COMPATIBILITY STUDIES IN MONOPODIAL ORCHIDS

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DECLARATION

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

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CONTENTS

	Page No.
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	4
3. MATERIALS AND METHODS	25
4. RESULTS	38
5. DISCUSSION	83
6. SUMMARY	100
7. REFERENCES	104
8. ABSTRACT	114

LIST OF TABLES

Table No.	Title	Page No.
3.1.1	Source of twelve parental genotypes of monopodial orchids	27
4.1.1	Mean performance of twelve parental genotypes of monopodial orchids for quantitative vegetative characters	40
4.1.2	Mean performance of twelve parental genotypes of monopodial orchids for quantitative floral characters	43
4.1.2.1	Variability parameters for morphological characters in twelve parental genotypes of monopodial orchids	45
4.1.2.2	Heritability and genetic advance for morphological characters in twelve parental genotypes of monopodial orchids	47
4.1.2.3a	Phenotypic correlation among different biometric characters in twelve parental genotypes of monopodial orchids	49
4.1.2.3b	Genotypic correlation among different biometric characters in twelve parental genotypes of monopodial orchids	50
4.1.2.3c	Environmental correlation among different biometric characters in twelve parental genotypes of monopodial orchids	51
4.1.3.1	Performance of twelve parental genotypes of monopodial orchids for qualitative floral characters	53
4.1.3.2	Pollen characters of twelve parental genotypes of monopodial orchids	55
4.2.1	Matrix showing diallel crossings attempted among twelve monopodial parental genotypes	57
4.2.2	Matrix showing compatibility relationships in diallel crossings among twelve monopodial parental genotypes	58

LIST OF TABLES CONTINUED

Table No.	Title	Page No.
4.2.3	Matrix of the 24 successful combinations that yielded seedlings	59
4.2.5a	Post pollination floral changes in twelve parental genotypes of monopodial orchids	66
4.2.5b	Stages of capsule development in twelve parental genotypes of monopodial orchids	68
4.2.5c	Duration to green capsule harvest (days) in successful combinations	69
4.2.5d	Average length (cm) of green capsules harvested from successful combinations	70
4.2.5e	Average width (cm) of green capsules harvested in successful combinations	72
4.2.5f	Capsule yield in successful combinations (per cent)	73
4.2.5g	Percentage of filled seeds over total seeds in successful combinations	75
4.3.1	Details of embryo culture of hybrid seeds obtained from 24 successful combinations	77 - 78
4.3.2	Observations on seedling morphology at deflasking	80 - 81
5.1	Ranking of twelve parental genotypes of monopodial orchids based on strength of incompatibility	92
5.2	Ranking of twelve parental genotypes of monopodial orchids based on extent of incompatibility	93
5.3	Ranking of twelve parental genotypes of monopodial orchids based on compatible crosses	94
5.4	Ranking of the 24 hybrid combinations based on seedling morphology at deflasking	98 - 99

LIST OF FIGURES

Fig. No.	Title	Between pages
1	GCV and PCV for twenty one traits in twelve parental genotypes of monopodial orchids	45-46
2	Heritability (H^2) and Genetic Advance (G.A.) for twenty one traits in twelve parental genotypes of monopodial orchids	47-48
3	Character distribution in terms of heritability and genetic advance	47-48
4	Phenotypic correlations among the characters	49-50
5	Genotypic correlations among the characters	50-51
6	Environmental correlations among the characters	51-52
7	Size of green capsules in twelve parental genotypes of monopodial orchids	70-71

LIST OF PLATES

Plate. No.	Title	Between pages
1	Floral characteristics of twelve parental genotypes of monopodial orchids used in the hybridization programme – single flower	53-54
2	Floral characteristics of twelve parental genotypes of monopodial orchids used in the hybridization programme – inflorescence	54-55
3	Floral biology	55-56
4	Stages of capsule development and incompatibility reactions	67-68
5	*In vitro* embryo culture of hybrid seeds and seedlings after deflasking	76-77

INTRODUCTION

1. INTRODUCTION

Orchids, with their bewildering range of flower colours, beautiful colour combinations, remarkable diversity in size, shape, fragrance and keeping quality and the striking resemblance of their flowers to various forms of animal life, capture the attention and admiration of every one. They undoubtedly form the royalty among ornamental plants and are considered as the embodiment of everything graceful and feminine, noble and refined.

Orchids belong to Orchidaceae, the most diverse family of plants known to man. It is the largest family of angiosperms comprising of over 800 genera and 35,000 species (Singh, 1986). This family accounts for above seven per cent of the species of flowering plants of the world (Pijl and Dodson, 1966). Orchids are worldwide in distribution with greater concentration in tropical and subtropical regions of high humidity.

To the plant scientist, Orchidaceae is a unique family. Fusion of androecium and gynoecium into a single structure–the gynandrium, packaging of pollen existing as tetrads into pollinia to prevent wastage, development of an incredibly large number of ovules in the ovary which require the stimulus of pollination for completing their development and the formation of numerous, dust-like, non-endospermous miniscule seeds which germinate in nature through fungal association are some of their peculiar adaptations. Another important aspect which considerably increases the value of orchids as cutflowers is their longevity, extending from two to three weeks on an average.

Orchids occupy a peak position in the evolution of monocots. In Orchidaceae, evolution was far too rapid for the establishment of an effective genetic hybridization barrier and intermingling of genomes is

possible even at the generic level (Abraham and Vatsala, 1981). This quality of the family has been of the greatest value to man in his horticultural pursuits and is the one very much exploited. The more than 1,00,000 orchid hybrids registered during the past century bear testimony to this fact.

Eventhough India is bestowed with a wealth of indigenous orchid flora, varied agroclimatic conditions, rich manpower and the desired technological advancement, orchid cultivation has not yet gained the attention and popularity that it deserves. Among the various constraints in this direction, the major constraint is the dearth of locally adapted and reasonably priced quality planting material. So we have to initiate orchid breeding and develop our own indigenous hybrid materials which have the adaptability to our agroclimatic conditions as well as novelty and quality enough to compete with international standards. Against this backdrop, the present study was initiated with the objective of developing new monopodial orchid hybrids with commercial cut flower qualities for export market. Many monopodial orchids are very showy, attractive and many have served as parents in hybridization programmes. These orchids have the comparative advantage over sympodials in that they are hardier, demanding lesser attention and care, but their market value is low, because the same old varieties are still cultivated and novelty is lacking. This problem can be addressed by taking up monopodial orchid breeding seriously to produce our own novel and adapted quality hybrids.

The breeding strategy to be followed for producing elite, new hybrids quickly should be by selecting parents with already proven commercial qualities, free-flowering habit and suitability to our climate and by intercrossing them with the aim of transferring and recombining characters of commercial importance. Specific breeding objectives looked for include improving the colour, size, shape and fullness of flowers. Several of the commercial floral attributes such as enhancement in inflorescence size

and in per plant inflorescence production are dependent on the healthy vegetative architecture of the plant. In view of this, twelve monopodial orchid genotypes comprising of popular and adapted monogeneric and bigeneric commercial varieties belonging to the genera *Arachnis, Aranda, Aranthera* and *Vanda* were selected as parents in the present hybridization programme and their vegetative and floral morphology was assessed. These genera with several proven hybrid varieties to their credit, provide ample scope and have immense potential for intra and intergeneric hybridization for the development of new, better adapted and acceptable hybrids for cultivation in our state.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Orchidaceae has attracted plant breeders due to the uninhibited intermingling of genomes, possible within the family. As a result, around one lakh orchid hybrids have been registered over the past one and a half centuries. Inspite of this, orchid cultivation for cut flower export is still in its infancy in Kerala. Eventhough monopodial orchids have the comparative advantage over sympodials because of their longer vase–life, their market value is low, because the same old varieties are still cultivated and novelty is lacking. It is essential to take up monopodial orchid breeding seriously to produce our own novel and adapted quality hybrids that are acceptable in the national and international markets. For developing hybrids, a thorough knowledge on floral biology, pollination, compatibility, *in vitro* seed germination and development, variability and hybrid development are highly essential. Hence an attempt has been made here to briefly review the pertinent literature on these aspects, based on studies conducted elsewhere.

2.1 FLORAL MORPHOLOGY

The details regarding the complex structure of the orchid flower were first cleared by Brown in 1833 and by Darwin in 1862 (Abraham and Vatsala, 1981).

In monopodials, the inflorescence is usually lateral (Abraham and Vatsala, 1981). Orchids bear flowers on an inflorescence which is either a spike as in *Oncidium* and *Phalaenopsis*, a raceme as in *Dendrobium moschatum* or a panicle as in *Cleisostoma racemiferum* or flowers are produced singly as in *Paphiopedilum* (Yadav and Bose, 1989).

Orchid flower exhibits a wide range of variation in its size and colour, but the basic flower pattern is remarkably constant. The

predominant shades are white, yellow, green and purple, occurring in pure form or in every possible combinations (Yadav and Bose, 1989).

Orchid flowers show a number of unique features not commonly found in other angiosperm species. The orchid flower is basically modelled according to the liliaceous pattern, each flower being constituted by 15 members–3 sepals + 3 petals + 6 stamens in 2 whorls of 3 each + 3 carpels (Abraham and Vatsala, 1981). Orchid flowers are zygomorphic, mostly bisexual or rarely unisexual as in *Catasetum*. The orchid flower consists of three sepals, three petals and the column or gynostemium bearing the reproductive parts. The sepals are alike in appearance in species like *Vanda*. But in a few species of *Bulbophyllum, Renanthera, Paphiopedilum* and many species of *Oncidium*, the dorsal sepal is of different size from the laterals. Though narrower, the sepals are coloured like the petals and hence termed 'petaloid' sepals. Of the three petals, two lateral ones are alike and the other one, called the 'lip' or 'labellum', which is the most prominent and reproductive part of the flower. The lip is attached to the base of the column and the colour, size and shape of the lip vary in different genera. The lip may or may not be spurred. The petals are usually more brightly coloured than the sepals, the lip being the brightest (Sheehan and Sheehan, 1979; Abraham and Vatsala, 1981).

The column / gynandrium / gynostemium / gynostegium situated in the centre of the flower, is the unique structure distinguishing the orchids from all other kinds of plants. This is formed by the fusion of male (stamen) and female (pistil) segments of the flower and is the reproductive part of orchid flower. The only fertile anther of the flower is borne on top of the column. In some genera of the *Vanda* tribe, the column is extended below beyond its attachment to the stalk of the flower, into a structure called 'foot'. In such genera, the lateral sepals and the lip are attached to this foot and together they form a sac-like

'mentum' which is an important characteristic of the above genera (Yadav and Bose, 1989; Abraham and Vatsala, 1981).

The pollen in orchids forms compact, waxy masses termed pollinia, which is an important character for orchid taxonomy (Dressler, 1981). Two pollen masses or pollinia are contained in a cavity known as clinandrium and covered by a deciduous operculum (anther cap). The pollinia, occur as two notched pollinia in *Vanda* to four pollinia applied to each other in pairs in *Arachnis, Phalaenopsis, Aerides, Renanthera* and *Angraceum.* In monopodial orchids, only the odd stamen of the outer whorl, situated opposite the labellum, is fertile. The two lateral stamens of the inner whorl form the sides of the clinandrium, while the odd stamen forms the front of the column. In *Cypripediae*, two stamens are fertile while in *Apostasiae*, three stamens are fertile (Abraham and Vatsala, 1981).

Just below the anther, on the ventral surface of the column, is a hollow cavity of sticky and viscid mass known as stigma or stigmatic surface. It is formed by the fusion of the two fertile stigmas of the flower. The partition wall between the stamen and stigma is called rostellum, which prevents self pollination and secretes a viscid substance to hold pollinia till they are mature for disbursal. The ovary is inferior, tricarpellary, one celled with three parietal placentations and consists of numerous minute ovules (Abraham and Vatsala, 1981; Mukherjee, 1990). The ovary has ridges in *Vanda* and is twisted due to a process called resupination (Bose and Bhattacharjee, 1980).

2.2 POLLEN STUDIES

Hybridization programme in orchids depends upon several factors such as pollen viability and germination, pollen production, dissemination and fertility. A brief summary of the salient research findings on these aspects is presented below.

2.2.1 Pollen Morphology

Moore and Webb (1978) reported that the pollen in Orchidaceae are found as polyads. Individual pollen grains of the group are tightly pressed together in such a way that their outlines become angular.

Sheehan and Sheehan (1979) reported that the pollen in Orchidaceae is not powdery as in most angiosperms but agglutinated into masses called pollinia. Depending on genus, two to eight pollinia occur per flower.

Abraham and Vatsala (1981) observed that pollen in Orchidaceae exists as tetrads. They are held together by means of elastic threads of tapetal origin giving rise to the condition termed mealy or granular and is seen in Neottieae and Epidendreae. In monopodials, pollinial tetrads are organised into many granular packets, prolongations of which form the caudicle. This is the situation termed sectile. In Vandeae, the pollen tetrads are collected into firm masses called waxy pollinia. In advanced subtribes like Oncidinae and Sarcanthinae, they have appendages like the stipe and the viscidium.

Johnson and Edwards (2000) reported that cohesive masses of pollen known as pollinia have evolved independently in two plant families *viz.*, Orchidaceae and Asclepiadaceae. Although a single hard pollinium contains more than a million pollen grains, the pollen : ovule ratio in orchids is much lower than in families with powdery pollen. This is sufficient since pollinia ensure the efficient removal of pollen from anther, minimal pollen wastage during transit and the deposition of large pollen loads on stigma to enable fertilization of the large number of ovules in orchid flowers.

2.2.2 Pollen Viability

Zirkle (1937) described a method for assessing the viability of pollen grains by mounting in acetocarmine. The grains which stained well and

looked plump and normal were considered as viable and the unstained shrivelled ones as non-viable.

Nair and Mathew (1986) reported that in *Vanilla*, pollen viability was reduced considerably one day after anthesis. Normal fruit set was noticed following self pollination just prior to the natural opening of the flower.

Devi and Deka (1992) observed that in terrestrial orchids like *Spathoglottis plicata* and *Phaius tankervilliae*, the pollen viability declined gradually after anthesis whereas in the epiphytic ones like *Aerides odoratum* and *Dendrobium amoenum* it showed an improvement, for three days after anthesis, before getting impaired.

Sobhana (2000) reported a low percentage of pollen fertility in *D. chrysanthum*.

Rani (2002) reported a high percentage of pollen fertility in *D.* Candy Stripe x Tomie Drake.

2.3 COMPATIBILITY ANALYSIS

A thorough understanding of the compatibility relationships of the genera under consideration is essential for successful hybrid development.

Many of the cases of apparent self incompatibility and cross sterility commonly encountered among cultivated orchid hybrids can be attributed to one of the two causes, hybrid sterility or polyploidy (Lenz and Wimber, 1959).

Abraham and Vatsala (1981) reported that sterility in polyploids often results from pairing abnormalities during meiosis. Sterility commonly encountered in most of the cultivated orchids is most frequently caused by triploidy.

Johansen (1990) demonstrated a unique incompatibility system in *Dendrobium* which also showed high incompatibility in interspecific pollination in contrast to any other orchid genus. Incompatibility response

was initiated by auxin content in pollinia. The compatibility substance was specifically recognised by the eleutherocytes produced in the stigmatic mucilage.

The failure of fruit development in many reciprocal crosses hints at the operation of a unidirectional incompatibility in orchids (Devi and Deka, 1992).

Devi and Deka (1994) performed 29 interspecific and 47 intergeneric crossings in orchids to determine cross compatibility. Percentage ovary drop after initial swelling was found to be high. Percentage fruit set ranged from 0 to 100 in interspecific and 0 to 75 in intergeneric crosses. Parthenocarpic fruit development without seed set was observed in several cases. Out of the 13 different hybrid capsules obtained, seeds of only three cross combinations germinated.

Chen *et al.* (2000) reported that out of 520 hybridizations conducted with the aim of developing white Taisuco *Phalaenopsis*, only 46.2 per cent cross combinations produced viable seeds.

Melendez-Ackerman and Ackerman (2001) reported self compatibility in *Listera cordata,* as all self pollinations produced fruits. Cross pollinations, however, differed significantly from the selfs, registering higher number of seeds per capsule and higher percentage of fertilized ovules.

Rani (2002) performed a total of 190 self and cross combinations in *Dendrobium* to determine cross compatibility out of which 84 combinations including seven selfs produced harvestable green capsules, the relative success being 44.21 per cent. Progeny from 67 hybrid combinations were established successfully in the greenhouse.

2.4 POLLINATION BIOLOGY

The phenomenon of pollination in orchids has intrigued men ever since they have been in cultivation and still continues to be one of the marvels of nature.

The relationship between orchids and their pollinators is one of the marvels of nature. Pijl and Dodson (1966) summarised all the known information on orchid flower pollination. Pollinators of several orchid species were listed, placing bees as the dominant pollinator. Other pollinators include a variety of insects, including flies, wasps, mosquitoes, moths, butterflies, beetles, ants, spiders and even birds. Northen (1970) reported that pollinator specificity has evolved to such an extend that the mechanisms will work only if an insect of just the right size and shape enters the flower

Abraham and Vatsala (1981) have reviewed the various contrivances by which orchids bring about allogamy. Mimicry is the mechanism in the genus *Ophrys*. In a species of the genus, the lip resembles the female of the pollinator wasp to the minute details including the odour. In another species, the lip resembles the natural prey of the pollinator, the common wasp.

Although orchid flowers have evolved so that cross fertilization is assured in most species, a considerable number of orchid species have been identified to be autogamous (Ridley, 1888;Abraham and Vatsala, 1981).

Arditti (1966) reported that orchids are known to attract pollinators by means of offer of food and smell (good or bad) as well as mimicry of prey, food and antagonists. The pollinator of *Vanda tricolor* may be attracted to the flower by its fragrance and visual light image.

2.4.1 Artificial Pollination and Hybridization

Artificial hybridization in orchids was started much later than in other angiosperm families. The main hurdles were the complexity of their flower structure and the consequent lack of understanding of the method of pollination.

The first man-made orchid hybrid was developed by Lindley in 1852. Evidences of natural crossing between wild members of the family have been observed by him as early as 1853 (Abraham and Vatsala, 1981). The first flowering interspecific hybrid was produced in 1856 by Dominy by crossing *Calanthe furcata* to *Calanthe masuca* and was named *Calanthe* Dominyi (Dressler, 1981).

In hybridization, selection of good and healthy parent plant and flower by visual observation accounts to a great extent. Very young plants or seedlings as well as plants with unhealthy looking canes blooming for the first time should not be selected as mother plant (Bose and Bhattacharjee, 1980).

Warren (1981) described different pollination mechanisms in orchids which varied widely depending on the floral morphology of the genera concerned.

Rhodehamel (1994) observed that cross pollination in monopodial orchids was effected by the deposition of pollinia from one flower into the stigmatic cup of another flower in another plant. Adherence of pollen to stigma is effected by wetness of pollen or stigma.

2.5 POST POLLINATION DEVELOPMENTS

In Orchidaceae, post pollination developments have to be viewed critically as the development of the female gametophyte occurs after pollination. Post pollination floral changes are an indication of the compatibility of a cross. Changes occurring to the orchid flower following pollination have been researched at great length by several workers, a concise account of which is presented below.

Post pollination phenomena in orchids have been studied in detail by Arditti (1979). Working mainly with *Cymbidium* flowers, he reported the effects of ethylene, excised floral segments, water and dry-weight relations, abscissic acid and effects of auxin, kinetin and gibberellic acid

and their interactions. Ethylene causes a number of phenomena in orchid blooms, including anthocyanin formation, fading, shortened flower life and dry sepal. Pollination or emasculation of flowers caused ethylene evolution, in addition to the transformation mentioned, and this can be autocatalytic in *Vanda* flowers (Arditti, 1979; Chadwick *et al.*, 1986).

Emasculation of various floral segments indicated that most post pollination phenomena were controlled by the rostellar- stigmatic region (Arditti, 1966).

Harrison and Arditti (1972) have noted that wilting of sepals, petals and labellum (lip), swelling of the gynostemium (column) which subsequently becomes green and increase in the diameter of ovaries are among the most easily observable post pollination phenomenon in orchid flowers.

From further studies, Strauss and Arditti (1980) concluded that the additional chemical compounds produced by the pollinated flowers or obtained from the pollen were responsible for the changes following pollination.

Yadav and Bose (1989) and Slater (1991) explained in detail the post pollination phenomena in orchids. It was found to include stigmatic closure, swelling and increase in fresh and dry weights of ovaries and gynostemia, hormone production, new biochemical pathways, synthesis and/or destruction of pigments, deresupination, nastic movements, cessation of scent evolution, breaking apart of pollinia due to tetrad dissociation, progressive dehydration of pollen grains and germination of pollen from the outside of pollinium to the inside.

Nadeau *et al.* (1993) observed that the activity of the ACC oxidase which catalyses the conversion of ACC to ethylene increased in the stigma after pollination.

Porat (1994) reported a rapid acceleration of the wilting process following successful pollination in several orchid genera. He also observed that wilting of flowers was accompanied by a loss of moisture from the cells of the upper layer of petals, leading to their upward folding.

2.6 HARVEST AND CULTURE OF GREEN CAPSULE

Green capsule/pod culture was a major advancement in increasing the germination of orchid seeds *in vitro* and reducing the time taken to reach flowering stage. Immature ovules from young capsules of orchids have been cultured *in vitro* in nutrient media to give rise to plants (Withner, 1943 and 1959).

2.6.1 Capsule Maturity

Assessment of the correct maturity stage is a major deciding factor in green capsule culture. Sauleda (1976) found that the pistillate parent was mainly responsible for determining the harvesting time when crosses were made between parents with different harvesting times.

Green capsules of *Paphiopedilum* harvested four months after pollination and that of *Cattleya, Cymbidium, Phalaenopsis* and *Eulophia* harvested eight to nine months after pollination germinated satisfactorily (Rosa and Laneri, 1977).

Nagashima (1982) obtained the highest germination in orchid genera such as *Cymbidium goeringii* and *Paphiopedilum insigne* var. Sanderae, when the green capsules were harvested at 115-120 days and 195-200 days, respectively after pollination.

Yadav and Bose (1989) considered capsules turning yellowish or brownish as a sign of maturity.

Seaton (1994) suggested harvest of seed capsules just a few days prior to the onset of dehiscence, the stage at which seeds will be fully mature and highly viable.

Sharma (1998) performed *in vitro* seed germination using seeds from capsules of *Vanda coerulea* at various stages of development. The germination percentage increased with the capsule age ranging from 180 days to 270 days. The seeds obtained from 270 days old capsules revealed the maximum seed germination in Knudson C medium.

2.6.2 Capsule Culture

Green capsule culture trials conducted on a wide range of orchids led to the conclusion that success is possible only after fertilization had been accomplished (Valmayor and Sagawa, 1967).

Abraham and Vatsala (1981) observed that good seed germination without fungal and bacterial contamination was obtained when the green capsules were harvested earlier.

Arditti *et al.* (1982) could germinate both mature and immature capsules of *Epipactis* asymbiotically. However, seeds from immature capsules germinated well and rapidly.

Experimental results obtained by Mitra (1986) indicate that seeds obtained from unripe capsules germinated readily in several orchid species. Reduced germination at capsule maturity was due to dormancy factors and changes in enzyme compliments. He further suggested that identification of the critical stage at which dormancy sets in would be beneficial.

The technique of green pod culture reduces the time lapse between germination and sowing of seeds, saves them from exposure to sterilizing agents and favours production of large number of seedlings (Pathak *et al.*, 1992).

According to Singh (1993), the difference in harvesting time between the dry seed culture process and the green pod culture process varied by as much as six to eight months. The reduction in harvesting time decreases the time required for flowering.

Results of the studies of Nagashima (1993) using 47 orchid species indicated that germination was the highest in seeds in which embryogenesis was almost complete.

Sharma (1998) observed a decrease in the germination of fully mature *Vanda* seeds with progressive age.

2.7 EMBRYO CULTURE STUDIES

In Orchidaceae, hybrid production is confronted with several hurdles at each step. The nature of orchid seed is one such hurdle. The seeds in orchids are very minute, without a functional endosperm and with specific nutritional requirements which have to be provided *in vitro* in hybrid development, through well- balanced culture media.

2.7.1 Nature of Orchid Seed

The first published description of an orchid seed is by Theophrastus (Salisbury, 1804).

Orchid seeds are unique in several aspects. They are minute, measuring from 0.25 to 1.20 mm in length (Hoene, 1949), 0.090 to 0.270 mm in width (Arditti, 1967) and weighing from 0.30 to 14.0 µg (Harley, 1951). They are produced in large numbers, ranging from 1,300 to 4,000,000 per capsule and the great majority of species have nonendospermous seeds with relatively undifferentiated embryos (Arditti, 1967).

The colour of orchid seeds may be white, cream, pale green, reddish orange or dark brown (Arditti, 1967).

The orchid embryo lies within a testa and consists of 80-100 cells which are relatively undifferentiated and mostly isodiametric with dense, granulated cytoplasm. The embryo is attached to the testa at the posterior end by means of a suspensor, having very large, vacuolated dead cells (Arditti, 1979). Single-celled suspensors were observed in certain species (Muralidhar and Mehta, 1986).

As the orchid seed matures, the suspensor shrinks, followed by changes in the structure and organisation of integuments. Cells of the testa are dead at maturity and are thick in epiphytes and thin in terrestrial orchids (Vijayaraghavan *et al.*, 1986).

Vij *et al.* (1992) identified several seed shapes, cylindrical, elliptical, filamentous, fusiform, ovoid, spathulate and their slight variations for orchids. Fusiform seeds are the most common and are considered basic in orchids. The seed size shows a direct correlation with the plant habit, the seeds being relatively larger in the ground growing taxa than in their epiphytic counterparts.

Cameron and Chase (1998) reported that in vanilloid orchids, seeds vary from being ovoid with sclerotic, multilayered outer integument in *Vanilla* to completely winged in *Galeola, Pseudovanilla* and *Erythroorchis*. Seed morphology easily distinguishes the genera of vanilloid orchids from one another and is clearly a useful tool in addressing phylogenetic relationships within the group.

2.7.2 Seed Germination

Although produced in very large numbers, orchid seeds lack metabolic machinery and functional endosperm, with the result that only 0.2–0.3 per cent seeds germinate in nature, with the association of mycorrhiza (Abraham and Vatsala, 1981).

Burgeff (1959) carried out detailed studies and demonstrated the association of various fungal mycelia with orchid roots at different stages of germination and plant growth.

The process of orchid seed germination, symbiotic or asymbiotic, essentially remains the same and differs from that of any other angiosperm. During germination, the embryo imbibed moisture, enlarged and burst out of the testa as an ovoid, top- shaped protocorm (Arditti *et al.*, 1981).

Mitra (1971) found that the protocorm differentiated into shoot and root meristems in opposite directions. A scale leaf developed first, followed by foliage leaves. Single celled rhizoids developed from the protocorm for absorption. After the two leaf stage, the protocorm and rhizoid lost their nutritive function and real roots were formed endogenously.

2.7.3 Changes During Germination

Ricardo and Alvarez (1971) reported that in the protocorms of *Vanda*, the parenchymal cells accumulated substantial quantities of lipid, protein and carbohydrate reserves, which disappeared gradually with the senescence of the parenchymatous region.

Sangama (1986) was of the opinion that germinating orchid seeds utilised lipids, proteins and carbohydrates in that order.

In the protocorms of *Vanilla planifolia* the cells were heavily laden with proteins and starch grains. Protein bodies disappeared during differentiation of meristem. Bipolar differentiation within the meristem produced the shoot and after formation of a few leaves the first root differentiated endogenously from the base of the meristem (Philip and Nainar, 1988).

Raghavan and Goh (1994) reported that the regulatory events in the embryo prior to seed maturity determined the fate of its proximal and distal parts during germination. Synthesis of DNA and cell division were confined to the proximal end, whereas cells at the distal end underwent enlargement.

2.7.4 Seed Germination and Development *In vitro*

Knudson (1946) showed that the seeds of *Cattleya, Laelia* and *Epidendrum* germinated freely on sugar and mineral containing agar medium under aseptic conditions without fungal association.

Arditti (1979) reported in four orchid genera including *Dendrobium* that only a few apical cells of protocorms divided to form a promeristem which gave rise to shoot apex and structures homologous to cotyledons.

Mathews and Rao (1985) reported that the differentiated protocorms had to be subcultured within a period ranging from 70 to 80 days for proper *in vitro* growth.

Yam and Weatherhead (1988) considered the seeds to have germinated when protocorms, either green or white, were observed in cultures.

Rubulo *et al.* (1989) defined germination as the presence of protocorms with one leaf primordium one month after culture.

Pathak *et al.* (1992) found that in *Goodyera biflora*, the protocorms, on emergence from the testa, were white and hairy. The first signs of chlorophyll development were apparent in leaf initials.

Singh (1992) reported that depending upon their genotype, the seeds develop chlorophyll within 10-20 days on the nutrient medium.

Singh (1993) found that inoculation of seeds into a nutrient medium under *in vitro* conditions not only improves the percentage of germination, but also reduces the time for differentiation of orchid seeds, both biochemically and morphologically.

Krishnan *et al.* (1993) observed visible protocorm formation from the embryos by the second and third weeks of culture in *Spathoglottis plicata*. He also found that the first leaf primordium was initiated between the fifth and sixth weeks of culture.

2.7.5 Effect of Culture Media on Seed Germination

The commonly used nutrient media for orchid seed culture are those proposed by Knudson (1946) (KC), Vacin and Went (1949) (VW),

Murashige and Skoog (1962) (MS), Raghavan and Torrey (1964), Nitsch (1969), Mitra *et al.* (1976) and Rosa and Laneri (1977) (RL).

Sangama (1986) conducted studies on seed germination morphogenesis in *Epidendrum radicans* and *Dendrobium* Jaquelyn Thomas clearly indicated the superiority of MS medium over KC and VW media.

Nagashima (1993) recorded that out of the forty seven orchid species tested, some were found to respond better to hyponex medium whereas others gave better germination and seedling growth in MS medium.

Since MS medium contained higher ionic concentration of nutrient salts, Zhang *et al.* (1993) found that half strength MS could adequately support rapid protocorm proliferation in orchids.

Rani (2002) stated that the basal medium MS half strength was found to be the best for early germination and rapid *in vitro* development as compared to MS quarter strength and MS, KC and VW full strengths.

2.8 GENETIC ANALYSIS AND VARIABILITY STUDIES

Work in these lines is very limited in orchids.

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

Genetic analysis studies were conducted by Sobhana (2000) in *Dendrobium* hybrids. High genetic variability was observed for number of flowers per spike, days for opening of florets and number of shoots per plant. Heritability was of moderate to high magnitude for most of the

characters. Flower size exhibited the highest heritability. Length of inflorescence exhibited high positive correlation with height of shoots and number of leaves.

Rani (2002) conducted genetic analysis studies in *Dendrobium* hybrids. Variability studies indicated high GCV and PCV for the characters number of nodes per cane, leaf area per cane, length of inflorescence, length of scape and number of flowers per inflorescence. Significant positive inter-correlation in all pair wise combinations at genotypic and phenotypic levels was observed between the seven characters *viz.*, number of leaves per clump, height of cane, leaf area per cane, age at first flowering, cane to flower first, vase life and length of inflorescence.

2.9 BREEDING OF HYBRIDS

The first flowering interspecific hybrid developed in 1852 by Dominy, by crossing *Calanthe masuca* to *Calanthe furcata* was termed *Calanthe* Dominyi and it flowered for the first time in 1856. In 1863, he developed the first intergeneric hybrid *Laeliocattleya* Exoniensis, crossing *Cattleya mossiae* with *Laelia crispa* (Dressler, 1981).

Evidences of natural hybridization occurring among wild members of the family have been noticed by Lindley as early as 1853 (Abraham and Vatsala, 1981).

Important orchid genera like *Cattleya, Cymbidium, Odontoglossum, Vanda, Dendrobium, Phalaenopsis* etc. undergone various interspecific and intraspecific combinations to yield improved hybrids (Abraham and Vatsala, 1981).

The first recognised *Vanda* hybrid, *Vanda* Miss Joaquim, flowered in the garden of Miss Agnes Joaquim of Singapore in 1893, was a cross between two terete-leaved species, *V. hookeriana* and *V. teres* (Abraham and Vatsala, 1981).

Many Indian species have earned world-wide recognition as parents in breeding programmes due to their inherent attractiveness coupled with their ability to transmit these characters to the hybrids. Some of the leading species are *Aerides multiflorum, Cymbidium devonianum, C. lowianum, C. traceanum, C. elagans, Dendrobium aggregatum, D. chrysotoxum, D. formosum, D. nobile, Paphiopedilum ventusum, Vanda coerulea* etc. (Bose and Bhattacharjee, 1980).

Singh (1986) has described the *Dendrobium* hybrids IIHR 38 (*D.* Pompadour x *D. superbiens*) and the *Vanda* hybrid IIHR 164 (*V. rothschildiana* x *V. coerulea)*

Philips (1986) while breeding with *Paphiopedilum rothschildianum* found that the species can add desirable floral qualities to the hybrid, while allowing the best of the other parent to be expressed.

Atwood (1989) identified a natural hybrid in *Paphiopedilum* and observed that barriers to hybridization breakdown occasionally, even among remotely related genera. The flowers are more massive than either parent, probably due to hybrid vigour. The hybrid also possesses several other attributes not observed in either parent.

Luer and Escobar (1989) identified natural hybrids within the genus *Dracula* in a cultivated collection. Ten natural hybrids of horticultural potential were described.

Porter (1989) reported that many strains of the primary hybrid *Paphiopedilum* M. Pearman (*P. bellatulum* x *P. delenatti*) have been developed and they have produced flowers larger than either parent with very full bloom, white colour and beautiful raspberry stippling.

Wallbrunn (1989) concluded that reciprocal hybridization within the hybrids yielded progeny with remarkable variation in flower characters.

McDonald (1991) has pointed out that any hybridization programme in orchids aims at increase in terms of flower size, flower number and spike length as well as improvement in flower quality and extended flowering season. Vegetative vigour of hybrids is important as it results in bigger, better blooms and more floriferous hybrids with greater flower substance.

Behar (1993) succeeded in combining the distinctive inflorescence shape of the miniature species *Lepanthopsis floripecten* with the bright red flower colour and better flower shape of *Lepenthes cochlearifolia* in the hybrid which flowered *in vitro.*

Wing (1993) pointed out the positive and negative attributes of *Vanda* Miss Joaquim and evaluated its role in hybridization, as it has been used extensively for developing exciting new vandas. *V.* Miss Joaquim featured as a parent in a mass hybridization programme generated a total of 83 first- generation, 152 second- generation, 108 third- generation, 31 fourth- generation, five fifth- generation and one sixth- generation hybrids by the end of 1990.

Davidson (1994) conducted intergroup hybridization between phalaenopsis type dendrobiums and those in section spathulata. The objective was to extend the flowering season, to expand the range of flower colours and shapes and to increase flowering in phalaenopsis type dendrobiums. Some outstanding intermediate hybrids with good flowering characteristics were obtained.

Nash (1995) presented historical information on the breeding of hybrid cattleyas, including the importance of *Brassovola* (*Rhyncolaelia*) *digbyana* as parental species.

Chen *et al.* (1995) conducted an extensive varietal improvement programme in *Phalaenopsis* using 29 wild species and 873 varieties. They succeeded in releasing 35 new hybrid varieties.

The details of selective hybridization that resulted in the development of *Vanda* Motes Gold Flake were published by Motes (1995).

Fuchs (1997) discussed the hybridizing trends of intergeneric vanda hybrids. He reported that *Vanda sanderiana* and *V. coerulea* are two important *Vanda* species found in the background of most of the vandaceous hybrids. *V. sanderiana* gives full form, whereas *V. coerulea* imparts rich blue-violet colour and lovely tessellations and also elongates the inflorescence of vandaceous hybrids.

He also pointed that a *Vanda* crossed with an *Ascocentrum* will make an *Ascocenda,* which is an intergeneric hybrid. He further observed that *Vanda* hybridize frequently with *Ascocenda. Aerides* when crossed with *Vanda* produces the man made genus '*Aeridovanda*'.

Mercy and Dale (1997) reported that majority of the commercially grown orchids today are hybrids derived from *Arachnis, Vanda, Renanthera, Ascocentrum, Cymbidium, Cattleya, Dendrobium, Oncidium, Phalaenopsis* and *Paphiopedilum.* They also observed that when species of extremely different flower sizes are crossed, the hybrid does not reach the mid point or average of the two parents but exhibits a size closer to their geometric means. When the flower sizes of the two parents are closer to each other, this difference is not apparent.

Sharma *et al.* (1998) evaluated the possibility of sustainable commercial exploitation of orchids and observed that several wild species possess blossom characters good enough to compete with the best hybrids and can be used as breeding material for the production of attractive, novel varieties.

Chen *et al.* (2000) observed the breeding behaviour of *Phalaenopsis equestris* and reported that hybrids with compact, multiple branched, inflorescence grew faster compared to those with larger flowers. They further reported that during the first ten years of the breeding

programme till 1998, a total of 30 hybrids could be registered with the Royal Horticulural Society.

Motes (2001) while hybridizing with lesser known vandas, commented on the propensity of *Vanda denisoniana* to confer full form on its progeny. making it a preeminent species for breeding successful hybrids.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigations were carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 2001-2003. The study formed a part of the DBT project entitled "Breeding for commercial orchid hybrids" (2002-2005) and was supported by a junior research fellowship. The parent material selected for the study was maintained in the greenhouse of the DBT project where the hybridization and compatibility studies were conducted. Green capsule (immature embryo) culture was done in the Plant Molecular Biology and Biotechnology Centre of the college.

The experiment consisted of the following major studies:

1. Evaluation of parent material
2. Hybridization and compatibility studies
3. Green capsule (immature embryo) culture of hybrid seeds

3.1 EVALUATION OF PARENT MATERIAL

3.1.1 Experimental Material

The experimental material consisted of twelve monopodial orchid varieties including six monogeneric and six bigeneric hybrids.

Monogeneric Hybrids

		Hereinafter referred to as
1	*Arachnis* Maggie Oei Red Ribbon	P_1
2	*Arachnis* Maggie Oei Yellow Ribbon	P_2
3	*Arachnis* Kapama	P_3

4	*Vanda* Miss Joaquim	P_4
5	*Vanda* Popoe Diana	P_5
6.	*Vanda* TMA Mandai	P_6

Bigeneric Hybrids

(i) ***Arachnis* x *Vanda***

7	*Aranda* Peg Lee You	P_7
8	*Aranda* Eric Mekie	P_8
9	*Aranda* Golden Sands	P_9

(ii) ***Arachnis* x *Renanthera***

10	*Aranthera* James Storei	P_{10}
11	*Aranthera* Mohammed Haniff	P_{11}
12	*Aranthera* Annie Black	P_{12}

The sources of the hybrid varieties used in the experiment are furnished in Table 3.1.1.

Statistical Details

Design	:	Completely Randomised Design (CRD)
Replications	:	6
Treatments	:	12
Plot size	:	Single plant

Planting material of the twelve monopodial orchid hybrids used in the study was terminal cuttings of the mature stem. Planting of parent material was done in rows on the ground in raised beds using dried coconut husk, brick pieces and coir pith as bedding materials. Crop management practices were carried out according to the package of practices recommendations of Kerala Agricultural University (KAU, 1997).

Table 3.1.1 Source of twelve parental genotypes of monopodial orchids

Parental genotype	Source of material	Type of material
1. *Arachnis* Maggie Oei Red Ribbon 2. *Arachnis* Maggie Oei Yellow Ribbon 3. *Arachnis* Kapama 4. *Vanda* Miss Joaquim 5. *Vanda* Popoe Diana	From the greenhouse, Department of Plant Breeding & Genetics, College of Agriculture, Vellayani.	Terminal cuttings
6. *Vanda* TMA Mandai 7. *Aranda* Peg Lee You 8.*Aranda* Eric Mekie 9. *Aranda* Golden Sands 10. *Aranthera* James Storei 11. *Aranthera* Mohammed Haniff 12. *Aranthera* Annie Black	From Seaside Farms, Puthenthoppe, Thiruvananthapuram	Terminal cuttings

3.1.2 Experimental Methods

Observations on vegetative and floral characters (both quantitative and qualitative) were recorded on the parent material.

Vegetative Characters

1. Length of Cane (cm)

The height of each mature cane was measured from the base to the tip of the cane.

2. Number of Leaves per Cane

Total number of laminate leaves per cane was recorded at maximum leaf stand.

3. Number of Aerial Roots

Total number of aerial roots per mature cane was recorded.

4. Length of Aerial Roots (cm)

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

5. Thickness of Stem (cm)

Thickness of stem was measured at the widest point using vernier calipers and was recorded.

6. Length of Internode (cm)

Distance between two consecutive leaves *i.e.*, internode was measured for the seventh, eighth and ninth internodes and was recorded.

7. Length of Leaf (cm)

Length of mature leaves was measured from the base to the tip and the value was recorded.

8. Width of Leaf (cm)

Width of mature leaves was measured at the widest region and the value was recorded.

9. Thickness of Leaf (cm)

Thickness of leaf was measured at the widest region on the lamina at a point equidistant from the margin and the midrib using vernier calipers and was recorded.

10. Leaf Area (cm^2)

Leaf area was measured graphically from 100 leaf samples representing the entire parent material. Since destructive sampling was not advisable and area measurement of standing leaves was cumbersome, a linear regression relationship of the form,

$y = a + bx$ was arrived at, where

y = leaf area (cm^2)

x = product of maximum length (cm) and breadth (cm) of leaf

a = constant, which is the y-intercept

b = regression coefficient of y on x

Floral Characters

Floral biology of the selected twelve monopodial orchid genotypes was studied and the following observations were recorded :

1. Flowering Nature – Free- Flowering / Seasonal

Flowering time was recorded and noted as free-flowering *i.e.*, flowering all round the year or seasonal *i.e.*, flowering at specific seasons.

2. Days to First Flower Opening from Inflorescence Emergence

Number of days from visible emergence of inflorescence to the opening of the first flower was counted and recorded.

3. Days to Last Flower Opening from First Flower Opening

Number of days from first flower opening to the opening of the last flower in the inflorescence was counted and recorded.

4. Number of Spikes per Cane

Total number of spikes produced per cane during the period was recorded.

5. Nature of Inflorescence Axis

Nature of inflorescence axis was recorded as :

a) erect – inflorescence axis held erect (0-30°)

b) arching – inflorescence axis held at an angle of 30-60°

6. Mode of Display of Flowers

Based on orientation of individual flowers, mode of display of flowers was recorded as given below.

a) Flowers arranged alternately on either sides of the inflorescence axis facing the opposite side so that flowers appear in two parallel rows.

b) Flowers arranged all around the peduncle so that inflorescence shows a bunched appearance.

7. Length of Inflorescence (cm)

The length of inflorescence was measured from the base of a fully opened inflorescence to the tip of the axis and recorded.

8. Length of Scape (cm)

The distance from the base of a fully opened inflorescence to the first flower was measured.

9. Diameter of Inflorescence Axis (cm)

Diameter of inflorescence axis was measured at the widest region using vernier calipers and recorded.

10. Number of Flowers per Inflorescence

Total number of flowers produced per inflorescence was counted and recorded.

11. Length of Internode of Inflorescence (cm)

Distance between two consecutive flowers i.e., internode was measured for the entire inflorescence and the mean was computed.

12. Length of Flower (cm)

Flower length was measured at the widest region from the tip of the labellum to the tip of the odd sepal and recorded.

13. Width of Flower (cm)

Flower width was measured at the widest region and the value was recorded.

14. Fullness Value

Fullness value of flower was calculated using the following formula developed by Leonhardt (1977).

$$F = \frac{W}{\frac{2S+2P+L+DS}{6}}$$

Where

F = Fullness value

W = Width of flower

S = Width of lateral sepal

P = Width of lateral petal

L = Width of labellum

DS = Width of dorsal sepal

15. Shape of Flower and Perianth Lobes

Shape of flower was noted and recorded as follows:

a. Full and rounded *vs.* narrow and horned

b. Flat surfaced *vs.* tips of sepals and petals incurved

c. Broad sepals and petals *vs.* narrow, spatulate sepals and petals

16. Vase Life (days)

Vaselife of the cut inflorescence in water as the holding solution was recorded. Fading of the first flower was recorded as the end of vase life.

17. Pollen Size (μ)

From the fully opened orchid flowers, pollinia were collected and pollen grains were stained in 1:1 glycerine-acetocarmine solution (2%). Diameter of ten normal-shaped and well-stained pollen grains was measured at random using a standard occular micrometer after calibrating the occular division under the high power (10 × 40x) of a microscope. The mean diameter was recorded in microns.

18. Pollen Fertility (%)

Acetocarmine staining technique was employed for studying pollen fertility. Pollen fertility was estimated by counting fertile and sterile pollen grains separately in the microscope field from a smear under the low power (10 × 10x) of a microscope. Pollen grains which were well-stained, normal-shaped and plumpy were considered as fertile. Unstained, small or shrivelled pollen grains were considered as sterile (Zirkle, 1937). Three slides were prepared and five random fields from each slide were observed in each variety and species. Fertility of pollen grains was expressed as percentage of the total number observed which was not less than 300 tetrads per treatment.

3.1.3 Statistical Analysis

The collected data were subjected to the analysis of variance to test for significant difference among the various orchid genera selected, following Panse and Sukhatme (1967).

3.2 HYBRIDIZATION AND COMPATIBILITY STUDIES

Compatibility analysis was done in detail by conducting all possible self and cross combinations including reciprocals. Self-compatibility was assessed in the varieties by using the pollen of the same plant. The pollinated flowers were tagged for identification.

3.2.1 Observations

1. Post Pollination Floral Changes (days)

Time required for the following post pollination floral changes was recorded:

i. Drooping of perianth

ii. Closure of stigma by overgrowth of column tip

iii. Covering of stigma by wilted sepals and petals

iv. Complete drying of sepals and petals

2. Stages of Capsule Development (days)

Stages of capsule development till harvest were sequenced and recorded with respect to the following changes.

i. Greening of ovary with slight swelling

ii. Swelling of ovary into capsule

iii. Prominent ribbing of capsule

iv. Slight flattening of capsule rib

v. Bursting of capsule beginning from tip

3. Days to Green Capsule Harvest in Successful Crosses

Number of days from pollination to green capsule harvest in compatible crosses was noted and recorded.

4. Length of Capsule (cm)

Length of capsule from base to tip was noted and recorded.

5. Width of Capsule (cm)

Width of capsule at the widest region was noted and recorded.

6. Percentage of Capsule Set

Number of green capsules harvested to total number of pollinations made was recorded and the percentage was calculated.

7. Percentage of Capsules with Germinating Seeds

Number of capsules with seeds that germinated on inoculation to the total number of capsules harvested was computed.

8. Percentage of Filled Seeds

Number of seeds with well-developed embryos to total number of seeds was worked out by scoring under the low power (10 × 10x) of a microscope.

3.3 EMBRYO CULTURE OF HYBRID SEEDS

The capsules from all successful parental combinations harvested at the green capsule stage formed the experimental material.

The following observations were recorded for seed germination and seedling growth at appropriate stages from seed inoculation in MS half strength basal medium to deflasking.

3.3.1 Observations on Rate of Growth

1. Days for Initiation of Germination

Number of days from inoculation to swelling and glistening of embryos *in vitro*, prior to protocorm formation was recorded.

2. Days for Protocorm Development

Number of days from inoculation to protocorm development was noted.

3. Days for Greening of Protocorms

Number of days from inoculation to pigment synthesis in germinating embryos/ protocorms was recorded.

4. Days for First Leaf Initiation

Number of days from inoculation to the visible emergence of leaf was recorded.

5. Days for Shoot Initiation

Number of days from inoculation to the visible differentiation of shoot was recorded.

6. Days for First Root Initiation

Number of days from inoculation to the development of first root initial was recorded.

7. Days for Deflasking

Number of days from inoculation to deflasking was recorded.

3.3.2 Observations on Seedling Morphology at Deflasking

The hybrid seedlings of the different parental combinations were deflasked at 2-3 leaves and 2-4 roots stage. Growth measurements were made at deflasking.

1. Height of Seedling (cm)

Height of seedling was measured using graph paper.

2. Number of Leaves

Number of leaves produced per seedling was counted.

3. Length of the Longest Leaf (cm)

Length of the longest leaf from base to tip was measured using graph paper.

4. Breadth of the Longest Leaf (cm)

Breadth of the longest leaf was measured at its widest region using graph paper.

5. Number of Roots

Number of roots per seedling was counted.

6. Length of the Longest Root (cm)

Length of the longest root was recorded with the aid of graph paper.

7. Diameter of the Longest Root (cm)

Diameter of the longest root was recorded using graph paper.

RESULTS

4. RESULTS

The results of the investigations on 'Compatibility studies in monopodial orchids' carried out are presented below :

4.1 Evaluation of parent material

4.1.1 Comparison of performance of parents

4.1.2 Estimation of genetic parameters of parents

4.1.3 Floral biology of parents

4.2 Hybridization and compatibility / incompatibility studies

4.3 Embryo culture of hybrid seeds

4.1 EVALUATION OF PARENT MATERIAL

Twelve monopidial orchid genotypes comprising of six monogeneric and six bigeneric hybrids belonging to the genera *Arachnis, Aranda, Aranthera* and *Vanda* were evaluated in the greenhouse, each being replicated six times.

4.1.1 Comparison of Performance of Parents Based on Quantitative Characters

The mean performance of the parental genotypes for vegetative and floral characters are presented in Tables 4.1.1 and 4.1.2. A wide range of variation was noticed for all the characters among the parental genotypes.

1. Length of Cane

Length of cane was seen highest for P_6 (116.20 cm) which was on par with P_2 (112.63 cm), P_3 (111.57 cm) and P_4 (105.77 cm). The mean

cane length was recorded lowest for P_7 (52.13 cm) which was on par with P_8 (60.63 cm).

2. Number of Leaves per Cane

The maximum number of leaves per cane was recorded for P_2 (45.17) and the minimum was for P_{11} (22.83) which was found to be on par with several of the remaining parental genotypes such as P_4 (23.33), P_{10} (24.33), P_7 (24.83), P_5 (26.67) and P_6 (27.33).

3. Number of Aerial Roots

The number of aerial roots was observed to be the highest in P_6 (17.00). The lowest number of aerial roots was recorded in P_{12} (3.67) which was on par with P_8 (5.17), P_9 (4.33), P_{10} (3.83) and P_{11} (4.33).

4. Length of Aerial Root

The mean aerial root length was significantly high in P_5 (60.18 cm) which was on par with P_6 (57.23 cm), P_4 (54.95 cm) and P_{10} (53.20 cm). The lowest mean aerial root length was recorded in P_1 (32.65 cm) which was on par with some of the remaining parental genotypes such as P_9, P_2, P_3, P_8 and P_{12}.

5. Thickness of Stem

The mean thickness of stem was recorded to be the maximum in P_{10} (1.75 cm) and the minimum was for P_{12} (0.48 cm).

6. Length of Internode

The length of internode was observed to be the highest in P_6 (4.06 cm) which was on par with P_4 (4.03 cm) and P_5 (3.87 cm). The lowest mean internodal length was recorded in P_1 (1.95 cm) which was on par with several of the remaining parental genotypes such as P_{10}, P_2, P_9, P_{12}, P_7, P_{11} and P_8.

Table 4.1.1 Mean performance of twelve parental genotypes of monopodial orchids for quantitative vegetative characters

Parental genotypes	Length of cane (cm)	No. of leaves per cane	No. of aerial roots	Length of aerial root (cm)	Thickness of stem (cm)	Length of internode (cm)	Length of leaf (cm)	Width of leaf (cm)	Thickness of leaf (cm)	Leaf area (cm^2)
P_1	93.25	35.00	7.67	32.65	1.14	1.95	11.25	2.63	0.20	23.54
P_2	112.63	45.17	9.17	34.23	1.13	2.24	10.77	2.55	0.22	22.04
P_3	111.57	33.00	6.17	34.93	1.06	2.91	11.15	3.08	0.19	25.07
P_4	105.77	23.33	14.33	54.95	0.70	4.03	9.03	0.53	0.10	5.84
P_5	101.20	26.67	14.67	60.18	0.72	3.87	8.13	0.48	0.10	5.29
P_6	116.20	27.33	17.00	57.23	0.74	4.06	13.45	0.52	0.11	7.20
P_7	52.13	24.83	7.17	51.52	1.51	2.50	14.60	4.03	0.16	43.47
P_8	60.63	28.17	5.17	36.38	1.28	2.62	14.55	2.88	0.20	32.14
P_9	75.47	28.83	4.33	33.92	1.02	2.25	12.15	3.02	0.15	28.44
P_{10}	77.02	24.33	3.83	53.20	1.75	2.18	10.90	3.07	0.19	26.24
P_{11}	68.43	22.83	4.33	47.50	0.83	2.51	10.93	2.62	0.22	22.83
P_{12}	89.37	28.17	3.67	37.60	0.48	2.32	9.30	2.30	0.18	17.79
SEm	4.441	1.640	0.820	2.871	0.058	0.282	0.478	0.125	0.010	1.665
CD (0.05)	12.561	4.639	2.320	8.121	0.165	0.797	1.353	0.354	0.027	4.709

7. Length of Leaf

The mean leaf length was recorded to be the highest in P_7 (14.60 cm) which was on par with P_8 (14.55 cm) and P_6 (13.45 cm). The lowest mean leaf length was recorded in P_5 (8.13 cm) which was found to be on par with P_4 (9.03 cm) and P_{12} (9.30 cm).

8. Width of Leaf

The width of leaf recorded was maximum in P_7 (4.03 cm) and the minimum was for P_5 (0.48 cm), P_6 (0.52 cm) and P_4 (0.53 cm) which were on par with each other.

9. Thickness of Leaf

Thickness of leaf ranged from a significantly high value of 0.22 cm in P_2 and P_{11} to 0.10 cm in P_4 and P_5. Not much variation with respect to this character was observed for the other genotypes, recording mean values ranging from 0.11 cm in P_6 to 0.20 cm in P_1 and P_8.

10. Leaf Area

The leaf area was observed to be significantly high in P_7 (43.47 cm^2). The lowest mean leaf area was seen in P_5 (5.29 cm^2) which was on par with P_4 (5.84 cm^2) and P_6 (7.20 cm^2).

11. Days to First Flower Opening from Inflorescence Emergence

The parent P_3 exhibited maximum time interval from inflorescence emergence to first flower opening (43.50 days) followed by P_2 (41.83 days). The shortest time interval was recorded in P_5 (28.00 days) which was on par with P_6 (28.17 days) and P_4 (29.50 days).

12. Days to Last Flower Opening from First Flower Opening

The time interval from the opening of the first flower to the last in the inflorescence was recorded high in P_2 (19.17 days) which was on par

with P_1 (17.33 days) and low in P_5 (7.67 days) which was on par with P_6 (8.00 days) and P_4 (8.67 days).

13. Number of Spikes per Cane

The mean total number of spikes per cane was recorded maximum in P_2 (12.50) followed by P_1 (9.83). The lowest mean number of spikes per cane was recorded in P_{11} (4.50).

14. Length of Inflorescence

Highest length of inflorescence was recorded in P_{10} (62.17 cm) which was on par with P_{11} (58.37 cm). Lowest mean length of inflorescence was observed in P_9 (25.67 cm) which was on par with P_3 (28.35 cm) and P_5 (28.63 cm).

15. Length of Scape

The mean length of scape was observed to be the highest in the parent P_{11} (24.00 cm) and lowest in the parent P_3 (7.57 cm).

16. Diameter of Inflorescence Axis

The diameter of inflorescence axis was recorded high in P_8 (0.78 cm). The inflorescence axis diameter was recorded low in P_{11} (0.39 cm), P_9 (0.40 cm) and P_{12} (0.44 cm) which were on par with each other.

17. Number of Flowers per Inflorescence

The parent P_{10} produced 18.17 flowers per inflorescence which was significantly high. The lowest mean number of flowers per inflorescence was expressed in P_4 and P_6 (4.50).

18. Length of Internode of Inflorescence

Highest length of internode of inflorescence was observed in P_1 (4.24 cm) which was on par with P_{11} (4.09 cm) and P_2 (3.65 cm). Lowest internodal length was observed in P_9 (2.04 cm) which was on par with P_{12} (2.13 cm), P_4 (2.58 cm), P_{10} (2.61 cm) and P_5 (2.77 cm).

Table 4.1.2 Mean performance of twelve parental genotypes of monopodial orchids for quantitative floral characters

Parental genotypes	Days to first flower opening from inflorescence emergence	Days to last flower opening from first flower opening	No. of spikes per cane	Length of inflorescence (cm)	Length of scape (cm)	Diameter of inflorescence axis (cm)	No. of flowers per inflorescence	Length of internode (cm)	Length of flower (cm)	Width of flower (cm)	Vase life (days)
P_1	40.50	17.33	9.83	50.90	17.53	0.52	8.00	4.24	8.08	7.12	17.83
P_2	41.83	19.17	12.50	46.22	16.55	0.53	8.17	3.65	8.27	7.63	16.83
P_3	43.50	14.83	5.33	28.35	7.57	0.47	7.00	3.11	6.22	6.05	20.17
P_4	29.50	8.67	5.33	31.18	20.25	0.53	4.50	2.58	7.12	6.57	6.50
P_5	28.00	7.67	7.00	28.63	14.55	0.54	5.17	2.77	6.45	6.02	6.67
P_6	28.17	8.00	6.00	32.68	19.95	0.43	4.50	3.00	7.08	7.00	5.67
P_7	36.67	13.00	6.83	33.58	12.37	0.49	7.17	3.32	5.85	6.63	14.67
P_8	34.67	15.67	5.67	36.88	19.02	0.78	6.00	3.03	7.47	6.28	13.67
P_9	34.17	12.17	6.33	25.67	12.22	0.40	6.67	2.04	4.52	5.32	11.83
P_{10}	36.50	14.00	5.67	62.17	17.33	0.51	18.17	2.61	6.78	5.23	13.00
P_{11}	39.50	14.33	4.50	58.37	24.00	0.39	8.50	4.09	7.05	6.13	13.00
P_{12}	37.83	12.83	5.67	46.47	16.20	0.44	14.33	2.13	6.48	5.15	14.00
SEm	1.301	1.046	1.004	1.510	1.032	0.019	0.664	0.266	0.096	0.081	1.101
CD (0.05)	3.680	2.959	2.839	4.272	2.919	0.051	1.877	0.753	0.273	0.230	3.114

19. Length of Flower

Length of flower ranged from 4.52 cm in P_9 to 8.27 cm in P_2. Significantly long flowers, second only to P_2 were observed in P_1 (8.08 cm).

20. Width of Flower

The width of flower was recorded maximum in P_2 (7.63 cm) and minimum in P_{12} (5.15 cm).

21. Vase Life

Highest vase life was recorded in P_3 (20.17 days). It was significantly high in P_1 (17.83 days) and P_2 (16.83 days) also. Lowest vase life of 5.67 days was observed in P_6.

4.1.2 Estimation of Genetic Parameters of Parents

Genetic parameters were estimated for the parents under the following heads:

4.1.2.1 Variability studies

4.1.2.2 Heritability and genetic advance

4.1.2.3 Correlation analysis

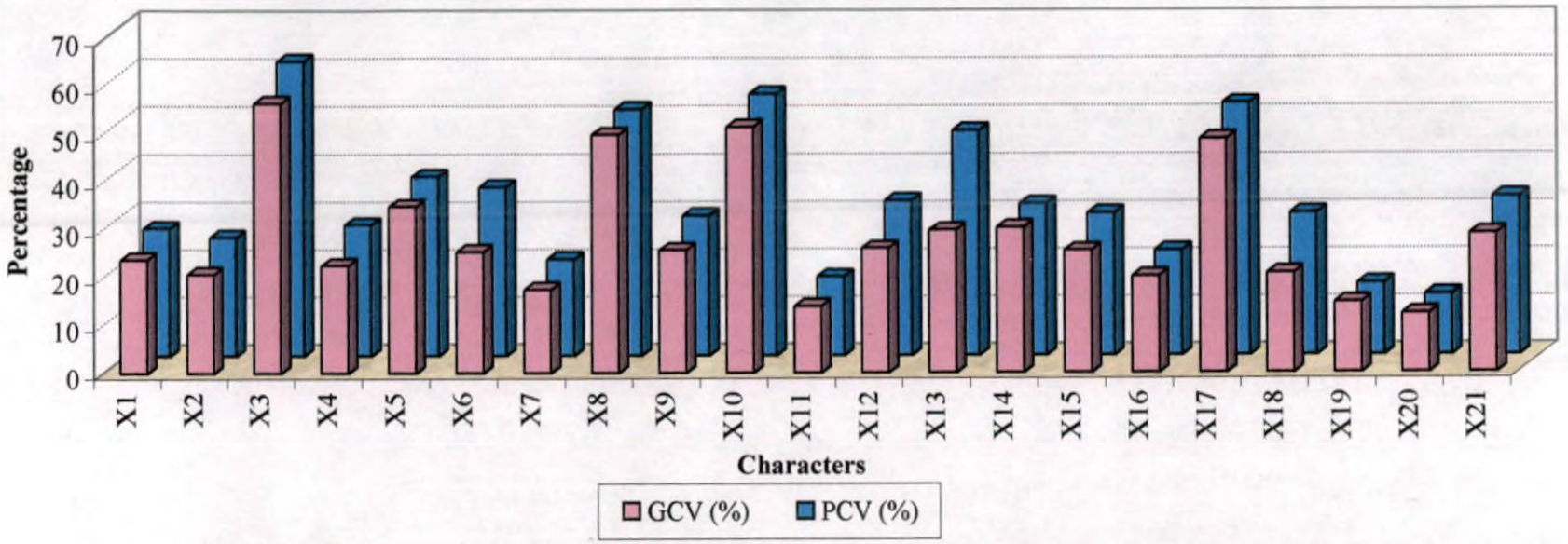
4.1.2.1 Variability Studies

The genotypic, phenotypic and environmental variances and coefficients of variation at genotypic and phenotypic levels were studied in monopodial orchids (Table 4.1.2.1 and Fig. 1).

The characters number of aerial roots (GCV= 56.83 %, PCV= 61.98 %), leaf area (GCV= 51.74 %, PCV= 55.06 %) and width of leaf (GCV= 50.16 %, PCV= 51.88 %) in the descending order exhibited the highest estimates of both genotypic and phenotypic variance.

Table 4.1.24 Variability parameters for morphological characters in twelve parental genotypes of monopodial orchids

Sl. No.	Morphological characters	Genotypic variance σ^2g	Phenotypic variance σ^2P	Environmental variance σ^2e	Genotypic coefficient of variation GCV (%)	Phenotypic coefficient of variation PCV (%)
1	Length of cane (cm)	450.072	568.414	118.342	23.93	26.90
2	Number of leaves per cane	36.607	52.746	16.139	20.88	25.07
3	Number of aerial roots	21.323	25.359	4.036	56.83	61.98
4	Length of aerial roots (cm)	102.127	151.587	49.460	22.70	27.65
5	Thickness of stem (cm)	0.130	0.151	0.021	35.14	37.79
6	Length of internode (cm)	0.506	0.983	0.477	25.55	35.60
7	Length of leaf (cm)	4.002	5.375	1.373	17.62	20.42
8	Width of leaf (cm)	1.342	1.436	0.094	50.16	51.88
9	Thickness of leaf (cm)	0.002	0.002	0.000	25.89	29.50
10	Leaf area (cm^2)	125.547	142.180	16.633	51.74	55.06
11	Days to first flower opening from inflorescence emergence	25.531	35.690	10.159	14.07	16.64
12	Days to last flower opening from first flower opening	11.885	18.451	6.566	26.24	32.69
13	Number of spikes per cane	4.080	10.125	6.045	30.05	47.33
14	Length of inflorescence (cm)	150.481	164.170	13.689	30.60	31.96
15	Length of scape (cm)	17.974	24.363	6.389	25.75	29.99
16	Diameter of inflorescence axis (cm)	0.010	0.012	0.002	20.39	22.18
17	Number of flowers per inflorescence	16.229	18.870	2.641	49.24	53.10
18	Length of internode of inflorescence(cm)	0.409	0.834	0.425	21.00	29.98
19	Length of flower (cm)	1.005	1.061	0.056	14.79	15.19
20	Width of flower (cm)	0.602	0.642	0.040	12.39	12.80
21	Vase life (days)	23.805	31.080	7.275	29.25	33.42



X1 Length of cane (cm)
X2 Number of leaves per cane
X3 Number of aerial roots
X4 Length of aerial roots (cm)
X5Thickness of stem (cm)
X6 Length of internode (cm)
X7 Length of leaf (cm)
X8 Width of leaf (cm)
X9 Thickness of leaf (cm)
X10 Leaf area (cm^2)
X11 Days to first flower opening from inflorescence emergence
X12 Days to last flower opening from first flower opening
X13 Number of spikes per cane
X14 Length of inflorescence (cm)
X15 Length of scape (cm)
X16 Diameter of inflorescence axis (cm)
X17 Number of flowers per inflorescence
X18 Length of internode (cm)
X19 Length of flower (cm)
X20 Width of flower (cm)
X21 Vase life (days)

Fig. 1 GCV and PCV for twenty one traits in twelve parental genotypes of monopodial orchids

4.1.2.2 Heritability and Genetic Advance

Estimates of heritability in the broad sense and genetic advance in the parental material were recorded (Table 4.1.2.2 , Fig. 2 and 3).

Heritability per cent was categorised as suggested by Allard (1960) *viz.*, low (<30), moderate (30-70) and high (>70). Accordingly, vegetative characters such as length of cane, number of aerial roots, thickness of stem, length of leaf, width of leaf, thickness of leaf and leaf area exhibited high heritability while the rest of the vegetative characters exhibited moderate heritability. Among floral characters, days to last flower opening from first flower opening, number of spikes per cane and length of internode exhibited moderate heritability while all the other floral characters studied showed high heritability.

Genetic advance in the range of 30-70 per cent was exhibited by majority of the characters considered.

High heritability (>70 %) combined with genetic advance greater than 70 per cent was observed for number of aerial roots, width of leaf, leaf area and number of flowers per inflorescence.

High heritability (>70 %) combined with 30-70 per cent genetic advance was observed for length of cane, thickness of stem, length of leaf, thickness of leaf, length of inflorescence, length of scape, diameter of inflorescence axis and vase life.

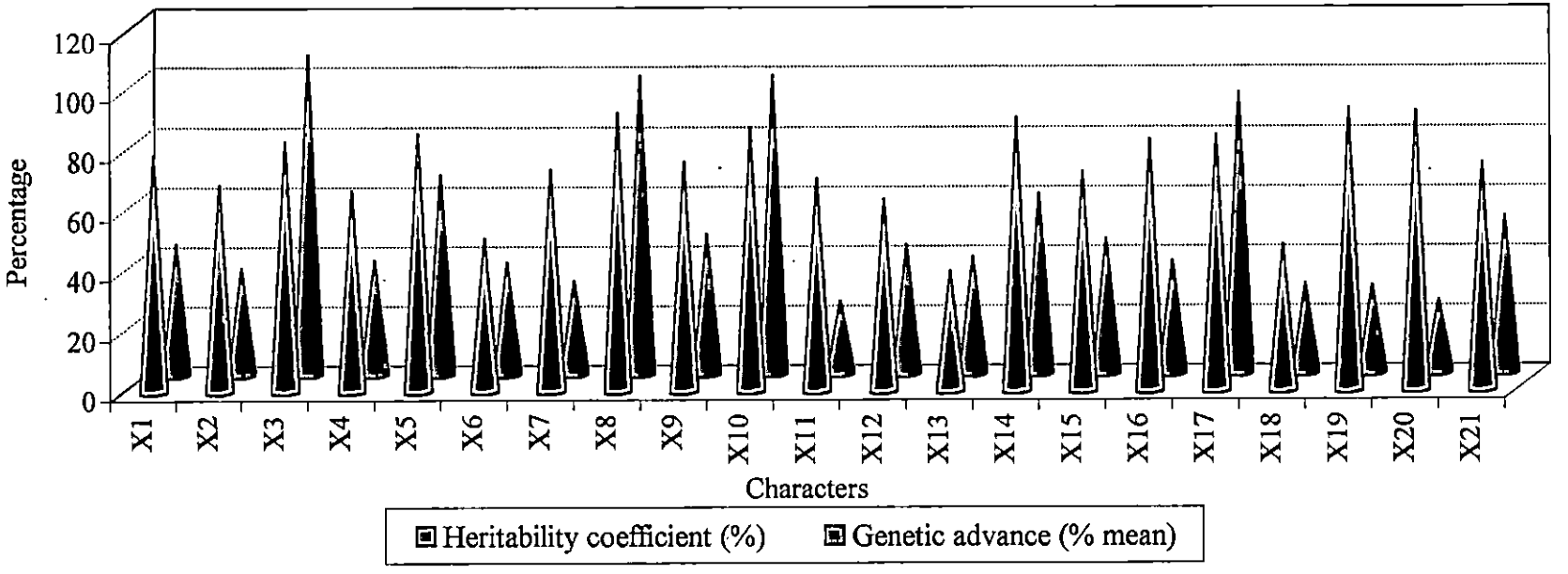
Although days to first flower opening from inflorescence emergence, length of flower and width of flower showed high heritability, their genetic advance was found to be less than 30 per cent. The rest of the vegetative and floral characters exhibited high heritability along with 30-70 per cent genetic advance.

4.1.2.3 Correlation Studies

Among the different quantitative characters studied in parental monopodial orchid genotypes, ten biometric characters were selected for

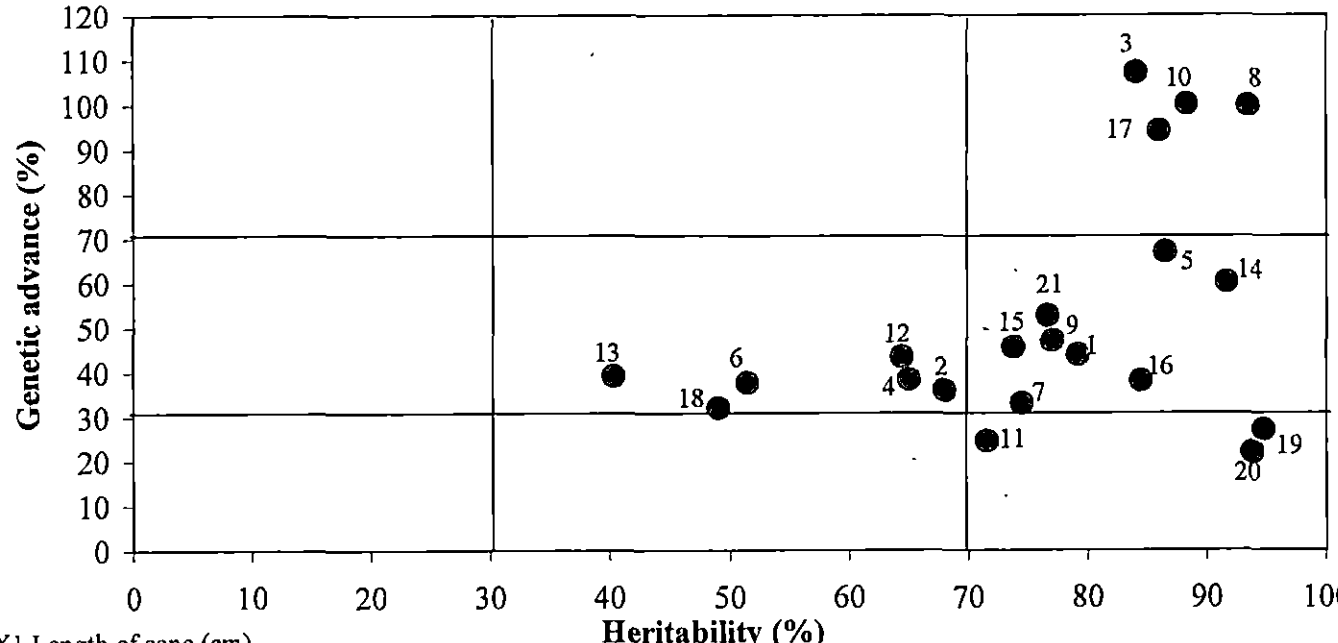
Table 4.1.2.2 Heritability and genetic advance for morphological characters in twelve parental genotypes of monopodial orchids

Sl. No.	Morphological characters	Heritability coefficient (%)	Genetic advance (at 5%)	Genetic advance (% mean)
1	Length of cane (cm)	79.18	38.89	43.87
2	Number of leaves per cane	69.40	10.38	35.83
3	Number of aerial roots	84.08	8.72	107.26
4	Length of aerial roots (cm)	67.37	17.09	38.38
5	Thickness of stem (cm)	86.46	0.69	66.99
6	Length of internode (cm)	51.50	1.05	37.63
7	Length of leaf (cm)	74.45	3.56	31.37
8	Width of leaf (cm)	93.46	2.30	99.83
9	Thickness of leaf (cm)	77.00	0.08	47.06
10	Leaf area (cm^2)	88.30	21.69	100.14
11	Days to first flower opening from inflorescence emergence	71.54	8.80	24.51
12	Days to last flower opening from first flower opening	64.41	5.70	43.38
13	Number of spikes per cane	40.30	2.64	39.29
14	Length of inflorescence (cm)	91.66	24.19	60.34
15	Length of scape (cm)	73.77	7.50	45.57
16	Diameter of inflorescence axis (cm)	84.47	0.19	38.00
17	Number of flowers per inflorescence	86.00	7.70	94.13
18	Length of internode of inflorescence (cm)	49.07	0.92	30.16
19	Length of flower (cm)	94.74	2.01	29.65
20	Width of flower (cm)	93.80	1.55	24.76
21	Vase life (days)	76.59	8.80	52.76



X1 Length of cane (cm)
X2 Number of leaves per cane
X3 Number of aerial roots
X4 Length of aerial roots (cm)
X5Thickness of stem (cm)
X6 Length of internode (cm)
X7 Length of leaf (cm)
X8 Width of leaf (cm)
X9 Thickness of leaf (cm)
X10 Leaf area (cm^2)
X11 Days to first flower opening from inflorescence emergence
X12 Days to last flower opening from first flower opening
X13 Number of spikes per cane
X14 Length of inflorescence (cm)
X15 Length of scape (cm)
X16 Diameter of inflorescence axis (cm)
X17 Number of flowers per inflorescence
X18 Length of internode (cm)
X19 Length of flower (cm)
X20 Width of flower (cm)
X21 Vase life (days)

Fig. 2 Heritability (H^2) and genetic advance(G.A.) for twenty one traits in twelve parental genotypes of monopodial orchids



X1 Length of cane (cm)
X2 Number of leaves per cane
X3 Number of aerial roots
X4 Length of aerial roots (cm)
X5Thickness of stem (cm)
X6 Length of internode (cm)
X7 Length of leaf (cm)
X8 Width of leaf (cm)
X9 Thickness of leaf (cm)
X10 Leaf area (cm^2)
X11 Days to first flower opening from inflorescence emergence
X12 Days to last flower opening from first flower opening
X13 Number of spikes per cane
X14 Length of inflorescence (cm)
X15 Length of scape (cm)
X16 Diameter of inflorescence axis (cm)
X17 Number of flowers per inflorescence
X18 Length of internode (cm)
X19 Length of flower (cm)
X20 Width of flower (cm)
X21 Vase life (days)

Fig3Character distribution in terms of heritability and genetic advance

the genotypic, phenotypic and environmental correlation studies. The ten characters selected for study were number of leaves per cane, number of aerial roots, leaf area, number of spikes per cane, length of inflorescence, length of scape, number of flowers per inflorescence, length of flower, width of flower and vase life. The significance of both phenotypic and environmental correlations was tested and the results are presented in Tables 4.1.2.3 (a, b, c) and Figs. 4 to 6.

In general, high positive correlations at genotypic and phenotypic levels were observed between most of the vegetative and floral characters considered. Maximum negative correlation with number of aerial roots was expressed by both leaf area (rg = -0.7486, rp = -0.6379) and length of inflorescence (rg = -0.4676, rp = -0.4297).

The character number of spikes per cane recorded significant positive correlation with number of leaves per cane (rg = 1.0017, rp = 0.5361).

Length of scape recorded significant positive correlation with length of inflorescence (rg = 0.5319, rp= 0.5202). Length of scape was found to be negatively correlated with number of leaves per cane (rg = -0.2892, rp = -0.2622) and leaf area (rg = -0.3545, rp = -0.2823).

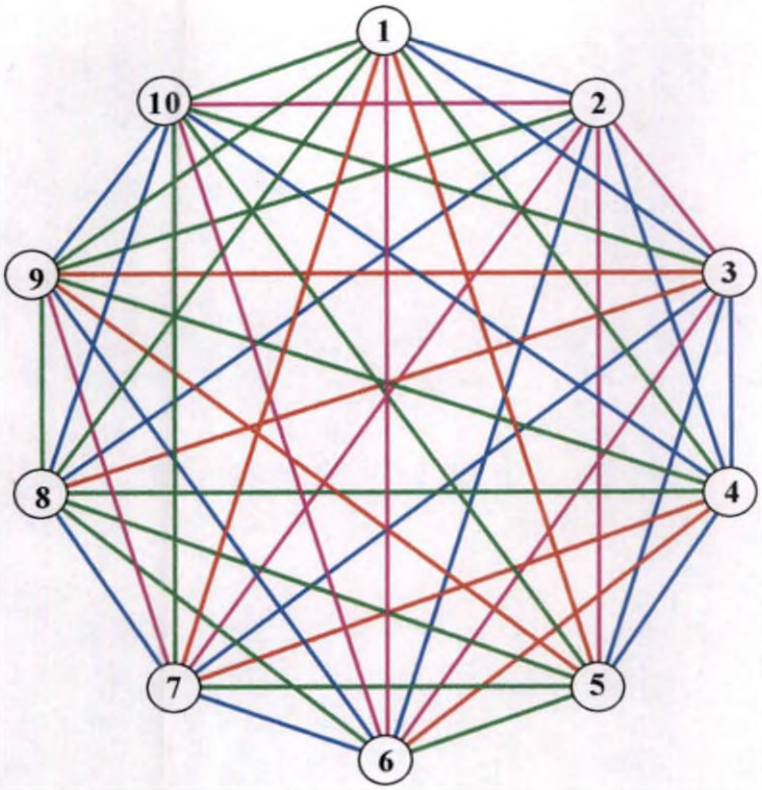
Number of flowers per inflorescence was positively correlated with the length of inflorescence (rg = 0.7479, rp = 0.6718) whereas it showed high negative genotypic and phenotypic correlation with number of aerial roots (rg = -0.6431, rp = -0.5366).

Length of flower expressed maximum positive correlation with length of scape (rg= 0.5758, rp = 0.4672) and number of spikes per cane (rg = 0.5551, rp = 0.3503). The other characters exhibiting high positive correlation with length of flower were length of inflorescence (rg = 0.5047, rp = 0.4568) and number of leaves per cane (rg = 0.4496, rp = 0.3795).

Table 4.1.2.3a Phenotypic correlation among different biometric characters in twelve parental genotypes of monopodial orchids

Characters	Number of leaves per cane	Number of aerial roots	Leaf area	Number of spikes per cane	Length of inflorescence	Length of scape	Number of flowers per inflorescence	Length of flower	Width of flower
Number of aerial roots	0.0318								
Leaf area	0.0856	-0.6379**							
Number of spikes per cane	0.5361**	0.0072	0.0400						
Length of inflorescence	-0.0016	-0.4297**	0.1497	0.1003					
Length of scape	-0.2622*	0.1364	-0.2823*	-0.0276	0.5202**				
Number of flowers per inflorescence	-0.0613	-0.5366**	0.2313	-0.0404	0.6718**	0.0326			
Length of flower	0.3795**	0.2314	-0.2225	0.3503**	0.4568**	0.4672**	0.0176		
Width of flower	0.4810**	0.4959**	-0.0825	0.4668**	-0.0615	0.1855	-0.4838**	0.6667**	
Vase life	0.3966**	-0.5885**	0.5691**	0.1679	0.2838*	-0.3252**	0.3041*	0.1226	0.0353

* Significant at 5 % level, ** Significant at 1 % level



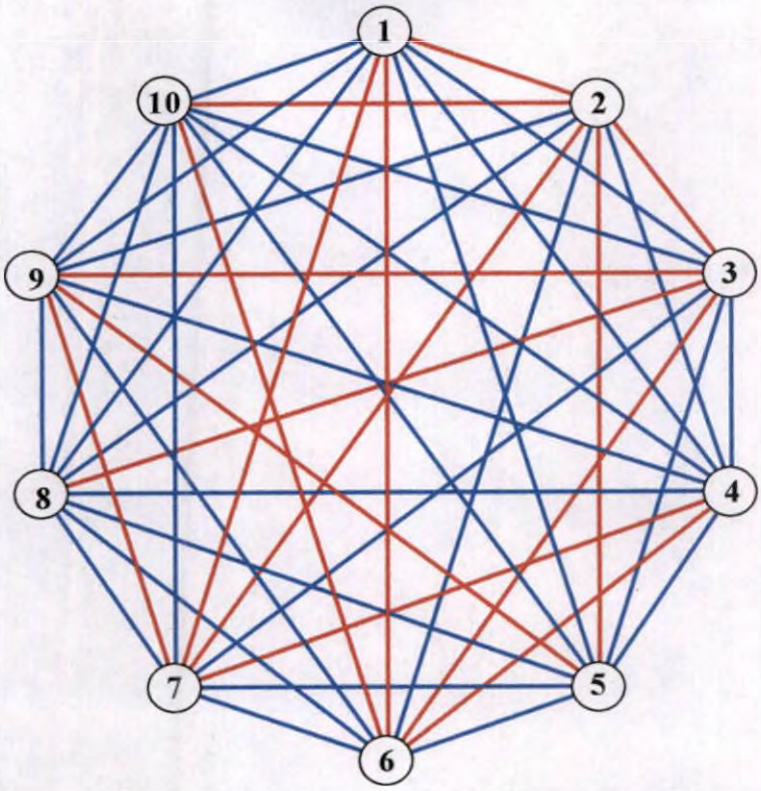
Positive
Negative
Positive significant
Negative significant

1. Number of leaves per cane
2. Number of aerial roots
3. Leaf area
4. Number of spikes per cane
5. Length of inflorescence
6. Length of scape
7. Number of flowers per inflorescence
8. Length of flower
9. Width of flower
10. Vase life

Fig. 4 Phenotypic correlations among the characters

Table 4.1.2.3b Genotypic correlation among different biometric characters in twelve parental genotypes of monopodial orchids

Characters	Number of leaves per cane	Number of aerial roots	Leaf area	Number of spikes per cane	Length of inflorescence	Length of scape	Number of flowers per inflorescence	Length of flower	Width of flower
Number of aerial roots	-0.0269								
Leaf area	0.0696	-0.7486							
Number of spikes per cane	1.0017	0.1876	0.0663						
Length of inflorescence	0.0236	-0.4676	0.1593	0.1321					
Length of scape	-0.2892	0.1899	-0.3545	-0.1301	0.5319				
Number of flowers per inflorescence	-0.0710	-0.6431	0.2453	-0.0806	0.7479	0.0161			
Length of flower	0.4496	0.2404	-0.2443	0.5551	0.5047	0.5778	0.0019		
Width of flower	0.5587	0.5372	-0.0912	0.7379	-0.0583	0.2237	-0.5612	0.6690	
Vase life	0.5539	-0.7721	0.7092	0.3616	0.3566	-0.4169	0.3641	0.1197	0.0260



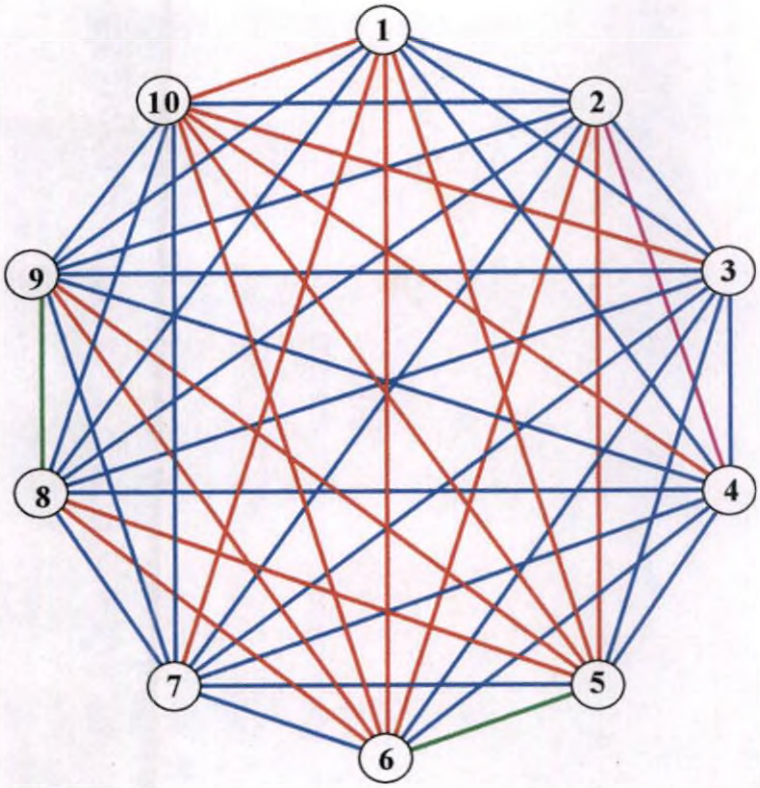
1. Number of leaves per cane
2. Number of aerial roots
3. Leaf area
4. Number of spikes per cane
5. Length of inflorescence
6. Length of scape
7. Number of flowers per inflorescence
8. Length of flower
9. Width of flower
10. Vase life

Fig. 5 Genotypic correlations among the characters

Table 4.1.2.3c Environmental correlation among different biometric characters in twelve parental genotypes of monopodial orchids

Characters	Number of leaves per cane	Number of aerial roots	Leaf area	Number of spikes per cane	Length of inflorescence	Length of scape	Number of flowers per inflorescence	Length of flower	Width of flower
Number of aerial roots	0.2371								
Leaf area	0.1642	0.0525							
Number of spikes per cane	0.0149	-0.3307*	0.0019						
Length of inflorescence	-0.1418	-0.1666	0.0647	0.0897					
Length of scape	-0.1951	-0.0648	0.0220	0.1096	0.5595*				
Number of flowers per inflorescence	-0.0311	0.0689	0.1372	0.0243	0.0721	0.1032			
Length of flower	0.1182	0.1843	0.0110	0.0416	-0.2047	-0.1204	0.1851		
Width of flower	0.2193	0.1898	0.0059	0.0685	-0.1044	-0.0049	0.2167	0.6305*	
Vase life	-0.0272	0.1610	-0.0857	-0.0884	-0.1072	-0.0395	0.0475	0.1857	0.1104

* Significant at 5 % level



Positive
Negative
Positive significant
Negative significant

1. Number of leaves per cane	6. Length of scape
2. Number of aerial roots	7. Number of flowers per inflorescence
3. Leaf area	8. Length of flower
4. Number of spikes per cane	9. Width of flower
5. Length of inflorescence	10. Vase life

Fig. 6 Environmental correlations among the characters

Width of flower recorded significant positive correlation with number of spikes per cane (rg = 0.7379, rp = 0.4668) length of flower (rg = 0.6690, rp= 0.6667), number of leaves per cane (rg = 0.5587, rp= 0.4810) and number of aerial roots (rg = 0.5372, rp = 0.4959). This character exhibited significant negative correlation with number of flowers per inflorescence (rg = -0.5612, rp = -0.4838).

Vase life showed maximum positive correlation with leaf area (rg = 0.7092, rp = 0.5691), number of leaves per cane (rg = 0.5539, rp= 0.3966), number of flowers per inflorescence (rg = 0.3641, rp = 0.3041) and length of inflorescence (rg = 0.3566, rp= 0.2838). This character showed high negative correlation with number of aerial roots (rg = -0.7721, rp = -0.5885) followed by length of scape (rg = -0.4196, rp = -0.3252).

Environmental correlation was found to be low in comparison with genotypic and phenotypic correlations for all pair-wise character combinations.

4.1.3 Floral Biology of Parents

Floral biology of parents was studied with respect to the following heads:

4.1.3.1 Flowering and floral morphology (Plate I, II and III)

4.1.3.2 Pollen characters (Plate III)

4.1.3.1 Flowering and Floral Morphology

Flowering and qualitative floral characters in the parental monopodial orchid genotypes were analysed in detail (Table 4.1.3.1).

1. Flowering Nature–Free-flowering/Seasonal

Free-flowering nature *i.e.*, flowering throughout the year was exhibited by P_1, P_2, P_{10}, P_{11} and P_{12}. Seasonal flowering was observed mainly from June to December in P_4, P_5, P_6, P_7, P_8 and P_9 whereas it was mainly confined to May to October in P_3.

Table 4.1.3.1 Performance of monopodial orchid genotypes for qualitative floral characters

Parental genotypes	Flowering nature- free- flowering/ seasonal	Nature of inflorescence axis	Mode of display of flowers	Shape of flower	Fullness value
P_1	Free-flowering	Arching	Alternate and facing opposite sides	Flat appearance, narrow, spatulate sepals and petals with tips slightly curved inwards	5.31
P_2	Free- flowering	Arching	Alternate and facing opposite sides	Flat appearance, narrow, spatulate sepals and petals with tips slightly curved in wards	5.29
P_3	Seasonal Mostly during May- October	Erect	Alternate and facing opposite sides	Flat appearance, narrow, spatulate sepals and petals with tips slightly curved inwards	3.06
P_4	Seasonal Mostly during June- December	Erect	Bunch type inflorescence	Full, flat look, broad sepals and petals	2.15
P_5	Seasonal Mostly during June- December	Erect	Bunch type inflorescence	Full, flat look, broad sepals and petals	2.22
P_6	Seasonal Mostly during June- December	Erect	Bunch type inflorescence	Full, flat look, broad sepals and petals	2.27
P_7	Seasonal Mostly during June- December	Erect	Alternate and facing opposite sides	Flat look, narrow spatulate sepals and petals with tips slightly curved inwards	5.27
P_8	Seasonal Mostly during June- December	Erect	Alternate and facing opposite sides	Flat look, narrow spatulate sepals and petals with tips slightly curved inwards	5.12
P_9	Seasonal Mostly during June- December	Erect	Alternate and facing opposite sides	Flat look, narrow spatulate sepals and petals with tips slightly curved inwards	5.26
P_{10}	Free- flowering	Arching	Alternate and facing opposite sides	Flat look, narrow spatulate sepals and petals with tips slightly curved inwards	4.93
P_{11}	Free- flowering	Arching	Alternate and facing opposite sides	Flat look, narrow spatulate sepals and petals with tips slightly curved inwards	5.20
P_{12}	Free- flowering	Arching	Alternate and facing opposite sides	Flat look, narrow spatulate sepals and petals with tips slightly curved inwards	4.83

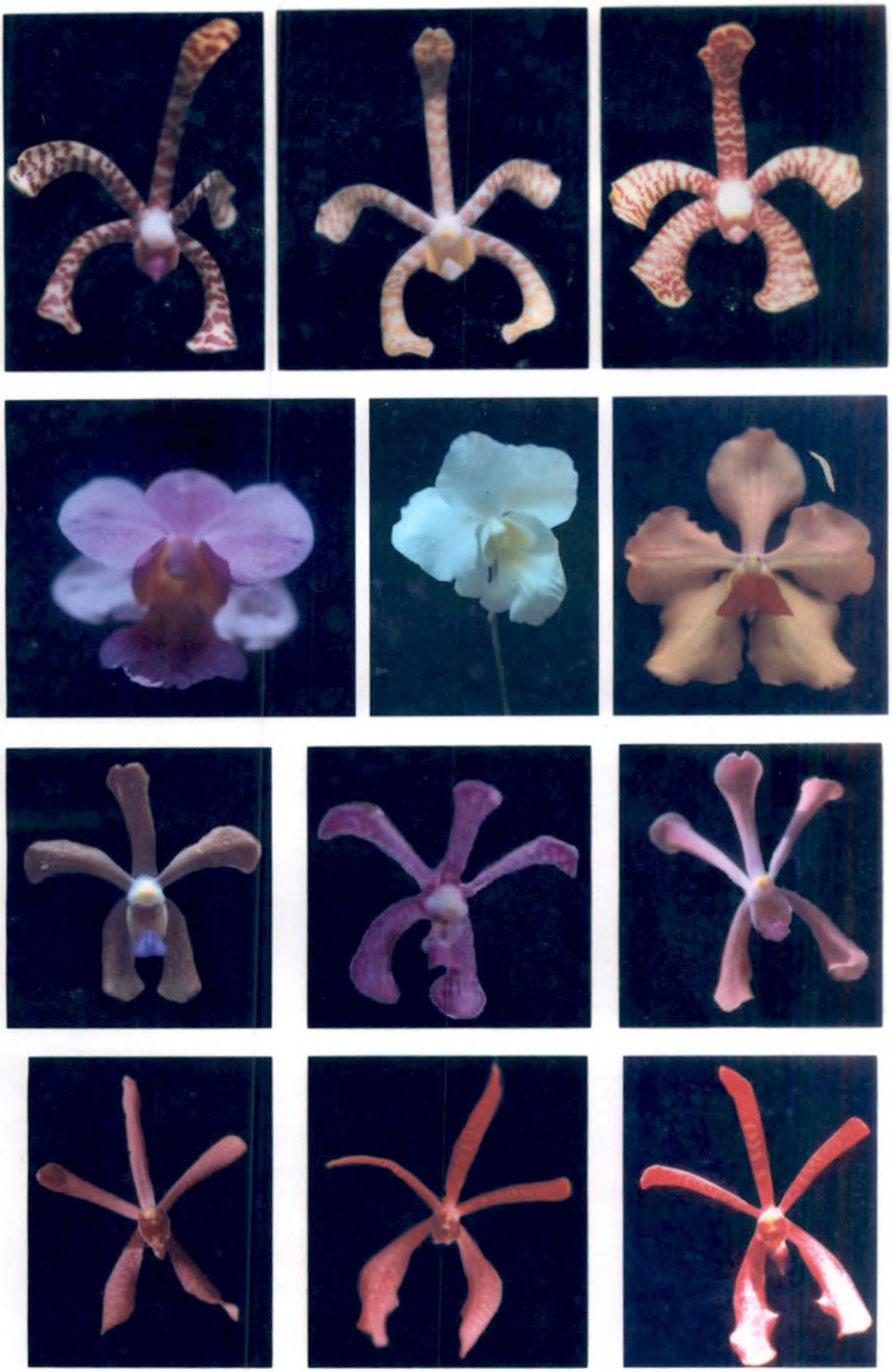


Plate I

2. Nature of Inflorescence Axis

Inflorescence axis was found to be arching in P_1, P_2, P_{10}, P_{11} and P_{12} while the rest of the parental genotypes exhibited erect inflorescence axis.

3. Mode of Display of Flowers

All the parental genotypes except P_4, P_5 and P_6 exhibited the mode of display of flowers as alternate and facing opposite directions. The parents P_4, P_5 and P_6 produced bunch type inflorescences.

4. Shape of Flower and Perianth Lobes

The flowers presented a flat appearance with narrow spatulate sepals and petals with the tips slightly incurved in almost all the parental genotypes except P_4, P_5 and P_6 where the flowers were full and flat with broad sepals and petals.

5. Fullness Value

Fullness value indicates the degree of fullness of a flower and the lower the value, the greater the fullness. Fullness values ranged from 2.15 in P_4 to 5.31 in P_1. Flowers were remarkably full in P_5 (2.22), P_6 (2.27) and P_3 (3.06).

4.1.3.2 Pollen Characters

4.1.3.2a Pollen Size (μ)

Pollen diameter of the selected twelve monopodial parental genotypes is presented in Table 4.1.3.2.

Pollen size was comparatively large in the parent P_3 (44.56μ) while the lowest pollen diameter was recorded in the parent P_6 (34.86μ).

4.1.3.2b Pollen Fertility (%)

Pollen fertility of the selected twelve parental genotypes was studied and results are presented in the Table 4.1.3.2.







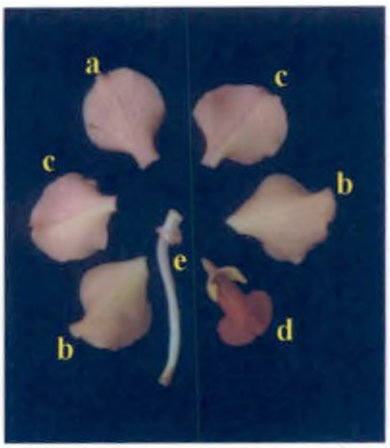


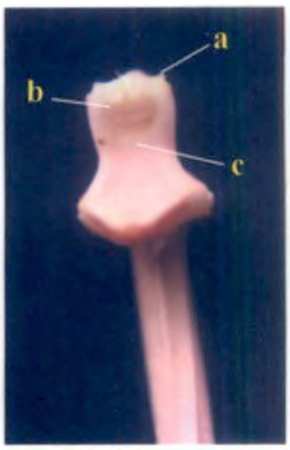


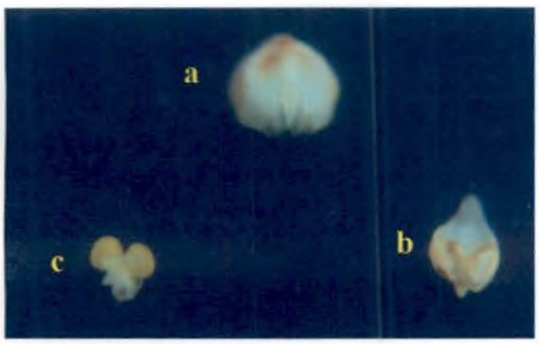

Plate II

Table 4.1.3.2 Pollen characters of twelve parental genotypes of monopodial orchids

Parental genotypes	Pollen size (μ)	Pollen fertility (%)
P_1	43.40	68.70
P_2	42.15	63.60
P_3	44.56	71.40
P_4	38.03	55.30
P_5	37.51	50.80
P_6	34.86	52.50
P_7	39.38	62.50
P_8	38.65	59.30
P_9	40.12	52.90
P_{10}	40.77	78.00
P_{11}	40.01	73.10
P_{12}	41.92	74.92
SE (m)	0.745	2.638
CV (%)	6.43	14.37







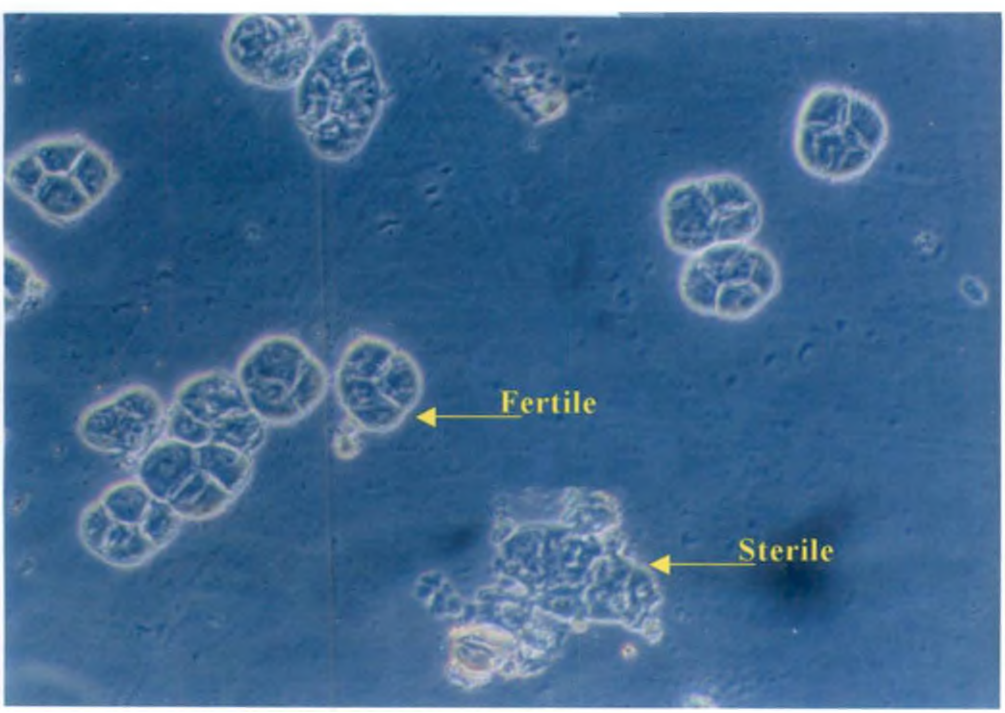


Plate III

The highest pollen fertility was recorded in P_{10} (78.0 %) while the lowest pollen fertility was recorded in P_5 (50.8 %).

4.2 HYBRIDIZATION AND COMPATIBILITY/INCOMPATIBILITY STUDIES

Among the selected twelve monopodial parental genotypes, depending upon the availability of receptive stigma and fertile pollen intercrossing in all possible combinations was done. This was done with the objective of studying the compatibility/incompatibility relationships between genotypes.

4.2.1 Diallel Crossings Attempted among the Genotypes of Monopodial Orchids

Out of the 144 (n^2) possible combinations, 116 diallel crossings have been undertaken (Table 4.2.1). These 116 combinations included 50 out of 66 crosses, 54 out of 66 reciprocals and 12 selfs. Some of the cross combinations and their reciprocals could not be attempted as the flowering of parents concerned did not synchronize.

4.2.2 Details of Diallel Crossings

Details of self and cross compatibility among the twelve monopodial parental genotypes have been analysed (Table 4.2.2).

Out of the 116 self and cross combinations attempted, 58 combinations succeeded in producing harvestable green capsules which included seven selfs and 51 crosses. The relative success of cross (51/104 = 49.04 %) and self (7/12 = 58.33 %) combinations did not differ considerably from the total estimate (58/116 = 50.00 %).

Among the 58 combinations yielding capsules, 12 combinations *viz.*, $P_3 \times P_8$, $P_8 \times P_{11}$, $P_9 \times P_6$, $P_9 \times P_9$, $P_{10} \times P_2$, $P_{11} \times P_2$, $P_{11} \times P_3$, $P_{11} \times P_9$, $P_{11} \times P_{10}$, $P_{12} \times P_1$, $P_{12} \times P_2$ and $P_{12} \times P_{10}$ did not yield any seeds in the capsule while 46 combinations were cultured axenically.

Table 4.2.1 Matrix showing diallel crossings attempted among twelve parental genotypes of monopodial orchids

♀ \ ♂	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}
P_1	X	X	X	X	X	X	X	NA	X	X	X	X
P_2	X	X	X	X	X	NA	NA	X	X	X	X	X
P_3	X	X	X	X	X	NA	X	X	X	X	NA	X
P_4	X	X	X	X	X	X	NA	X	X	X	X	X
P_5	X	X	NA	X	X	X	NA	X	NA	X	NA	X
P_6	X	X	X	X	X	X	X	NA	NA	X	X	X
P_7	X	X	NA	NA	X	X	X	NA	X	X	X	X
P_8	X	X	NA	X	X	NA	NA	X	X	X	X	NA
P_9	X	X	X	X	X	X	X	NA	X	NA	NA	X
P_{10}	X	X	X	X	X	X	NA	NA	NA	X	X	X
P_{11}	X	X	X	NA	X	X	X	X	X	X	X	NA
P_{12}	X	X	X	X	X	X	X	NA	X	X	X	X

NA- Not attempted

Table 4.2.2 Matrix showing compatibility relationships in diallel crossings among twelve parental genotypes of monopodial orchids

♀ \ ♂	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}
P_1	8	8	3	7	2	3	8	1	3	3	7	3
P_2	8	6	3	4	8	1	1	8	8	4	7	7
P_3	8	4	7	8	2	1	7	5	4	4	1	3
P_4	3	2	2	3	3	8	1	6	4	2	6	2
P_5	2	2	1	8	3	3	1	6	1	2	1	4
P_6	2	3	7	8	8	6	8	1	1	4	2	3
P_7	2	3	1	1	4	2	4	1	6	3	8	4
P_8	3	8	1	2	3	1	1	7	6	3	5	1
P_9	8	3	7	8	2	5	8	1	5	1	1	3
P_{10}	8	5	3	8	2	7	1	1	1	4	6	4
P_{11}	8	5	5	1	8	6	4	7	5	5	8	1
P_{12}	5	5	4	4	4	3	6	1	4	5	7	3

1- Combinations where pollination not attempted
2- Combinations where pollinated flowers abscised without any change
3- Combinations where pollinated flowers abscised within two months
4- Combinations where pollinated flowers abscised after two months growth
5- Combinations with no seeds in capsule
6- Combinations registering no seed germination
7- Combinations lost while in culture
8- Combinations successfully deflasked

Table 4.2.3 Matrix of the 24 successful combinations that yielded seedlings

♀ \ ♂	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}
P_1	X	X					X					
P_2	X				X			X	X			
P_3	X			X								
P_4						X						
P_5				X								
P_6				X	X		X					
P_7											X	
P_8		X										
P_9	X			X			X					
P_{10}	X			X								
P_{11}	X				X						X	
P_{12}												

Among the 46 combinations inoculated *in vitro*, no germination was obtained with seeds from 10 combinations *viz.*, $P_2 \times P_2$, $P_4 \times P_8$, $P_4 \times P_{11}$, $P_5 \times P_8$, $P_6 \times P_6$, $P_7 \times P_9$, $P_8 \times P_9$, $P_{10} \times P_{11}$, $P_{11} \times P_6$ and $P_{12} \times P_7$. Successful seed germination was obtained in 36 combinations.

Out of the 36 combinations that germinated successfully, 12 combinations showed arrested development of the culture *viz.*, $P_1 \times P_4$, $P_1 \times P_{11}$, $P_2 \times P_{11}$, $P_2 \times P_{12}$, $P_3 \times P_3$, $P_3 \times P_7$, $P_6 \times P_3$, $P_8 \times P_8$, $P_9 \times P_3$, $P_{10} \times P_6$, $P_{11} \times P_8$ and $P_{12} \times P_{11}$.

Thus out of the total 46 combinations inoculated *in vitro*, 24 combinations yielded seedlings which were deflasked successfully (Table 4.2.3).

4.2.3 Analysis of Compatibility

Based on compatibility analysis, the monogeneric hybrid P_2 turned out to be the best female parent generating hardened seedlings in four combinations followed by P_1, P_6, P_9 and P_{11} generating hardened seedlings in three combinations each (Table 4.2.3).

The monogeneric hybrid P_1 turned out to be the best male parent generating successfully hardened seedlings in six combinations followed by P_4 yielding five successful combinations.

Selfed seedlings obtained from two varieties *viz.*, P_1 and P_{11} were deflasked successfully.

None of the combinations with P_3, P_{10} and P_{12} as male parents yielded any viable seedlings for deflasking. Similarly no combination with P_{12} as the female parent could generate seedlings which reached deflasking stage.

4.2.4 Analysis of Incompatibility

The levels of incompatibility [Plate IV(b)] were grouped under six heads as follows:

1. Instances where pollination attempted, but flowers abscised before the onset of any visible post pollination change.

Female parent in cross combination	Number of combinations
Arachnis Maggie Oei Red Ribbon (P_1)	1
Arachnis Maggie Oei Yellow Ribbon (P_2)	-
Arachnis Kapama (P_3)	1
Vanda Miss Joaquim (P_4)	4
Vanda Popoe Diana (P_5)	3
Vanda TMA Mandai (P_6)	2
Aranda Peg Lee You (P_7)	2
Aranda Eric Mekie (P_8)	1
Aranda Golden Sands (P_9)	1
Aranthera James Storei (P_{10})	1
Aranthera Mohammed Haniff (P_{11})	-
Aranthera Annie Black (P_{12})	-

2. Instances where pollinated flowers abscised within two months (after initial swelling and greening of ovary)

Female parent in cross combination	Number of combinations
Arachnis Maggie Oei Red Ribbon (P_1)	5
Arachnis Maggie Oei Yellow Ribbon (P_2)	1
Arachnis Kapama (P_3)	1
Vanda Miss Joaquim (P_4)	3
Vanda Popoe Diana (P_5)	2
Vanda TMA Mandai (P_6)	2
Aranda Peg Lee You (P_7)	2
Aranda Eric Mekie (P_8)	3
Aranda Golden Sands (P_9)	2
Aranthera James Storei (P_{10})	1
Aranthera Mohammed Haniff (P_{11})	-
Aranthera Annie Black (P_{12})	2

3. Instances where pollinated flowers with swelling ovaries abscised after two months growth

Female parent in cross combination	Number of combinations
Arachnis Maggie Oei Red Ribbon (P_1)	-
Arachnis Maggie Oei Yellow Ribbon (P_2)	2
Arachnis Kapama (P_3)	3
Vanda Miss Joaquim (P_4)	1
Vanda Popoe Diana (P_5)	1
Vanda TMA Mandai (P_6)	1
Aranda Peg Lee You (P_7)	3
Aranda Eric Mekie (P_8)	-
Aranda Golden Sands (P_9)	-
Aranthera James Storei (P_{10})	2
Aranthera Mohammed Haniff (P_{11})	1
Aranthera Annie Black (P_{12})	4

4. Instances where green capsules were harvested at normal stage, but did not contain seeds

Combinations
$P_3 \times P_8$
$P_8 \times P_{11}$
$P_9 \times P_6$
$P_9 \times P_9$
$P_{10} \times P_2$
$P_{11} \times P_2$
$P_{11} \times P_3$
$P_{11} \times P_9$
$P_{11} \times P_{10}$
$P_{12} \times P_1$
$P_{12} \times P_2$
$P_{12} \times P_{10}$

5. Instances where green capsules contained seeds which did not germinate

Combinations

$P_2 \times P_2$

$P_4 \times P_8$

$P_4 \times P_{11}$

$P_5 \times P_8$

$P_6 \times P_6$

$P_7 \times P_9$

$P_8 \times P_9$

$P_{10} \times P_{11}$

$P_{11} \times P_6$

$P_{12} \times P_7$

6. Instances where seeds germinated, but aborted while in culture

Combinations

$P_1 \times P_4$

$P_1 \times P_{11}$

$P_2 \times P_{11}$

$P_2 \times P_{12}$

$P_3 \times P_3$

$P_3 \times P_7$

$P_6 \times P_3$

$P_8 \times P_8$

$P_9 \times P_3$

$P_{10} \times P_6$

$P_{11} \times P_8$

$P_{12} \times P_{11}$

In 16 combinations (13.79 %), flower abscission was observed without any visible post pollination floral changes. Incompatibility was of the highest degree in these combinations where even the initial swelling of ovary following pollination was not detected.

In 24 combinations (20.69 %), pollinated flowers abscised within two months after pollination. The abscised flowers exhibited initial greening and swelling of ovary into capsules which indicates a reduction in the strength of incompatibility from the first level.

In 18 combinations (15.52 %), capsules abscised showing yellowing and decay before maturity, after two months of capsule growth. This again indicates a lesser degree of incompatibility reaction.

In 12 combinations (10.34 %) the capsules reached the correct stage of maturity, but they were found to be empty, without seeds.

In 10 combinations (8.62 %), green capsules were harvested at the correct stage of maturity, but they failed to germinate when inoculated *in vitro*.

In 12 combinations (10.34 %) green capsules harvested at the correct stage of maturity germinated *in vitro*, but in the later stages, the protocorms initiated showed declined growth rate and gradually degenerated.

The extent and strength of incompatibility reaction varied among the selected twelve monopodial orchid parental genotypes (Table 4.2.2).

The strength of incompatibility was of the highest degree with P_5 as the male parent. Complete flower drop within two months from pollination was observed in seven combinations out of which four showed flower abscission without any post pollination change.

The strength of incompatibility was of the highest degree when P_4 was used as the female parent, recording complete flower drop within

two months from pollination in all seven unsuccessful combinations out of which four showed flower abscission without any post pollination change.

The extent of incompatibility was of the highest degree when P_5, P_{10} and P_{12} were used as male parents, in which nine out of the twelve combinations failed to produce capsules.

The extent of incompatibility was high with P_4 as the female parent. Here, eight out of the twelve combinations failed to produce harvestable capsules.

4.2.5 Details of Post Pollination Developments

The details of post pollination developments are summarized below under eight heads.

1. Post pollination floral changes
2. Stages of capsule development [Plate IV(a)]
3. Days to green capsule harvest in successful crosses
4. Length of capsule
5. Width of capsule
6. Percentage of capsule set
7. Percentage of capsules with germinating seeds
8. Percentage of filled seeds

1. Post Pollination Floral Changes

Following successful pollination, the sepals, petals, column and ovary underwent a series of changes during the early stages of capsule development (Table 4.2.5a). Post pollination floral changes include drooping of perianth followed by closure of stigma by overgrowth of column tip. Complete covering of the stigma was effected by the wilted

Table 4.2.5a Post pollination floral changes in twelve parental genotypes of monopodial orchids

Parental genotypes	Number of days from pollination for			
	drooping of sepals and petals	closure of stigma by over growth of column tip	covering of stigma by wilted sepals and petals	complete drying of sepals and petals
P_1	4-7	3-6	7-13	10-15
P_2	4-6	5-8	5-10	11-14
P_3	4-8	4-10	7-12	10-13
P_4	2-4	3-7	5-10	12-14
P_5	2-6	3-6	5-10	12-15
P_6	2-4	4-8	6-11	10-12
P_7	3-5	5-10	7-12	10-14
P_8	2-5	4-6	6-14	13-16
P_9	3-6	3-8	4-10	13-15
P_{10}	4-6	4-9	7-11	12-16
P_{11}	3-5	5-9	6-12	13-17
P_{12}	4-7	5-8	6-10	10-14

sepals and petals. Complete drying of sepals and petals resulted in about two weeks after pollination.

2. Stages of Capsule Development

Following successful pollination, the capsule underwent a series of changes till harvest. These changes were initiated with the greening of the ovary with slight swelling followed by swelling of the ovary into capsule (Table 4.2.5b). The capsule attained maximum length at 60-75 per cent maturity. At this stage, the ribs along the length of the capsule swelled into prominence. The capsules were harvested at 75-90 per cent maturity, which is ideal for green capsule harvest. If left unharvested, the capsules burst open by separation of capsule wall from ribs, beginning near the tip of the capsule and proceeding backwards.

3. Days to Green Capsule Harvest in Successful Crosses

Time taken for the harvest of green capsules in compatible combinations (58/116) was analysed (Table 4.2.5c).

Duration to green capsule harvest ranged from 66 days in $P_6 \times P_5$ to 135 days in $P_{11} \times P_5$ in individual crosses.

The time taken for the harvest of green capsules ranged from 86.00 (P_5) to 118.20 (P_3) days when the genotypes were used as female parents and the variability ranged between 8.39 (P_8) and 27.37 (P_7) per cent. When the same genotypes were used as male parents, the duration ranged from 89.50 (P_{10}) to 112.00 (P_7 and P_{12}) days and the variability ranged from 7.96 (P_4) to 28.29 (P_5) per cent.

4. Length of Capsule

Length of green capsule ranged from 4.20 cm in $P_2 \times P_8$ to 9.80 cm in $P_1 \times P_{11}$ in individual crosses (Table 4.2.5d and Fig. 7).

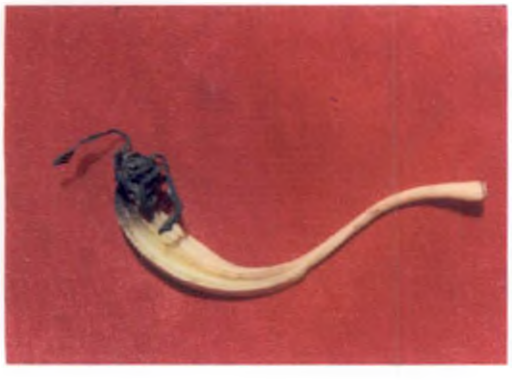


Plate IV

Table 4.2.5 b Stages of capsule development in parental genotypes of monopodial orchids

Parental genotypes	Number of days from pollination for				
	greening of ovary with slight swelling	capsule formation	prominent ribbing of capsule	slight flattening of capsule rib (green capsule harvest stage)	bursting of capsule beginning from tip
P_1	4-6	95-120	110-115	127-135	140-148
P_2	4-7	84-110	105-110	113-120	126-135
P_3	3-5	85-105	95-105	110-128	130-140
P_4	3-6	55-84	82-85	94-102	108-115
P_5	4-6	65-80	74-80	87-96	103-110
P_6	5-8	70-85	81-85	97-105	112-120
P_7	4-7	50-85	79-88	96-118	118-128
P_8	6-8	75-100	95-100	105-113	115-125
P_9	6-9	64-82	76-82	90-105	106-115
P_{10}	4-6	53-72	67-73	78-89	100-105
P_{11}	5-8	68-90	87-90	102-110	115-130
P_{12}	6-8	83-100	93-100	110-125	130-142

Table 4.2.5c Duration to green capsule harvest (days) in successful combinations

♀ \ ♂	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}	$\bar{x}$	C.V. (%)
P_1	97	85		108			94				120		100.80	11.99
P_2	113	118			98			122	105		94	112	108.86	8.76
P_3	128		89	121			123	130					118.20	12.66
P_4						102		80			94		92.00	9.88
P_5				98				74					86.00	13.95
P_6			113	95	66	89	125						97.60	20.83
P_7									121		69		95.00	27.37
P_8		87						98	96		110		97.75	8.39
P_9	121		125	106		130	115		92				114.83	11.08
P_{10}	89	110		110		121					111		108.20	9.67
P_{11}	94	90	98		135	86		120	98	105	96		102.44	14.38
P_{12}	109	115					103			74	87		97.60	15.41
$\bar{x}$	107.29	100.83	106.25	106.33	99.67	105.60	112.00	104.00	102.40	89.50	97.63	112.00		
C.V. (%)	12.55	13.66	13.00	7.96	28.29	16.43	10.59	20.65	9.97	17.32	15.34	0.00		

Table 4.2.5d Average length (cm) of green capsules harvested from successful combinations

♀ \ ♂	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}	$\bar{x}$	C.V. (%)
P_1	8.3	8.1		8.9			7.3				9.8		8.48	9.85
P_2	7.5	8.4			5.5			4.2	5.7		5.9	5.4	6.09	21.43
P_3	8.8		8.1	5.4			6.4	7.0					7.14	16.89
P_4						8.5		6.5			7.0		7.33	11.59
P_5				7.9				6.0					6.95	13.67
P_6			7.4	9.4	7.7	7.5	5.0						7.40	18.98
P_7									4.3		5.1		4.70	8.51
P_8		5.8						4.5	5.4		4.6		5.08	10.74
P_9	6.0		6.2	5.1		5.7	6.3		7.7				6.17	12.83
P_{10}	5.4	6.4		8.2		8.7					4.4		6.62	24.63
P_{11}	8.7	7.5	7.8		5.4	5.2		6.9	8.2	5.8	6.2		6.87	17.48
P_{12}	5.6	5.8					7.1			6.5	6.9		6.38	9.27
$\bar{x}$	7.19	7.00	7.38	7.48	6.20	7.12	6.42	5.85	6.26	6.15	6.24	5.40		
C.V. (%)	19.22	15.05	9.80	22.08	17.12	20.11	12.59	19.00	23.40	5.69	26.09	0.00		

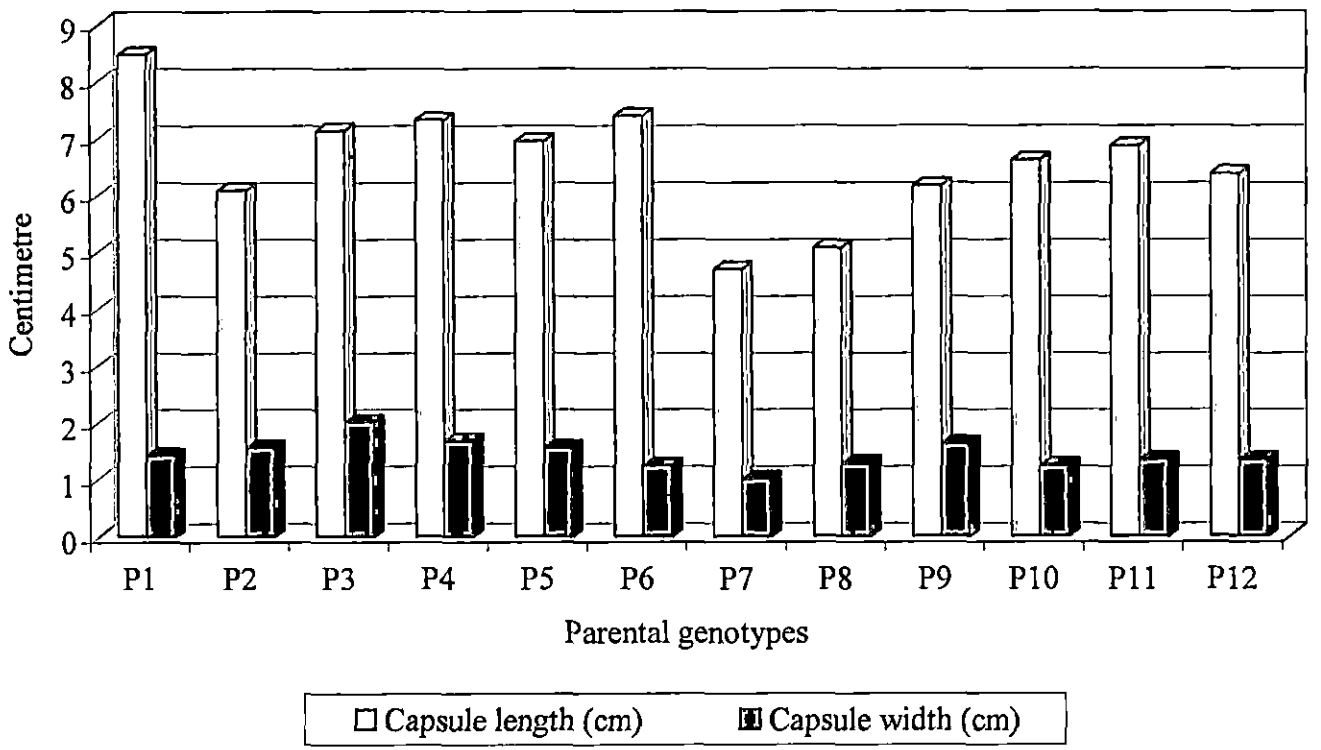


Fig. 7 Size of green capsules in twelve parental genotypes of monopodial orchids

Mean length of green capsules ranged from 4.70 cm in P_7 to 8.48 cm in P_1 when the genotypes were used as female parents. The variability ranged from 8.51 (P_7) to 24.63 (P_{10}) per cent.

When the same genotypes were used as male parents, capsule length ranged from 5.40 cm in P_{12} to 7.48 cm in P_4 and the variability ranged from 5.69 (P_{10}) to 26.09 (P_{11}) per cent.

5. Width of Capsule

Width of green capsule ranged from 0.80 cm in $P_7 \times P_9$ and $P_{10} \times P_{11}$ to 2.30 cm in $P_2 \times P_2$ (Table 4.2.5e).

Mean width of green capsules ranged from 1.00 cm in (P_7) to 2.00 cm (P_3) when the genotypes were used as female parents. Coefficient of variation was observed to range from 7.07 (P_3) to 29.50 (P_2) per cent for the character.

When the same genotypes were used as male parents, width of capsule registered a range from 1.00 cm in P_{12} to1.60 cm in P_1 and the variability ranged from 6.62 (P_6) to 27.19 (P_3) per cent.

6. Percentage of Capsule Set

Percentage capsule set in individual combinations ranged from eight in $P_{12} \times P_{11}$ to 38 in $P_2 \times P_1$ (Table 4.2.5f).

Mean percentage capsule set ranged from 13.80 in P_{12} to 23.57 in P_2 with a coefficient of variation ranging from 12.50 in P_5 to 42.56 per cent in P_4 when the genotypes were used as female parents. When the genotypes were used as male parents, mean percentage capsule yield ranged from 17.60 in P_7 to 25.43 in P_1 and the coefficient of variation ranged from zero in P_{12} (single entry in P_{12}) to 45.45 per cent in P_9.

Table 4.2.5e Average width (cm) of green capsules harvested in successful combinations

♀ \ ♂	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}	$\bar{x}$	C.V. (%)
P_1	1.7	1.7		1.3			1.0				1.4		1.42	18.58
P_2	1.9	2.3			1.5			1.5	0.9		1.8	1.0	1.56	29.50
P_3	1.8		2.2	2.1			2.0	1.9					2.00	7.07
P_4						1.5		1.8			1.7		1.67	7.48
P_5				1.4				1.7					1.55	9.68
P_6			1.1	0.9	1.3	1.6	1.3						1.24	18.81
P_7									0.8		1.2		1.00	20.00
P_8		1.4						1.4	1.3		1.0		1.28	12.86
P_9	1.7		1.5	1.9		1.5	1.8		1.3				1.62	12.58
P_{10}	1.2	1.1		1.4		1.7					0.8		1.24	24.25
P_{11}	1.5	1.2	1.3		1.6	1.4		1.1	1.6	1.1	1.3		1.34	13.63
P_{12}	1.4	1.6					1.3			1.3	1.1		1.34	12.13
$\bar{x}$	1.60	1.55	1.53	1.50	1.47	1.54	1.48	1.57	1.18	1.20	1.29	1.00		
C.V. (%)	14.17	25.47	27.19	26.39	8.50	6.62	24.70	17.15	24.79	8.33	24.70	0.00		

Table 4.2.5f Capsule yield in successful combinations (per cent)

♀ \ ♂	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}	$\bar{x}$	C.V. (%)
P_1	25	24		10			28				11		19.60	38.53
P_2	38	18			19			33	11		28	18	23.57	37.87
P_3	22		10	29			13	20					18.80	35.82
P_4						22		9			30		20.33	42.56
P_5				14				18					16.00	12.50
P_6			17	18	17	11	14						15.40	16.73
P_7									25		11		18.00	38.89
P_8		13						22	17		24		19.00	22.64
P_9	30		22	17		22	20		10				20.17	29.81
P_{10}	28	25		20		14					25		22.40	21.98
P_{11}	24	22	25		17	20		12	33	29	17		22.11	27.67
P_{12}	11	17					13			20	8		13.80	30.88
$\bar{x}$	25.43	19.83	18.50	18.00	17.67	17.80	17.60	19.00	19.20	24.50	19.25	18.00		
C.V. (%)	30.01	21.28	30.70	32.55	5.34	25.22	33.05	40.54	45.45	18.37	41.70	0.00		

7. Percentage of Capsules with Germinating Seeds

Out of the 46 combinations producing green capsules containing filled seeds, seeds from all capsules harvested from 36 combinations germinated (Table 4.2.2). Zero per cent capsules with germinating seeds was registered in twelve combinations *viz.*, $P_3 \times P_8$, $P_8 \times P_{11}$, $P_9 \times P_6$, $P_9 \times P_9$, $P_{10} \times P_2$, $P_{11} \times P_2$, $P_{11} \times P_3$, $P_{11} \times P_9$, $P_{11} \times P_{10}$, $P_{12} \times P_1$, $P_{12} \times P_2$ and $P_{12} \times P_{10}$ where no seed obtained from green capsules germinated.

8. Percentage of Filled Seeds

Percentage of filled seeds ranged from 13 in $P_{12} \times P_7$ to 78 in $P_9 \times P_7$ in individual combinations (Table 4.2.5g).

Mean percentage of filled seeds ranged from 25.50 in P_{12} to 58.75 in P_3 and the coefficient of variation ranged from 4.88 per cent in P_7 to 67.11 per cent in P_{10} when the parental genotypes were used as female parents.

When used as male parents, mean percentage of filled seeds ranged from 29.50 in P_6 to 54.00 in P_{12} and the coefficient of variation ranged from zero per cent in P_{12} (single entry) to 48.64 per cent in P_4.

4.3 EMBRYO CULTURE OF HYBRID SEEDS

The details of embryo culture of hybrid seeds obtained from successful combinations were analysed (Table 4.3.1, 4.3.2 and Plate V). Significant differences could be observed between the hybrid combinations with respect to the time taken to attain each of the different *in vitro* developmental stages.

4.3.1 Observations on Rate of Growth

1. Days for initiation of germination

The number of days taken for germination initiation was found to be significantly low in $P_{11} \times P_1$(15.50 days). This was observed to be on par

Table 4.2.5g Percentage of filled seeds over total seeds in successful combinations

♀ ♂	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}	P_{11}	P_{12}	$\bar{x}$	C.V. (%)
P_1	31	71		68			63				22		51.00	39.93
P_2	35	34			54			65	59		57	54	51.14	21.66
P_3	63		47	76			49						58.75	19.94
P_4						42		32			34		36.00	12.00
P_5				35				22					28.50	22.81
P_6			33	41	65	30	54						44.60	29.49
P_7									43		39		41.00	4.88
P_8		32						47	38				39.00	15.60
P_9	41		23	25			78						41.75	52.84
P_{10}	76			18		22					25		35.25	67.11
P_{11}	65				23	24		32			68		42.40	47.04
P_{12}							13				23		18.00	27.77
$\bar{x}$	51.83	47.67	34.33	43.83	47.33	29.50	51.40	39.60	46.67		38.29	54.00		
C.V. (%)	32.63	39.27	28.67	48.64	37.57	26.42	41.99	37.88	19.19		43.34	0.00		

with the combination P_6 x P_5 (17.00 days) whereas it was found to be significantly high in P_9 x P_4 (40.50 days).

2. Days for protocorm development

The time taken for the development of protocorm was recorded lowest in P_{11} x P_1 (24.83 days). The number of days taken for development of protocorm in P_1 x P_1 (56.83 days) was found to be significantly high. This was found to be on par with P_9 x P_4 (55.17 days) and P_2 x P_1 (54.33 days).

3. Days for greening of protocorms

The mean duration taken for development of chlorophyll ranged from 34.83 days in P_6 x P_5 to 75.50 days in P_6 x P_4, which was observed to be the longest.

4. Days for first leaf initiation

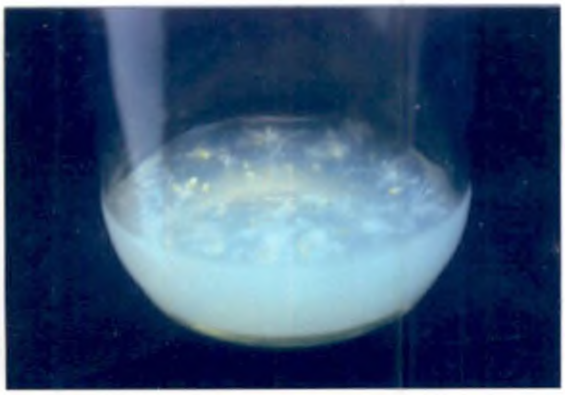
The time taken for formation of first leaf primordium was recorded lowest in P_{11} x P_1 (58.17 days). This was observed to be on par with P_6 x P_5 (58.33 days) whereas the combination P_{10} x P_1 recorded the longest duration of 95.50 days for development of first leaf primordium which was found to be on par with P_6 x P_4 (94.83 days).

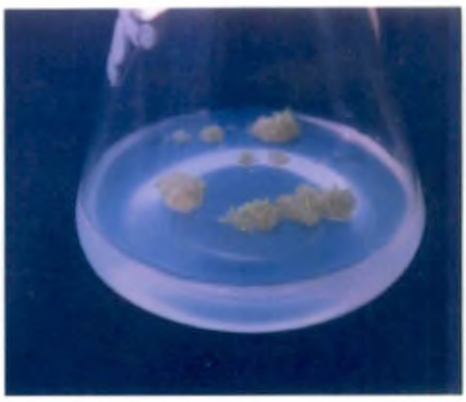
5. Days for shoot initiation

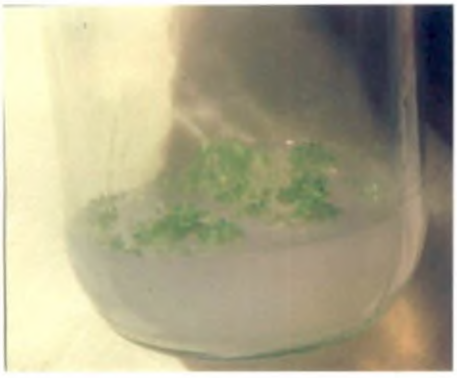
The time taken for the development of first shoot primordium was found to be significantly low in P_6 x P_5 (85.17 days) whereas it was found to be the highest in P_{11} x P_5 (143.67 days).

6. Days for first root initiation

Number of days taken for the initiation of first root was observed to be the lowest in P_6 x P_5 (113.50 days) which was on par with P_5 x P_4 (113.67 days). The longest duration taken for development of root







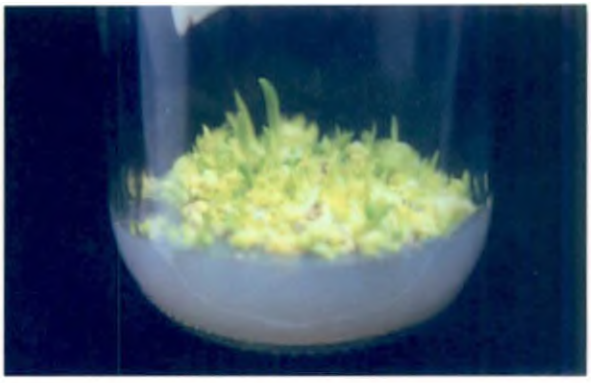


Plate V

Table 4.3.1 Details of embryo culture of hybrid seeds obtained from 24 successful combinations

Sl. No.	Cross combination	Number of days to germination initiation	Number of days taken for the development of					Number of days taken for planting out
			Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1	$P_1 \times P_1$	33.17	56.83	71.33	88.50	135.33	174.50	276.17
2	$P_1 \times P_2$	26.33	43.50	64.50	77.00	99.00	126.67	178.33
3	$P_1 \times P_7$	22.00	40.17	55.67	80.00	119.17	146.50	240.00
4	$P_2 \times P_1$	32.33	54.33	61.67	84.00	132.00	156.50	249.17
5	$P_2 \times P_5$	29.00	44.50	59.50	73.50	96.00	149.17	217.33
6	$P_2 \times P_8$	21.83	39.33	44.33	65.00	109.17	138.50	225.83
7	$P_2 \times P_9$	33.00	50.67	63.00	83.50	119.67	162.67	243.33
8	$P_3 \times P_1$	19.50	37.33	55.50	65.33	101.83	129.50	207.17
9	$P_3 \times P_4$	25.33	47.17	66.00	83.50	129.17	163.17	259.83
10	$P_4 \times P_6$	20.17	34.00	41.00	64.67	100.83	123.17	208.50
11	$P_5 \times P_4$	26.83	48.17	63.67	76.67	95.00	113.67	186.83
12	$P_6 \times P_4$	33.00	51.83	75.50	94.83	133.00	154.67	267.83
13	$P_6 \times P_5$	17.00	30.00	34.83	58.33	85.17	113.50	155.83
14	$P_6 \times P_7$	24.83	42.67	64.00	79.17	125.50	154.50	267.67

Table 4.3.1 Continued

Sl. No.	Cross combination	Number of days to germination initiation	Number of days taken for the development of					Number of days taken for planting out
			Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
15	$P_7 \times P_{11}$	22.67	35.00	48.17	63.67	106.50	124.00	200.17
16	$P_8 \times P_2$	29.17	45.00	57.83	81.50	134.50	158.33	239.33
17	$P_9 \times P_1$	19.17	43.00	51.00	74.33	116.33	151.53	200.67
18	$P_9 \times P_4$	40.50	55.17	60.67	76.00	129.00	172.00	263.17
19	$P_9 \times P_7$	23.33	38.17	54.00	74.50	107.00	121.67	223.17
20	$P_{10} \times P_1$	28.17	47.83	69.33	95.50	116.33	139.33	220.17
21	$P_{10} \times P_4$	29.50	49.50	64.83	91.00	124.33	148.67	256.17
22	$P_{11} \times P_1$	15.50	24.83	42.33	58.17	90.17	124.33	183.67
23	$P_{11} \times P_5$	26.00	42.00	56.17	84.00	143.67	164.83	263.17
24	$P_{11} \times P_{11}$	19.17	35.00	52.33	68.50	114.83	133.17	195.00
	SEm	1.02	1.269	1.179	1.286	1.267	1.045	1.541
	CD (0.05)	3.085	3.552	3.303	3.601	3.547	2.926	4.316

primordia was observed in $P_1 \times P_1$ (174.50 days) which was found to be on par with $P_9 \times P_4$ (172.00 days).

7. Days for deflasking

Deflasking required 155.83 days in $P_1 \times P_1$ which was found to be significantly low, whereas the seedlings of $P_6 \times P_5$ recorded longest duration taken for deflasking (276.17 days).

4.3.2 Observations on seedling morphology at deflasking

1. Height of seedling

Height of seedling at deflasking was observed to range from 1.77 cm in $P_2 \times P_1$ to 5.22 cm in $P_1 \times P_7$. The hybrid combination $P_2 \times P_9$ with a height of 5.18 cm was found to be par with the highest value.

2. Number of leaves

Mean number of leaves per seedling was observed to range from 1.83 in $P_3 \times P_4$ to 4.83 in the hybrid $P_9 \times P_4$. Six hybrids recorded values on par with the highest for number of leaves. These six hybrids comprised of $P_9 \times P_7$ registering number of leaves of 4.33, $P_{10} \times P_4$ with 4.17 and $P_2 \times P_9$, $P_6 \times P_4$, $P_{10} \times P_1$ and $P_{11} \times P_1$ with 4.00 leaves each. Values on par with the lowest were recorded by four hybrids viz., $P_2 \times P_8$ (2.00), $P_6 \times P_5$ (2.17), $P_4 \times P_6$ (2.67) and $P_2 \times P_5$ (2.67).

3. Length of the longest leaf

Length of the longest leaf was recorded maximum in the hybrid combination $P_5 \times P_4$ (4.37 cm). The hybrid combination $P_{11} \times P_{11}$ produced the shortest leaf (1.22 cm) among the different combinations.

4. Breadth of the longest leaf

The breadth was recorded high in $P_1 \times P_1$ (0.48 cm) whereas it was found to be the lowest in $P_2 \times P_5$ (0.19 cm).

Table 4.3.2 Observations on seedling morphology at deflasking

Sl. No.	Cross combination	Height of seedling	Number of leaves	Length of the longest leaf	Breadth of the longest leaf	Number of roots	Length of the longest root	Diameter of the longest root
1	$P_1 \times P_1$	4.32	3.83	3.64	0.48	4.00	3.38	0.24
2	$P_1 \times P_2$	4.76	2.83	4.22	0.26	2.00	2.45	0.22
3	$P_1 \times P_7$	5.22	3.67	3.24	0.35	4.50	1.63	0.25
4	$P_2 \times P_1$	1.77	3.83	1.47	0.34	2.33	0.75	0.22
5	$P_2 \times P_5$	3.84	2.67	3.24	0.19	3.33	1.34	0.22
6	$P_2 \times P_8$	2.42	2.00	1.75	0.26	2.50	1.15	0.22
7	$P_2 \times P_9$	5.18	4.00	3.80	0.35	4.17	3.23	0.28
8	$P_3 \times P_1$	3.84	3.00	2.64	0.24	2.83	1.77	0.22
9	$P_3 \times P_4$	3.14	1.83	1.79	0.26	2.17	1.14	0.21
10	$P_4 \times P_6$	3.85	2.67	3.17	0.22	3.00	3.25	0.22
11	$P_5 \times P_4$	5.13	3.67	4.37	0.22	2.67	0.73	0.19
12	$P_6 \times P_4$	2.35	4.00	1.85	0.20	2.50	0.63	0.15
13	$P_6 \times P_5$	2.70	2.17	2.26	0.25	1.17	1.93	0.21
14	$P_6 \times P_7$	2.19	3.17	1.70	0.25	2.33	1.55	0.23

Table 4.3.2 Continued

Sl. No.	Cross combination	Height of seedling	Number of leaves	Length of the longest leaf	Breadth of the longest leaf	Number of roots	Length of the longest root	Diameter of the longest root
15	$P_7 \times P_{11}$	2.84	3.50	2.49	0.23	3.67	1.25	0.16
16	$P_8 \times P_2$	2.53	3.00	1.60	0.25	3.50	1.15	0.18
17	$P_9 \times P_1$	2.45	3.83	1.86	0.33	2.67	1.28	0.21
18	$P_9 \times P_4$	2.14	4.83	2.61	0.25	2.00	0.85	0.27
19	$P_9 \times P_7$	2.84	4.33	1.91	0.22	3.50	0.95	0.29
20	$P_{10} \times P_1$	5.13	4.00	4.31	0.35	3.00	0.72	0.21
21	$P_{10} \times P_4$	2.92	4.17	1.58	0.28	3.00	0.82	0.27
22	$P_{11} \times P_1$	3.83	4.00	2.60	0.22	3.00	1.16	0.19
23	$P_{11} \times P_5$	3.63	3.00	3.00	0.30	2.67	0.46	0.22
24	$P_{11} \times P_{11}$	1.82	3.83	1.22	0.22	3.50	0.61	0.18
	SEm	0.015	0.308	0.012	0.010	0.349	0.012	0.009
	CD (0.05)	0.042	0.864	0.035	0.028	0.977	0.033	0.025

5. Number of roots

Number of roots was found to be significantly high in the seedlings of P_1 x P_7 (4.5). This was found to be on par with P_2 x P_9 (4.17), P_1 x P_1 (4.00) and P_7 x P_{11} (3.67). P_6 x P_5 recorded the lowest number of roots in the seedling (1.17) which was noted to be on par with P_1 x P_2 (2.00) and P_9 x P_4 (2.00).

6. Length of the longest root

Root length in P_1 x P_1 recorded the maximum value with 3.38 cm whereas the root length in P_{11} x P_5 (0.46 cm) was found to be the lowest.

7. Diameter of the longest root

The diameter of the longest root was found to be maximum in the hybrid P_9 x P_7 (0.29 cm). This was found to be on par with P_2 x P_9 (0.28 cm), P_9 x P_4 (0.27 cm) and P_{10} x P_4 (0.27 cm). The hybrid combination P_6 x P_4 recorded the lowest root diameter with 0.15 cm which was found to be on par with P_7 x P_{11} (0.16 cm).

DISCUSSION

5. DISCUSSION

Orchids which are distinctive plants among monocotyledons constitute one of the most precious groups of great floricultural value. The highly promiscuous nature of this group of plants permitting uninhibited intermingling of genomes even at the generic level has rendered them an excellent material for combination breeding. Breeders have exploited this quality to the fullest extent, registering more than 100,000 orchid hybrids during the past 100 years and the quest for novelty is still continuing. The present study was conducted to analyse the compatibility among twelve monopodial orchids, resulting in 24 hybrid combinations. Salient findings in the course of their development are discussed below.

COMPARISON BETWEEN PARENTS BASED ON VEGETATIVE CHARACTERS

Although the ultimate success of an orchid hybrid is decided by the beauty of its bloom, vegetative vigour is also important as has been pointed out by McDonald (1991). He stressed the importance of vegetative vigour, stating that vigorous hybrids result in bigger, better blooms and more floriferous nature with greater flower substance. Also while selecting parents for any hybridization programme, the general health and superior vegetative qualities of the plants are of great importance. Hence a comparative analysis of the vegetative characters of the parents utilized in the present study is presented below.

Parent material was evaluated with respect to vegetative characters one year after planting. A wide range of variation for vegetative biometric characters was observed among the parents. The range was prominent for most of the vegetative characters like length of cane (52.13 to 116.20 cm), number of leaves per cane (22.83 to 45.17), number of

aerial roots (3.67 to 17.00), length of aerial root (32.65 to 60.18 cm), length of leaf (8.13 to 14.60 cm), width of leaf (0.48 to 4.03 cm) and leaf area (5.29 to 43.47 cm). This wide range of variations in parents may be due to the fact that the majority of the parental genotypes employed in the present study are themselves higher order monogeneric and bigeneric hybrids. This finding is in conformity with the reports of Hurst (1898) that in orchids higher order multigeneric hybrids showed a far wider range of character variation as compared to lower order primary hybrids. Similar results have been observed in orchids by McConnel and Kamemoto (1983). They found that even reciprocal crossings in multigeneric hybrids yielded offsprings differing in cane height, pseudobulb production and flower yield, bearing evidence to their highly heterozygous nature. In the present study characters like thickness of stem (0.48-1.75 cm) and length of internode (1.95-4.06 cm) also showed a high range of variation. This wide variation in external vegetative morphology bears evidence to their diverse genetic makeup, which when combined will give rise to a wide array of variants from which the chances of selecting a desired hybrid are more.

COMPARISON BETWEEN PARENTS BASED ON FLORAL CHARACTERS

A clear background knowledge on floral biology is of special significance in the breeding of orchids due to the structural and functional peculiarities of their flowers. The important biometric characters deciding the size and nature of inflorescence are length of inflorescence, length of scape, number of flowers per inflorescence, length of internode of inflorescence and thickness of inflorescence axis. Days to first flower opening from inflorescence emergence is primarily decided by the length of inflorescence and its rate of growth (Rani, 2002). In the present study, this character was found to follow the same trend as the length of inflorescence in all varieties, showing that the rate of inflorescence growth did not vary much between varieties. Flowering time is decided by the

number of flowers per inflorescence and the rate of flower opening. Correlation was not observed between flowering time and number of flowers per inflorescence in the parental varieties studied denoting that the rate of flower opening varied with the variety.

Length of inflorescence has been pointed out as a character of prime importance in any orchid breeding programme (McDonald, 1991). Proper balancing between the length of inflorescence and length of scape is important in deciding the elegance and grace of an inflorescence. Diameter of inflorescence axis also has a major say in deciding the shape and nature of the inflorescence axis. Length of internode of inflorescence should be optimum for proper display of flowers; ample clearance between successive flowers is essential to prevent overcrowding of flowers whereas more clearance leads to the ungainly, prominent exposure of inflorescence axis.

Number of flowers per inflorescence is a character of prime importance in orchid breeding, as has been pointed out by Kamemoto (1983), McConnel and Kamemoto (1983), Singh (1986) and McDonald (1991). As all the parents used in the present study are themselves higher order hybrids the increased average flower number of seven to eighteen exhibited by the majority of the parents is in accordance with the observations of Singh (1982) that in orchids, higher order hybrids show increased number of flowers per spike. Bobisud and Kamemoto (1982) arrived at the same conclusion that flower production in *Dendrobium* hybrids was primarily influenced by parental genotypes. The inheritance of the character number of blooms per inflorescence has been reported by several scientists. Tippit (1997) was of the opinion that in a new hybrid, the number of flowers per inflorescence was the geometric mean of the two genetically dissimilar parents involved in the cross.

Among the twelve parental genotypes, flower length was found to be greater than flower width in all the varieties excluding P_7 and P_9. Atwood

(1989) and Porter (1989) encountered a similar situation where flowers in *Paphiopedilum* hybrids were found to be generally longer and wider when parents having different length : breadth ratios were crossed. Oakeley (1991) in *Lycaste* species has described in detail the advantages of breeding for reduced size by selecting as parents varieties having reduced flower length and width.

In monopodials, flowering is either throughout the year or seasonal. In the present study, out of the 12 parental genotypes, five were free-flowering and seven were seasonal, flowering from May to October (P_3) and June to December (P_4, P_5, P_6, P_7, P_8, P_9). Sobhana (2000) conducted similar parental evaluation in *Dendrobium* prior to hybridization. She studied ten commercial hybrids of *Dendrobium* and observed that five out of the ten flowered round the year while the other five were seasonal flowering in the same season as mentioned above. Rani (2002) conducted similar works in 14 parental genotypes of *Dendrobium* and reported that five were free-flowering. Seasonal flowering is not as advantageous as free-flowering because this restriction of flowering to certain months or seasons limits the availability of flowers of varieties like P_3, P_4, P_5 etc to certain periods/ months of the year, limiting their market share, while the flowers of such as P_1, P_2, P_{10}, P_{11} and P_{12} which are free-flowering are available throughout the year. The general trend observed on demand of flowers is related to their constant availability in the market which is observed in free-flowering hybrid varieties. Free-flowering nature should be kept in mind while new, prospective hybrids from the present study are selected for developing into commercial hybrids. Five of the parents used in the present programme exhibited free-flowering character. This indicates the high possibility for this character to be reflected among their progeny hybrids also.

Nature of inflorescence axis in monopodials may be erect or arching. The mode of display of flowers is alternate and facing opposite directions

or whorled. In the present study, the parents exhibited erect as well as arching inflorescence axes and the mode of display was alternate, flowers facing opposite directions or whorled, presenting a bunched appearance. Both these modes of display are advantageous as the inflorescence presents the same appearance when viewed from either sides. In majority of the parents used in the present study, the inflorescence axes were arching with the flowers alternate and facing opposite directions. This is in accordance with findings of Rani (2002) in *Dendrobium*. Davidson (1994) reported that while selecting parents for hybridization programmes in orchids, the distinctive shape of the inflorescence axis and attractive mode of display were important.

Fullness value gives a clue about the degree of fullness or the perfection in shape and arrangement of sepals and petals. Leonhardt (1977), while breeding with *Cymbidium* and related genera, made use of fullness value to obtain an estimate of the degree of fullness. Fullness value in diploid *Cymbidium* was 4.7 and that in tetraploid was 4.1, indicating that the tetraploid was comparatively fuller. In the present study, parent P_4 produced remarkably full flowers with a fullness value of 2.15 whereas flowers were observed to be full in parents like P_5 (2.22), P_6 (2.27) and P_3 (3.06). Oakeley (1991) observed that slight reflexing and overlapping of sepals and petals are desirable attributes in orchids. In the present breeding programme, except in P_4, P_5 and P_6, all the other parental genotypes showed the curving inwards of petal and sepal tips.

In order to minimize wastage, pollen in Orchidaceae is agglutinated in masses called pollinia (Sheehan and Sheehan, 1979). Abraham and Vatsala (1981) reported that pollen in orchids existed as tetrads, held together by elastic bands of tapetal origin. In the present study pollen size was found to range from 34.86 μ in P_6 to 44.56 μ in P_3 and pollen fertility percentage was observed to range from 50.80 per cent in P_5 to 78.00 per cent in P_{10}. Varghese (1995), Sobhana (2000) and Rani (2002) also

conducted detailed studies on pollen size and pollen fertility in orchids. Rani (2002) observed that pollen size was related to pollen fertility and pollen germination. Low pollen fertility and germination were found to reflect to some extent on the low percentage of hybrid seed set and hence pollen studies are important in orchid breeding programmes.

COMPATIBILITY ANALYSIS

Although uninhibited intermingling of genomes has been pointed out as a characteristic feature of Orchidaceae, several cases of incompatibility have been reported, particularly in monopodials. Leonhardt (1977) found that incompatibility systems in orchids are of two types–exogenous barriers such as geographical isolation, pollinator specificity and seasonal flowering habit and endogenous barriers of a genic or chromosomal nature. Exogenous barriers can be easily overcome by hybridization under controlled conditions whereas endogenous system may offer permanent barriers to hybridization.

Endogenous incompatibilities of a genic origin may be associated with an inability of pollen to germinate on a given stigmatic surface or an inability of the pollen tubes to grow down the column and reach the ovules. In a comparatively milder form of incompatibility, inhibition may occur in the ovary, preventing fertilization. All these forms of incompatibility are manifested externally as initial flower drop following pollination. In the present investigation, high ovary drop (20.70 %) after initial swelling following pollination in the pre and post-zygotic phases was observed, which may be due to the above mentioned genic incompatibility. Devi and Deka (1994), Varghese (1995), Sobhana (2000) and Rani (2002) have also reported very high initial ovary drop in orchids like *Dendrobium*. Changes following pollination and the control exerted by the rostellar stigmatic region in bringing about these changes have been studied at length by Harrison and Arditti (1972), Arditti (1979) and Slater (1991). In the present study, post pollination floral changes comprising of

the changes happening to the perianth, column and ovary have been analysed in detail. The time taken for attaining each of these developmental stages till capsule harvest has been studied for all the crosses conducted. A female parent dependent variation was observed for the onset of these changes in the varieties studied. In *Cymbidium*, Leornhadt (1977) encountered 89.30 per cent initial ovary drop. He further observed that in analysing inter and intra groups compatibilities, the following four parameters were the most useful :

1)Percentage capsule yield

2)Percentage capsules with filled / apparently normal seeds

3)Percentage filled seeds per capsule

4)Percentage capsules with germinating seeds

Leonhardt (1977) in *Cymbidium* reported that a total of 2466 pollinations were made with 265 (10.75 %) fruits harvested of which 182 (68.68 %) contained an average of 31.30 per cent seeds with apparently viable embryos. Of these, seeds from 142 fruits (53.58 %) germinated, producing seedlings.

In the present investigation on monopodials, out of the 116 cross combinations attempted 58 (50 %) succeeded in producing harvestable green capsules. Out of these 58 combinations capsules from 46 (79.31 %) combinations contained an average of 43.28 per cent filled/apparently normal seeds. Out of these 46 combinations cultured *in vitro* capsules from 36 (78.26 %) combinations contained germinating seeds. Similar findings were reported by Rani (2002) in *Dendrobium*. She carried out 1696 pollinations in 84 combinations with 218 (12.58 %) green capsules harvested, of which 211 (96.78 %) in 81 combinations contained an average of 33.54 per cent seeds with apparently viable embryos. Out of these, seeds from 197 green capsules (93.36 %) belonging to 76 combinations germinated *in vitro*.

Parthenocarpic fruit development without seed set has been reported in orchids by several workers (Devi and Deka, 1992; Varghese, 1995; Sobhana, 2000; Rani, 2002). Some pre or post-fertilization barriers may be operational in such cases. In the present study with monopodial orchids, this type of parthenocarpic fruit development was observed in 12 cross combinations. The production of non-germinating, yet apparently viable / filled seeds is another incompatibility system which was found operational in ten cross combinations. Such embryos may be dormant or non-functional due to various reasons such as chemical inhibitors, structural hybridity, genic and chromosomal imbalances etc. Additional research and supporting data are essential to pinpoint the exact reason. In the present study, incompatibility was found to strike at later stages also. Out of the 36 germinating combinations, 24 combinations provided mature seedlings for deflasking. In 12 combinations incompatibility was found to strike at different stages in the post zygotic phase. In these combinations, growth rate slowed down considerably prior to degeneration *in vitro*, suggesting perhaps the involvement of genic and chromosomal incompatibility mechanisms.

Failure of fruit development has been observed in many crosses especially in cases where the varieties P_3 and P_{10} were used as male parents. The same problem had been reported by Devi and Deka (1992), Varghese (1995) and Rani (2002) in *Dendrobium*. This bears evidence to the operation of a unidirectional incompatibility system within the family. Leonhardt (1977) has reported a similar incompatibility system in hybridization between *Cymbidium* and *Ansellia* whereby seedlings were easily produced when *Cymbidium* was used as the female parent and rarely produced when *Ansellia* was used as the female parent. Another probable reason for the incompatibility noted when large flowers are pollinated using pollen from small flowers is purely physical rather than genetic. The pollen tubes may not have had the physical capacity to grow down the length of the column to reach the unfertilized ovules.

A detailed account of the extent and strength of incompatibility reaction is presented. Incompatibility reaction is strongest when it strikes at an early stage *i.e.*, flower drop after pollination before the onset of any post pollination floral change. So when a particular parent as male or female presents this symptom after pollination in several cross combinations, the strength of its incompatibility reaction can be considered to be high. Ranking of the twelve parental genotypes of monopodial orchids was done based on strength of incompatibility (Table 5.1). The parent P_8 ranked first exhibiting the strongest incompatibility denoting that majority of the unsuccessful crosses with P_8 succumbed to incompatibility in the early stages following pollination. The parents P_7 and P_{12} ranked second and P_{10} ranked third for the strength of incompatibility. Strength of incompatibility was the lowest in P_{11} followed by P_1.

Extent of incompatibility reaction denotes the number of cross combinations in which incompatibility reaction is presented (at any stage) when a particular parent is used as male or female. The more the number of crosses presenting incompatible reaction, the greater the extent of incompatibility. Ranking of the twelve parental genotypes of monopodial orchids was done based on the extent of incompatibility (Table 5.2). Extent of incompatibility was the highest in P_{12} denoting that the highest number of crosses expressed incompatibility reaction at various stages from pollination to deflasking in this parent. Incompatibility reaction was presented by all the twelve combinations were P_{12} was used as either the female or the male parent. The parents P_3, P_8 and P_{10} ranked second for the extent of incompatibility. Extent of incompatibility was the lowest in P_1 followed by P_2 and P_4.

Ranking of the 12 parental genotypes was done based on compatibility, *viz.*, the number of successful combinations that were deflasked (Table 5.3). The parent P_1 ranked first, providing six and three

Table 5.1 Ranking of twelve parental genotypes of monopodial orchids based on strength of incompatibility

Parental genotypes	Mean incompatibility		Rank assigned
	as female parent	as male parent	
P_1	4.67	5.42	10
P_2	5.42	4.50	9
P_3	4.50	3.67	6
P_4	2.83	5.17	5
P_5	3.50	4.08	4
P_6	4.42	3.83	7
P_7	3.25	4.17	2
P_8	3.42	3.75	1
P_9	4.33	4.00	8
P_{10}	4.17	3.33	3
P_{11}	5.25	4.91	11
P_{12}	4.25	3.17	2

Table 5.2 Ranking of twelve parental genotypes of monopodial orchids based on extent of incompatibility

Parental genotypes	Number of combinations presenting incompatibility reaction		Rank assigned
	as female parent	as male parent	
P_1	9	6	6
P_2	8	10	5
P_3	10	12	2
P_4	11	7	5
P_5	11	9	3
P_6	9	11	3
P_7	11	9	3
P_8	11	11	2
P_9	9	11	3
P_{10}	10	12	2
P_{11}	9	10	4
P_{12}	12	12	1

Table 5.3 Ranking of twelve parental genotypes of monopodial orchids based on compatible crosses

Parental genotypes	Number of combinations providing hardened seedlings		Rank assigned
	as female parent	as male parent	
P_1	3	6	1
P_2	4	2	2
P_3	2	-	5
P_4	1	5	2
P_5	1	3	4
P_6	3	1	4
P_7	1	3	4
P_8	1	1	5
P_9	3	1	4
P_{10}	2	-	5
P_{11}	3	2	3
P_{12}	-	-	6

successful combinations as male and female parents respectively; P_2 and P_4 came second with six successful combinations each to their credit, P_2 with two and four combinations as male and female parents respectively and P_4 with five and one combination as male and female parents respectively; P_{11} ranked third with two and three successful combinations as the male and female parents, respectively, to its credit. The bigeneric hybrid variety P_{12} turned out to be the poorest combiner, ranking last. No combination turned out to be successful when P_{12} was used as either male or female parent. Self compatibility was noticed in two genotypes while the remaining ten were self incompatible.

IN VITRO EMBRYO CULTURE

Green capsule culture was a major advancement in increasing the germination of orchid seeds *in vitro*. Withner (1959) was of opinion that very young as well as fully mature ovules did not form good explants *in vitro* due to dormancy, pH, inhibitory and other metabolic factors. Sauleda (1976) found that the pistillate parent was mainly responsible for determining the correct capsule maturity stage. Sobhana (2000) harvested green capsules of *Dendrobium* at 75-90 per cent maturity, *viz.*, 90-140 days after pollination. Rani (2002) too confirmed the findings and the capsules were harvested at 62-130 days after pollination *viz.*, between 75-90 per cent maturity. In the present study also, capsules were harvested at 74-135 days after pollination with very high success in terms of *in vitro* germination.

The inherent genetic and physiological features were found to play a direct role in *in vitro* seed germination and differentiation of organs. Hazarika and Sarma (1995) in *Dendrobium transparens* observed seed germination 16-18 days after inoculation and Krishnan *et al.* (1993) observed the same in *Spathoglottis plicata* around two weeks of culture. The present study revealed a wide range in duration from 15.50 to 40.50 days for *in vitro* germination. The variety P_6 germinated early,

taking 17.00 days. Varieties P_9 and P_{11} also germinated early, taking 19.17 days for the same, which is in conformity with the above mentioned reports. On the other hand the highly bred bigeneric hybrid varieties included in the parentage exhibited a range extending upto 40.50 days. The same general trend of slow growth was followed by the complex hybrids throughout *in vitro* growth, till deflasking.

SEEDLING MORPHOLOGY AT DEFLASKING

Post deflasking survival of plantlets depended greatly on the number of leaves present at the time of deflasking and the rate of leaf growth. Sutter *et al.* (1985) expressed the view point that the stage at which deflasking is done is important in determining the further survival. The results of the present study were also in full conformity with his findings that survival was the highest with seedlings that had developed a minimum 2-3 leaves and 2-4 roots. The lesser the time taken to reach this stage, the more vigorous the hybrid combination was considered to be. Thus the duration to deflasking is important in that it is an indication of the vegetative vigour or heterotic potential of the hybrid.

Rani (2002) reported that fast growing cultures yielding vigorous seedlings continued the rapid rate of growth in the greenhouse also, reaching flowering stage much earlier than slow growing cultures. Hence a detailed analysis of days taken for deflasking and seedling morphology at deflasking are important because they reflect further growth and development in the greenhouse.

In the present study, seedling morphology at deflasking was analysed with respect to height of seedling, number of leaves per seedling, length and breadth of the longest leaf, number of roots per seedling and length and diameter of the longest root. Seedling height at deflasking showed a highly significant wide range (1.77 cm in $P_2 \times P_9$ to 5.22 cm in $P_1 \times P_7$) of variation between combinations, indicating the effect of genotype in character expression. Length of the longest leaf ranged from

1.22 cm in P_{11} selfed to 4.37 cm in P_5 x P_4 and length of the longest root ranged from 0.46 cm in P_{11} x P_5 to 3.38 cm in P_1 x P_1. In general, an enhanced seedling height at deflasking stage indicates higher root length and leaf length. Diameter of the longest root is another character having a bearing on seedling vigour particularly in monopodial orchids. Root diameter ranged from 0.15 cm in P_6 x P_4 to 0.29 cm in P_9 x P_7. In most of the combinations, thick and vigorous root initials were observed.

The 24 hybrid combinations deflasked were ranked based on seedling morphology at deflasking (Table 5.4). The two most important characters *viz.*, number of leaves and number of roots per seedling at deflasking were considered for the same. The combination P_2 x P_9 ranked first with 4.00 leaves and 4.17 roots per seedling. The seven combinations P_1 x P_1, P_1 x P_7, P_7 x P_{11}, P_9 x P_7, P_{10} x P_1, P_{10} x P_4 and P_{11} x P_1 ranked second producing 3.50 to 4.33 leaves and 3.00 to 4.50 roots per seedling. The eight combinations P_3 x P_1, P_5 x P_4, P_6 x P_4, P_8 x P_2, P_9 x P_1, P_9 x P_4, P_{11} x P_5 and P_{11} x P_{11} ranked third.

The objective of the present study was to undertake intra and intergeneric hybridization and *in vitro* embryoculture in monopodial orchids. With this objective in focus, hybridization in all possible combinations was carried out among the selected parentals. Hybrid seeds were cultured *in vitro*, providing all the necessary culture conditions. They were deflasked at the appropriate stage and seedling morphology was studied. Detailed analysis of compatibility / incompatibility relations was done at the different stages starting immediately after pollination till deflasking. Out of the 116 cross combinations, seedlings from 24 combinations were successfully deflasked, for *ex vitro* establishment. Further hybridization works are essential for the detailed analysis of pre and post zygotic incompatibility systems encountered at various stages in monopodial orchid breeding.

Table 5.4 Ranking of the 24 hybrid combinations based on seedling morphology at deflasking

Parental genotypes	Position assigned based on		Rank assigned
	number of leaves per plant	number of roots per plant	
$P_1 \times P_1$	2	1	2
$P_1 \times P_2$	3	3	5
$P_1 \times P_7$	2	1	2
$P_2 \times P_1$	2	3	4
$P_2 \times P_5$	3	2	4
$P_2 \times P_8$	3	3	5
$P_2 \times P_9$	1	1	1
$P_3 \times P_1$	2	2	3
$P_3 \times P_4$	4	3	6
$P_4 \times P_6$	3	2	4
$P_5 \times P_4$	2	2	3
$P_6 \times P_4$	1	3	3
$P_6 \times P_5$	3	4	6
$P_6 \times P_7$	2	3	4

Table 5.4 continued

Parental genotypes	Number of combinations presenting incompatibility reaction		Rank assigned
	as female parent	as male parent	
$P_7 \times P_{11}$	2	1	2
$P_8 \times P_2$	2	2	3
$P_9 \times P_1$	2	2	3
$P_9 \times P_4$	1	3	3
$P_9 \times P_7$	1	2	2
$P_{10} \times P_1$	1	2	2
$P_{10} \times P_4$	1	2	2
$P_{11} \times P_1$	1	2	2
$P_{11} \times P_5$	2	2	3
$P_{11} \times P_{11}$	2	2	3

SUMMARY

6. SUMMARY

The present study was conducted in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during 2001–2003 to assess the compatibility among monopodial orchids.

- Twelve genotypes of monopodial orchids comprising of six monogeneric (P_1 to P_6) and six bigeneric (P_7 to P_{12}) hybrid varieties were evaluated adopting completely randomized design with six replications.
- Data were collected for vegetative characters and floral characters from the selected parental genotypes.
- Analysis of variance revealed significant differences among the parental genotypes with respect to all the 21 (ten vegetative + eleven floral) biometric characters studied.
- High phenotypic and genotypic coefficients of variation were observed for number of aerial roots, leaf area and width of leaf indicating high variability for these characters and scope for improvement through selection.
- Heritability estimates were high (>70 %) for all the vegetative characters except number of leaves per cane, length of aerial root and length of internode and floral characters like days to last flower opening from first flower opening and number of spikes per cane suggesting very little influence of the environment in the expression of these characters.
- High values of genetic advance (>70 %) were recorded for number of aerial roots, width of leaf, leaf area and number of flowers per inflorescence. Genetic advance in the range of 30-70 per cent was expressed by majority of the characters studied.

- High heritability coupled with high genetic advance was observed for number of aerial roots, width of leaf, leaf area and number of flowers per inflorescence indicating additive gene action for these characters. This suggests that permanent improvement could be attained by practicing selection on the above traits.
- The genotypic, phenotypic and environmental correlations of the twelve parents were studied for ten biometric characters. High positive correlation at genotypic and phenotypic levels was observed between most of the vegetative and floral characters studied.
- Significant positive inter-correlation at genotypic and phenotypic levels was observed for length of flower and width of flower with number of leaves per cane, number of aerial roots, number of spikes per cane, length of inflorescence and length of scape.
- Environmental correlation was observed to be low in comparison with genotypic and phenotypic correlations for all pair-wise character combinations.
- Flowering and floral quality of the parental genotypes were analysed with respect to five characters.
- Pollen was found to exist as tetrads which were spherical to rectangular in shape and agglutinated in masses called pollinia. Although similar in shape, pollen diameter ranged from 34.86 μ in P_6 to 44.56 μ in P_3.
- Pollen fertility percentage showed much variation among the parental genotypes. The highest pollen fertility of 78.0 per cent was recorded by P_{10} and lowest was recorded by P_5 (50.8 %).
- Intercrossing in all possible combinations involving the twelve parental genotypes of monopodial orchids was done, depending on the availability

of receptive stigma and fresh pollen. Out of the 144 possible combinations, 116 diallel crossings were undertaken. These 116 combinations included 50 crosses, 54 reciprocals and 12 selfs. Some of the cross combinations and their reciprocals could not be attempted as their flowering did not synchronize.

- Out of the 116 self and cross combinations attempted, 58 succeeded in producing harvestable green capsules. Out of these, no seeds were obtained from the capsules of 12 combinations. Seeds from the remaining 46 combinations were cultured axenically.
- Percentage capsule yield ranged from 8 to 38 in the various hybrid combinations.
- Percentage of filled seeds ranged from 18 to 76 in the different combinations.
- No germination was obtained with seeds from ten out of the 46 combinations inoculated *in vitro*. Successful seed germination was observed in 36 combinations.
- Further development was found to be arrested in 12 combinations at various stages of *in vitro* development. Mature seedlings were obtained from 24 combinations.
- The levels of incompatibility reactions were grouped under six heads ranging from flower abscission before the onset of any visible post pollination change to instances where seeds germinated but aborted in culture. A total of 58 combinations attempted succumbed to incompatibility at these different stages from pollination to deflasking.
- Immature embryos from 24 cross combinations were cultured *in vitro* in MS half strength basal medium and taken through three to four subculture

passages. Seedlings having 2-3 leaves and 2-4 roots were deflasked. Time taken for attaining this stage varied from 66 days to 135 days. Significant differences among the combinations were observed with respect to number of days taken for germination initiation, number of days for development of protocorms, chlorophyll, first leaf and first root primoridia and for deflasking.

- Significant differences in seedling morphology were observed among the 24 combinations at deflasking with respect to all the seven vegetative characters studied.

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* Originals not seen

COMPATIBILITY STUDIES IN MONOPODIAL ORCHIDS

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ABSTRACT

A research programme "Compatibility studies in monopodial orchids." was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during 2001-2003 with the objective of undertaking intra and intergeneric hybridization and *in vitro* embryo culture in monopodial orchids.

Twelve monopodial orchid genotypes comprising of six monogeneric and six bigeneric hybrids were selected as parents after initial evaluation. They were evaluated adopting completely randomized design with six replications.

Analysis of variance revealed significant differences for almost all the characters studied. Genotypic and phenotypic coefficients of variation were high for number of aerial roots, leaf area and width of leaf. Characters like number of aerial roots, width of leaf, leaf area and number of flowers per inflorescence had high heritability coupled with high genetic advance.

Significant positive inter-correlation at genotypic and phenotypic levels was observed for length of flower and width of flower with number of leaves per cane, number of aerial roots, number of spikes per cane, length of inflorescence and length of scape.

The 12 parental genotypes were crossed in all possible combinations (144) after preliminary studies on floral biology. A total of 116 crosses were done including 50 crosses, 54 reciprocals and 12 selfs. Incompatibility reaction was noticed at different stages ranging from flower abscission before the onset of any visible post pollination change to instances where seeds geminated but aborted in culture. A total of 58 combinations attempted succumbed to incompatibility at these different

stages from pollination to deflasking. Harvestable green capsules were obtained from 58 combinations and they were inoculated in MS half strength basal medium. Percentage capsule yield ranged from 8 to 38 in the various hybrid combinations. Percentage filled seeds ranged from 18 to 76 in the various combinations. Capsules from twelve combinations did not contain seeds and seeds from ten combinations did not germinate on inoculation. Protocorms of developing seedlings from twelve combinations aborted at various stages of *in vitro* development.

The remaining 24 cross combinations were taken through three to four subculture passages. Seedlings having 2-3 leaves and 2-4 roots were deflasked. Significant differences among the combinations were observed with respect to number of days taken for germination initiation, number of days taken for development of protocorms, chlorophyll, first leaf and first root primordia and for deflasking.

Significant differences in seedling morphology were observed among the 24 hybrid combinations at deflasking with respect to all the seven vegetative characters studied.