerianum and P. venustum) the rest of the species (P. fairrieanum, P. hirsutissimum, P. insigne and P. villosum) have 13 pairs of somatic chromosomes. This is an indication of the closeness between the species. However, speciation could be attributed to the gene mutation, chromosome repatterning (without a change in number) and the variation in chromosome morphology in terms of relative arm length and secondary constriction etc.

The chromosome number of Paphiopedilum species of North-East India have been discussed earlier by many workers (Table 9). Mehra and Sehgal (1980) reported that P. fairrieanum has got 13 pairs of chromosomes. The present study has also revealed the same and is in accordance with the earlier reports. According to Mehra and Sehgal (1975, 1976) P. hirsutissimum was reported to have chromosome number n= 13, which supports present findings. In the case of P. insigne, Mehra and Sehgal (1976) reported 14 pairs of chromosomes, whereas the present study revealed that there are only 13 pairs of chromosomes. There are no concrete reports on the basic chromosome number of P. spicerianum. In the present study, it is found that P. spicerianum has got 15 pairs of chromosomes (2n = 30). Biswas and Sharma (1979) has also been reported as 2n = 30 for P. spicerianum.

A variety of reports are there regarding the chromosome number of P. venustum. Mehra and Sehgal (1980) reported only 19 pairs of chromosomes in P. venustum. However, the present investigation revealed that this species has got 21 pairs of chromosomes (2n = 42). In P. villosum, Mehra and Sehgal (1980)

reported 13 pairs of chromosomes. This report supports the observations in the present study.

In all the species of **Paphiopedilum** under present investigation, the chromosomes are found to be very large. Maekawa (1971), Atwood (1984) and Kondo (1994) reported that among the members of the Orchidaceae, **Paphiopedilum** have the largest chromosome.

Different species of **Paphiopedilum** showed a striking resemblance with regard to the chromosome, at first sight. The genus has got a basic chromosome number, n = 13. However, **P. spicerieanum** and **P. venustum** differed from the other species with 2n = 26 and 2n = 42 respectively. Chromosomes in all the species examined are of large size. Each species has got distinct karyotype formula and chromosomal structure. Out of the six species studied, **P. hirsutissimum** and **P. villosum** have got quite large and most distinct chromosomes. The variation in existing reports may be because of the usage of cultivars as study material.

CHAPTER – VIII

GENERAL CONCLUSION

The North-Eastern region of India is one of the richest geographical zone of India for orchid with its diverse topography: altitude, climate and many other phytogeographical factors. This region has got immense treasure of orchid taxa which grow in profusion and also at the zenith with regard to their ornamental importance (Joy et al., 1994). However, various destructive activities and ruthless exploitation by the inhabitants disturb the delicately balanced natural habitat of orchids. Several orchid species have become extinct, many are at the verge of extinction and some are endangered.

Orchids are of tremendous horticultural interest, very promissing as well as challenging for various botanical investigations because of their characteristic and unique mode of life and reproduction. The genus Paphiopedilum is commercially most immportant group of orchids, with marvellous, beautiful, long lasting and large flowers.

In India, all the species of Paphiopedilum except P. druryi are found in North-Eastern region between 60-2220 m altitude. These are terrestrial or occassionally epiphytic, sympodial herbs with horizontally spreading thick roots. Leaves are conduplicate, oblong, elliptic, distichous, two to seveal, coriaceous, green or green mottled with light green or purple markings. Inflorescence terminal, terete. Flowers one or two per inflorescence, waxy in apprearance. Dorsal sepal erect, large, lateral sepals, united to form a synsepalum. Petals spreading, horizontal or pendent. Lip is slipper shaped, side lobes incurved. Column horizontal short with fleshy staminode at

apex infront of two fertile, ventral anther; pollinia two, glutinous. Stigma large, ventral, fleshy, short stalked, more or less hidden by side lobes of lip.

North-Eastern region of India is a trijunction - a meeting place of (a) Himalayan element, high altitude, (b) South, South-East Asian and far-east flora and (c) Peninsular India. Out of the seven existing species, six are confined to North-East, whereas P. druryi is only found in the Travancore hills of South India. This shows a discontinuous distribution of the species. Various ecological and adaphic factors could be the possible reasons for the distribution pattern of these species.

There are eight species reported from this region, viz., P. charlesworthii, P. fairrieanum, P. hirsutisimum, P. insigne, P. spicerianum, P. venustum, P. villosumand P. wardii. Out of these species, P. charlesworthii and P. wardii are supposed to be extinct from the nature. Every species has got distinct characteristics for identification, such as size and colour of leaf, leaf apex, lip and dorsal sepal. There are many variations and varieties among each species of Paphiopedilum of this region. Species diversity can be defined with satisfaction, the same may not be true in case of ecosystem diversity. Still, the ecosystem diversity/regional diversity can be identified on the basis of the increase or decrease in the number of species. Among all the species of Paphiopedilum, P. venustum showed the maximum number of variations, especially within the populations of Meghalaya. Hence, it may be suggested that Meghalaya could be considered as the centre of origin and dispersion of P. venustum.

The incessant sequence of biological diversity and gene pool should be efficiently utilized for the conservation of these species. The increasing importance of in vitro multiplication and perpetuation of species requires pure/natural species (biodiversity) for basis breeding stock.

Phenological observations are pre-requisite for sceintific multiplication and hybridization. The concept and significance of phenological investigations have been discussed in detail by Lieth (1970), Lieth and Radford (1971). Most of the characteristics are found species specific. The leaf apex may be considered as a very distinguishing character of species, for the identification even in vegetative stage. Various genetical as well as ecological factors influenced the phenology of **Paphiopedilum** species of this region. Out of six species studied, five species except **P. hirsutissimum** has got the flowering season from October-March i.e., from autumn to the end of winter. The floral longivity was minimum in **P. hirsutissimum** (blooming, March-June). This observation indicate that temperature, atomospheric humidity, intensity of light etc., have the direct impact on the flowering and longivity. Less relative humidity, high temperatue, high intensity of light have a negative impact on the floral longivity of **Paphiopedilum** species. The periodicity of different phenophases reflects seasonal distribution of specific kind of resources such as flower, pollen, fruit, seeds etc. Regular seasonal pattern observed in all the species may be due to the conducive climate of the region. This kind of informations would be a great help in

implementing sceintific multiplication/developmental programmes, proper utilization and management of resources in the orchid industry as well as for the conservation of the species. Since, the Paphiopedilum species are facing threat to their survival in nature, propagation and conservation of the species are the need of the time.

Epidermal and cuticular characters of the leaf have remarkable value in the field of palaeobotany, palaeoecology, pharmacognosy and taxonomy (Stace, 1966; Hardin, 1979; Wurdack, 1986; Singh and Dube, 1991). Paphiopedilum species under present investigation has got specific characteristics of leaf surface morphology. The cuticular sculpturing was different in all the species. The sculpturing of cuticle may be corelated with the light intensity of the environment. Absence of epidermal hairs observed in all the species is a general feature of Orchidaceae. Hyperstomatic chamber as well as lack of stomates on upper surface of the leave observed in the present study may function as water reservoir. The structural pattern of stoma present in all the species of Paphiopedilum supporting the premitive nature of the group. The overall similarity observed in the structure and only one type of stomata (elliptical) suggest that the group represent one phyletic line and might have evolved under a common environmental factors. Cuticular sculpturing, presence/absence of glandular hairs or trichomes, distribution, size , shape and structure of stomata, stomatal opening etc., are much significant in elucidating the phylogeny of the species. Leaf topology could be used as a secondary or supporting characteristic in tracing

out the taxonomic position/phylogeny of Paphiopedilum species along with the available information from other aspects of botany.

Orchid seeds are smallest among the seeds produced by the flowering plants and Paphiopedilum seeds are very small devoid of endosperm. Usually the number of seeds per capsule is ranging from 1,300 to 10,00,000. In the case of Paphiopedilum species this number is in the range of 42,150 to 1,03,250. Seeds of all the species studied are of floating nature.

The viability test is usually conducted by germinating the seeds. In the present study, a standradised staining method with Triphenyl Tetra Chloride and Malachite green has been described for the viability test of the Paphiopedilum species. With this method, the red stained embryos with green reticulations are considered as viable; whereas the wholly green stained ones are considered to be sterile.

Seeds of Paphiopedilum species were viable even after 4-6 months of bursting the capsule. This may be due to the appropriate maintenance of optimum temperature (4 C) required for the viability of seeds. Seeds are variously adapted with their structure to perform the function of propagation in nature. However, the rate of germination in nature is very meagre, because of non-availability of the specific fungal requirements and suitable atmospheric conditions. Singh (1992) reported that only 0.2%-0.3% of the seeds germinate in nature. Hadley (1970), Harvais (1974), Arditti et al., (1981), Fast (1982) reported that

the terrestrial orchids are very difficult to germinate.

The *in vivo* germination of **Paphiopedilum** species in the present study has only been partially successful with a very low percentage of germination. This may be due to the lack of proper endomycorrhizal association (an essential requirement) or various other factors like soil and atmospheric conditions. Other possible reasons may be the presence of germination inhibitors, onset of dormancy and the light intensity. These aspects are need to be further investigated to have a better conclusion. However, propagation through *in vivo* seed germination if followed properly will be of great advantage.

Cytology of **Paphiopedilum** species was very fascinating but a difficult task. Chromosomes of different species of **Paphiopedilum** showed a striking resemblance. The genus has got a basic chromosome number, n = 13. ***P. fairrieanum, P. hirsutissimum, P. insigne*** and ***P. villosum*** are represented by 2n = 26; whereas ***P. spicerianum*** has got 15 pairs of chromosomes (2n = 30) and ***P. venustum*** has 21 pairs of chromosomes (2n = 42). Karyotype of each species showed distinct characteristics in the shape as well as the position of centromeres. Chromosomes of all the species are large and distinct. However, ***P. hirsutissimum*** and ***P. villosum*** have quite large and distinct chromosomes out of all the six species studied.

There are lot of variations and varieties among each species of **Paphiopedilum** of North-Eastern region and have many natural hybrids. This incessant series of biological diversity and gene

pool should be properly utilized in various multiplication, propagation and conservation programmes. The knowledge of germ plasm, distribution, phenology, leaf topology, seed viability and germination, and cytologyetc., are the remarkable requirements for the scientific approach to the conservation of the species. The present study on **Paphiopedilum** species of North-Eastern region of India is a modest attempt to contribute some relevant informations towards the academic as well as applied venture.

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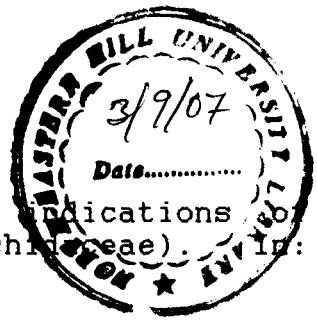

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