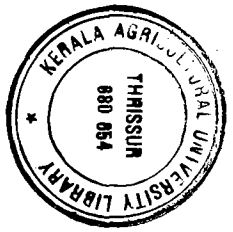
Intra and interspecific hybridization in *Dendrobium* spp.



By

C. LEKHA RANI

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VELLAYANI, THIRUVANANTHAPURAM

2002

DECLARATION

I hereby declare that this thesis entitled **"Intra and interspecific hybridization in *Dendrobium* spp."** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Lekha Rani

Vellayani,
18-4-2002.

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CERTIFICATE

Certified that this thesis entitled **"Intra and interspecific hybridization in *Dendrobium* spp."** is a record of research work done independently by **Smt. C. Lekha Rani** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Lekha Rani C

LEKHA RANI. C.

CONTENTS

Page No.

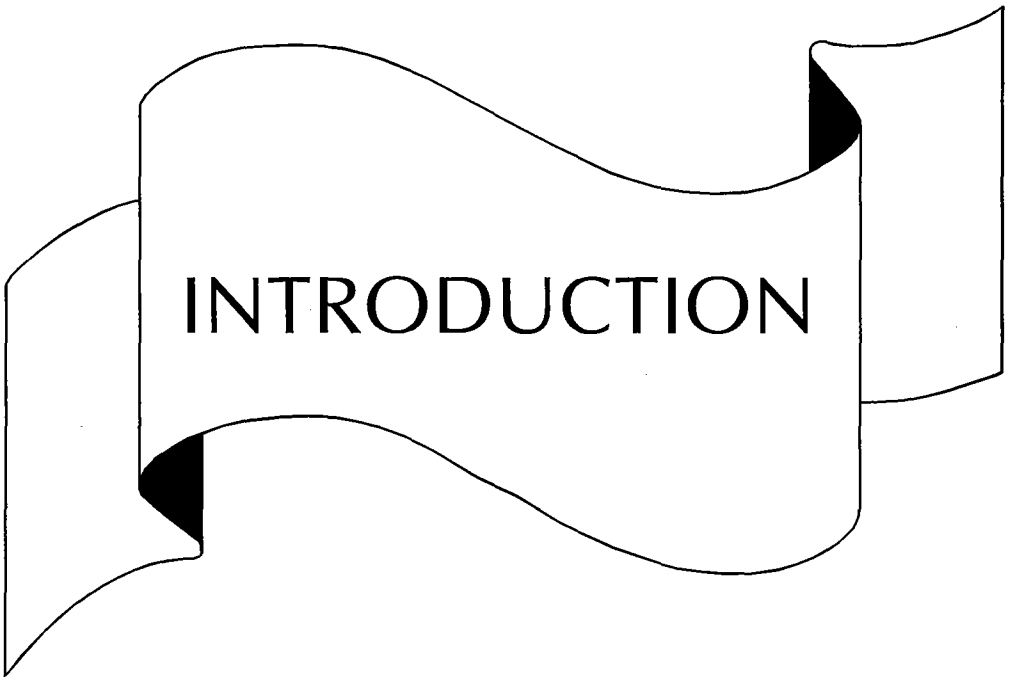
LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

1.	2,4-D	-	2,4-dichlorophenoxy acetic acid
2.	ANOVA	-	Analysis of variance
3.	BA	-	Benzyladenine
4.	BAP	-	Benzylaminopurine
5.	BP	-	Banana pulp
6.	CD	-	Critical difference
7.	CW	-	Coconut water
8.	EH	-	Economic heterosis
9.	GA	-	Genetic advance
10.	gca	-	General combining ability
11.	GCV	-	Genotypic coefficient of variation
12.	h^2	-	Heritability
13.	HB	-	Heterobeltiosis
14.	IAA	-	Indole-3-acetic acid
15.	IBA	-	Indole-3-butyric acid
16.	NAA	-	Naphthalene acetic acid
17.	PCV	-	Phenotypic coefficient of variation
18.	RH	-	Relative heterosis
19.	sca	-	Specific combining ability
20.	SE	-	Standard error


INTRODUCTION

INTRODUCTION

Orchids have reigned supreme in floriculture ever since man tried to appreciate nature and tap its beautiful resources for adornment. Association of mankind with this group of plants began way back in the history of civilization. The oriental man got enchanted by the beauty and fragrance of the orchid flower and started to cultivate it as early as 500 B.C (Abraham and Vatsala, 1981). They considered the orchid as the embodiment of everything graceful and feminine, noble and refined. The famous Chinese philosopher Confuscius equated the acquaintance with good men to "entering into a room full of fragrant orchids".

The Greek philosopher Theophrastus (327-287 B.C) and the Swedish botanist Carl Linnaeus (1707-1778 A.D) are considered to be the pioneers in Orchidology. It was Theophrastus who first used the Greek word 'orchis' while referring to the twin tubers of a medicinal plant coming under the present family Orchidaceae (Fanfani and Rossi, 1988). Linnaeus adopted the name 'orchid' in his book '*Species Plantarum*' which marked the beginning of modern plant taxonomy (Fanfani and Rossi, 1988). Orchids have been well-known to horticulturists and herbalists for the past 400 years (Arditti, 1966; Garay, 1974). The British botanist, John Lindley introduced the name 'Orchidaceae' to the family in 1836 (Fanfani and Rossi, 1988). Orchidaceae is the largest family of

angiosperms comprising of over 800 genera and 35,000 species (Singh, 1986). The family accounts for above seven per cent of the species of flowering plants of the world (Pijl and Dodson, 1966) and enjoys a world wide distribution.

Most of the orchids are epiphytic while some are terrestrial. This epiphytic habitat of orchid genera has contributed to a large extent to their stable nature, wide distribution and survival in competition with other plants. Due to this habit, the orchids are not affected by continuous alterations of soil conditions to which the terrestrial plants are subjected to and therefore show comparative stability in their constitution.

To the taxonomist and the plant breeder, Orchidaceae maintains an individuality of its own. The various morphological, physiological and genetic peculiarities displayed by this group of plants have stimulated extensive research so that orchidology is at present one of the most dynamic branches of botany. The construction of the orchid flower resembling various forms of animal life as well as the efficiency with which they carry out cross pollination successfully have made this group one of the marvels of nature. 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, usually extending from two to three weeks.

Orchidaceae represents a peak in the evolution of monocots. Evolution has been so rapid in this family that it was impossible to establish effective physiological barriers to hybridization such that even distant crosses are possible. Species identity is maintained through the action of a comparatively weak mechanical isolation barrier - pollinator specificity. This excessive promiscuity makes it an excellent material for combination breeding and the development of new hybrids. Through assisted pollination, genomes of different species and genera can be combined in this entomophilous family. Artificial hybridization in orchids was attempted much later than in other angiosperm families due to complexity of flower structure. The first flowering interspecific hybrid was developed in 1852 by Dominy by crossing *Calanthe masuca* to *Calanthe furcata*. 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). The first sexageneric hybrid in the whole plant and animal kingdom, '*Brilliandeara* Gary' was an orchid hybrid registered in 1982. In nature too, the isolation barrier has failed in several instances and distant crosses have succeeded. This high permissibility has enabled the orchids to go up the ladder of evolution in leaps and bounds. The extent of variation that can be brought about in orchids through segregation and recombination is tremendous. This quality of the family has been of the greatest value to man in his horticultural pursuits, and is one (which he has probably) exploited to

the fullest extent. The 100,000 or more man-made orchid hybrids registered during the past 100 years bear evidence to this fact.

Cultivation of orchids on a commercial scale is fast emerging as an absorbing and rewarding vocation the world over. Of late, South East Asia has developed into a major supplier of orchid hybrids. Orchid culture has been identified as a lucrative agri-business in India. Kerala, with its varied agro climatic conditions, rich man power and technological advancement has been earmarked by the Government of India as a zone for intensification of orchid cultivation. However, the orchid industry in India is still in its infancy. A critical analysis of the reasons points to the dearth of quality planting material at reasonable prices. At present, the planting material is imported from other countries at an exorbitant cost. Moreover, the hybrids that they are prepared to spare will be the ones that have lost their competitive relevance. Planting materials of the latest hybrids reigning in the international auction markets will never be exported to buyer countries. So the non-availability of locally adapted and reasonably priced novel varieties is the major bottle neck which restricts commercial orchid cultivation in India. At the same time, we are blessed with a wealth of indigenous orchid flora, offering excellent scope for improvement through hybridization. About 1300 orchid species have been reported from India (Maheshwari, 1980) scattered all over North Eastern Himalayas (600 species), North Western Himalayas (300 species), Maharashtra (130 species), Andaman and Nicobar Islands (70 species) and Western Ghats (200 species). Many Indian species have earned world-wide recognition in breeding programmes due to their

inherent attractiveness coupled with their ability to transmit these characters to their hybrids. Hybrids of Indian orchids like *Aerides multiflorum, Cymbidium devonianum, C. lowianum, C. traceanum, C. elegans, Dendrobium aggregatum, D. chrysotoxum, D. formosum, D. nobile, Paphiopedilum venustum, Vanda coerulea, V. spathulata* etc. have no peers in the world of orchids (Bose and Bhattacharjee, 1980). So we have to initiate orchid breeding and develop our own indigenous hybrid material which have the adaptability to our agroclimatic conditions as well as novelty and quality enough to compete with international standards. Moreover, considering the new international patent policies on plants and plant products, the import of planting materials from other countries may very soon become an obsolete practice and so we must be ready with our own hybrids urgently.

Against this back drop, the present study has been initiated with the objective of developing new hybrids of *Dendrobium* with commercial cutflower qualities for export market. The genus *Dendrobium* is a renowned sympodial epiphytic orchid currently enjoying very high popularity among the commercial orchids in Kerala. Many species of the genus are very showy, attractive and of great ornamental value and many have served as parents in hybridization programmes. Being the second largest genus in the family with considerable genetic diversity, it provides ample scope and has immense potential for intervarietal and inter specific hybridization. The breeding strategy to be followed for producing elite, new hybrids quickly should be by using species and varieties 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.

In view of this, nine commercial hybrid varieties, three semi-commercial hybrids and two species, of which one enjoys a semi-commercial status were selected as parents in the present hybridisation programme. The objective is to produce elite, first generation hybrids with commercial cutflower qualities for export market based on floral characters such as free-flowering nature, long arching inflorescence with more number of flowers, novel blending of colours, larger flower size and ideal mode of display of flowers.


REVIEW OF
LITERATURE

2. REVIEW OF LITERATURE

Orchids have been considered important in horticulture ever since man tried to appreciate nature. They have intrigued taxonomists as they represent a peak in the evolution of monocots. Orchidaceae has attracted plant breeders due to the uninhibited intermingling of genomes, possible even at the generic level, within the family. As a result, around one lakh orchid hybrids have been registered over the past one and a half centuries. Several hundred hybrids, mainly from Thailand and Singapore are on record within the genus *Dendrobium*, which is the most popular sympodial genus suited for commercial cultivation in the tropical belt. However the dearth of indigenous hybrids in *Dendrobium* is a major problem. In a field where novelty is cherished, breeding our own indigenous, novel, adapted hybrids is the only solution. 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 biology

A thorough understanding of the floral biology is an essential pre-requisite to any plant breeding programme. This background

knowledge is of special significance in the breeding of orchids, mainly due to the structural and functional peculiarities of the flowers in Orchidaceae.

2.1.1. Floral morphology

In epiphytic orchids, inflorescence is usually terminal indicating the end of vegetative phase or some times lateral. Flowers are produced either singly as in *Paphiopedilum*, in groups of two to five as in *Cattleya*, in the form of an umbel as in *Cirrhopetalum*, in a spike as in *Oncidium, Phalaenopsis* etc. or in a raceme as in *Dendrobium* (Yadav and Bose, 1989).

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).

Flowers exhibit much variation in 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 combination (Abraham and Vatsala, 1981).

Orchid flowers are zygomorphic, mostly bisexual or rarely unisexual as in *Catasetum*. The flower consists of three sepals, three petals and the column or gynostemium bearing the reproductive parts. The sepals are alike in appearance except in a few species. Though narrower, the sepals are coloured like the petals and hence termed

'petaloid' sepals. Two of the petals are similar, but the odd petal is highly modified to form the 'lip' or 'labellum' which is the most prominent and distinctive part of the flower. 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).

Situated in the centre of the flower, is a waxy structure known as the column/gynandrium/gynostemium/gynostegium. This is the reproductive part of the orchid flower and is the primary feature distinguishing Orchidaceae from all other families of plants. In the evolution of orchids, the male (stamen) and female (pistil) segments of the flower fused together to form the column. The only fertile anther of the *Dendrobium* flower is borne on top of the column.

Pollen forms compact, waxy masses termed pollinia contained in a cavity known as clinandrium and covered over by a deciduous operculum (anther cap). The pollina occur as two pairs of two each or simply as a group of four in *Dendrobium*. The pistillate part consists of the stigma which is a shiny depression filled with sticky mucilage and a slender, elongated ovary. The partition wall between the stamen and stigma is called rostellum, which prevents self pollination and secretes a viscid substance to hold pollina till they are mature for disbursal. The ovary is inferior, tricarpellary and single celled with three parietal placentas and numerous ovules (Abraham and Vatsala, 1981; Mukherjee, 1990).

The flower of most orchids is in an upside down position or resupinated, having turned through 180° on its pedicel (Abraham and Vatsala, 1981). According to Nyman *et al.* (1984) in *Dendrobium,* flowers were borne with the labellum uppermost, at inflorescence emergence. The buds became resupinate just before or during opening, by a twisting of the pedicel. The degree of twisting depended on the orientation of the inflorescence axis relative to the ground and the position of the pedicel on it. Individual flowers at successive nodes along the inflorescence alternated in twisting clockwise and counter clockwise. Resupination of orchids has also been reported by Bose and Bhattacharjee (1980).

The orchid flower is modelled according to the liliaceous pattern, being constituted by 15 members - 3 sepals + 3 petals + 6 stamens (in two whorls of three each) + 3 carpels. In *Dendrobium,* only the odd stamen of the outer whorl situated opposite the labellum is fertile. The two lateral stamens of this whorl appear to have merged with the labellum, contributing to its two lateral lobes. 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. All three stigmas are fertile in *Cypripediae* and *Apostasiae* (Abraham and Vatsala, 1981).

2.1.2. Anthesis

According to Croat (1980) the modes of flowering behaviour had a direct influence on pollination biology and thereby on evolution. The processes related to anthesis varied with species and environment.

In *Vanilla* Nair and Mathew (1986) observed that flower opening commenced between 10.30 am and 1.00 pm and was completed by 6.00 pm. On an average, 49 days were taken from flower bud initiation to the anthesis of the first flower and 74 days for completion of anthesis in an inflorescence.

Christenson (1992) in *Stelis argentata* reported that in sunny weather, new flowers opened primarily in the mornings and during rainy weather, in the late afternoons. The flowers lasted upto nine days on the inflorescence, but most pollinia were removed (by pollinators) during the first two days of anthesis.

Anthesis occurred between 8.30 am and 5.30 pm with peaks between 9 am and 10 am and also between 3 pm and 4 pm in *Dendrobium* hybrids (Varghese, 1995). She further reported that the flowers opened in acropetal succession and retained their freshness for 45-50 days on the inflorescence.

Sobhana (2000) came across a variety dependent variation in the time of anthesis in *Dendrobium* hybrids. In most of the varieties, anthesis commenced from 7.30 to 8.30 am and extended to 11.00 to 11.30 am while in some others, the onset of anthesis was delayed till 9.00 to 11.00 am and extended upto 2.30 pm.

2.1.3. Stigma receptivity

The wet stigmas of Orchidaceae differ markedly from the wet stigmas of other families by the presence of detached secretory cells (eleutherocytes) in the former in a mucilaginous matrix (Johansen, 1990).

Yeung (1988) concluded that cells within the stigma of orchids separate from one another, followed by a large accumulation of mucilaginous material. He further observed that a lipid layer covered the mature stigma of *Epidendrum ibaguense* at anthesis.

Studies of Devi and Deka (1992) revealed that the stigma remained receptive for four consecutive days following anthesis in *Spathoglottis plicata,* for five days in *Aerides odoratum,* for six days in *Dendrobium amoenum* and for 12 days in *Phaius tankervilleae.*

According to Varghese (1995), the stigma in *Dendrobium* hybrids remained receptive from the first day of anthesis to the ninth day. Maximum stigma receptivity was observed between the fourth and sixth days after anthesis.

Sobhana (2000) observed maximum stigma receptivity in *Dendrobium* hybrids from the second to the fifth day after anthesis.

2.2. Pollen studies

Successful hybridization in orchids depends upon several factors such as pollen viability and germination, pollen production, dissemination

and fertilization. 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. In the opinion of Sheehan and Sheehan (1979) the pollen in Orchidaceae is not powdery as in most angiosperms but agglutinated into masses called pollina. Depending on genus, two to eight pollinia occur per flower. In *Dendrobium*, two pairs of ovoid, tightly pressed pollinia are seen under the anther cap.

From studies on similar lines, Abraham and Vatsala (1981) found that pollen in Orchidaceae exists as tetrads. They are held together by elastic threads of tapetal origin.

The tetrad nature of pollen in orchids was also reported by Das and Ghoshal (1988) and Varghese (1995).

From her studies in *Dendrobium* Sobhana (2000) concluded that the pollen existed as tetrads and were spherical to rectangular in shape. The pollen from the various hybrids and the wild species were almost similar in shape but differed in size. The species had comparatively small pollen.

Johnson and Edwards (2000) reported that cohesive masses of pollen known as pollinia have evolved independently in two plant families viz., Orchidaceae and Asclepiadaceae. They further observed that though 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) observed 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.

Das and Ghoshal (1988) reported a low percentage of pollen sterility in *Dendrobium chrysotoxum* and *D. transparens*.

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

2.2.3. Pollen germination

The stimulating effect of boron in low concentrations (1.0 to 40.0 ppm) on pollen germination was reported by several scientists (Schumucker, 1935; Munzen, 1960; Johri and Vasil, 1961).

Rao and Chin (1973) reported that the pollen culture media containing sucrose and stigmatic extract were more effective, compared to inorganic salts and growth substances in promoting pollen germination of orchid hybrids.

Experiments by Varghese (1995) in *Dendrobium* resulted in successful germination of pollen in a medium comprising of two per cent sucrose, one per cent agar and 75 mg l^{-1} boric acid.

In a study by Latha and Namboodiri (1999) pollen of *Spathoglottis plicata* was found to germinate after 5-6 hours of incubation in Brewbaker medium with 10 per cent sucrose. Germination continued upto 30-36 hours. The pollen of *Cymbidium ensifolium* required 14 hours of incubation for germination initiation, but thereafter the rate of germination increased progressively for another 12 hours.

Sobhana (2000) concluded from her studies that pollen germination was lower in the wild species of *Dendrobium* compared to the hybrids, in a medium containing sucrose (2 %), agar (1 %) and boric acid (75 mg l^{-1}).

2.3. Compatibility analysis

Although uninhibited intermingling of genomes is a characteristic feature of Orchidaceae, several cases of incompatibility have been pointed out. 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).

Duncan and Curtis (1943) observed that the self incompatible orchids always have homomorphic, gametophytic, polyallelic incompatibility with stigmatic inhibition of pollen germination.

In the opinion of Abraham and Vatsala (1981) infertility in polyploids often results from pairing abnormalities during meiosis. Sterility is most frequently caused by triploidy, commonly encountered in many of the cultivated orchids.

Kamemoto *et al.* (1989) conducted studies on cross compatibility and meiotic pairing in some *Dendrobium* species and found that sections Phalaenanthe and Ceratobium are closely related to each other while section Latourea is distantly related to them.

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).

Owens and Alquini (1993) could not detect any correlation between stigma form and the presence or absence of a self-incompatibility system in *Dendrobium*.

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.

Sobhana (2000) conducted self compatibility studies in different *Dendrobium* varieties and found that selfing was not successful only in one out of ten hybrids studied.

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 self, registering higher number of seeds per capsule and higher percentage of fertilized ovules.

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.

Darwin (1904) has discussed in detail the floral modifications that have evolved to ensure cross pollination of orchids (in most cases) and to ascertain that the insect will be properly positioned to bring about pollination.

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.

The relationship between orchids and their pollinators is one of the marvels of nature. 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 (Northen, 1970).

In order to ensure cross-pollination, the pollinia are so positioned that they get stuck to the specific pollinator insect as it leaves the flower. Pollen is deposited on the stigma of the next flower it visits, as it moves down to the nectary (Northen, 1970).

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.

On the other hand, in *Dendrobium speciosum* pollinator insects were guided into the flower by the colour gradation of the perianth (Slater and Calder, 1988).

A considerable number of orchid species have been identified to be autogamous (Ridley, 1888; Abraham and Vatsala, 1981).

Catling (1980) reported on rain-assisted autogamy in *Liparis loeselii* where autogamy increased from 17 to 70 per cent when flowers were watered with a rain-like spray.

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.

In 1852, Dominy performed the first successful hand - pollination in orchids by crossing *Calanthe masuca* to *Calanthe furcata* (Dressler, 1981).

In hybridization, selection of good and healthy 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 plants (Bose and Bhattacharjee, 1980).

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

Slater (1991) observed that cross pollination in *Dendrobium speciosum* was effected by the deposition of pollinia from one flower into the stigmatic cup of another flower in another plant. The pollinia were submerged into the viscuous liquid of the stigmatic cup, containing detached stigmatic cells (eleutherocytes) and mucilage. Adherence of pollen to stigma is effected by wetness of pollen or stigma.

2.5. Post pollination phenomena

In Orchidaceae, post pollination phenomena 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).

Removal of various floral segments indicated that most post-pollination phenomena were controlled by the rostellar stigmatic region. However, a certain interdependence was noted among all the various floral segments exhibiting post pollination phenomena (Harrison and Arditti, 1972). In their opinion wilting of floral parts such as sepals, petals and labellum and changes such as swelling and greening of gynostemium as well as ovary were among the most easily observable post pollination phenomena 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.

Ethylene evolution following emasculation or pollination resulted in post pollination phenomena in orchid blooms, including anthocyanin formation, fading, reduced flower life and wilting of sepal tips (Arditti, 1979; Chadwick *et al.*, 1986).

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.

Slater (1991) further reported a more intense perianth colour and closure of perianth as post pollination phenomena in *Dendrobium speciosum*. The detached cells of stigma located near the entrance to the stylar canal were found to have lost starch from the amyloplasts after the pollen tubes had passed.

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.

A rapid acceleration of the wilting process followed successful pollination in several genera including *Dendrobium* (Porat, 1994). He

also noted 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 to reach flowering stage. Immature ovules from young pods of orchids have been cultured *in vitro* in nutrient media to give rise to plants (Withner, 1943; Withner, 1959).

Very young as well as fully mature ovules do not form good explants *in vitro* due to dormancy, pH, inhibitory and other metabolic factors (Withner, 1953).

2.6.1. Capsule maturity

Assessment of the correct maturity stage is a major deciding factor in green capsule culture. Sagawa and Valmayor (1966) found that the earliest culture of *Dendrobium nobile* capsule was possible only 80-85 days after pollination.

In the opinion of Sauleda (1976), 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).

Pods of *Cyripedium reginae* harvested a week before dehiscence germinated well. Very early harvest reduced germination greatly (Harvais, 1982).

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.

Hegde (1984) found that the pods of *Dendrobium* species matured in nine to 17 months.

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

Johansen (1990) found that the variation in maturation time for the capsules in different species was very much pronounced, ranging from 43 to 441 days in *Dendrobium salaccense* and *D. heterocarpum*, respectively. It was further observed that in reciprocal crosses, capsules of the same parents differed in size at 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.

2.6.2. Capsule culture

Green pod 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.

In the opinion of Harvais (1982) the capacity of *Cypripedium reginae* seeds to germinate varied from capsule to capsule, place to place or from season to season.

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.

Ballard (1987) in *Cypripedium reginae* found that fertilization remained incomplete even beyond the sixth week after pollination, delayed perhaps by weather conditions.

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), depending on genera, 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) could observe 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).

The series of events leading to the development of egg is a post-pollination phenomenon in orchids and the time interval from pollination to fertilization may range from ten days to six months (Sagawa and Valmayor, 1966).

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).

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).

Depending on the genotype of seed, its quality and culture conditions, protocorms developed chlorophyll within 10-30 days after inoculation (Shoushtari *et al.*, 1994).

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 (Mitra, 1971).

2.7.3. Changes during germination

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 (Ricardo and Alvarez, 1971).

In *Cattleya*, lipid reserves were utilized slowly when seeds were sown on medium lacking carbohydrate source, but utilized rapidly on medium containing sucrose (Harrison, 1977). Further studies of Harrison and Arditti (1978) indicated that during germination of *C. aurantiaca*, the levels of chlorophyll and specific activity of ribulose 1-5 diphosphate carboxylase increased in sucrose containing media.

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).

Krishnan *et al.* (1993) found that the stored lipids and proteins were entirely adequate for the development of protocorms in *Spathoglottis* during initial stages. The accumulated starch was used up for organogenesis during later stages.

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/capsule sterilisation

Sterilisation prior to inoculation is inevitable as orchid seeds are cultured under completely aseptic conditions. Since mature orchid seeds have tough seed coats, chemical treatments for sterilization can be safely employed (Jordan, 1965).

Mature seeds of *Vanda* Miss. Joaquim pretreated with 5 per cent chlorox for 10 minutes and rinsed with sterile water prior to inoculation produced seedlings in 10-12 weeks whereas mature seeds without pretreatment were lost due to contamination. Green pod culture proved to be the best, since seeds directly transferred to the medium without exposure to the outside germinated well and produced strong seedlings within 8-10 weeks (Rao and Avadhani, 1964).

Mitra (1971) used chlorine water to sterilise capsules and seeds. Pods were dipped in absolute alcohol (12 seconds) and chlorine water (45 minutes) whereas seeds folded in filter paper were dipped in chlorine water for 10 minutes and rinsed with three changes of sterile water.

Rosa and Laneri (1977) successfully used 70 per cent ethanol and 1.7 per cent sodium hypochlorite for sterilising pods and 7.0 per cent calcium hypochlorite for sterilising seeds of five orchid genera.

Immature capsules of *Epipactis*, when sterilised by immersing in saturated calcium hypochlorite solution (7 g/1000 ml water) for 10 minutes gave good germination without contamination (Arditti *et al.*, 1982).

Pyati and Murthy (1995) achieved pod sterilization in *Dendrobium ovatum* by dipping in alcohol followed by flaming. Pod sterilization of *Vanda coerulea* was effected by pre-treatment in 0.1 per cent mercuric chloride for five minutes followed by alcohol dip and flaming.

2.7.5. Seed germination and development *in vitro*

Curtis (1943) studied germination and seedling development in five species of *Cypripedium*. It was observed that the protocorms were initially non-chlorophyllous and chlorophyll development was initiated in the leaf tips.

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.

Olivia and Arditti (1984) found that roots and shoots generally appeared together in most *Cypripedium* seedlings.

According to Mathews and Rao (1985), the differentiated protocorms had to be subcultured within a period ranging from 70 to 80 days for proper *in vitro* growth. Overcrowding without transfer resulted in stunted growth.

Muralidhar and Mehta (1986) reported that *Cymbidium longifolium* embyros exhibited a prominent zone of pro meristematic

cells by the fiftieth day of culture from which an unequal pair of first embryonic photosynthetic leaves developed. Simultaneously, marginal cells gave rise to unicellular rhizoids.

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. Adult plantlet stage *in vitro* was attained when the seedlings developed pseudobulbs, roots and leaves of at least 30 mm length.

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

From their studies on four orchid genera, Reddy *et al.* (1992) found that the inherent genetic and physiological features in a species play a direct role in the differentiation of organs.

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

Nagashima (1993) studied seeds of 47 orchid species and reported that germination rate ranged from 0.8 to 100 per cent and the number of days from sowing to germination ranged from 3 to 305, depending on stage of embryogenesis.

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.

Nagashima (1994) reported in temperate *Cymbidium* species that the terminal bud of the protocorm elongated downward and formed the rhizome. After the elongation of rhizome, the terminal bud grew upward and differentiated into shoots and roots.

Hazarika and Sarma (1995) reported that immature seeds of *Dendrobium transparens* showed signs of swelling 16-18 days after inoculation. The embryos emerged out and developed into distinct, globular, yellowish-green protocorms within 25 days of culture. Ninety per cent germination was observed after 25 days of inoculation.

2.8. Culture media, components and media supplements

Culture media and culture conditions are equally important in the development of hybrids in orchids. Since the plants are exacting in their requirements, very often, modifications are to be made to suit specific

situations. Several attempts were made in the past for developing and refining *in vitro* culture techniques for seedling production in orchids. The pertinent information gathered in these aspects through research works is reviewed below.

2.8.1. Effect of culture media on seed germination

Many media have been used for the axenic germination of terrestrial and epiphytic orchids. However, none of these media is universal.

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).

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

Devi *et al.* (1990) pointed out that the preferred medium for *Dendrobium* seed germination varied with the species. *D. farmeri* and *D. primulinum* gave 50-60 per cent higher germination on VW medium. On the other hand, Nitsch medium gave better results with *D. moschatum* and *D. fimbriatum* as compared to other media.

Kumaria and Tandon (1991) were of opinion that high ionic concentration of nutrient salts and vitamins in the medium was inevitable

for the germination of *Dendrobium fimbriatum* var. *oculatum* seeds. On inoculating four-month old seeds, highest germination (91 %) was obtained on Nitsch medium followed by MS (85 %). Protocorm stage was reached in four to five weeks on MS, Nitsch and VW media.

In vitro studies on seed germination and seedling development in four species of South Indian tropical orchids showed a significant interaction between the media and the species. *Dendrobium crepidatum* yielded better results in MS and RL media than in KC medium (Reddy *et al.*, 1992).

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.

The effect of media, however, was found to be inconsistent by Pauw and Remphrey (1993). They found that it varied depending on the year and season of collection of capsules as well as the species cultured.

Hazarika and Sarma (1995) conducted *in vitro* germination studies in *Dendrobium transparens* Lindl. and reported that best growth of seedlings was obtained in supplemented MS medium.

Since MS medium contained high ionic concentration of nutrient salts, Zhang *et al.* (1993) found that half strength MS could adequately

support rapid protocorm proliferation in orchids. Bhasker (1996) found that supplemented quarter strength MS could produce seedlings with maximum number of shoots, leaves and roots in *Phalaenopsis* after a 12 week culture period.

2.8.2. Effect of complex additives on seed germination

A large and bewildering number of complex additives have been routinely used for orchid seed and seedling cultures.

The most frequently used complex additive in orchid seed culture is coconut water (CW), the liquid endosperm of coconut. It induces cell division in otherwise non-dividing cells and promotes morphogenesis and mass multiplication of protocorms in orchids (Intuwong and Sagawa, 1973).

Morel (1974) has enumerated the beneficial effects of coconut water in bringing about rapid protocorm multiplication in orchids.

McIntyre *et al.* (1974) found that addition of coconut water (15%) to KC medium led to increased growth of both epiphytic and terrestrial orchids. Vigorous root growth resulted in epiphytes.

Coconut water (10 %) when added to KC medium along with micronutrients, gave satisfactory germination in five orchid genera (Rosa and Laneri, 1977).

Flamee (1978) concluded that no one substance stimulated germination and there were variations in germination between species on the same medium.

Enhanced growth in different orchids has been reported to occur in the presence of coconut water (CW), banana pulp (BP), peptone, apple juice and peptone, fish extract and peptone, pineapple and tomato fruit. The approximate composition of coconut water and banana pulp has been provided (Arditti and Ernst, 1993).

According to Mathews and Rao (1980) peptone promotes protocorm growth and proliferation in *Vanda*. Yeast extract was successfully used for seed germination and protocorm proliferation in many orchid species.

Sahid (1980) reported that growth rate of *Dendrobium* hybrids could be improved by adding potato and pea extracts to KC medium.

Mitra (1986) stated that a growth-stage dependent variation in nutritional requirement was evinced in orchid cultures. The nutritional requirement of the seedling stage differed from those of germination and protocorm stages.

Addition of peptone along with vitamins and casein hydrolysate to VW medium enhanced germination of *Acampe praemorsa* (Krishnamohan and Jorapur, 1986).

Bopaiah and Jorapur (1986) found that a nutrient medium supplemented with coconut milk (CW), peptone, casein hydrolysate and banana pulp along with vitamins and kinetin was most suitable for normal and healthy growth in *Cymbidium aloifolium*.

Addition of potato cubes to culture medium gave more than 95 per cent germination in *Cypripedium reginae* (Ballard, 1987).

Pierik (1987) observed that supplementing germination medium with banana pulp should be avoided in *Paphiopedilum* since this completely stops the growth after germination.

Soediono (1988) found that supplemented VW medium (CW 15% + NAA 10 ppm) led to rapid protocorm proliferation followed by enhanced seedling growth in *Dendrobium* Jaquelyn Thomas.

Choi and Chung (1989) reported that *Cymbidium* seeds germinated well in a medium containing hyponex, peptone, NAA and kinetin.

According to Rubulo *et al.* (1989), supplementing KC medium with 10 per cent coconut water gave the best germination in *Bletia urbana*. All seedlings developed to the adult stage, forming leaves, pseudobulbs and roots after 90 days of *in vitro* culture.

Das and Ghoshal (1989) found that modified KC medium when supplemented with NAA (1 mg l^{-1}) could promote further growth of *Dendrobium chrysotoxum* and *D. pierardii* x *D. crepidatum* seedlings at two leaf stage.

Addition of 15 per cent CW and 5 per cent BP enhanced germination and accelerated seedling growth in *Dendrobium farmeri* and *D. primulinum* (Devi *et al.*, 1990).

Immature seeds of *Rhyncostylis retusa* and *Vanda coerulea* gave 20 per cent enhanced germination when VW medium was supplemented with CW, BP, pineapple juice and vitamin stock of Nitsch medium. First leaf was observed in nine to ten weeks and first root in 14 to 16 weeks from inoculation. The seedlings were well formed within 20 weeks (Nath *et al.*, 1991).

Sharon *et al.* (1992) used the basal medium supplemented with 15 per cent CW for raising protocorms of *Dendrobium* Snowfire from immature seeds.

Independent of basal medium, the presence of potato improved the survival of *Phalaenopsis* protocorms (Tsai *et al.*, 1993).

The relative efficiency of peptone (0.5 %) and auxins (IAA 1 mg l^{-1} and NAA 1 mg l^{-1}) in enhancing the growth rate of *Dendrobium* hybrid seedlings *in vitro* was reported by Devi and Deka (1994).

For *Cattleya, Encyclia* and *Oncidium,* 25 per cent CW and for *Stanhopea*, 60 g l^{-1} BP were the best additives (Villolobos and Munoz, 1994).

Bhasker (1996) has pointed out the beneficial effects of peptone and CW on *in vitro* seedling growth in *Phalaenopsis*. Peptone (1000

mg l^{-1}) along with BA (20 mg l^{-1}) and NAA (1 mg l^{-1}) maximised shoot, leaf and root production after 12 weeks of culture. Foliar growth was enhanced by the addition of CW.

2.8.3. Effect of growth regulators on seed germination

In order to promote seed germination and seedling growth in orchids, many plant growth regulators have been tried.

Withner (1959) reported that several orchids reacted positively to IAA, IBA or NAA. Auxins such as IAA, IBA, NAA and 2,4-D are commonly used in orchid culture. Their effects are variable with orchid species.

Hadley and Harvais (1968) emphasized the interaction of cytokinin and auxin in maintaining the shoot/root balance in orchids. They further reported that GA_3 enhanced protocorm survival, but caused abnormal elongation of the emergent shoot.

It was found that IAA 1 mg l^{-1} or 2 mg l^{-1} effectively promoted growth of orchid seedlings (Hayes, 1969).

Harvais (1972) found that kinetin enhanced shoot characters and chlorophyll formation but suppressed the root characters of *Dactylorrhiza* protocorms.

Fonnesbech (1972a) reported that the protocorms of *Cymbidium* proliferated when grown on a medium supplemented with IAA whereas 2,4-D inhibited protocorm formation.

Payawal and 'de Guzman (1972) observed the optimal concentration of NAA in *Vanda* to be 1.25 mg l^{-1}.

Ichihashi and Kako (1973) found that NAA 0.1 mg l^{-1} was sufficient in *Cattleya* cultures for inducing optimum growth.

Prasad and Mitra (1975) reported that IAA 0.1 mg l^{-1} enhanced seed germination in *Cymbidium mastersii* upto 80 per cent. They further observed that the addition of NAA to the basal medium resulted in protocorm proliferation in *Cymbidium madidum* and species and hybrids of *Bletilla* and *Vanda*.

The growth regulator NAA stimulated germination and seedling growth in several species like *Cattleya aurantiaca, Cymbidium madidum* and *Bletilla* spp. (Strauss and Reisinger, 1976).

Kusumoto (1978) found in some orchid species that the combined effect of 2,4-D and kinetin or BAP favours protocorm formation.

Arditti (1979) was of opinion that experiments with auxins, cytokinins and gibberellins on orchid seed germination have given inconsistent and therefore inconclusive results. He further observed that 2,4-D inhibited germination and induced callus formation in *Vanda* Miss Joaquim. 2 mg l^{-1} of IAA and NAA induced callus formation and reduced the percentage of normal seedlings.

The growth hormone NAA 1 mg l^{-1} when used along with other additives resulted in better protocorm proliferation in three interspecific hybrids of *Vanda* (Mathews and Rao, 1980).

Various parameters controlling the growth of orchid protocorms were investigated in the hybrid embryos of *Vanda* by Mathews and Rao (1985). All the auxins except 2,4-D favoured protocorm multiplication in the concentration range 0.5 to 4.0 mg l^{-1}. IAA and NAA were found to be the most favourable auxins for protocorm multiplication.

Mitra (1986) observed that GA_3 is rarely used in seed germination as it caused abnormal elongation of the emergent shoot.

Sangama (1986) was of the opinion that GA_3 1 mg l^{-1} reduced the time taken for germination in *Spathoglottis, Epidendrum* and *Dendrobium* Jaquelyn Thomas.

Muralidhar and Mehta (1986) found that in *Cymbidium longifolium* VW medium supported 80 per cent seed germination when supplemented with IAA (0.2 mg l^{-1}) and kinetin (0.4 mg l^{-1}) in addition to vitamins and aminoacids.

A kinetin : NAA ratio of 10:1 favoured early germination and subsequent growth in *Coelogyne punctulata* (Sharma and Tandon, 1986).

The auxin NAA 1 mg l^{-1} resulted in enhanced germination and accelerated seedling growth in *Dendrobium fimbriatum* and *D. moschatum* (Devi *et al.*, 1990).

Lim *et al.* (1993) found that IBA and kinetin gave enhanced shoot production in the seed cultures of *Dendrobium*.

A combination of kinetin and IBA (0.5 ppm each) produced best seedling development of *Bletilla striata*. Best results were obtained in *Spiranthes* with 3 ppm kinetin (Suner, 1995).

Hazarika and Sarma (1995) found that a combination of kinetin, IBA (0.1 mg l^{-1} each) and NAA (1.0 mg l^{-1}) was best for enhanced germination and seedling growth in *Dendrobium transparens* Lindl.

Maximum number of shoots and leaves in *Phalaenopsis* cultures resulted from a combination of 8 ppm adenine and 16 ppm BA (Bhasker, 1996).

2.8.4. Effect of carbon source on seed germination

Orchids must have an external supply of carbohydrates to continue their growth and differentiation. Orchid seeds and young seedlings have the ability to utilize various carbohydrates. However, different species have their own preferences (Arditti, 1967).

Glucose, fructose, or oligosaccharides containing these sugars alone could adequately satisfy the energy requirements of *Phalaenopsis* protocorms (Ernst *et al.*, 1971). In *Dactylorrhiza purpurella*, the results with dextrose and sucrose were essentially similar (Harvais, 1972).

Of the sugars tested on the growth of *Cymbidium* protocorms, sucrose was better than maltose, glucose and fructose. The optimum concentrations of sucrose ranged from 3.0 to 4.0 per cent (Fonnesbech, 1972b).

Harrison and Arditti (1978) found that sucrose induced germination and enhanced chlorophyll development in certain species that failed to germinate on sugar-free medium. Sucrose could be replaced by glucose.

In hybrid *Vanda*, Mathews and Rao (1985) tested different carbon sources and found that 2.0 per cent sucrose was the best source. Absence of sucrose stopped growth of protocorms and 10.0 per cent sucrose caused tissue necrosis.

Ballard (1987) in *Cypripedium reginae* reported that sucrose was better compared to dextrose or trehalose and 2.0 per cent sucrose was better compared to 1.5 or 1.0 per cent.

In *Paphiopedilum ciliolare,* Pierik *et al.* (1988) concluded that an extra ordinary low sugar concentration was optimal for germination, higher concentration being inhibitory.

High sucrose concentration (4.0 %) reduced germination in *Bletia urbana*, but no significant difference could be observed in the response between 2.0 per cent and 3.0 per cent sucrose (Rubulo *et al.*, 1989).

Among various carbon sources tested, sucrose, fructose and glucose at 2.0 to 3.0 per cent gave best germination and seedling growth in *Cymbidium elegans* and *Coelogyne* spp. In sugar-free medium, germination and growth were negligible (Sharma and Tandon, 1990).

Ernst and Arditti (1990) recorded that although *Phalaenopsis* seeds and seedlings can utilize many sugars as C source, seedling fresh weight and survival decreased with the increased polymerisation and increased molecular weight.

2.8.5. Effect of charcoal on seed germination

Ernst (1974) recorded that *Paphiopedilum* seedlings grew well on culture media to which activated charcoal was added.

According to Rosa and Laneri (1977), the addition of charcoal to the culture medium helped in the rapid and better development of shoots and roots in seedlings of *Phalaenopsis*.

Fridborg *et al.* (1978) attributed the beneficial effects of activated charcoal to its adsorption of inhibitory phenolic and carboxylic compounds produced by the tissues in culture. They further observed

that charcoal has the tendency to adsorb hormones and vitamins and thereby inhibit growth. Hence it should be used with caution in culture media.

The initial formulations of charcoal containing medium for orchid seed germination gained wide acceptance (Yam and Weatherhead, 1988).

Pierik *et al.* (1988) found that significant increase of shoot and root development resulted in *Paphiopedilum ciliolare* when activated charcoal at 2 gl^{-1} was added to the medium after protocorm formation, but was inhibitory during seed germination.

According to Hinnen *et al.* (1989) activated charcoal strongly enhanced the growth and development of *Phalaenopsis* seedlings.

Yam *et al.* (1990) observed that activated charcoal exerted a beneficial effect on culture media by adsorption and removal of phytotoxic metabolites. They further pointed out that it can also be detrimental due to the removal of additives such as auxins and/or cytokinins.

2.8.6. Effect of pH of media on seed germination

Knudson (1951) noted the inability of *Cattleya* seeds to germinate if the initial pH of medium is below 4.5.

Dendrobium nobile germinated better within a pH range of 4.0 - 5.0 (Ito, 1955) whereas many other orchid species responded favourably to

media with pH between 5.0 and 6.0 (Scott and Arditti, 1959; Kotomori and Murashige, 1965).

Maintaining the pH at 5.2 to 5.5 was favourable for successful germination in *Cymbidium mastersii* (Prasad and Mitra, 1975).

Rosa and Laneri (1977) observed that a pH of 5.2 for *Cattleya* and *Phalaenopsis* and 6.0 for *Cymbidium* and *Paphiopedilum* was satisfactory for germination.

Reyburn (1978) recorded in *Cymbidium* that germination in the dark was optimal at pH 5.5-6.0 and a pH of 7.0 was strongly inhibitory.

Orchid seeds germinated well within a pH range of 4.8 to 5.2 with germination commencing at pH 3.6 and tapering off at 7.6 (Arditti, 1979).

Maximum germination and optimal growth of protocorms at pH 5.0 was reported in *Dendrobium chrysanthum* and *Sarcanthus pallidus* (Raghuwanshi *et al.*, 1986).

Optimal germination of *Paphiopedilum ciliolare* occurred at a pH of 6.0 (Pierik *et al.*, 1988).

Ichihashi (1990) obtained good germination of *Bletilla striata* seeds when the pH was adjusted to 5.1 $\pm$ 0.1.

George (1997) found that optimal growth of protocorms in *Dendrobium osterholt* resulted when the pH was adjusted to 5.8.

2.9. Deflasking, planting out and acclimatization

Once the seedlings are developed *in vitro*, cogenial environment is to be provided to acclimatize them to the harsh external conditions. Post deflasking mortality can be considerably reduced by minimizing transplanting shock. Here again, special skill and care is the key to success. The salient research findings on these aspects are briefly summarized below.

Dunstan and Turner (1984) were of opinion that post deflasking mortality is mainly caused by desiccation.

Sutter *et al.* (1985) found that post deflasking survival of plantlets depended greatly on the number of leaves present at the time of planting out and the rate of leaf growth.

Ziu (1986) observed that pre transfer culture conditions were equally important as post transfer growth conditions in deciding the success in acclimatization of *in vitro* cultured plants.

Kim *et al.* (1988) obtained 30 per cent increase in post planting out survival of *Cymbidium kanran* plantlets by soaking the potting medium comprising of moss peat compost, for 30 minutes in a disinfectant solution.

Wainwright (1988) pointed out that the difference in relative humidity, light levels and temperature between the *in vitro* and *in vivo* environments are mainly responsible for post deflasking mortality.

Vij and Pathak (1989) found that a period of humidity acclimatization was necessary to enable the seedlings to undergo morphological and physiological adaptations prior to exposure to the harsh external conditions.

Seeni and Latha (1990) observed that *Phalaenopsis* hybrid seedlings got easily established within two weeks of planting out, recording 100 per cent survival. Broken tiles was found to be the best medium followed by charcoal, cassava pith and rubber seed husk in that order.

Sureshkumar (1992) reported that *Dendrobium* hybrid seedlings registered the best survival in pure charcoal, followed by cassava pith and rubber seed husk. They got established in one month after transplanting.

Lakshmidevi (1992) used a mixture of brick and charcoal in equal proportions as the potting medium for *Dendrobium fimbriatum* and *D. moschatum* plantlets and obtained 20 and 40 per cent survival, respectively.

Sharma and Tandon (1992) observed 65 per cent survival of *Dendrobium* hybrid seedlings when transferred to a potting medium comprising of charcoal fragments, brick pieces and coconut fibre.

Singh (1993) recorded that orchid seedlings established well when given a post deflasking wash in running water and planted in community pots in 1:1 mixture of tree fern fibre and charcoal.

Lim *et al.* (1993) found that for *Dendrobium moniliforme* peat moss was the best potting medium for improving aerial growth of seedlings whereas perlite was best for increasing root numbers.

Sharma and Chauhan (1995) observed that *in vitro* raised hybrid seedlings of *Dendrobium* and *Paphiopedilum* are generally kept either in community pots or in individual thumb pots of 5.0 cm diameter. A potting mixture comprising of broken brick, broken charcoal, tree-fern, bark pieces, leaf mould and dry sphagnum in 1:1:1:1:1:2 ratio with green sphagnum on top supported maximum survival in *Dendrobium*. *Ex vitro* establishment of the seedlings took three months in Shillong.

Gangaprasad (1996) found that seedlings and tissue cultured plants of different orchid species such as *Dendrobium aquem, Ipsea malabarica, Vanda spathulata* etc. got established well within three to six months of transplanting into a brick + charcoal medium.

2.10. 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.

2.11. Breeding of hybrids

The first flowering interspecific hybrid developed in 1852 by Dominy, by crossing *Calanthe masuca* to *Calanthe furcata* was termed *Calanthe* Dominii 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).

Hundreds of intergeneric, interspecific or intraspecific natural hybrids of *Dendrobium, Odontoglossum, Cattleya, Laelia, Oncidium, Phalaenopsis* etc. have been reported from different parts of the world (Abraham and Vatsala, 1981).

One of the earliest scientific accounts on orchid hybrids was presented by Hurst (1898). Out of the 800 hybrids then on record, about 500 were primary hybrids, including 100 intergeneric hybrids. A total of 270 secondary hybrids and about 30 tertiary hybrids had flowered till then. Primary hybrids were found to be intermediate between, yet specifically distinct from either parents in morphological characters. Secondary hybrids showed a far wider range of character variation.

Many Indian species have earned world-wide recognition 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 venustum, Vanda coerulea* etc. (Bose and Bhattacharjee, 1980).

Vacherot and Lecouffle of France were the pioneers of *Dendrobium* breeding. The nobile type (narrow petals) dendrobiums of Eastern Himalayas and *D. phalaenopsis* (rounded petals) of Eastern Asia were the most frequently used parents. Colour has always been of prime importance in *Dendrobium* breeding, ranging from chalky white to yellow, brown and intense crimson (Abraham and Vatsala, 1981).

Bobisud and Kamemoto (1982) evaluated inbred progenies developed from the amphidiploid *Dendrobium* Jaquelyn Thomas through

selfings, sibmatings and back crosses. Selection and inbreeding were effective in increasing flower size and improving colour purity. The characters like flower size, flower colour, flower production, vase life and bud drop were primarily influenced by parental genotypes since inbreeding decline was not apparent.

While breeding with yellow *Phalaenopsis*, Singh (1982) observed that the further from the species, the better becomes the flower shape, flower size and number of flowers per spike, but the lighter becomes the colour.

Kamemoto (1983) evaluated the *Dendrobium* cultivars Louis Bleriot and Pompadour. Data on spray yields of five seed propagated amphidiploid cultivars were tabulated.

Ando (1983) conducted breeding trials involving reciprocal crosses between *Dendrobium nobile* and eight other species and between *D. moniliforme* and 21 other species. It was found that *D. nobile* was reciprocally compatible only with *D. haniffii, D. linowianum* and *D. moniliforme*. With *D. moniliforme* all crosses were fertile, the seed set varying between 17 and 100 per cent.

McConnel and Kamemoto (1983) reported that reciprocal crosses involving two accessions of *Dendrobium canaliculatum* yielded offspring differing in cane height, pseudobulb production and flower yield. Reciprocal crosses of *Dendrobium schulleri* and *D.* Sunset differed in

flower quality, whereas reciprocal matings of two amphidiploid *D.* Jaquelyn Thomas selections did not differ in vegetative or floral characters.

Singh (1984) observed that fragrance is a character most sought after by *Cymbidium* breeders since majority of the species lack that attribute. The scented *C. munronianum* has been used as parent in several breeding programmes.

Cheng *et al.* (1985) concluded from a study on 22 species and six hybrids of diploid and triploid *Dendrobium* that no correlation existed between chromosome number and flower size.

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*). The *Dendrobium* hybrid is robust with 35-40cm long flower spikes bearing 12-15 flowers per spike. The flowering season is between mid February and late May.

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

Stewart (1986) found that the intergeneric hybrid *Eulocymbidiella (Eulophiella* x *Cymbidiella)* was intermediate in shape between the two

parents. In colouration, the flowers were quite different from either parent.

Thammasiri *et al.* (1987) found that colour fading in yellow flowered *Dendrobium* hybrids was due to the degradation of flower pigments at different stages of maturity, from bud stage.

Das and Ghoshal (1988) studied the breeding behaviour of *Dendrobium chrysotoxum, D. crepidatum, D. pierardii, D. primulinum* and *D. transparens*. Successful reciprocal crosses were achieved between *D. crepidatum* and *D. transparens* and between *D. crepidatum* and *D. pierardii* while unidirectional crosses were successful with *D. primulinum* x *D. crepidatum* and *D. transparens* x *D. pierardii*.

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.

Kamemoto *et al.* (1989) evaluated 16 seed propagated amphidiploid *Dendrobium* progenies for cut flower production. Variations were observed in flowering season, flower yield, plant height and floral characters. Bud drop percentage varied from 2.5 to 10.5 and vase life from 12.8 to 22.2 days. A cross displaying several desirable characteristics was released under the number UH 800 as a white, seed propagated, amphidiploid cultivar.

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. delenatii*) have been developed and they have produced flowers larger than either parent with very full bloom, white colour and beautiful raspberry stippling.

Takasaki (1989) reported that out of the hybrids obtained by crossing the striped *Phalaenopsis* Kathleen Ali with the spotted *P.* Frisson, some had good spots while others had stripes with spots.

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

Alcorn (1990) experimented with selfing in *Lycaste* Macama Jocelyn and obtained surprising variations in colours and shapes.

McDonald (1991) has pointed out that any hybridization program 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. Scent may be transmitted to the progeny by the careful selection of perfumed pod parents. Vase life is most often attributable to the substance of the flower and tetraploids are usually of thicker substance. Vegetative vigour of hybrids is important as it results in

bigger, better blooms and more floriferous hybrids with greater flower substance.

Oakeley (1991) observed that clear colours are preferred in *Lycaste* breeding. An attractive feature noted is that the reverse of floral segments often have a different colour to the face. Fading of colours on standing is a problem. The pink colour gene of *Lycaste skinneri* is fragile and easily lost. However, breeding for small size and *L. skinneri* shape was considered as an attractive proposal.

He also noted that slight reflexing of petals and overlapping along their upper margins are attractive attributes. Commonly seen demerits in shape include an excessively short dorsal sepal, twisting or reflexing of sepal tips, curling up of the lower edge of lateral sepals and excessive tapering of sepals at their base to give a 'gappy' appearance.

He further observed that *Lycaste cruenta* was found not to transmit its cinnamon fragrance to its hybrid *L.* Imshootiana, although the fragrance was transmitted to its hybrid with *Lycaste* Brugensin, viz., *L.* Hera.

Rogerson (1991) reported that the higher order hybrid, *Paphiopedilum* F.C. Puddle with six species in its parentage plays a predominant role in breeding for white flower colour in the genus. The high fertility of the variety, its tendency to inhibit and suppress colours in hybrids and the relatively recessive nature of its poor shape mainly account for its popularity as a parent.

Rittershausen (1991) has described *Dendrobium phalaenopsis* and its four hybrids *D. Dale* Takiguchi, *D.* Ekapol, *D.* Hawaiian Beauty and the miniature, *D.* Orchidwood. In most cases, the plants have been line bred from *D. phalaenopsis* and its varieties like *bigibbum, schroederae* and *hololeuca* with no other species added.

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) summarized the positive and negative attributes of *Vanda* Miss Joaquim and evaluated its role in hybridization. Miss Joaquim is a cross between *Vanda hookeriana* and *V. teres* and is the first *Vanda* hybrid to be registered.

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.

Moses (1994) described the progressive development of semi alba *Phalaenopsis* with their full,flat flowers and white sepals and petals contrasting with the deep red lips. Their breeding began in 1986 by

crossing the lavender pink lipped *P. equestris* with the solid white *P. aphrodite.*

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

Yam (1994) discussed the progress made in improving flower colour in the genus *Paraphalaenopsis* by considering intra and intergeneric hybridizations.

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. Studies on protoplast fusion, isoenzyme electrophoresis and DNA finger printing to assist in varietal identification were performed.

Griesbach (1995) described peloric mutations in orchids whereby the flower becomes actinomorphic by the replacement of the labellum by a petal. Inheritance of peloria was studied and details of prize winning peloric orchids were enumerated.

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

While breeding for yellow colour in *Phalaenopsis,* Norton *et al.* (1995) observed that only a limited number of hybrids proved fertile enough to continue the breeding programme.

Coleman and Glicenstein (1997) found the pale whitish-green flower colour of a natural primary hybrid to be intermediate between its parents, the pure white *Platanthera dilatata* and the green *P. hyperborea*. The shape of lip was also intermediate between the two parents.

Fuchs (1997) 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 the rich blue-violet colouration, lovely tesselation as well as the long inflorescence.

According to Mercy and Dale (1997), 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.

Tippit (1997) reported that in a new hybrid, certain characteristics such as growth habit, natural spread of the flowers, number of blooms,

length of inflorescence etc. are the geometric means of the two parents involved in the cross with genetically dissimilar parents; wide variations in colour and form result, regardless of the genus.

Islam *et al.* (1998) analysed floral pigments in 18 *Calanthe* species and hybrids and pointed out that such data can be successfully used to raise progenies with desirable flower shades using target species as parents.

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.

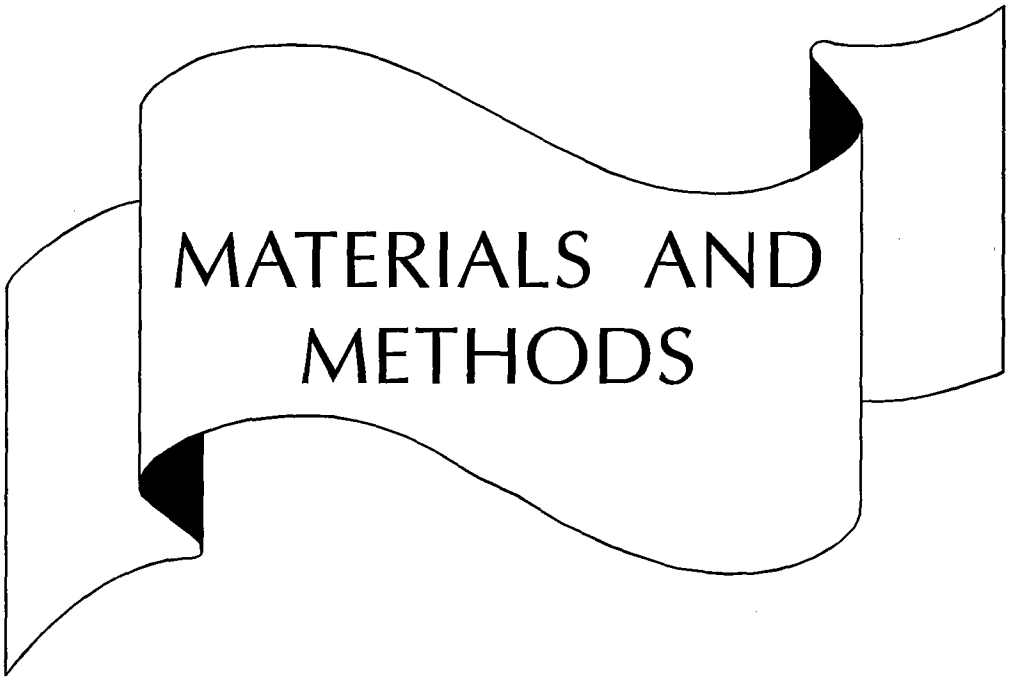
Catling and Brownell (1999) observed that the natural hybrids between *Platanthera lacera* and *P. leucophaea* were intermediate between the two parental species for 11 quantitative characters.

Chen *et al.* (2000), while discussing the breeding behaviour of *Phalaenopsis equestris* observed that hybrids with compact, multiple branched inflorescence grew faster compared to those with large flowers. Studies on the influence of parents on fertility and the inheritance of pink and white floral colours have been presented. 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 Horticultural Society. Currently, 12 hybrids are in production for the market.

Knyasev *et al.* (2000) confirmed the hybrid status of *Cypripedium ventricosum* by statistical analysis of morphological characters, which were intermediate in expression, between the two parents. Allozyme data are fully consistent with hybrid origin.

Motes (2001), while hybridizing with lesser known vandas, commented on the propensity of *V. denisoniana* to confer full form on its progeny, making it a pre eminent 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 1998-2001. The study forms a part of the DBT project entitled "Breeding for interspecific and intergeneric hybrids of orchids for commercial cultivation" (1997-2001) and was supported by a senior research fellowship. The parent and hybrid materials were maintained in the green house of the project where the hybridization and compatibility studies were conducted. Green capsule (immature embryo) culture was done in the Biotechnology Laboratory of the college. Hybrid seedlings were hardened in the humidity controlled chamber fabricated within the green house.

The experiment consisted of the following major studies :

1. Evaluation of parent material
2. Hybridization and compatibility studies
3. Green capsule (immature embryo) culture and refinement of the culture medium
4. Hardening techniques of the hybrid seedlings
5. Evaluation of the hybrid material

3.1. Evaluation of parent material

3.1.1. Experimental material

The experimental material consisted of the following nine leading commercial hybrid varieties, three semi-commercial hybrids and two species of *Dendrobium* (Plate I):

Commercial hybrids

	Hereafter referred to as
1. *Dendrobium* (Candy Stripe x Tomie Drake)	P_1
2. *D.* Chiangmai Pink	P_2
3. *D.* Nagoya Pink	P_3
4. *D.* Pramot 3	P_4
5. *D.* Renapa Red 3	P_5
6. *D.* Rinabha	P_6
7. *D.* Sakura	P_7
8. *D.* Sonia 16	P_8
9. *D.* White Fairy	P_9

Semi-commercial hybrids

10. *D.* Caesar Pink	P_{10}
11. *D.* Uniwai Pink	P_{11}
12. *D.* Walter Oumae	P_{12}

Species

13. *D. barbatulum* (wild)	P_{13}
14. *D. philippica* (semi-commercial)	P_{14}

The source of the varieties and species used in the experiment are furnished in Table 3.1.1.

Table 3.1.1. Source of *Dendrobium* parental genotypes

Parental genotype	Source of material	Type of material
1. *D.* [Candy Stripe x Tomie Drake] 2. *D.* Chiangmai Pink 3. *D.* Nagoya Pink 4. *D.* Pramot 3 5. *D.* Renapa Red 3 6. *D.* Rinabha 7. *D.* Sakura 8. *D.* Sonia 16 9. *D.* White Fairy	Imported from Thailand through Meena nursery, Thiruvananthapuram	Mericloned plantlets
10. *D.* Caesar Pink 11. *D.* Uniwai Pink 12. *D.* Walter Oumae 13. *D. philippica*	Personal collection of Dr. S.T. Mercy, Principal Investigator of DBT project	New divisions from established clumps
14. *D. barbatulum*	Idukki arch dam site	New divisions from wildly growing clumps in the forest

Statistical details

Design	:	Completely Randomised Design (CRD)
Replications	:	10
Treatments	:	14
Plot size	:	Single plant

Planting material of the nine commercial hybrids used in the study were meristem cultured clones of six months age. New divisions having healthy, young shoots separated from the established clumps were used as planting material of the three semi-commercial hybrids and the two species included in the study. Planting was done in clay orchid pots of 15 cm diameter using a mixture of charcoal, brick pieces and dried coconut husk (2:2:1) as the potting medium. Timely management practices were followed as per the package of practices recommendations of Kerala Agricultural University (KAU, 1997).

3.1.2. Experimental methods

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

Growth parameters were observed and recorded from mature canes of plants of age three years and above.

1. Number of shoots per clump

Total number of shoots produced per clump was counted and recorded.

2. Number of leaves per clump

Total number of laminate leaves produced per clump was recorded.

3. Height/Length of cane (cm)

The height of each mature cane from a clump was measured from the base to tip and the mean was computed.

4. Number of nodes per cane

Total number of nodes per mature cane was counted and the mean was computed.

5. Number of leaves per cane

Total number of laminate leaves observed at maximum leaf - stand per cane was counted and the average was computed.

6. Leaf area per cane (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

7. Length of leaf (cm)

Length of mature leaves was measured and the values were recorded.

8. Width of leaf (cm)

Width of mature leaves was measured at the widest point and the values were recorded.

9. Age at first flowering (months)

Age of the clump at the time of visible emergence of first inflorescence head was recorded.

10. Cane to flower first

Serial number of the cane in a clump on which the first inflorescence head emerged was recorded.

11. Days to first flower opening

Time duration in days from visible emergence of inflorescence to the opening of the first flower was recorded.

12. Flowering time (days)

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

13. Vase life (days)

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

14. Days for wilting of all flowers

Number of days for the wilting of all flowers from first flower opening in an *in situ* inflorescence was recorded.

15. 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.

16. Length of scape (cm)

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

17. Number of flowers per inflorescence

Total number of flowers produced in individual inflorescences was counted and recorded.

18. Internodal length of inflorescence (cm)

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

19. Diameter of inflorescence axis (cm)

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

20. Length of flower (cm)

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

21. Width of flower (cm)

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

22. Flowering nature - free flowering/seasonal

Flowering time was recorded, noting whether blooming occurred round the year or in specific seasons.

23. Time taken for single flower opening (days)

Time taken for the opening of a single flower was computed by dividing the flowering time by number of flowers.

24. Nature of inflorescence axis

Nature of inflorescenc axis was recorded as :

a) erect - inflorescence axis held erect (0-30°)
b) arching - inflorescence held at an angle of 30-60°
c) pendulous - inflorescence axis curving down

25. Mode of display of flowers on the inflorescence axis

Mode of display of flowers was recorded based on their orientation as follows:

a) whorled - flowers arranged around the inflorescence axis

b) alternate - flowers arranged on either side of the inflorescence axis facing the dorsal side so that flowers appear in two parallel rows or facing lateral sides so that a row of flowers can be seen from each side.

26. Colour of flower

The colour of flowers and blending of colours were noted and recorded. Colour gradation and colour blending in the sepal/petal and the labellum in particular were observed.

27. Texture of flower

The texture of the flower was closely observed and recorded as follows:

a) thick and substantial vs. thin and fragile.

b) glossy vs. slightly glossy and leathery.

28. Shape of flower

Shape of flower was noted and recorded as follows:

a) full and rounded vs. narrow and horned.

b) flat-surfaced vs. reflexed

c) broad sepals and petals vs. narrow, spatulate sepals and petals.

29. Size of flower (cm)

Circumference of flower (cm) was taken as a measure of its size.

30. Fullness of flower

Fullness 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

31. Fragrance

Presence or absence of fragrance in flowers was observed and recorded.

3.1.3. Statistical analysis

The collected data were subjected to the analysis of variance to test for significant difference among the varieties and species following Panse and Sukhatme (1967).

3.2. Floral biology studies of the parent material

Detailed floral biology studies were conducted on all the fourteen parental varieties. The following observations were recorded :

3.2.1. Flower opening time

Mature buds were tagged separately at full-bud stage when the buds attained maximum size prior to the dipping of the bud. The buds were observed at hourly intervals till flower opening commenced.

3.2.2. Anthesis

Emergence of anther and shedding of pollen do not occur in orchids as the pollen are aggregated into sticky masses called pollinia which are held within the anther cavity, covered and protected by the anther cap. Hence the time at which the pollen became mature enough to achieve successful fertilization was taken as the correct time of anthesis.

To judge the time of maturity of pollen and time of anthesis, pollinia were extracted in the morning (8-10 am) and selected flowers in each treatment were hand-pollinated immediately with this fresh pollen. This was continued for five consecutive days from the first day of flower opening. Initiation of capsule development following pollination was taken as indicative of pollen maturity and the day on which pollen extraction was done was taken as the day of anthesis.

3.2.3. Stigma receptivity

The flowers were hand-pollinated from the day of anthesis to ten consecutive days to find out the correct stigma receptivity period. Pollination was done three times during the day, viz., morning, noon and evening. Initiation of fruit development following pollination denoted stigma receptivity.

3.2.4. Pollen studies

Pollen morphology, pollen fertility and pollen germination studies were conducted.

3.2.4.1. Pollen morphology

Pollinia were collected from fully opened flowers three days after opening and pollen grains were stained in 1:1 glycerine : acetocarmine solution (2%) for 24 hours. Diameter of ten normal-shaped and well-stained pollen grains from each smear was measured at random using a standard occular micrometer under the high power (400 x) of a microscope. The mean diameter was recorded in microns.

The shape of pollen grains was studied under high power magnification (400 x).

3.2.4.2. Pollen fertility

Acetocarmine staining technique mentioned earlier was employed to study pollen fertility. Pollen fertility was estimated by counting fertile

and sterile pollen grains separately from a smear under the low power (100 x) 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.

3.2.4.3. Pollen germination

Good pollen germination in *Dendrobium* was reported in a medium of two per cent sucrose + one per cent agar + 75 ppm boric acid (Varghese, 1995). Germination studies were conducted to get a more dependable estimate of pollen fertility.

Pollinia were collected on the third day after flower opening. A drop of the germination medium was placed on a cover glass. Fresh pollen grains from the pollinia were introduced into the medium. The medium was then allowed to rest as a hanging drop by inverting the cover slip on a cavity slide. Prepared slides were kept at room temperature for incubation in a desiccator containing water.

After 24 hours of incubation, germination counts were made under the low power (100 x) of a microscope. The observations were made in five different microscope fields on not less than 100 tetrads per treatment and the mean percentage of germination was worked out.

3.3. Hybridization and compatibility studies

To study compatibility, all possible self and cross combinations, depending on the availability of flowers were made, including reciprocals. Since flowering was seasonal in the wild species, some of the crosses involving these species could not be attempted under this study.

According to conventional practice followed in orchid hybridisation, the first and last flowers of each inflorescence were not used for crossing. Likewise, as the pollinia are held within the clinandrium and protected by the operculum which effectively prevents self pollination, emasculation of flowers was not attempted. To prevent pollination through natural insect pollinators, the parent plants used for crossing were protected *en bloc* by insect-proof netting. Hand-pollination was carried out at the correct stage of stigma receptivity of each variety. Self compatibility was assessed in the varieties by using the pollen grains of the same plant. The flowers were tagged for identification.

3.3.1. Observations

1. Post pollination floral changes (days)

Time required for the following post pollination changes was recorded :

i) Drooping of sepals and petals
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) Flattening of capsule rib

v) Bursting of capsule beginning from tip

3. Maximum flower retention period in unsuccessful combinations (days)

Number of days from pollination to abscission of last flower in incompatible crosses was noted and recorded.

4. Duration to green capsule harvest in successful combinations (days)

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

5. Size of green capsules at harvest (cm)

Length of capsule from base to tip and width of capsule at the widest point were recorded.

6. Capsule yield (percentage)

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

7. Capsules with/without seeds (percentage)

Number of green capsules with seeds to total number of green capsules harvested was recorded and the percentage was computed.

8. Filled seeds over total seeds (percentage)

Number of seeds with well developed embryos to total number of seeds was worked out by scoring under the low power (100 x) of a microscope.

9. Capsules with germinating seeds (percentage)

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

3.4. Immature embryo (green capsule) culture

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

3.4.1. Stage of green capsule harvest

All the capsules were harvested for embryo culture in the green capsule stage at 75-90 per cent maturity.

3.4.2. Preparation and surface sterilisation of capsule

The harvested green capsules were prepared by removing adhering wilted perianth parts and extra length of pedicel. A preliminary wash in running tap water was given. The intact capsules were cleaned by soaking in one per cent solution of labolene detergent (Glaxo India Ltd., Bombay) in distilled water for 20 minutes. The capsules were then rinsed three to four times with distilled water.

All surface sterilization operations were carried out inside a laminar airflow chamber (Klenzaids; horizontal model). After initial cleaning, the capsules were transferred to a sterile beaker. Surface sterilization was performed by soaking in freshly prepared 0.1 per cent mercuric chloride solution (in sterile double distilled water) for 10 minutes. All traces of the chemical were then removed by washing four to five times with sterile double distilled water. The capsules were finally dipped in 70 per cent ethanol and flamed thoroughly for 10-15 seconds. Ethanol dip and flaming was repeated once again.

3.4.3. Inoculation and incubation

All inoculation operations were carried out inside the laminar air flow chamber.

After surface sterilization, the two ends of the capsules were cut using a sharp sterile blade. The capsules were then slit open logitudinally. The exposed seeds were scraped and inoculated on to sterilized semi-solid culture medium using sterile forceps/blade. These first flasks are termed master flasks.

To study germination percentage, the seeds were transferred to 10 ml sterile distilled water and shaken to form a uniform suspension. Aliquots of seed suspension were viewed under the low power (100 x) of a microscope. Counts of seeds with well developed embryos and total seed counts were taken. Equal volumes of the fresh seed suspension were pipetted into sterilized culture tubes containing 15 ml of the medium.

The cultures were incubated in a culture room for further development. Controlled conditions of light, temperature and humidity were provided.

Darkness was provided initially after inoculation by covering the culture racks with black muslin cloth. Once germination was initiated, a 12 - hour photoperiod with a light intensity of 3000 lux under fluorescent tube lights was provided. A uniform temperature of 26 $\pm$ 2°C and a relative humidity of 95 per cent was maintained in the culture room.

3.4.3.1. Sub-culture

Sub-culturing was done periodically to prevent overcrowding of seedlings in the flasks. All sub-culture operations were carried out in the laminar airflow chamber.

During first sub-culture, approximately equal numbers of developing protocorms from each master flask were transferred into three to 12 new flasks. The developing seedlings were then taken through two to three further sub-culture passages, as and when needed.

3.4.4. Culture media

The basal media used for the study were MS (Murashige and Skoog, 1962) full strength, half strength (50 % concentration of inorganic salts) and quarter strength (25 % concentration of inorganic salts), VW (Vacin and Went, 1949) full strength and KC (Knudson, 1946) full strength. Chemical composition of the media are presented in Appendix I.

3.4.4.1. Media preparation

The chemicals used for media preparation were of analytical grade, purchased from the British Drug House (BDH, Bombay), Merck (Bombay), Sigma (USA) and Sisco Research Laboratory (SRL, Bombay). All glasswares used were manufactured by Borosil Co. Ltd., Bombay. They were all washed with detergent and sterilised before use.

Standard procedures were followed for the preparation of culture media (Thorpe, 1980). Stock solutions of major and minor nutrients were prepared by dissolving the required quantity of chemicals in specific volume of double distilled water. Plant growth substances were first dissolved in dilute acid/alcohol. The volume was then made up with double distilled water. The stock solutions were stored under refrigerated conditions (4°C).

Stock solutions of plant growth substances were prepared fresh every week. Other stocks were maintained under refrigerated conditions in appropriate containers for upto one month.

For preparation of MS medium, required quantities of the stock solutions were pipetted out into a 1000 ml beaker. Sucrose and myo inositol were weighed out, added fresh and dissolved. For specific treatment requirements, phytohormones and organic additives were added fresh at this stage. The volume was made up to 950 ml using double distilled water. The pH of the solution was checked and adjusted to 5.8 using 0.1 N HCl or 0.1 N NaOH with the help of an electronic pH meter (Global; model DPH 500). The gelling agent, agar was added to the medium and the final volume was made up to 1000 ml.

The media VW and KC were prepared fresh every time. Nutrient salts were weighed out in required quantity and were directly dissolved in double distilled water. The pH and final volume were adjusted after addition of sucrose and agar.

The medium was heated till the agar melted. It was dispensed into sterilized culture flasks/tubes while still hot. Corning brand culture tubes (25 x 150 mm) and Erlenmeyer flasks (250 ml) were the containers used. About 15 ml of the medium for each tube and 60 ml of the medium for each flask were dispensed with. The culture vessels were then tightly plugged with non-absorbant cotton and sterilized by autoclaving (National Steel Equipment Pvt. Ltd., Bombay) at 121°C and 1.1 kg/cm^2 pressure for 20 minutes.

3.5. Refinement of *in vitro* culture medium

The basal medium (half strength MS) was supplemented with different phytohormones and organic additives to study their effects on

protocorm development and seedling growth. The various concentrations were fixed based on the previous reports and preliminary trials.

3.5.1. Effect of media

Different basal media such as MS full strength, MS half strength, MS quarter strength, VW full strength and KC full strength were tried.

3.5.2. Effect of phytohormones

Different combinations of auxins (IAA & IBA) and cytokinins (BA & kinetin) were tried. The concentrations were fixed as 2,4,6 and 8 ppm for each hormone.

3.5.3. Effect of organic additives

The complex additives used singly were coconut water 100, 200 and 300 ml l^{-1} (CW 10, 20 and 30 % V/V), peptone 0.5 and 1.0 g l^{-1} (0.05 and 0.1 % W/V) and banana pulp 25, 50 and 75 gl^{-1} (BP 2.5, 5 and 7.5 % W/V). Coconut water was collected from freshly harvested tender coconuts (eight months old) of the local West Coast Tall variety, filtered and added to the medium. Banana pulp was obtained from fully ripened fruits of the variety Palayankodan. Appropriate quantities of the pulp were weighed, homogenised in a blender and added to the medium.

3.5.4. Effect of sucrose

The effect of sucrose on seed germination and seedling growth was studied in half strength MS medium. Varying levels of sucrose viz., 0.5, 1.0, 2.0 and 3.0 per cent were tried.

3.5.5. Effect of charcoal

The influence of charcoal on germination and further growth of seedlings was studied using different concentrations of charcoal, viz., 0.05, 0.10, 0.15 and 0.20 per cent in half strength MS medium.

3.5.6. Observations

The following observations were recorded for seed germination and seedling growth trials at appropriate stages from seed inoculation to deflasking and planting out. Growth measurements were made on seedlings of six months age.

1. Filled seed (percentage)

Number of seeds with well developed embryos out of the total number of seeds was worked out by scoring under the low power (100 x) of a microscope.

2. Seed germination (percentage)

Number of seeds that germinated out of the total number of seeds inoculated was computed after four to six weeks.

3. Number of days to germination initiation

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

4. Number of days for development of protocorm

Number of days from inoculation to protocorm development was noted.

5. Number of days for development of chlorophyll

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

6. Number of days for development of first leaf primordium

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

7. Number of days for development of shoot

Number of days from inoculation to visible differentiation of shoot was computed.

8. Number of days for development of first root primordium

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

9. Height of seedling (cm)

Height of seedling was measured using graph paper.

10. Number of leaves per seedling

Number of leaves produced per seedling was counted.

11. Length of leaf (cm)

Length of longest leaf from base to tip was measured on a graph paper.

12. Number of roots per seedling

Number of roots per seedling was counted.

13. Length of root (cm)

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

14. Number of days to deflasking and planting out

Number of days from inoculation to deflasking and planting out was recorded.

3.6. *Ex vitro* establishment of hybrids

The hybrid seedlings of the different parental combinations were deflasked for hardening.

3.6.1. Deflasking

Deflasking was done when the *in vitro* raised seedlings attained sufficient growth for transplanting. Flasks containing seedlings having at least three to four leaves and three to five roots were selected. In order to reduce transplanting shock, the selected culture vessels were

transferred and kept in the preparation room for two weeks prior to deflasking.

For deflasking, each flask was opened and half filled with tap water. The rooted seedlings were then loosened by shaking the flask. The contents were poured into a clean beaker. The deflasked seedlings were washed clean under the tap without damaging the roots and then soaked in tap water for 30 minutes to remove all adhering pieces of medium. Seedlings were then treated with the fungicide Indofil M_{45} (2% solution) for 10 minutes for disinfection.

3.6.2. Potting media and planting out

The deflasked seedlings were transplanted in plastic cups or clay community pots with holes.

Different types of sterilized potting media were tried.

T_1 - Coconut husk (2 x 2 x 2 cm pieces)

T_2 - Coconut fibre rolled into a loose ball

T_3 - Soilrite

T_4 - Broken tiles (2 x 2 x 2 cm pieces) + charcoal (2 x 2 x 2 cm pieces) + Soilrite in 2:2:1 ratio

T_5 - Broken tiles (2 x 2 x 2 cm pieces) + charcoal (2 x 2 x 2 cm pieces) + tree fern root in 2:2:1 ratio.

3.6.3. Acclimatization

The following techniques were tried for *ex vitro* establishment of hybrid seedlings :

T_1 - Direct planting out into the green house.

In the green house, 50 per cent shade (using plastic black agro shade net) with misting for 15 minutes thrice daily was provided.

T_2 - Planting out into the green house under 75 per cent shade where rain/mist proofing using clear plastic sheet and irrigation using a hand sprayer with fine mist nozzle at an interval of three hours were provided.

T_3 - Planting out into a humidity controlled chamber. For this, an improvised, tunnel shaped 4.00 x 1.65 x 1.50 m humidity chamber made of curved iron frame tightly covered over with clear polythene sheet was fabricated. The chamber floor had a single layer of loose bricks arranged in a zig-zag manner and covered over by a 3-6 cm thick layer of loose sand. A high relative humidity ranging from 85-95 per cent could be maintained when the sand was thoroughly moistened and the chamber kept covered with the polythene sheet. The seedlings in community pots were kept in the chamber and irrigation was done on every alternate day using a hand sprayer with fine mist nozzle. The sand flooring was moistened as and when needed.

3.6.4. Hardening

The acclimatised seedlings in community pots were transferred to orchid stands of 75 cm height kept in the green house (30 x 12 x 4 m) in 50 per cent shade under plastic black agro shade net. Timely management practices were followed as per the Package of Practices Recommendations of Kerala Agricultural University (KAU, 1997).

Individualisation from community pots and repotting into orchid pots of 10 cm diameter was done after four to six months. The seedlings were repotted into orchid pots of 15 cm diameter after another four to six months.

3.6.5. Observations

Survival at two and four weeks after transplanting was recorded during acclimatization.

Survival at one and three months after acclimatization were recorded during hardening.

The following observations were recorded six months after planting out :

1. Number of shoots per seedling
2. Height of seedling (cm)
3. Number of leaves per seedling
4. Length of leaf (cm)
5. Width of leaf (cm)
6. Number of roots per seedling

3.7. Evaluation of hybrid material based on morphological characters

3.7.1. Experimental material

The treatments consisted of the hybrid seedlings belonging to successful combinations.

Statistical details

Design	:	Completely Randomised Design (CRD)
Treatments	:	All successful combinations
Plot size	:	Single plant

3.7.2. Experimental methods

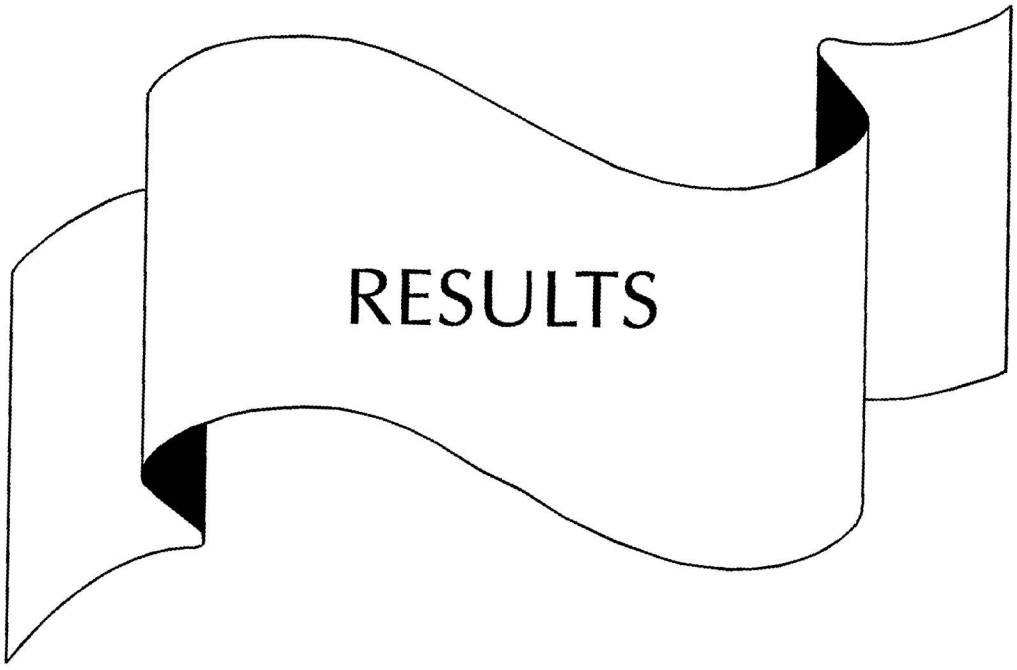
Observations on vegetative characters of the hybrid material were recorded at 1.5 - 2.0 years after transplanting. Vegetative characters such as number of shoots per clump, number of leaves per clump, height/length of cane, number of nodes per cane, number of leaves per cane, leaf area per cane, length of leaf and width of leaf were the characters evaluated. Observations on these characters were recorded in the same manner as described earlier under sub head 3.1.2.

Observations on floral characters (both quantitative and qualitative) were recorded on the hybrids that flowered during the course study. Quantitative floral characters observed were age at first flowering, cane to flower first, days to first flower opening, flowering time, days

for wilting of all flowers, length of inflorescence, length of scape, number of flowers per inflorescence, length of internode of inflorescence, diameter of inflorescence axis, length of flower and width of flower. Qualitative floral characters evaluated were time taken for single flower opening, nature of inflorescence axis, mode of display of flowers, colour of flower, texture of flower, shape of flower, flower size, fullness value and fragrance. Observations on floral characters were recorded in the same manner as described earlier under sub head 3.1.2.

3.8 Combining ability studies

The 14 parental genotypes were crossed in all possible combinations. Based on the results, the possibility of establishing a diallel/half diallel/partial diallel was explored. Based on the particular mating design available, gene action in terms of gca and sca was studied.


RESULTS

4. RESULTS

The results of the investigations on "Intra and interspecific hybridization in *Dendrobium* spp." carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani during 1998-2001, are presented below under four major heads :

4.1. Evaluation of parental 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 *in vitro* embryo culture of hybrids

4.2.1. Hybridization and compatibility/incompatibility studies

4.2.2. Immature embryo culture of hybrids

4.2.3. Refinement of *in vitro* culture medium

4.3. Evaluation of hybrid material

4.3.1. *Ex vitro* establishment of hybrids

4.3.2. *Ex vitro* survival during acclimatization

4.3.3. *Ex vitro* survival during hardening

4.3.4. Comparison of performance of hybrids six months after transplanting

4.3.5. Comparison of performance of hybrids 1.5-2.0 years after transplanting

4.3.6. Combining ability analysis

4.4. Estimation of heterosis

4.1. Evaluation of parental material

Fourteen genotypes of *Dendrobium* comprising of nine leading commercial varieties, three semi-commercial varieties and two species were evaluated in the green house, each being replicated ten times.

4.1.1. Comparison of performance of parents based of quantitative characters

The mean performance of parental genotypes have been analysed based on different quantitative vegetative and floral characters (Tables 4.1.1.1 and 4.1.1.2). Significant differences among the parental genotypes were observed with respect to all the biometric characters studied.

1. Number of shoots per clump

The parent P_4 recorded the highest mean number of 10.9 shoots per clump. Shoot numbers on par with the highest value were observed in the two species, P_{13} (10.3) and P_{14} (10.1) and in the varieties, P_6 (10.1) and P_{10} (10.0). Parents P_2 and P_{12} recorded the lowest mean number of 8.0 shoots per clump.

Table 4.1.1.1. Mean performance of parental genotypes of *Dendrobium* for quantitative vegetative characters

Parental genotypes	No. of shoots per clump	No. of leaves per clump	Height / length of cane (cm)	No. of nodes per cane	No. of leaves per cane	Leaf area per cane (cm^2)	Length of leaf (cm)	Width of leaf (cm)
P_1	9.3	30.4	20.25	6.8	6.2	217.20	11.75	3.38
P_2	8.0	32.3	19.70	7.1	6.6	224.40	11.30	3.37
P_3	8.9	32.5	20.30	7.3	6.1	247.00	11.15	3.55
P_4	10.9	34.5	25.70	7.5	6.5	257.65	13.10	3.48
P_5	9.7	36.2	38.40	8.0	7.7	335.05	13.35	3.73
P_6	10.1	37.0	28.10	7.5	6.6	291.15	13.45	3.75
P_7	9.6	40.2	35.35	9.4	7.4	365.06	12.90	4.56
P_8	9.1	34.3	25.45	7.4	6.5	273.60	15.05	3.42
P_9	9.3	42.4	34.55	9.5	7.8	411.85	15.10	4.27
P_{10}	10.0	34.0	25.95	7.5	7.0	130.90	8.55	2.15
P_{11}	9.0	34.2	25.45	9.4	7.8	275.55	11.40	3.75
P_{12}	8.0	27.8	19.10	8.8	6.9	209.65	11.70	2.91
P_{13}	10.3	34.0	20.00	15.1	9.4	118.60	6.70	1.86
P_{14}	10.1	45.7	39.90	18.7	9.7	276.60	12.55	2.65
SE_m	0.361	1.829	1.193	0.492	0.314	13.087	0.479	0.092
CD (0.05)	1.020	5.483	3.374	1.391	0.888	37.018	1.436	0.280

2. Number of leaves per clump

Total number of leaves per clump was observed to be the highest in the species P_{14} (45.7) which was on par with P_9 (42.4). The lowest mean number of leaves per clump was recorded in the variety P_{12} (27.8) which was on par with P_1 (30.4), P_2 (32.3) and P_3 (32.5).

3. Height/length of cane

The mean height/length of the mature cane ranged from 19.10 cm in P_{12} to 39.90 cm in P_{14}. The cane heights recorded in P_7 (35.35 cm) and P_9 (34.55 cm) were on par with the highest while the cane heights recorded in P_2 (19.70 cm), P_1 (20.25 cm) and P_3 (20.30 cm) were on par with the lowest (19.10 cm in P_{12}).

4. Number of nodes per cane

The two species P_{13} and P_{14} were found to be significantly superior to all other genotypes for number of nodes per mature cane. The highest mean number of 18.7 nodes was observed in P_{14}, followed by 15.1 nodes in P_{13}. Several of the remaining parental genotypes were on par with each other with respect to this character.

5. Number of leaves per cane

The parents P_{13} and P_{14} recorded the highest number of leaves per cane at maximum leaf stand. At this stage, 9.7 leaves were observed

in P_{14} followed by 9.4 leaves in P_{13}. Not much variation was observed for this character among the other parental genotypes.

6. Leaf area per cane

The parent P_9 recorded the highest total leaf area per flowering cane (411.85 cm^2). No other variety was found to be on par with P_9 for this character. Significantly high leaf areas were recorded by P_7 (365.06 cm^2) and P_5 (335.05 cm^2) also. The lowest mean leaf area was seen in P_{13} (118.60 cm^2) which was on par with P_{10} (130.90 cm^2).

7. Length of leaf

The mean leaf length was significantly high in P_9 (15.10 cm) and P_8 (15.05 cm) which were on par with each other. The lowest leaf length was recorded in P_{13} (6.70 cm).

8. Width of leaf

Leaf width was significantly high in P_7 (4.56 cm) and P_9 (4.27 cm) which were on par with each other. The lowest leaf width was recorded in P_{13} (1.86 cm).

9. Age at first flowering

Early onset of the reproductive phase was observed in P_1, flowering at a mean age of 18.71 months. Early flowering was noted in the two species *viz.*, P_{13} (19.05 months) and P_{14} (19.10 months) as well

Table 4.1.1.2. Mean performance of parental genotypes of *Dendrobium* for quantitative floral characters

Parental genotypes	Age at first flowering (months)	Cane to flower first	Days to first flower opening	Flowering time (days)	Vase life (days)	Days for wilting of all flowers	Length of inflorescence (cm)	Length of scape (cm)	No. of flowers/ inflorescence	Length of internode (cm)	Diameter of inflorescence axis (cm)	Length of flower (cm)	Width of flower (cm)
P_1	18.71	6.2	31.0	17.7	8.0	42.1	41.65	15.05	9.1	3.29	0.336	6.63	6.81
P_2	21.00	6.1	34.4	14.9	10.9	44.3	30.15	9.66	11.0	2.26	0.479	5.46	6.24
P_3	21.90	6.0	30.4	14.8	9.5	40.0	33.55	12.25	6.6	3.20	0.333	6.20	6.62
P_4	21.00	6.9	34.1	17.7	17.7	53.3	47.60	19.50	12.6	2.65	0.482	5.46	5.90
P_5	19.85	6.7	47.4	17.3	18.4	52.3	36.00	14.80	8.2	3.00	0.485	6.05	6.27
P_6	24.10	7.8	36.2	15.2	15.9	55.2	40.75	17.15	7.2	3.42	0.467	5.83	6.78
P_7	22.95	7.9	48.3	16.9	13.0	48.2	46.60	17.70	12.1	2.60	0.404	7.27	7.95
P_8	23.95	6.8	34.3	13.7	14.8	54.6	41.85	15.40	10.1	2.90	0.387	6.71	6.39
P_9	24.55	7.8	42.3	18.3	13.7	51.8	48.80	16.75	14.4	2.43	0.380	4.87	5.91
P_{10}	22.85	7.8	32.4	11.5	9.6	37.8	25.20	12.05	7.1	2.20	0.377	4.18	5.14
P_{11}	18.90	6.2	32.5	10.9	9.8	39.7	27.85	14.10	8.7	2.21	0.361	4.12	5.16
P_{12}	18.85	6.0	27.6	6.1	10.5	40.2	15.50	5.99	6.0	2.10	0.395	3.58	4.98
P_{13}	19.05	6.8	30.4	7.6	6.6	25.7	12.00	3.54	19.7	1.44	0.412	2.95	3.04
P_{14}	19.10	6.9	37.3	22.2	15.4	66.8	62.65	21.55	33.9	1.90	0.446	3.19	4.00
$S.E_m$	0.255	0.239	0.723	0.823	0.961	1.626	1.250	0.641	0.945	0.049	0.005	0.088	0.087
CD (0.05)	0.723	0.676	2.045	2.327	2.882	4.886	3.534	1.812	2.674	0.138	0.014	0.249	0.246

as in the two semi-commercial hybrids P_{12} (18.85 months) and P_{11} (18.90 months). Flowering was late in P_9 requiring a mean vegetative growth period of 24.55 months to flower.

10. Cane to flower first

The first inflorescence was produced on the sixth cane on an average in both P_3 and P_{12} which recorded the lowest value for this character. Not much variation was observed for this character among the other genotypes, recording mean values ranging from 6.1 in P_2 to 7.9 in P_7.

11. Days to first flower opening

The parent P_7 exhibited the maximum time interval from inflorescence emergence to first flower opening (48.3 days) closely followed by P_5 (47.4 days). The duration was the shortest in P_{12} recording 27.6 days.

12. Flowering time

Flowering time did not vary much among the commercial hybrids, ranging from 13.7 days in P_8 to 18.3 days in P_9. Time interval from opening of the first flower to the last in the inflorescence was significantly high in P_{14} (22.2 days) and significantly low in P_{12} (6.1 days) and P_{13} (7.6 days).

13. Vase life

Highest vase life was noted in P_5 (18.4 days). It was significantly high in P_4 (17.7 days) and P_6 (15.9 days) also. Lowest vase life of 6.6 days was observed in P_{13} which was on par with P_1 (8.0 days).

14. Days for wilting of all flowers

Days for wilting of all flowers in the inflorescence ranged from a significantly high value of 66.8 days in P_{14} to a significantly low value of 25.7 days in P_{13}. Not much variation with respect to this character was observed for the other genotypes, recording mean values ranging from 37.8 days in P_{10} to 55.2 days in P_6.

15. Length of inflorescence

The parent P_{14} displayed the highest inflorescence length (62.65 cm) which exceeded the measurement in all the other genotypes. The mean inflorescence length was significantly high in P_9 (48.80 cm), P_4 (47.60 cm) and P_7 (46.60 cm), preceded only by P_{14}. Inflorescence length was the lowest in P_{13} (12.00 cm) followed by P_{12} (15.50 cm).

16. Length of scape

The species P_{14} recorded the highest scape length of 21.55 cm. Next to P_{14}, the highest scape length was registered in P_4 (19.50 cm) which was on par with the mean expressed in P_7 (17.70 cm). The species P_{13} recorded the lowest scape length of 3.54 cm.

17. Number of flowers per inflorescence

The highest mean value of 33.9 for number of flowers per inflorescence was observed in P_{14}, which superseded all the other genotypes for this character. The species P_{13} produced 19.7 flowers per inflorescence, second only to P_{14}. Number of flowers per inflorescence was significantly high in P_9 (14.4), P_4 (12.6) and P_7 (12.1) which were on par with each other. The lowest mean number of flowers per inflorescence was expressed in P_{12} (6.0).

18. Length of internode of inflorescence

The mean internodal length between the flowers in the inflorescence ranged from 1.44 cm in P_{13} to 3.42 cm in P_6. Considerable clearance between successive flowers was observed in P_1 (3.29 cm) which was on par with P_5 (3.00 cm).

19. Diameter of inflorescence axis

Sturdy inflorescences with significantly thick inflorescence axes were observed in P_5 (0.485 cm), P_4 (0.482 cm) and P_2 (0.479 cm). The inflorescence axis diameter was significantly low in the varieties P_3 (0.333 cm) and P_1 (0.336 cm).

20. Length of flower

Length of flower ranged from 2.95 cm in P_{13} to 7.27 cm in P_7. Significantly long flowers, second only to P_7 were observed in P_8 (6.71 cm) and P_1 (6.63 cm).

21. Width of flower

Width of flower ranged from 3.04 cm in P_{13} to 7.95 cm in P_7. Considerably broad flowers were produced in P_1 (6.81 cm), P_6 (6.78 cm) and P_3 (6.62 cm).

4.1.2. Estimation of genetic parameters of parents

Genetic parameter analysis of parents have been conducted under the following heads :

4.1.2.1. Variability studies

4.1.2.2. Heritability and genetic advance

4.1.2.3. Correlation analysis

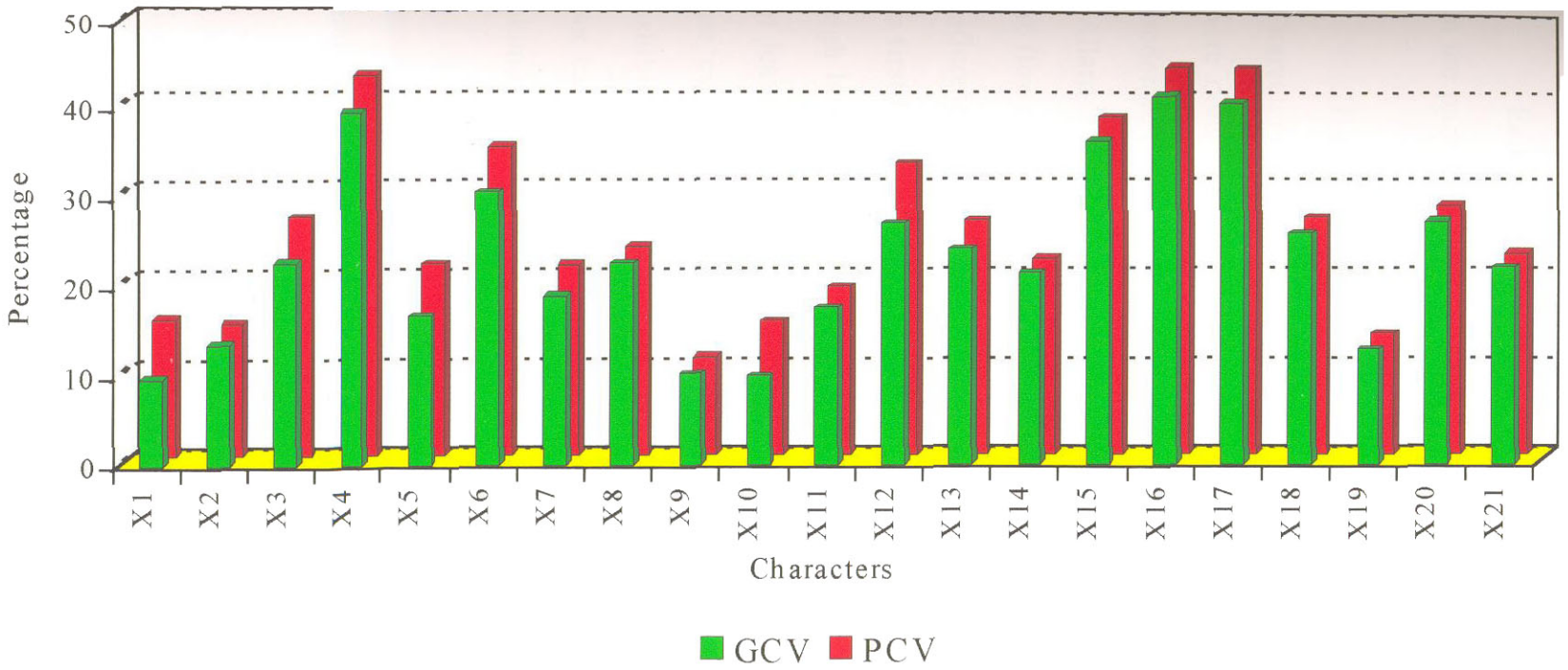
4.1.2.1. Variability studies

The genotypic and environmental components of phenotypic variance and the coefficients of variation at genotypic and phenotypic levels in *Dendrobium* parental material have been studied (Table 4.1.2.1.; Fig. 1).

The characters length of scape (GCV = 41.47 %, PCV = 43.78 %), number of flowers per inflorescence (GCV = 40.58 %, PCV = 43.68 %), number of nodes per cane (GCV = 39.46 %, PCV = 42.63 %), length of inflorescence (GCV = 36.21 %, PCV = 37.77 %) and leaf area per cane (GCV = 30.53 %, PCV = 34.44 %) in the descending order exhibited the highest estimates of variance. Both at genotypic and phenotypic levels, these characters maintained the same trend in the estimation of variance.

Table 4.1.2.1. Variability parameters for morphological characters in parental genotypes of *Dendrobium*

Sl. No.	Morphological characters	Genotypic variance σ^2_g	Phenotypic variance σ^2_p	Environmental variance σ^2_e	Genotypic coefficient of variation GCV (%)	Phenotypic coefficient of variation PCV (%)
1.	Number of shoots per clump	0.895	2.196	1.301	9.76	15.29
2.	Number of leaves per clump	23.511	27.484	3.973	13.59	14.69
3.	Height / length of cane (cm)	37.320	51.546	14.227	22.61	26.58
4.	Number of nodes per cane	14.479	16.897	2.417	39.46	42.63
5.	Number of leaves per cane	1.527	2.513	0.987	16.63	21.34
6.	Leaf area per cane (cm^2)	6280.116	7993.031	1712.915	30.53	34.44
7.	Length of leaf (cm)	5.139	6.482	1.343	18.83	21.15
8.	Breadth of leaf (cm)	0.564	0.604	0.040	22.48	23.27
9.	Age at first flowering (months)	4.644	5.297	0.653	10.17	10.86
10.	Cane to flower first	0.458	1.029	0.571	9.88	14.81
11.	Days to first flower opening	38.949	44.178	5.229	17.52	18.66
12.	Flowering time (days)	14.967	21.738	6.771	26.97	32.51
13.	Vase life (days)	7.810	9.239	1.429	23.95	26.05
14.	Days for wilting of all flowers	94.634	99.079	4.446	21.25	21.74
15.	Length of inflorescence (cm)	177.739	193.354	15.615	36.21	37.77
16.	Length of scape (cm)	35.982	40.088	4.106	41.47	43.78
17.	Number of flowers per inflorescence	23.636	27.325	3.689	40.58	43.68
18.	Length of internode of inflorescence (cm)	0.483	0.507	0.024	25.73	26.36
19.	Diameter of inflorescence axis (cm)	0.0028	0.0030	0.0002	12.90	13.37
20.	Length of flower (cm)	1.928	2.005	0.077	27.06	27.60
21.	Width of flower (cm)	1.618	1.694	0.076	21.86	22.37



X1 Number of shoots per clump
X2 Number of leaves per clump
X3 Height / length of cane (cm)
X4 Number of nodes per cane
X5 Number of leaves per cane
X6 Leaf area per cane (cm^2)
X7 Length of leaf (cm)
X8 Width of leaf (cm)
X9 Age at first flowering (months)
X10 Cane to flower first
X11 Days to first flower opening
X12 Flowering time (days)
X13 Vase life (days)
X14 Days for wilting of all flowers
X15 Length of inflorescence (cm)
X16 Length of scape (cm)
X17 Number of flowers per inflorescence
X18 Length of internode of inflorescence (cm)
X19 Diameter of inflorescence axis (cm)
X20 Length of flower (cm)
X21 Width of flower (cm)

Fig. 1. GCV and PCV for twenty one traits in parental genotypes of *Dendrobium*

4.1.2.2. Heritability and genetic advance

Estimates of heritability in the broad sense and genetic advance in the parental material have been recorded (Table 4.1.2.2.; Figs. 2 and 3).

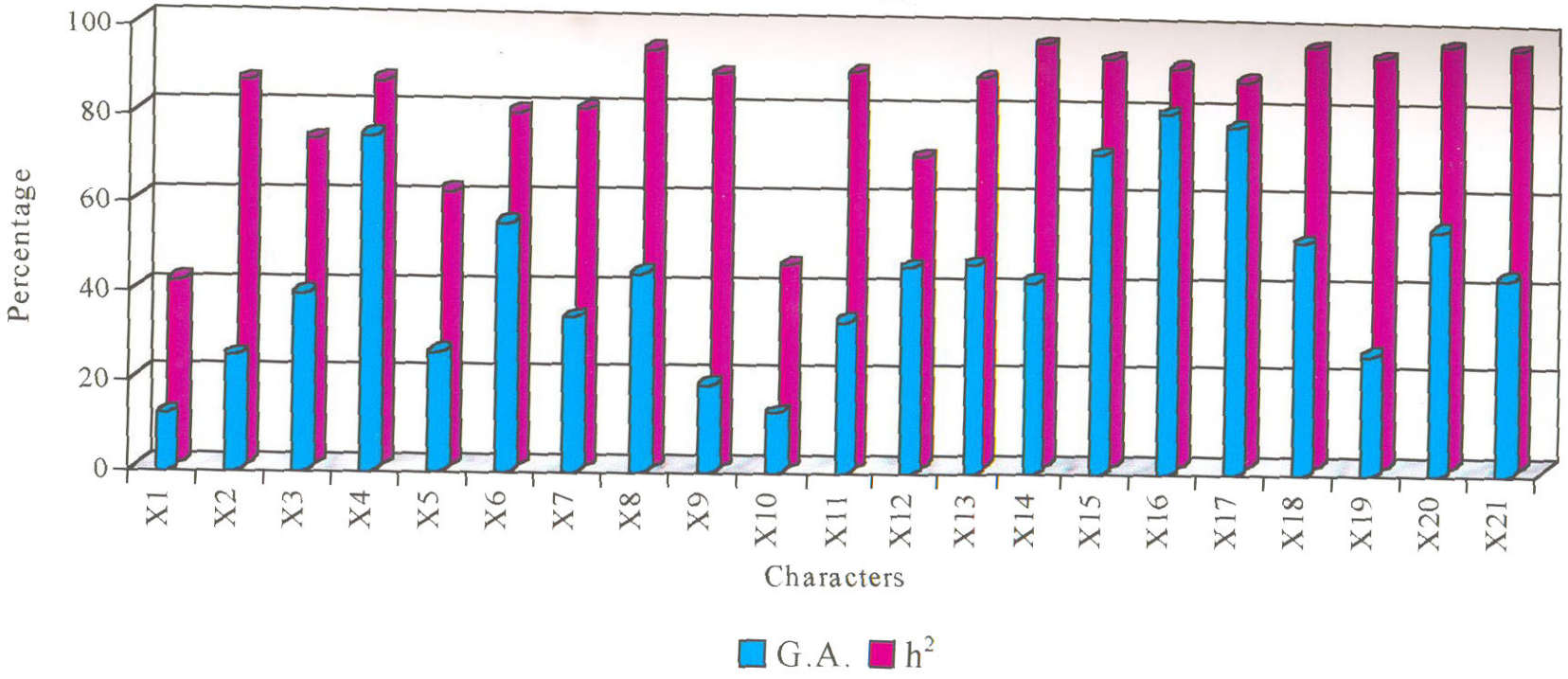
Heritability estimates were high for most of the characters. Characters exhibiting heritability estimates of greater than 70 per cent were considered as having high heritability, 30-70 per cent as having moderate heritability and less than 30 per cent as having low heritability (Allard, 1960). Accordingly, the floral characters such as length and width of flower, length of internode, diameter of inflorescence axis, length of inflorescence, length of scape, number of flowers per inflorescence, days to first flower opening, vase life and days for wilting of all flowers showed high heritability (> 70 %). Important vegetative characters like number of leaves per clump, height of cane, number of nodes per cane, leaf area per cane, length of leaf, width of leaf and age at first flowering also exhibited high heritability. Heritability was moderate for number of leaves per cane, flowering time, cane to flower first and number of shoots per clump (30 to 70 %).

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 length of scape, length of inflorescence, number of flowers per inflorescence and number of nodes per cane. Important floral characters such as days to first flower opening, vase life, days for wilting of all flowers, length of internode, length of flower and

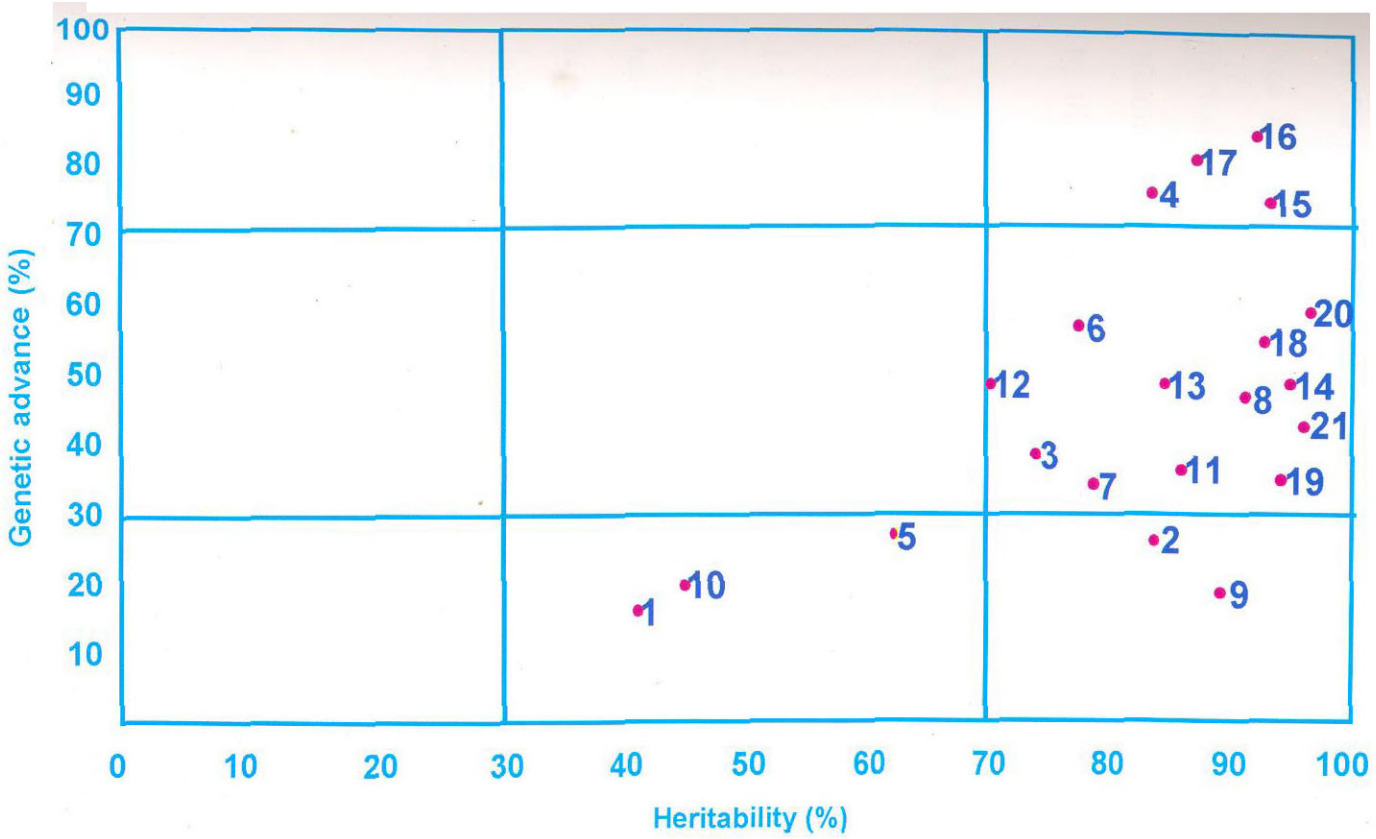
Table 4.1.2.2. Heritability and genetic advance for morphological characters in parental genotypes of *Dendrobium*

Sl. No.	Morphological characters	Heritability coefficient (%)	Genetic advance (at 5 %)	Genetic advance (% mean)
1.	Number of shoots per clump	40.76	1.24	12.80
2.	Number of leaves per clump	85.54	9.24	25.90
3.	Height / length of cane (cm)	72.40	10.71	39.65
4.	Number of nodes per cane	85.69	7.26	75.31
5.	Number of leaves per cane	60.74	1.98	26.64
6.	Leaf area per cane (cm^2)	78.57	144.70	55.74
7.	Length of leaf (cm)	79.29	4.16	34.55
8.	Width of leaf (cm)	93.30	1.49	44.60
9.	Age at first flowering (months)	87.68	4.16	19.62
10.	Cane to flower first	44.49	0.93	13.58
11.	Days to first flower opening	88.16	12.07	33.89
12.	Flowering time (days)	68.85	6.61	46.09
13.	Vase life (days)	86.97	5.45	46.70
14.	Days for wilting of all flowers	95.51	19.59	42.78
15.	Length of inflorescence (cm)	91.92	26.33	71.53
16.	Length of scape (cm)	89.76	11.71	80.98
17.	Number of flowers per inflorescence	86.79	9.35	77.90
18.	Length of internode of inflorescence (cm)	95.29	1.40	51.85
19.	Diameter of inflorescence axis (cm)	93.11	0.11	26.82
20.	Length of flower (cm)	96.15	2.80	54.58
21.	Width of flower (cm)	95.33	2.56	43.99



X1 Number of shoots per clump
X2 Number of leaves per clump
X3 Height / length of cane (cm)
X4 Number of nodes per cane
X5 Number of leaves per cane
X6 Leaf area per cane (cm^2)
X7 Length of leaf (cm)
X8 Width of leaf (cm)
X9 Age at first flowering (months)
X10 Cane to flower first
X11 Days to first flower opening
X12 Flowering time (days)
X13 Vase life (days)
X14 Days for wilting of all flowers
X15 Length of inflorescence (cm)
X16 Length of scape (cm)
X17 Number of flowers per inflorescence
X18 Length of internode of inflorescence (cm)
X19 Diameter of inflorescence axis (cm)
X20 Length of flower (cm)
X21 Width of flower (cm)

Fig. 2. Heritability (h^2) and Genetic Advance (G.A.) for twenty one traits in parental genotypes of *Dendrobium*



1 Number of shoots per clump
2 Number of leaves per clump
3 Height / length of cane (cm)
4 Number of nodes per cane
5 Number of leaves per cane
6 Leaf area per cane (cm^2)
7 Length of leaf (cm)
8 Width of leaf (cm)
9 Age at first flowering (months)
10 Cane to flower first
11 Days to first flower opening
12 Flowering time (days)
13 Vase life (days)
14 Days for wilting of all flowers
15 Length of inflorescence (cm)
16 Length of scape (cm)
17 Number of flowers per inflorescence
18 Length of internode of inflorescence (cm)
19 Diameter of inflorescence axis (cm)
20 Length of flower (cm)
21 Width of flower (cm)

Fig. 3. Character distribution in terms of heritability and genetic advance

width of flower possessed high (> 70 %) heritability along with 30-70 per cent genetic advance. All the four characters possessing moderate heritability *viz.,* number of leaves per cane, flowering time, cane to flower first and number of shoots per clump recorded less than 30 per cent or 30-70 per cent genetic advance. Although number of leaves per clump, age at first flowering and diameter of inflorescence axis had high heritability, their genetic advance was found to be less than 30 per cent. Number of shoots per clump and cane to flower first were more influenced by the environment in comparison with the other characters.

4.1.2.3. Correlation studies

The genotypic, phenotypic and environmental correlations of the 14 parents were studied for 12 biometric characters, *viz.,* number of shoots per clump, number of leaves per clump, height of cane, number of leaves per cane, leaf area per cane, age at first flowering, cane to flower first, vase life, length of inflorescence, number of flowers per inflorescence, length of flower and width of flower (Tables 4.1.2.3.a to 4.1.2.3.c; Figs. 4 to 6).

In general, high positive correlation at genotypic and phenotypic levels were observed between most of the vegetative and floral characters considered. 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.

Table 4.1.2.3.a. Genotypic correlations among different biometric characters in parental genotypes of *Dendrobium*

	No. of shoots/ clump	No. of leaves/ clump	Height/ length of cane	No. of leaves/ cane	Leaf area/ cane	Age at first flowering	Cane to flower first	Vase life	Length of inflore-scence	No. of flowers/ inflorescence	Length of flower
No. of leaves/clump	0.3707										
Height/length of cane	0.5565	0.8988									
No. of leaves/cane	0.2048	0.5042	0.6190								
Leaf area/cane	-0.0715	0.6495	0.3433	-0.2074							
Age at first flowering	0.4164	0.2760	0.2669	-0.3385	0.4303						
Cane to flower first	0.7007	0.6348	0.7593	0.2225	0.3719	0.7370					
Vase life	0.4212	0.7528	0.5266	0.0010	0.6540	0.3019	0.3948				
Length of inflorescence	0.3689	0.7210	0.5817	-0.0902	0.6454	0.3320	0.4216	0.7717			
No. of flowers per inflorescence	0.2191	0.5949	0.5923	0.8428	-0.0913	-0.2717	0.1223	0.2733	0.3326		
Length of flower	0.0951	-0.0358	-0.2952	-0.7551	0.4822	0.4452	0.1887	0.3664	0.3592	-0.5539	
Width of flower	0.0300	-0.0250	-0.2743	-0.3070	0.5448	0.4736	0.2281	0.3277	0.4087	-0.6337	0.9561

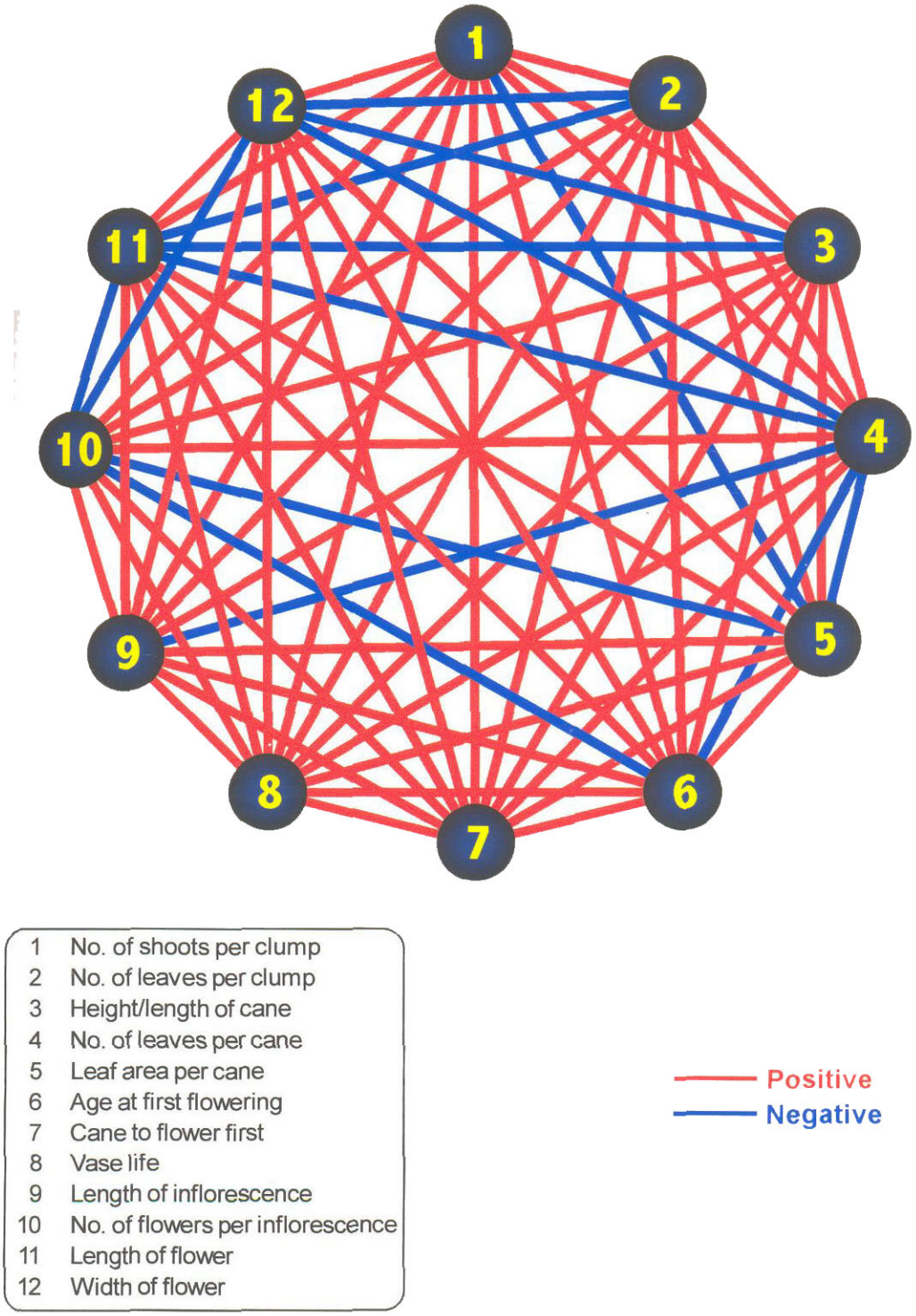


Fig. 4. Genotypic correlations among the characters

Table 4.1.2.3.b. Phenotypic correlations among different biometric characters in parental genotypes of *Dendrobium*

	No. of shoots/ clump	No. of leaves/ clump	Height/ length of cane	No. of leaves/ cane	Leaf area/ cane	Age at first flowering	Cane to flower first	Vase life	Length of inflore-scence	No. of flowers/ inflorescence	Length of flower
No. of leaves per clump	0.1742*										
Height/length of cane	0.2614**	0.7315**									
No. of leaves per cane	0.0919	0.3524**	0.4380**								
Leaf area per cane	0.0053	0.5280**	0.2244**	-0.1281							
Age at first flowering	0.2858**	0.2518**	0.2263**	-0.2760**	0.3784**						
Cane to flower first	0.2847**	0.3855**	0.4269**	0.1882*	0.1890*	0.4829**					
Vase life	0.2457**	0.6442**	0.4175**	0.0074	0.5494**	0.2443**	0.2533**				
Length of inflorescence	0.2156**	0.6476**	0.4967**	-0.0759	0.5479**	0.2989**	0.2602**	0.6891**			
No. of flowers per inflorescence	0.1040	0.5173**	0.4958**	0.5680**	-0.0806	-0.2415**	0.0328	0.2239**	0.3094**		
Length of flower	0.0512	-0.0314	-0.2488**	-0.5882**	0.4113**	0.4118**	0.1286	0.3281**	0.3422**	-0.5083**	
Width of flower	0.0108	-0.0237	-0.2018*	-0.6336**	0.4787**	0.4301**	0.1548	0.3099**	0.3776**	-0.5838**	0.9242**

** - Significant at 1 per cent level * - Significant at 5 per cent level

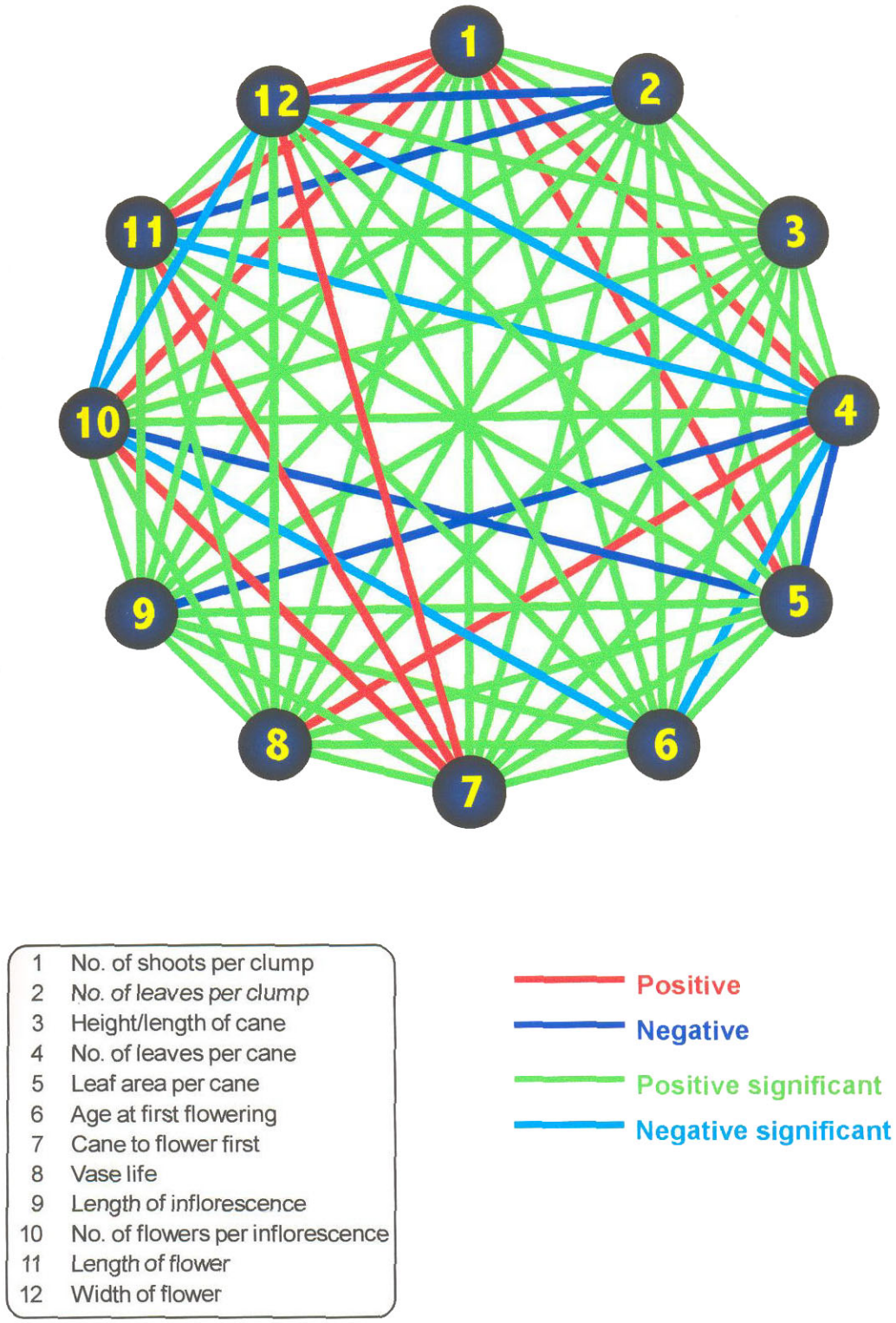


Fig. 5. Phenotypic correlations among the characters

Table4.1.2.3.c. Environmental correlations among different biometric characters in parental genotypes of *Dendrobium*

	No. of shoots/ clump	No. of leaves/ clump	Height/ length of cane	No. of leaves/ cane	Leaf area/ cane	Age at first flowering	Cane to flower first	Vase life	Length of inflore-scence	No. of flowers/ inflorescence	Length of flower
No. of leaves per clump	-0.1528										
Height/length of cane	-0.1011	0.1213									
No. of leaves per cane	-0.0306	-0.0462	0.0834								
Leaf area per cane	0.1285	-0.0253	-0.1425	0.0522							
Age at first flowering	0.1365	0.0954	0.0743	-0.1314	0.1311						
Cane to flower first	-0.0239	-0.0219	-0.0104	0.1555	-0.0896	0.0864					
Vase life	0.0116	-0.0375	-0.0020	0.0299	0.0528	-0.1524	0.0286				
Length of inflorescence	-0.0467	0.0763	0.1485	-0.0480	-0.0040	0.0080	-0.0446	-0.0094			
No. of flowers per inflorescence	-0.0941	0.0339	0.1378	-0.1926*	-0.0312	-0.0346	-0.1595	-0.1037	0.1186		
Length of flower	-0.0551	0.0140	-0.0242	-0.0910	-0.0859	0.0430	0.0359	-0.0981	0.0814	-0.0322	
Width of flower	-0.0448	-0.0127	-0.0590	-0.0998	0.0691	-0.0446	0.0389	0.1470	-0.0893	-0.0844	0.1844*

* - Significant at 5 per cent level

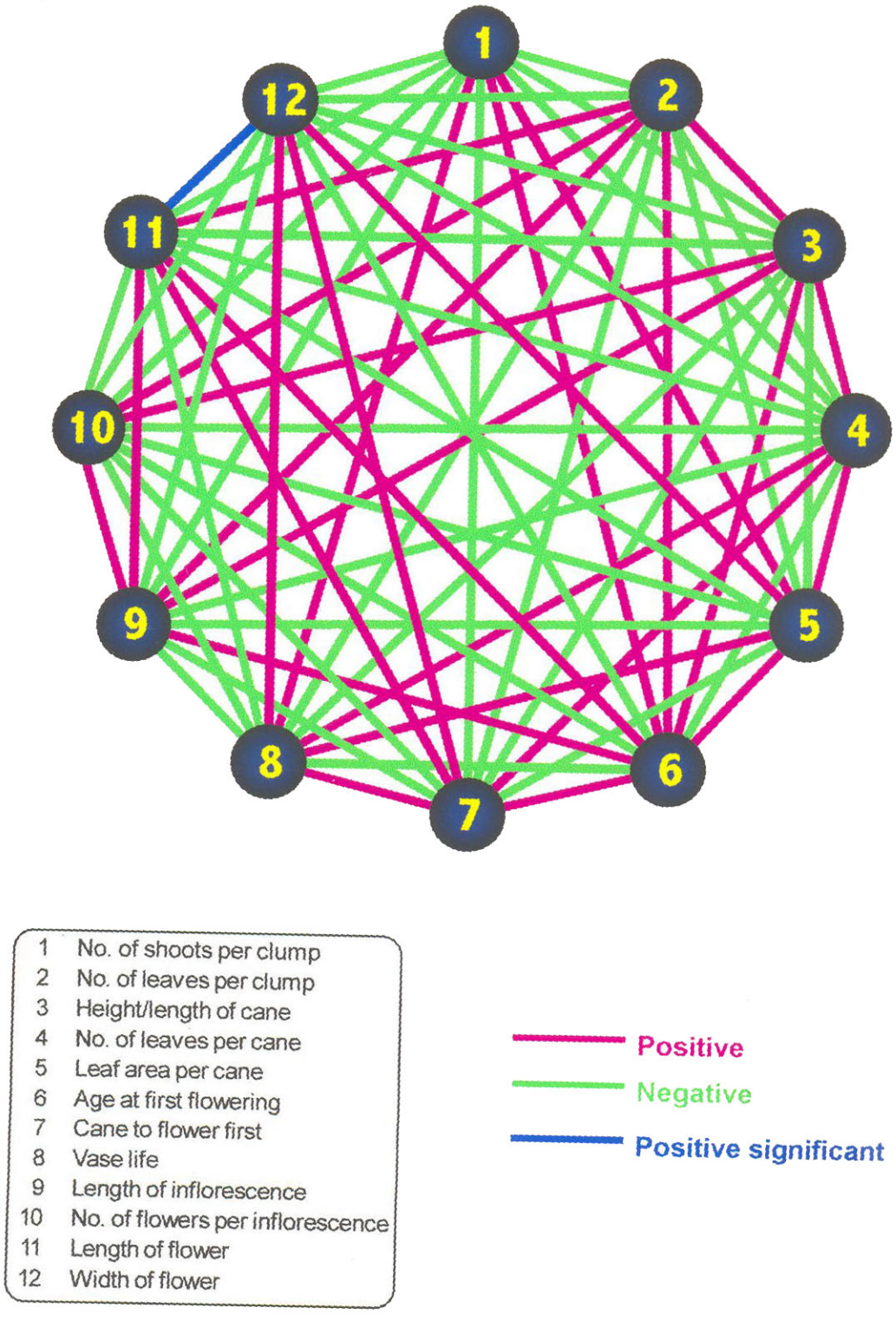


Fig. 6. Environmental correlations among the characters

Age at first flowering was positively correlated with number of shoots per clump (rg = 0.4164, rp = 0.2858), number of leaves per clump (rg = 0.2760, rp = 0.2518), height of cane (rg = 0.2669, rp = 0.2263), leaf area per cane (rg = 0.4303, rp = 0.3784) and cane to flower first (rg = 0.7370, rp = 0.4829). This character showed high negative genotypic and phenotypic correlations with number of leaves per cane (rg = -0.3385, rp = -0.2760) and number of flowers per inflorescence (rg = -0.2717, rp = -0.2415).

Cane to flower first recorded significant positive correlation with number of shoots per clump (rg = 0.7007, rp = 0.2847), number of leaves per clump (rg = 0.6348, rp = 0.3855), height of cane (rg = 0.7593, rp = 0.4269), number of leaves per cane (rg = 0.2225, rp = 0.1882), leaf area per cane (rg = 0.3719, rp = 0.1890), age at first flowering (rg = 0.7370, rp = 0.4829), vase life (rg 0.3948, rp = 0.2533) and length of inflorescence (rg = 0.4216, rp = 0.2602).

Vase life expressed maximum positive correlation with length of inflorescence (rg = 0.7717, rp = 0.6891) followed by number of leaves per clump (rg = 0.7528, rp = 0.6442). The other characters exhibiting high positive correlation with vase life were leaf area per cane (rg = 0.6540, rp = 0.5494), height of cane (rg = 0.5266, rp = 0.4175), length of flower (rg = 0.3664, rp = 0.3281), width of flower (rg = 0.3277, rp = 0.3099), number of shoots per clump (rg = 0.4212, rp = 0.2457) and number of flowers per inflorescence (rg = 0.2733, rp = 0.2239). Vase life was found to be positively correlated with age at first

flowering (rg = 0.3019, rp = 0.2443) and cane to flower first (rg = 0.3948, rp = 0.2533).

Length of inflorescence recorded high and significant positive correlation with number of leaves per clump (rg = 0.7210, rp = 0.6476), leaf area per flowering cane (rg = 0.6454, rp = 0.5479), height of cane (rg = 0.5817, rp = 0.4967) and number of shoots per clump (rg = 0.3689, rp = 0.2156). The other characters expressing high positive correlation with length of inflorescence were age at first flowering (rg = 0.3320, rp = 0.2989), cane to flower first (rg = 0.4216, rp = 0.2602), length of flower (rg = 0.3592, rp = 0.3422) and width of flower (rg = 0.4087, rp = 0.3776).

Number of flowers per inflorescence showed high positive correlation with number of leaves per cane (rg = 0.8428, rp = 0.5680), number of leaves per clump (rg = 0.5949, rp = 0.5173), height of cane (rg = 0.5923, rp = 0.4958) and length of inflorescence (rg = 0.3326, rp = 0.3094). This character exhibited significant negative correlation with length of flower (rg = -0.5539, rp = -0.5083), width of flower (rg = -0.6337, rp = -0.5838) and age at first flowering (rg = -0.2717, rp = -0.2415).

Length of flower showed maximum positive correlation with width of flower (rg = 0.9561, rp = 0.9242) followed by age at first flowering (rg = 0.4452, rp = 0.4118), leaf area per cane (rg = 0.4822, rp = 0.4113), length of inflorescence (rg = 0.3592, rp = 0.3422) and vase life (rg = 0.3664, rp = 0.3281). This character showed high negative correlation with number of leaves per cane (rg = -0.7551, rp = -0.5882), height of cane

(rg = -0.2952, rp = -0.2488) and number of flowers per inflorescence (rg = -0.5539, rp = -0.5083).

Width of flower recorded maximum positive correlation with length of flower followed by leaf area per cane (rg = 0.5448, rp = 0.4787), age at first flowering (rg = 0.4736, rp = 0.4301), length of inflorescence (rg = 0.4087, rp = 0.3776) and vase life (rg = 0.3277, rp = 0.3099). High negative correlations were exhibited by width of flower with number of leaves per cane (rg = -0.3070, rp = -0.6336), number of flowers per inflorescence (rg = -0.6337, rp = -0.5838) and height of cane (rg = -0.2743, rp = -0.2018).

Environmental correlation was observed 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 have been studied with respect to the following heads :

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

4.1.3.2 Flower opening - anthesis; stigma receptivity

4.1.3.3 Pollen characters (Plate II)

4.1.3.1 Flowering and floral morphology

Flowering and qualitative floral characters in the parental genotypes of *Dendrobium* have been analysed in detail (Table 4.1.3.1).

1. Flowering nature - free-flowering/seasonal

Free-flowering nature ie., flowering throughout the year was exhibited by P_4, P_6, P_8, P_9 and P_{14}. Flowering was seasonal, occurring mostly from June to December in P_1, P_2 and P_3. Blooming was observed from May to October P_5, P_7, P_{11} and P_{12}. Seasonal flowering was observed from April to November in P_{10} whereas it was confined to November to January in P_{13}.

2. Time taken for single flower opening

The average time taken for opening of a single flower ranged from 0.39 day in P_{13} to 2.24 days in P_3. A single flower took approximately 2.11 days to complete opening in P_5 and P_6. All the other varieties required one to two days to complete flower opening except P_{14} in which a bloom opened in 0.65 day.

3. Nature of inflorescence axis

Inflorescence axis was found to be arching in all varieties except P_2, P_9 and P_{13}. In addition to arching nature, the inflorescence axis in P_3 sometimes exhibited pendulous nature whereas P_2, P_9 and P_{13} had erect inflorescence axes.

Table 4.1.3.1: Performance of parental genotypes of *Dendrobium* for qualitative floral characters

Parental genotypes	Flowering nature-free flowering / seasonal	Average time taken for single flower opening (days)	Nature of inflorescence axis	Mode of display of flowers	Colour of flower	Texture of flower	Shape of flower	Flower size (circumference) (cm)	Fullness value	Fragrance
P_1	Seasonal Mostly during June-December	1.95	Arching	Alternate and facing opposite sides	Pink and striped; light pink with dark pink stripes throughout; central, hairy thickening or labellum - white	Moderately thick and glossy	Perfectly shaped with full, flat look; broad and rounded sepals and petals	21.10	2.67	Nil
P_2	Seasonal Mostly during June-December	1.35	Erect	Basal portion of inflorescence - whorled; top-alternate and facing opposite sides	Light pink with green tinge; sepals - greenish white; petals - greenish white towards the inside; central, hairy thickening of labellum - greenish white	Moderately thick and glossy	Full; rounded sepals and petals with slight reflexing	18.37	2.70	Nil
P_3	Seasonal Mostly during June-December	2.24	Arching/ pendulous	Alternate and facing opposite sides/ alternate and facing dorsal side	Pink and white; sepals and petals - white towards the inside and light pink towards the outside; central, hairy thickening of labellum - white	Moderately thick, substantial looking and glossy	Perfectly shaped with full, rounded sepals and petals -slight reflexing	20.13	2.28	Nil

P_4	Free-flowering	1.40	Arching	Alternate and facing opposite sides	Purple and white; sepals and petals- white towards the inside and purple towards the outside; central, hairy thickening of labellum-white	Very thick, substantial looking and glossy	Slightly reflexed, spatulate looking sepals and petals of almost equal size	17.84	3.18	Nil
P_5	Seasonal Mostly during May-October	2.11	Arching	Alternate and facing opposite sides	Solid deep purple; central, hairy thickening of labellum -white	Very thick, substantial looking and glossy	Full and flat; broad and rounded sepals and petals	19.34	3.05	Nil
P_6	Free-flowering	2.11	Arching	Alternate and facing opposite sides	Solid deep magenta; white operculum; central, hairy thickening of labellum -deep velvety magenta	Very thick, substantial looking and glossy	Perfectly shaped with a full, flat look; broad and rounded sepals and petals	19.80	2.39	Nil
P_7	Seasonal Mostly during May-October	1.40	Arching	Alternate and facing opposite sides	Solid pale purple throughout	Thick, substantial looking and glossy	Flat with stellar appearance; sepals and petals- spatulate, narrow and pointed	23.90	3.23	Nil
P_8	Free-flowering	1.36	Arching	Alternate and facing opposite sides	Deep purple and white; sepals and petals - white towards the inside and deep purple towards the outside; central, hairy thickening of labellum -white	Very thick, substantial looking and glossy	Flat, with stellar appearance, sepals and petals- pointed	20.57	3.08	Nil

P_9	Free-flowering	1.27	Erect	Basal portion of inflorescence - whorled; top alternate and facing opposite sides	Solid white tinged with light green	Thick substantial looking and glossy	Full; rounded sepals and petals	16.92	3.02	Nil
P_{10}	Seasonal Mostly during April-November	1.62	Arching	Alternate and facing opposite sides	Light purple with deeper purple criss-cross stripes; central, hairy thickening of labellum – light purple	Thick leathery and slightly glossy	Semi-horned appearance; very narrow, spatulate, pointed sepals and petals	14.63	4.61	Nil
P_{11}	Seasonal Mostly during May-October	1.25	Arching	Alternate and facing opposite sides	Light pink and white; central hairy thickening of labellum-white	Thick, leathery and slightly glossy	Slightly reflexed with narrow, spatulate sepals and petals	14.57	3.88	Fragr-ant
P_{12}	Seasonal Mostly during May-October	1.02	Arching	Alternate and facing opposite sides	White with very light pink tinge	Thin, leathery and slightly glossy	Slightly reflexed with narrow, spatulate sepals and petals	13.44	3.32	Nil
P_{13}	Seasonal Mostly during November-January	0.39	Erect	Whorled	Solid creamy white with yellow center; central, hairy thickening of labellum - yellow	Thin, fragile - looking and glossy	Small, full and flat; sepals - narrow, pointed; petals -broad, tapering	9.40	3.09	Nil
P_{14}	Free-flowering	0.65	Arching	Alternate and facing opposite sides	Light purple with deeper purple tips of sepals and petals	Thick, leathery and slightly glossy	Slightly reflexed with narrow, spatulate sepals and petals	11.29	4.54	Nil

4. Mode of display of flowers

Mode of display was alternate and facing opposite directions in all varieties except P_2, P_9 and P_{13}. In P_3, when the inflorescence axis was pendulous, the flowers were alternate and facing the dorsal side. The arrangement was whorled at the base and alternate towards the top in P_2 and P_9, whereas the entire inflorescence was whorled in P_{13}.

5. Colour of flower

The pigmentation of the flower in general and of each petal in particular is explained (Table 4.1.3.1.) (Plate I). Single solid colour was noted in six varieties, being solid white tinged with light green in P_9, white with faint pink tinge in P_{12}, creamy-white with yellow centre in P_{13}, solid pale purple in P_7, deep purple in P_5 and deep magenta in P_6. Double colour was observed in six varieties *viz.,* light pink with green tinge in P_2, light pink and white in P_{11}, pink and white in P_3, light purple with deep purple sepal and petal tips in P_{14}, purple and white in P_4 and deep purple and white in P_8. Light pink pigmentation with deeper pink regular stripes was noted in P_1, whereas light purple with deeper purple criss-cross stripes was observed in P_{10}.

6. Texture of flower

Very thick and substantial looking glossy flowers were observed in P_4, P_5, P_6 and P_8. The flowers were thick, substantial looking and glossy in P_7 and P_9. P_1, P_2 and P_3 produced moderately thick and glossy flowers. The flowers appeared to be thick, leathery and slightly glossy

Plate I. Floral characteristics of parental genotypes of *Dendrobium* used in the hybridization programme (from left to right, row-wise)

1. *Dendrobium* Sakura (P_7) Large flowers, white with purple tinge. Sepals and petals thick and glossy, narrow and pointed with stellar appearance.	2. *D.* barbatulum (P_{13}) Solid creamy white with yellow centre. Flowers very small, thin, fragile looking and glossy.	3. *D.* Walter Oumae (P_{12}) Small flowers, white with very faint pink tinge. Slightly reflexed in appearance with narrow, spatulate sepals and petals.
4. *D.* Candy Stripe x Tomie Drake (P_1) Pink and striped; large flowers with full appearance. Sepals and petals medium thick and glossy.	5. *D.* philippica (P_{14}) Flowers small, light purple, slightly reflexed; narrow, thick and leathery sepals and petals.	6. *D.* Uniwai Pink (P_{11}) Small flowers, light pink and white, slightly reflexed; narrow sepals and petals, thick and glossy.
7. *D.* Rinabha (P_6) Flowers large with solid deep magenta colour, broad petals and full appearance. Sepals and petals very thick and glossy.	8. *D.* Renapa Red 3 (P_5) Flowers large, solid deep purple with full appearance. Sepals and petals very thick and glossy.	9. *D.* White Fairy (P_9) Flowers medium large, solid white, fully rounded sepals and petals which are thick and glossy.
10. *D.* Nagoya Pink (P_3) Large flowers; pink and white with full, rounded appearance. Sepals and petals moderately thick and glossy.	11. *D.* Caesar Pink (P_{10}) Small flowers, light purple with deeper purple stripes. Sepals and petals slightly twisted with semi-horned appearance. Sepals and petals thick and leathery.	12. *D.* Chiangmai Pink (P_2) Large flowers; pink and white with green tinge; full, rounded appearance. Sepals and petals moderately thick and glossy.
13. *D.* Sonia 16 (P_8) Large flowers; white with deep purple tipped sepals and petals; stellar appearance. Sepals and petals thick and glossy.	14. *D.* Pramot 3 (P_4) Medium sized flowers; purple and white with semi-stellar appearance. Sepals and petals spatulate, thick and glossy.	

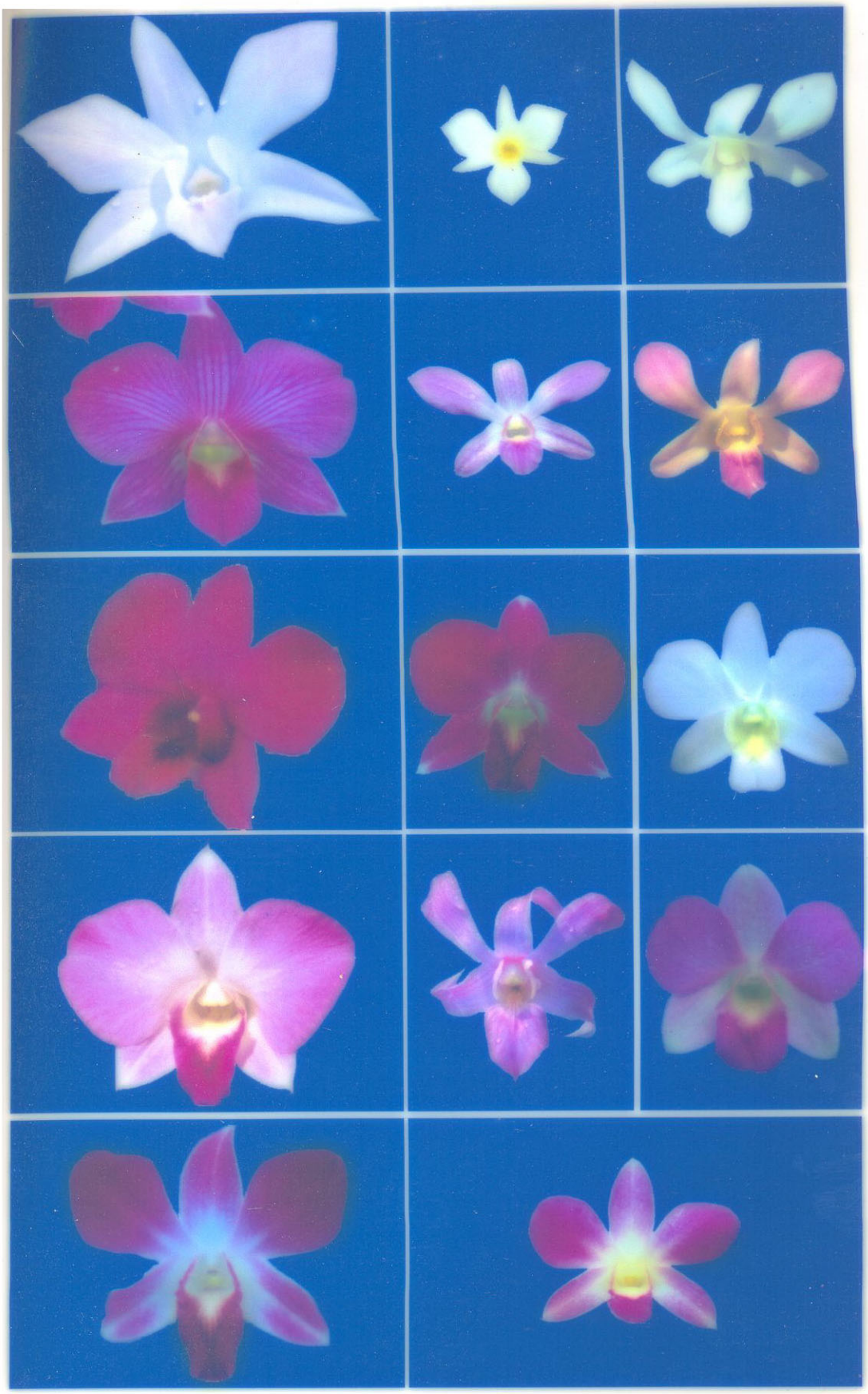


Plate I

in P_{10}, P_{11} and P_{14}, whereas in P_{12} they were thin, leathery and slightly glossy. Thin textured, glossy and fragile flowers were found in P_{13}.

7. Shape of flower

The flowers were full, with broad sepals and petals in P_1, P_2, P_3, P_5, P_6 and P_9. Slight reflexing of sepals and petals was noticed in P_2 and P_3. In the species P_{13}, the small flower presented a full appearance with broad petals and labellum. The variety P_8 with its broad, pointed sepals and petals exhibited a stellar appearance. Slightly reflexed, spatulate sepals and petals were noticed in P_4 whereas the sepals and petals were spatulate, narrow and pointed in P_7. In P_{12} and P_{14}, the sepals and petals were slightly reflexed, spatulate and narrow. The semi-commercial hybrid P_{10} presented a semi-horned appearance with spatulate, very narrow and pointed sepals and petals.

8. Flower size

Size of flower ranged from 9.40 cm in P_{13} to 23.90 cm in P_7. The smallest flowers were found in the species P_{13} and P_{14} followed by the semi-commercial hybrids P_{10}, P_{11} and P_{12}. The largest flowers were recorded in the commercial hybrids. The flowers in P_1 (21.10 cm), P_8 (20.57 cm), P_3 (20.13 cm), P_6 (19.80 cm) and P_5 (19.34 cm) were considerably large compared to the rest of the parental genotypes.

9. Fullness value

Fullness value indicates the degree of fullness of a flower; the lower the value, the greater the fullness. Fullness values ranged from

2.28 in P_3 to 4.61 in P_{10}. Flowers were remarkably full in P_6, P_1 and P_2. While considering the semi-commercial hybrids and species, the small flowers of P_{13} exhibited remarkable fullness, the value being 3.09.

10. Fragrance

No fragrance was observed in any of the commercial hybrids and species, whereas a faint, pleasant aroma was associated with the flower of P_{11}.

4.1.3.2. Flower opening and stigma receptivity

4.1.3.2.a. Flower opening time

In all the *Dendrobium* genotypes studied, flowers opened during the day-time, in acropetal succession (Table 4.1.3.2). Flower opening commenced one to one and a half months after inflorescence emergence and one to three weeks were required for the opening of all flowers in the inflorescence (Table 4.1.1.2). Each flower in an inflorescence opened almost during the same time of the day (Table 4.1.3.2) at a uniform time interval (Table 4.1.3.1).

Flower opening in *Dendrobium* genotypes commenced from 7.30 am on sunny days, but was delayed till 12.30 pm on rainy days. Flower opening commenced early in the morning in the species and the semi-commercial hybrids evaluated, with weather-dependent variations. In this group, flower opening commenced between 7.30 and 9.00 am in P_{13}, whereas in P_{12}, the flower started opening between 7.30 and 11.30 am.

Table 4.1.3.2. Flower opening time, anthesis time and stigma receptivity period in parental genotypes of *Dendrobium*

Parental genotypes	Flower opening time	Anthesis time (days)	Maximum stigma receptivity period
P_1	8.00 am - 12.00 noon	3.8	3rd - 9th day
P_2	8.30 am - 11.30 am	2.4	3rd - 9th day
P_3	9.00 am - 12.00 noon	3.7	3rd - 8th day
P_4	8.30 am - 11.30 am	2.9	3rd - 9th day
P_5	8.30 am - 12.30 pm	3.6	4th - 10th day
P_6	9.00 am - 12.30 pm	3.5	4th - 10th day
P_7	8.30 am - 11.30 am	3.3	3rd - 9th day
P_8	8.00 am - 11.00 am	2.4	3rd - 9th day
P_9	7.30 am - 11.00 am	2.6	3rd - 8th day
P_{10}	8.00 am - 11.00 am	2.8	2nd - 6th day
P_{11}	8.00 am - 11.00 am	2.5	2nd - 6th day
P_{12}	7.30 am - 11.30 am	3.3	3rd - 8th day
P_{13}	7.30 am - 9.00 am	2.0	1st - 3rd day
P_{14}	7.30 am - 9.30 am	2.3	2nd - 5th day

Among the nine commercial hybrids tested, flower opening commenced between 7.30 and 11.00 am in P_9, while it was delayed till 9.00 am to 12.30 pm in P_6.

4.1.3.2.b. Anthesis time

Anthesis time (time of maturity of pollen after flower opening) based on the capacity of pollinia to effect successful capsule set after pollination was studied in each of the parental genotypes (Table 4.1.3.2).

Mean anthesis time ranged from 2.0 days after flower opening in P_{13} to 3.8 days in P_1. Anthesis was observed to be comparatively early in the two species P_{13} and P_{14} (2.3 days). Anthesis was slightly later in the three semi-commercial hybrids, being 2.5 days after flower opening in P_{11}, 2.8 days in P_{10} and 2.9 days in P_{12}. In the commercial hybrids, anthesis time ranged from 2.4 days after flower opening in P_2 and P_8 to 3.8 days in P_1; anthesis was comparatively early in P_4 (2.9 days) and P_9 (2.6 days) and comparatively late in P_3 (3.7 days), P_5 (3.6 days), P_6 (3.5 days) and P_7 (3.3 days).

4.1.3.2.c. Maximum stigma receptivity period

Stigma receptivity after flower opening based on successful capsule set was studied in each of the parental genotypes (Table 4.1.3.2).

In all genotypes tested, successful capsule set was obtained irrespective of the time of the day at which pollination was carried out *viz.*, morning, noon or evening.

Maximum stigma receptivity period ranged from first to third day after flower opening in P_{13}, to fourth to tenth day after flower opening in P_5 and P_6. In several of the parental genotypes tested *viz.*, P_1, P_2, P_4, P_7 and P_8, maximum stigma receptivity was observed from third to ninth day after flower opening. The parents P_3, P_9 and P_{12} recorded high stigma receptivity from third to eighth day whereas P_{14} recorded maximum stigma receptivity from second to fifth day. The semi-commercial hybrids P_{10} and P_{11} registered maximum stigma receptivity from second to sixth day after flower opening.

4.1.3.3. Pollen characters

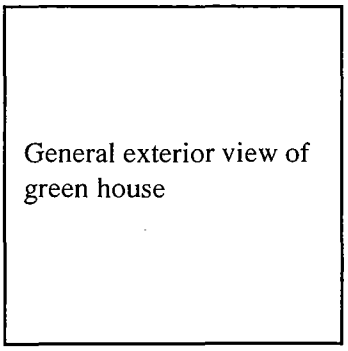
4.1.3.3.a. Pollen morphology

Pollen in *Dendrobium* appeared to be agglutinated in masses called pollinia (Plate II). Each flower possessed a pair of pollinia and each pollinium consisted of two oval-shaped lobes. Pollinia in all *Dendrobium* genotypes were yellow in colour. Pollinia appeared to be tightly compressed in depressions known as clinandria at the tip of the column, and closely covered over by a lid or operculum in the shape of a pair of attached hemispheres.

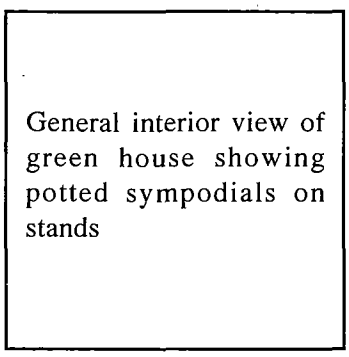
Pollen existed as tetrads which were spherical to rectangular in shape (Plate II). The pollen of the various hybrids and the species were almost similar in shape.

Significant differences in pollen diameter was observed among the *Dendrobium* genotypes (Table 4.1.3.3.). Pollen diameter ranged from 19.84 μ in P_{13} to 48.64 μ in P_1. Significantly large pollen tetrads were

Plate II. General view and Floral biology

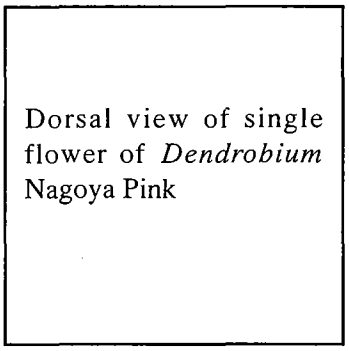


General exterior view of green house

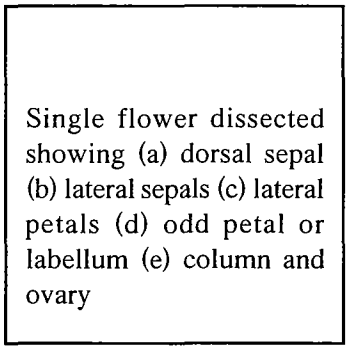


General interior view of green house showing potted sympodials on stands

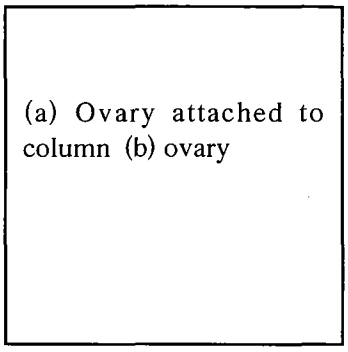
Floral biology



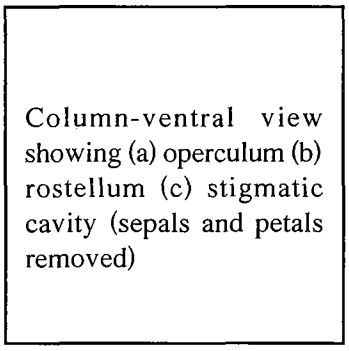
Dorsal view of single flower of *Dendrobium* Nagoya Pink



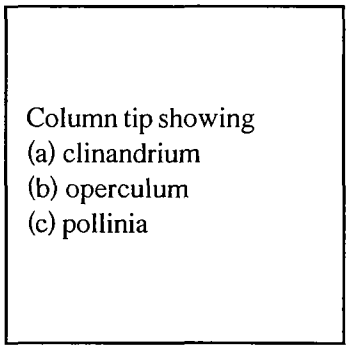
Single flower dissected showing (a) dorsal sepal (b) lateral sepals (c) lateral petals (d) odd petal or labellum (e) column and ovary



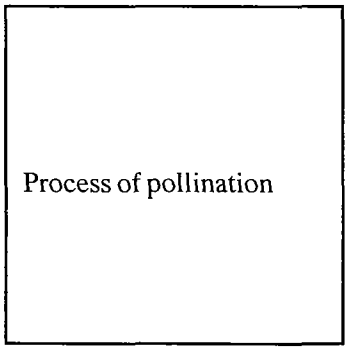
(a) Ovary attached to column (b) ovary



Column-ventral view showing (a) operculum (b) rostellum (c) stigmatic cavity (sepals and petals removed)

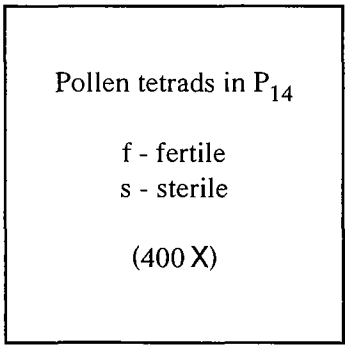


Column tip showing
(a) clinandrium
(b) operculum
(c) pollinia



Process of pollination

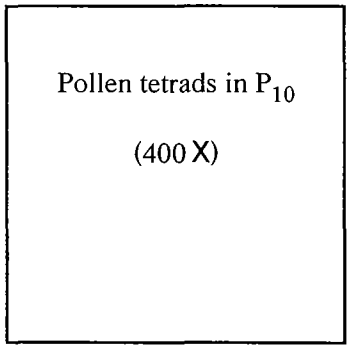
Pollen morphology



Pollen tetrads in P_{14}

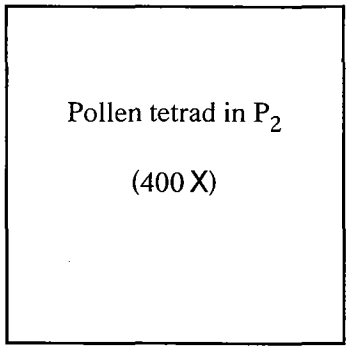
f - fertile
s - sterile

(400 X)



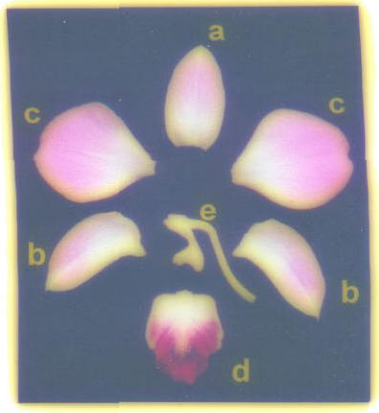
Pollen tetrads in P_{10}

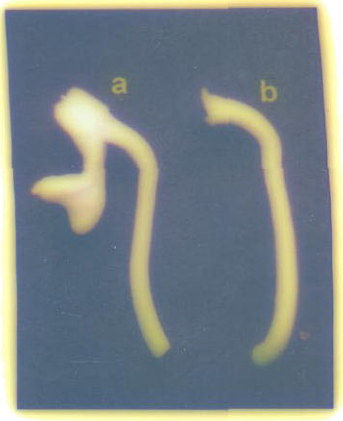
(400 X)

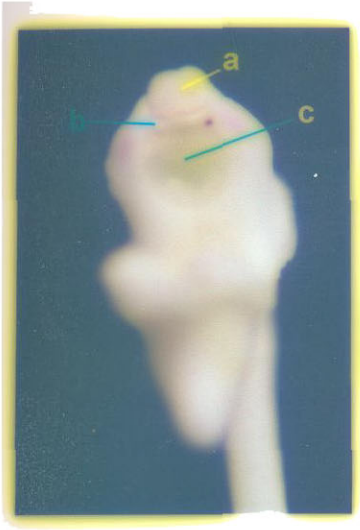


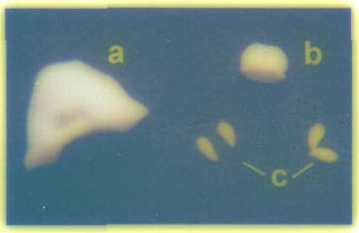
Pollen tetrad in P_2

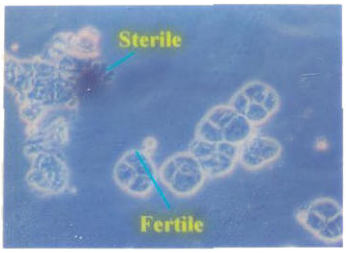
(400 X)

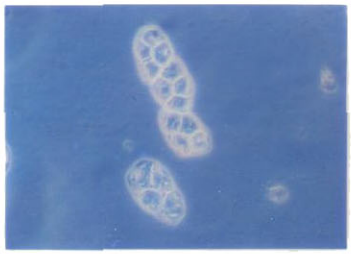












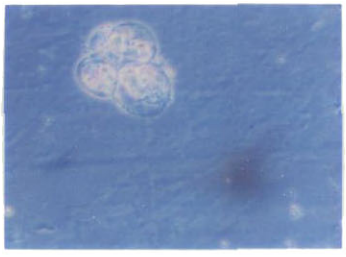


Plate II

Table 4.1.3.3. Pollen characters in parental genotypes of *Dendrobium*

Parental genotypes	Pollen diameter (μ)	Pollen fertility (%)	Pollen germination (%)
P_1	48.64	80.8	77.1
P_2	31.84	55.5	48.3
P_3	34.40	76.1	64.9
P_4	40.32	68.0	62.9
P_5	40.64	65.4	53.4
P_6	44.64	59.1	46.7
P_7	34.40	62.2	55.1
P_8	38.08	62.5	59.6
P_9	29.60	58.7	42.5
P_{10}	25.92	60.2	46.5
P_{11}	28.88	70.5	52.6
P_{12}	31.02	73.4	61.5
P_{13}	19.84	46.2	16.1
P_{14}	21.44	52.4	21.0
SE_m	1.297	2.154	2.090
CD(0.05)	3.631	6.033	5.853

found in P_1 and P_6 (44.64 μ). Pollen size was comparatively large in the commercial hybrids ranging from 29.60 μ in P_9 to 48.64 μ in P_1, medium in the semi-commercial hybrids with a range from 25.92 μ (P_{10}) to 31.02 μ (P_{12}) and small in the species *viz.,* 19.84 μ in P_{13} and 21.44 μ in P_{14}.

4.1.3.3.b. Pollen fertility

Pollen fertility varied significantly among the genotypes tested (Table 4.1.3.3.). The highest pollen fertility of 80.8 per cent was recorded in P_1 which was on par with P_3 producing 76.1 per cent fertile pollen. High pollen fertility was exhibited by P_{12} (73.4 %), P_{11} (70.5 %) and P_4 (68.0 %). Pollen fertility was the lowest in P_{13} (46.2 %) preceded by P_{14} (52.4 %).

4.1.3.3.c. Pollen germination

Pollen germination varied significantly among the varieties tested, with P_1 recording the highest germination of 77.1 per cent, followed by P_3 (64.9 %), P_4 (62.9 %), P_{12} (61.5 %) and P_8 (59.6 %) (Table 4.1.3.3.). Percentage pollen germination was the lowest in the species P_{14} (21.0) and P_{13} (16.1).

4.2. Hybridization and *in vitro* embryo culture of hybrids

4.2.1. Hybridization and compatibility/incompatibility studies

Intercrossing in all possible combinations involving the 14 parental genotypes of *Dendrobium* was done, depending on the availability

of receptive stigma and fresh pollen. This was done with the objective of studying the compatibility/incompatibility between genotypes.

4.2.1.1. Diallel crossings attempted among the genotypes of *Dendrobium*

A total of 1696 pollinations were done, covering 190 out of the 196 (n^2) possible combinations (Table 4.2.1.1). These 190 combinations included 88 crosses and their reciprocals (88) and 14 selfs. Three cross combinations involving the species P_{13}, *viz.*, $P_5 \times P_{13}$, $P_7 \times P_{13}$ and $P_{12} \times P_{13}$ and their reciprocals could not be attempted as their flowering seasons did not synchronize.

4.2.1.2. Details of diallel crossings

Details of self and cross compatibility among the 14 parental genotypes of *Dendrobium* have been analysed in detail (Table 4.2.1.2.).

Out of the 190 self and cross combinations attempted, 84 combinations succeeded in producing harvestable green capsules. These 84 combinations included seven selfs and 77 crosses (including reciprocals). The relative success of cross (77/176 = 43.75 %) and self (7/14 = 50 %) combinations did not differ considerably from the total estimate (84/190 = 44.21 %).

Out of the 84 combinations successfully yielding green capsules, no seeds were obtained from the capsules of three combinations *viz.*, $P_3 \times P_{11}$, $P_8 \times P_6$ and $P_9 \times P_{12}$. Seeds from the remaining 81 combinations were cultured axenically.

Table 4.2.1.1. Matrix showing diallel crossings attempted among 14 parental genotypes of *Dendrobium*

♀ ♂	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_{13}	P_{14}
P_1	x	x	x	x	x	x	x	x	x	x	x	x	x	x
P_2	x	x	x	x	x	x	x	x	x	x	x	x	x	x
P_3	x	x	x	x	x	x	x	x	x	x	x	x	x	x
P_4	x	x	x	x	x	x	x	x	x	x	x	x	x	x
P_5	x	x	x	x	x	x	x	x	x	x	x	x	NA	x
P_6	x	x	x	x	x	x	x	x	x	x	x	x	x	x
P_7	x	x	x	x	x	x	x	x	x	x	x	x	NA	x
P_8	x	x	x	x	x	x	x	x	x	x	x	x	x	x
P_9	x	x	x	x	x	x	x	x	x	x	x	x	x	x
P_{10}	x	x	x	x	x	x	x	x	x	x	x	x	x	x
P_{11}	x	x	x	x	x	x	x	x	x	x	x	x	x	x
P_{12}	x	x	x	x	x	x	x	x	x	x	x	x	NA	x
P_{13}	x	x	x	x	NA	x	NA	x	x	x	x	NA	x	x
P_{14}	x	x	x	x	x	x	x	x	x	x	x	x	x	x

NA - Not attempted

Table 4.2.1.2. Matrix showing compatibility relationships in diallel crossings among 14 parental genotypes of *Dendrobium*

♀ \ ♂	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_{13}	P_{14}
P_1	❀	❀	❀	❀	②	❀	⓪	❀	❀	❀	①	⑦	⓪	①
P_2	❀	③	❀	❀	⑦	❀	❀	②	❀	⓪	①	①	⓪	⑦
P_3	❀	②	❀	❀	❀	❀	❀	❀	⓪	❀	⑤	⓪	①	⓪
P_4	❀	②	❀	❀	❀	❀	②	⑥	①	⓪	①	②	⓪	①
P_5	❀	①	⓪	⑥	②	❀	❀	❀	⑦	①	⓪	❀	⊗	⓪
P_6	❀	⓪	❀	❀	①	②	②	③	❀	①	①	❀	⓪	⓪
P_7	❀	❀	❀	❀	③	④	❀	①	❀	①	⓪	②	⊗	①
P_8	❀	❀	⑥	②	①	⑤	❀	③	❀	②	⑦	⓪	①	⓪
P_9	❀	⑦	⑥	①	❀	❀	❀	⓪	①	⓪	①	⑤	⓪	①
P_{10}	❀	❀	①	⓪	⑥	①	❀	①	①	②	⓪	①	⓪	①
P_{11}	⓪	❀	③	❀	②	⓪	⑦	①	①	⓪	❀	❀	①	⓪
P_{12}	②	②	⓪	❀	❀	②	④	⓪	①	③	❀	⓪	⊗	①
P_{13}	①	⓪	①	⓪	⊗	⓪	⊗	❀	❀	①	⓪	⊗	○	①
P_{14}	❀	❀	❀	⓪	①	❀	⓪	①	①	⓪	①	⓪	⓪	○

⊗ combinations where pollination not attempted
⓪ combinations where pollinated flowers abscised without any change
① combinations where pollinated flowers abscised within two weeks
② combinations where pollinated flowers abscised during the third and fourth weeks
③ combinations where pollinated flowers abscised during the fifth and sixth weeks
④ combinations where pollinated flowers abscised during the seventh and eighth weeks
⑤ combinations with no seed in capsule
⑥ combinations registering no seed germination
⑦ *combinations lost while in culture*
○ combinations lost while hardening
❀ successful combinations maintained in the green house

No germination was obtained with seeds from five (P_4 x P_8, P_5 x P_4, P_8 x P_3, P_9 x P_3 and P_{10} x P_5) out of the 81 combinations inoculated *in vitro*. Successful seed germination was obtained in 76 combinations.

Although seeds from 76 combinations germinated, further development was found to be arrested in seven combinations *viz.*, P_1 x P_{12}, P_2 x P_5, P_2 x P_{14}, P_5 x P_9, P_8 x P_{11}, P_9 x P_2 and P_{11} x P_7 at various stages of *in vitro* development. Mature seedlings were obtained from 69 combinations.

Out of the 69 combinations from which seedlings were deflasked and planted out, progeny from 67 combinations (Table 4.2.1.3.) were established successfully in the green house. Selfed seedlings obtained from the two species *viz.*, P_{13} and P_{14} did not survive.

4.2.1.3. Analysis of compatibility

The variety P_1 turned out to be the best female and male combiner generating hardened seedlings in eight combinations as the female parent and 11 combinations as the male parent (Table 4.2.1.3.). The variety P_3 was the second best combiner with eight and seven successfully hardened combinations as female and male parents, respectively. Other superior combiners as female parent were P_2 and P_7 with six successful combinations each and P_4, P_5 and P_6 with five successful combinations each, per parent. As male parents other notable genotypes were P_4 with

Table 4.2.1.3. Matrix of the 67 successful combinations with hardened seedlings established in the green house

	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_{13}	P_{14}
P_1	x	x	x	x		x		x	x	x				
P_2	x		x	x		x	x		x					
P_3	x		x	x	x	x	x	x		x				
P_4	x		x	x	x	x								
P_5	x					x	x	x				x		
P_6	x		x	x					x			x		
P_7	x	x	x	x			x		x					
P_8	x	x					x		x					
P_9	x				x	x	x							
P_{10}	x	x					x							
P_{11}		x		x							x	x		
P_{12}				x	x						x			
P_{13}								x	x					
P_{14}	x	x	x			x								

eight, P_6 and P_7 with seven each and P_2 and P_9 with six each successful combinations.

Selfed seedlings obtained from five varieties *viz.*, P_1, P_3, P_4, P_7 and P_{11} were successfully established in the green house.

None of the combinations with P13 and P14 as male parents resulted in successful establishment of seedlings in the green house. Both the species were found to be poor combiners. Selfed seedlings from both these species died during hardening. When P_{13} was used as the female parent, successful *ex vitro* establishment of seedlings was obtained in two combinations with P_8 and P_9 as pollen parents. When P_{14} was used as the female parent, four combinations with P_1, P_2, P_3 and P_6 as male parents yielded hardened seedlings.

4.2.1.4. Analysis of incompatibility

Out of the 190 cross combinations attempted, hardened seedlings were not obtained from 123 combinations (64.74 %). These 123 unsuccessful combinations were lost at different stages in the pre and post zygotic phases, ranging from flower abscission before the onset of any post pollination change to failure to survive during hardening in the greenhouse (Table 4.2.1.2.).

The levels of incompatibility (Plate III) reactions were grouped under nine 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		No. of combinations
P_1	*D.* [Candy Stripe x Tomie Drake]	2
P_2	*D.* Chiangmai Pink	2
P_3	*D.* Nagoya Pink	3
P_4	*D.* Pramot 3	2
P_5	*D.* Renapa Red 3	3
P_6	*D.* Rinabha	3
P_7	*D.* Sakura	1
P_8	*D.* Sonia 16	2
P_9	*D.* White Fairy	3
P_{10}	*D.* Caesar Pink	3
P_{11}	*D.* Uniwai Pink	4
P_{12}	*D.* Walter Oumae	3
P_{13}	*D. barbatulum*	4
P_{14}	*D. philippica*	5
	Total	40

2. Instances where pollinated flowers abscised after initial greening and swelling of ovary (within 14 days after pollination)

Female parent in cross combination		No. of combinations
P_1	*D.* [Candy Stripe x Tomie Drake]	2
P_2	*D.* Chiangmai Pink	2
P_3	*D.* Nagoya Pink	1
P_4	*D.* Pramot 3	3
P_5	*D.* Renapa Red 3	2
P_6	*D.* Rinabha	3
P_7	*D.* Sakura	3
P_8	*D.* Sonia 16	2
P_9	*D.* White Fairy	4
P_{10}	*D.* Caesar Pink	6
P_{11}	*D.* Uniwai Pink	3
P_{12}	*D.* Walter Oumae	2
P_{13}	*D. barbatulum*	4
P_{14}	*D. philippica*	4
	Total	41

3. Instances where pollinated flowers with swelling ovaries abscised during the third and the fourth weeks

Female parent in cross combination		No. of combinations
P_1	*D.* [Candy Stripe x Tomie Drake]	1
P_2	*D.* Chiangmai Pink	1
P_3	*D.* Nagoya Pink	1
P_4	*D.* Pramot 3	3
P_5	*D.* Renapa Red 3	1
P_6	*D.* Rinabha	2
P_7	*D.* Sakura	1
P_8	*D.* Sonia 16	2
P_9	*D.* White Fairy	-
P_{10}	*D.* Caesar Pink	1
P_{11}	*D.* Uniwai Pink	1
P_{12}	*D.* Walter Oumae	3
P_{13}	*D. barbatulum*	-
P_{14}	*D. philippica*	-
	Total	17

4. Instances where developing capsules abscised during the fifth and the sixth weeks

Combination
1. P_2 selfed
2. $P_6 \times P_8$
3. $P_7 \times P_5$
4. P_8 selfed
5. $P_{11} \times P_3$
6. $P_{12} \times P_{10}$

5. Instances where capsules yellowed and decayed before maturity, abscising during the seventh and the eighth weeks

Combination
1. $P_7 \times P_6$
2. $P_{12} \times P_7$

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

Combination
1. $P_3 \times P_{11}$
2. $P_8 \times P_6$
3. $P_9 \times P_{12}$

7. **Instances where green capsules contained seeds which did not germinate**

Combination
1. $P_4 \times P_8$
2. $P_5 \times P_4$
3. $P_8 \times P_3$
4. $P_9 \times P_3$
5. $P_{10} \times P_5$

8. **Instances where seeds germinated, but aborted while in culture**

Combination
1. $P_1 \times P_{12}$
2. $P_2 \times P_5$
3. $P_2 \times P_{14}$
4. $P_5 \times P_9$
5. $P_8 \times P_{11}$
6. $P_9 \times P_2$
7. $P_{11} \times P_7$

9. **Instances where mature seedlings were deflasked successfully, but failed to survive during hardening**

Combination
1. P_{13} selfed
2. P_{14} selfed

Cent per cent flower abscission without expressing any visible post pollination floral change was observed in 40 (21.05 %) unsuccessful combinations. All flowers in these combinations were shed within the first two weeks after pollination. Incompatibility was of the highest degree in these combinations where even the initial swelling of ovary following pollination was not detected.

In 41 combinations (21.58 %) the pollinated flowers abscised after initial greening and swelling of ovary. Although these flowers were also shed within the first two weeks after pollination, a distinct reduction in the strength of incompatibility from the first level was evident in that they manifested a visible response to the stimulus of pollination.

In 17 combinations (8.90 %) the pollinated flowers with ovaries swelling into capsules abscised during the third and the fourth weeks after pollination.

The developing capsules abscised during the fifth and the sixth weeks after pollination in six combinations (3.16 %), *viz.*, P_2 selfed, $P_6 \times P_8$, $P_7 \times P_5$, P_8 selfed, $P_{11} \times P_3$ and $P_{12} \times P_{10}$.

In two combinations (1.05 %) *viz.*, $P_7 \times P_6$ and $P_{12} \times P_7$, the capsules developed normally till the sixth week after pollination, whereafter they yellowed and decayed, abscising within the eighth week.

In three combinations (1.58 %) *viz.*, $P_3 \times P_{11}$, $P_8 \times P_6$ and $P_9 \times P_{12}$, green capsules were harvested at the correct stage of maturity, but were found to be empty, without seeds.

In five combinations (2.63 %) the green capsules contained seeds but they failed to germinate when inoculated *in vitro*.

In seven combinations (3.68 %) *viz.*, $P_1 \times P_{12}$, $P_2 \times P_5$, $P_2 \times P_{14}$, $P_5 \times P_9$, $P_8 \times P_{11}$, $P_9 \times P_2$ and $P_{11} \times P_7$, the seeds germinated *in vitro*, but degenerated later at various stages, while still in culture. The rate of growth of these developing protocorms/seedlings gradually slowed down, whereafter they degenerated.

The final and the mildest level of incompatibility was observed in the selfings of P_{13} and P_{14}, where mature seedlings were deflasked successfully, but failed to survive during hardening in the greenhouse.

Much variation was observed in the extent and strength of the incompatibility reaction among the 14 parental genotypes of *Dendrobium* during the early stages, till capsule harvest (Table 4.2.1.2.). The number of combinations resulting in complete flower abscission, out of the total crosses attempted was considered as indicative of the extent of incompatibility at this stage. The time taken for last flower abscission after pollination was taken as indicative of the strength of incompatibility.

The extent of incompatibility was the highest when P_{10} and P_{12} were used as female parents. In both the genotypes ten combinations failed to produce capsules. In P_6, P_{11} and P_{14} (as female parent) nine each out of the 14 combinations attempted resulted in complete flower abscission. When P_{13} was taken as the female parent, eight out of the 11 combinations attempted met with failure during capsule development.

The strength of incompatibility reaction was of the highest degree with P_{14}, P_{13} and P_9 as female parents. Complete flower drop within two weeks from pollination was observed in all incompatible combinations, which were nine in P_{14} , eight in P_{13} and seven in P_9.

The extent of incompatibility was high with P_{10}, P_{11}, P_{13} and P_{14} as male parents. In the semi-commercial hybrids P_{10} and P_{11}, 12 and ten combinations, respectively failed to yield harvestable green capsules. In the species P_{13}, all the ten cross combinations attempted, except the self, registered complete flower drop. In the species P_{14}, all the 12 combinations, except the self and the cross using P_2 as the female parent, failed to yield harvestable green capsules.

The strength of incompatibility was the highest when P_{11}, P_{13} and P_{14} were used as male parents, recording complete flower drop within two weeks from pollination in all unsuccessful combinations.

4.2.1.5. Details of post pollination developments

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

1. Post pollination floral changes
2. Stages of capsule development (Plate III)
3. Maximum flower retention period in unsuccessful combinations
4. Duration to green capsule harvest in successful combinations
5. Length of green capsules at harvest
6. Width of green capsules at harvest

7. Percentage capsule yield
8. Percentage capsules with/without seeds
9. Percentage filled seeds over total seeds
10. Percentage capsules with germinating 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 4.2.1.5.a.). The first noticeable change following pollination was slight wilting and drooping of sepals and petals. Almost simultaneously, the tip of the column swelled rapidly, protruding downwards, leading to partial stigmatic closure. Complete covering of the stigma was effected by the wilted sepals and petals. The labellum was drawn up from below to cover the stigma, while the wilted petals and odd sepal arched over the column. The sides of the column were covered by the side sepals. Complete drying of sepals and petals resulted in about two weeks after pollination.

2. Stages of capsule development

The first visible indication in the gynoecium of the success of pollination was the greening of ovary with slight swelling (Table 4.2.1.5.b.). Ovaries turned green in 3-8 days from pollination, depending on the genotype (Plate III). Rapid swelling of ovary into capsule followed. The capsule attained maximum length at 60-75 per cent maturity. At this stage, the three ribs along the length of the capsule swelled into

Table 4.2.1.5.a. Post pollination floral changes in parental genotypes of *Dendrobium*

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	2-4	2-6	5-10	10-12
P_2	2-4	2-6	5-10	10-12
P_3	3-5	3-8	6-12	12-14
P_4	4-6	4-10	7-12	13-16
P_5	4-6	4-10	7-12	13-16
P_6	4-6	4-10	7-12	13-16
P_7	3-5	3-8	6-12	12-14
P_8	4-6	4-10	7-12	13-16
P_9	3-5	3-8	6-12	12-14
P_{10}	3-5	3-8	6-12	12-14
P_{11}	3-5	3-8	6-12	12-14
P_{12}	3-5	3-8	6-12	12-14
P_{13}	2-4	2-6	5-10	10-12
P_{14}	2-4	2-6	5-10	10-12

Table 4.2.1.5.b. Stages of capsule development in parental genotypes of *Dendrobium*

Parental genotypes	Number of days from pollination for				
	greening of ovary with slight swelling	capsule formation	prominent ribbing of capsule	flattening of capsule rib (green capsule harvest stage)	bursting of capsule beginning from tip
P_1	3-5	85-105	100-105	118-128	130-140
P_2	3-5	66-83	78-83	87-98	105-110
P_3	4-6	47-58	53-58	62-80	85-90
P_4	3-5	78-98	93-98	109-116	120-130
P_5	5-8	80-100	95-100	110-120	125-135
P_6	5-8	65-80	75-80	86-96	103-108
P_7	4-6	57-70	65-70	76-86	90-95
P_8	4-6	70-85	80-85	94-102	105-115
P_9	3-5	48-82	77-82	87-96	103-108
P_{10}	4-6	51-65	60-65	68-75	80-85
P_{11}	4-6	75-95	90-95	105-113	115-125
P_{12}	3-5	75-95	90-95	109-115	115-125
P_{13}	5-8	87-108	103-108	112-130	135-145
P_{14}	5-8	63-80	75-80	80-90	100-105

Plate III. Stages of capsule development, incompatibility reactions and seed morphology

Stages of capsule development

Wilting and drooping of sepals and petals and swelling of ovary; P_5 x P_{12} seven days after pollination.

Complete wilting of sepals and petals and greening of swollen ovary; P_7 x P_4 14 days after pollination

Rapid swelling of ovary into capsule; P_1 selfed at 40 per cent maturity.

General view of capsules in P_1 at various stages of development.

Prominent swelling of capsule ribs; P_1 selfed at 60 per cent maturity.

Flattening of ribs and swelling near capsule tip; ideal stage for harvest of green capsule; P_1 selfed at 85 per cent maturity.

Bursting of capsule from tip; P_1 selfed at 100 per cent maturity.

Incompatibility reactions

Yellowing and abscision of flower in P_4 x P_2 three weeks after pollination.

L.S. of abscised empty capsule in P_2 selfed, five weeks after pollination.

L.S. of mature, empty green capsule in P_9 x P_{12}

Seed morphology

P_{11} x P_2 (200X)

t - testa
e - embryo

P_2 x P_1

f - fertile
s - sterile

P_{11} x P_2 (40X)

P_{14} selfed (40X)

Plate III

prominence. Following this stage the capsule wall swelled, stretching the ribs which lost their angular prominence, becoming flattened. The entire capsule enlarged further taking on a rounded appearance. This stage (75-90 % maturity) was 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. Maximum flower retention period in unsuccessful combinations

Maximum flower retention period observed in combinations where harvestable green capsules were not obtained (106/190) was analysed (Table 4.2.1.5.c.).

Maximum flower retention period ranged from four days in P_{10} x P_3 and P_{10} x P_8 to 44 days in P_7 x P_6, in individual crosses.

The flowers were retained from 6.9 (P_{13}) to 19.9 (P_7) days with the coefficient of variation ranging from 12 (P_9) to 69 (P_{10}) per cent when the genotypes were used as female parents. When the same genotypes were used as male parents, the flowers were retained from 9.1 (P_2) to 22.8 (P_7) days with a coefficient of variation ranging from 22 (P_{14}) to 98 (P_3) per cent.

4. Duration to green capsule harvest

Time taken for the harvest of green capsules in compatible combinations (84/190) was analysed (Table 4.2.1.5.d.).

Table 4.2.1.5.c. Maximum flower retention period (days) in unsuccessful 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}	P_{13}	P_{14}	$\bar{x}$	C.V.(%)
P_1					27		11				14		9	11	14.4	50
P_2		36						27		12	9	10	10		16.0	59
P_3		26							14			13	11	12	15.2	40
P_4		17					28		12	13	13	26	14	11	16.8	39
P_5		12	11		24					9	10			8	12.3	48
P_6		14			11	18	24	36		14	11		12	9	16.6	52
P_7					32	44		13		10	13	18		9	19.9	66
P_8				22	14			37		18		14	12	9	18.0	52
P_9				10				9	11	9	11		9	12	10.1	12
P_{10}			4	9		13		4	7	26	9	9	5	8	9.4	69
P_{11}	14		32		27	8		12	9	8			6	7	14.2	64
P_{12}	22	24	7			5	43	11	6	32		9		6	19.8	65
P_{13}	7	5	5	8		6				7	5			12	6.9	34
P_{14}				9	11		8	10	6	7	9	8	9		8.6	18
$\bar{x}$	14.3	9.1	11.8	11.6	20.9	19.0	22.8	17.7	9.3	14.2	10.4	13.4	9.7	9.5		
C.V. (%)	52	54	98	51	42	74	62	70	34	54	25	46	28	22		

Table 4.2.1.5.d. 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}	P_{13}	P_{14}	$\bar{x}$	C.V. (%)
P_1	121	118	120	123		124		121	121	119		128			122.0	3
P_2	94		90	92	95	88	87		96					98	92.5	4
P_3	74		68	65	70	64	67	62		76	80				69.6	9
P_4	109		110	111	112	116		114							112.0	2
P_5	120			118		112	115	116	113			110			114.9	3
P_6	96		96	86					92			94			92.8	4
P_7	80	84	78	82			86		76						81.0	5
P_8	96	94	100			102	95		98		98				97.6	3
P_9	91	87	90		92	88	94					95			91.0	3
P_{10}	68	69			75		72								71.0	4
P_{11}		110		106			105				108	113			108.4	3
P_{12}				109	112						115				112.0	3
P_{13}								130	112				120		120.7	7
P_{14}	89	90	85			83								80	85.4	5
$\bar{x}$	94.0	93.1	93.0	99.1	92.7	97.1	90.1	108.6	101.1	97.5	100.3	108.0	120.0	89.0		
C.V. (%)	18	18	17	19	19	21	18	25	15	31	15	13	--	14		

Duration to green capsule harvest ranged from 62 days in P_3 x P_8 to 130 days in P_{13} x P_8, in individual crosses.

When each one of the 14 parental genotypes were used as the recurring female parent, no significant variability (3-9 %) was observed in duration to green capsule harvest. The time taken for the harvest of green capsules ranged from 69.6 (P_3) to 122.0 (P_1) days when the genotypes were used as female parents. When the same genotypes were used as male parents, the duration ranged from 89.0 (P_{14}) to 120.0 (P_{13}) days, but the variability increased upto 31 per cent (P_{10}).

5. Length of green capsules

Length of green capsule ranged from 2.4 cm in P_{14} selfed capsules to 5.0 cm in P_1 x P_4 capsules, in individual crosses (Table 4.2.1.5.e.; Fig. 7).

Mean length of green capsules ranged from 2.5 cm in P_{13} and P_{14} to 4.6 cm in P_1 when the genotypes were used as female parents. No significant variability for length of capsules (2 % in P_{13} to 7 % in P_2) was observed when each one of the 14 parental genotypes was used as the recurring female parent. When the same genotypes were used as male parents, capsule length ranged from 2.5 cm in P_{13} to 4.3 cm in P_{10} and the variability increased upto 22 per cent (P_2 and P_8).

When used as both the male and female parents, P_{13} registered the lowest capsule length, followed by P_{14}. The two species P_{13} and P_{14} produced shorter green capsules as both the male and the female parents.

Table 4.2.1.5.e. 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}	P_{13}	P_{14}	$\bar{x}$	C.V. (%)
P_1		4.5	4.6	5.0		4.5		4.6	4.4	4.5		4.2			4.6	5
P_2	3.5		3.7	3.4	3.2	3.3	3.8		3.6					3.2	3.5	7
P_3	4.2		4.1	4.1	3.8	3.9	4.0	4.1		4.1	3.6				4.0	5
P_4	4.4		4.3	4.9	4.3	4.2		4.2							4.4	6
P_5	4.9			4.2		4.5	4.4	4.6	4.7			4.4			4.5	5
P_6	3.9		3.8	3.7					3.8			3.5			3.7	4
P_7	3.3	3.2	3.4	3.2			3.3		3.0						3.2	4
P_8	4.2	4.4	4.2			3.8	4.3		4.0		4.0				4.1	5
P_9	4.2	4.1	4.1		4.3	4.5	4.3					4.2			4.2	3
P_{10}	3.1	3.2			3.0		3.2								3.1	3
P_{11}		4.6		4.3			4.6				4.5	4.2			4.4	4
P_{12}				4.3	4.5						4.2				4.3	4
P_{13}								2.5	2.6				2.5		2.5	2
P_{14}	2.5	2.5	2.6			2.7								2.4	2.5	5
$\bar{x}$	3.9	3.8	3.9	4.1	3.9	3.9	4.0	4.0	3.7	4.3	4.1	4.1	2.5	2.8		
C.V. (%)	19	22	15	15	16	17	13	22	20	7	9	8	0	20		

Although P_1 produced the longest capsules when used as the female parent, comparatively shorter capsules resulted when used as the male parent. The parents P_4, P_8, P_{11} and P_{12} produced comparatively longer capsules when used as both male and female parents. Longer capsules were produced by P_7 when used as the male parent as compared to when used as the female parent.

6. Width of green capsules

Width of green capsules (Table 4.2.1.5.f) ranged from 0.6 cm in P_{14} selfed to 1.8 cm in P_1 x P_4 producing the shortest and the longest capsules, respectively (Table 4.2.1.5.e.) in individual combinations. Capsules having a width of 1.8 cm were produced by P_1 x P_6, P_9 x P_6 and P_9 x P_7 also.

A comparison of the array means revealed the following on the average width of green capsules. Mean width of green capsules ranged from 0.8 cm in P_{10}, P_{13} and P_{14} to 1.6 cm in P_1 and P_9 when the genotypes were used as female parents. Coefficient of variation was observed to range from four (P_{12}) to 18 (P_8) per cent for the character. When the same genotypes were used as male parents, width of capsule registered a range from 0.8 cm in P_{13} to 1.5 cm in P_4 and the variability increased upto 47 per cent.

The two species produced narrower capsules as compared to the 12 varieties. A significant variation in width of green capsule was however not noticed in the genotypes when used as male or female parents. The variability increased when the genotypes were used as male parents.

Table 4.2.1.5.f. Average width (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}	P_{13}	P_{14}	$\overline{x}$	C.V. (%)
P_1			1.7	1.8		1.8		1.7	1.5	1.4		1.3			1.6	12
P_2	1.7		1.6	1.5	1.5	1.5	1.7		1.4					1.4	1.5	8
P_3	1.4		1.3	1.3	1.1	1.2	1.2	1.1		1.1	0.8				1.2	15
P_4	1.5		1.4	1.7	1.5	1.4		1.2							1.5	11
P_5	1.6			1.3		1.5	1.6	1.4	1.6			1.5			1.5	8
P_6	1.4		1.3	1.3					1.4			1.2			1.3	6
P_7	1.4	1.4	1.4	1.3			1.3		1.2						1.4	6
P_8	1.3	1.3	1.2			0.7	1.2		1.3		1.2				1.2	18
P_9	1.6	1.4	1.3		1.5	1.8	1.8					1.5			1.6	12
P_{10}	0.8	0.9			0.7		0.9								0.8	12
P_{11}		1.6		1.5			1.5				1.4	1.3			1.5	8
P_{12}				1.4	1.5						1.4				1.4	4
P_{13}								0.7	0.8				0.8		0.8	8
P_{14}	0.7	0.8	0.8			0.8								0.6	0.8	7
$\overline{x}$	1.4	1.3	1.3	1.5	1.3	1.3	1.4	1.2	1.3	1.3	1.2	1.4	0.8	1.1		
C.V. (%)	25	24	20	13	26	31	22	30	20	17	24	10	0	47		

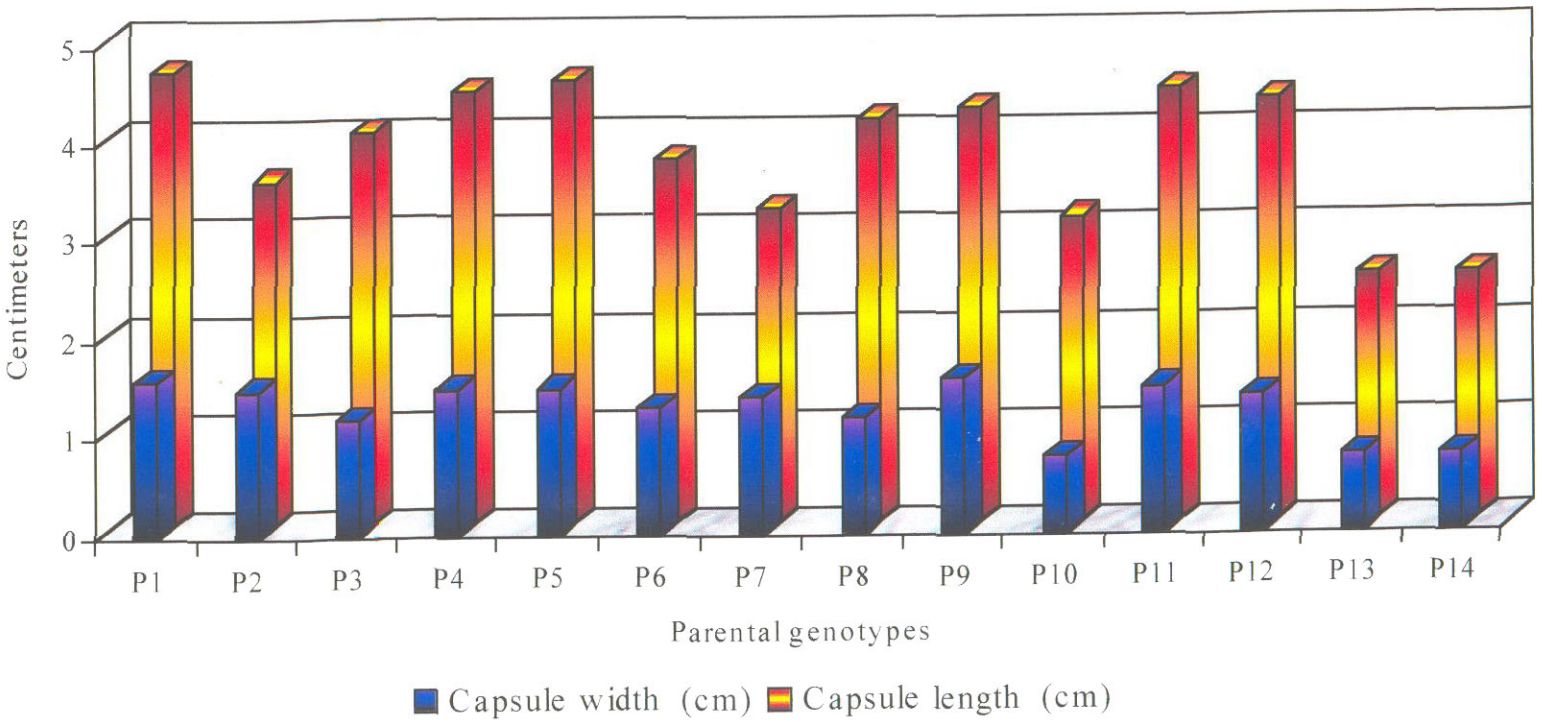


Fig. 7. Size of green capsules in parental genotypes of *Dendrobium*

7. Percentage capsule yield

Percentage capsule yield in individual combinations ranged from 8 in P_2 x P_{14}, P_3 x P_1, P_4 x P_3 and P_{14} selfed to 33 in P_6 x P_4 (Table 4.2.1.5.g.).

Mean percentage capsule yield ranged from 11.0 in P_{13} to 22.4 in P_6 with a coefficient of variation ranging from zero in P_{12} to 49 per cent in P_2 when the genotypes were used as female parents. When the genotypes were used as male parents, mean percentage capsule yield ranged from 8.0 in P_{14} to 22.8 in P_1 and the coefficient of variation ranged from zero in P_{13} and P_{14} (single entry in P_{13}) to 56 per cent in P_2.

8. Capsules with/without seeds

Out of the 84 combinations producing green capsules, no seed was observed in capsules from three combinations *viz.*, P_3 x P_{11}, P_8 x P_6 and P_9 x P_{12} (Table 4.2.1.2., Plate III). The remaining 81 combinations yielded green capsules containing seeds.

9. Percentage of filled seeds

Percentage of filled seeds ranged from 11 in P_{10} x P_5 to 76 in P_2 x P_1 and P_6 x P_1 in individual combinations (Table 4.2.1.5.h.; Plate III).

Mean percentage of filled seeds ranged from 22.7 in P_9 to 55.4 in P_6 and the coefficient of variation ranged from 31 per cent in P_{12} to 56 per cent in P_5 when the parental genotypes were used as female parents.

Table 4.2.1.5.g. 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}	P_{13}	P_{14}	$\bar{x}$	C.V. (%)
P_1	20	18	22	13		22		17	18	10		13			17.0	25
P_2	30		18	10	17	20	9		10					8	15.3	49
P_3	8		18	20	10	22	13	11		22	13				15.2	35
P_4	27		8	20	18	20		11							17.3	40
P_5	25			17		29	14	25	11			17			19.7	34
P_6	29		13	33					17			20			22.4	37
P_7	20	18	10	22			11		10						15.2	36
P_8	25	11	11			11	20		25		11				16.3	42
P_9	22	10	11		9	18	18					10			14.0	37
P_{10}	25	11			11		22								17.3	42
P_{11}		25		22			11				11	25			18.8	38
P_{12}				20	20						20				20.0	0
P_{13}								10	13				10		11.0	16
P_{14}	20	13	13			10								8	12.8	36
$\bar{x}$	22.8	13.7	13.8	19.7	14.2	19.0	14.8	14.8	14.8	16.0	13.8	19.6	10.0	8.0		
C.V. (%)	27	56	33	33	33	32	32	43	37	53	31	28	0	0		

Table 4.2.1.5.h. 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}	P_{13}	P_{14}	$\bar{x}$	C.V. (%)
P_1	49	18	56	65		42		34	24	16		13			25.2	54
P_2	76		54	47	45	39	25		22					25	41.6	44
P_3	63		37	59	49	42	33	18		19					40.0	42
P_4	68		38	43	30	22		13							35.7	54
P_5	64			15		32	21	30	20			57			34.1	56
P_6	76		49	43					75			34			55.4	34
P_7	71	30	54	32			43		20						41.7	44
P_8	65	53	15				29		46		20				38.0	52
P_9	21	16	14		29	33	23								22.7	32
P_{10}	35	41			11		35								30.5	44
P_{11}		56		43			29				38	21			37.4	35
P_{12}				43	32						28				32.7	31
P_{13}								21	25				32		26.0	42
P_{14}	41	29	21			17								37	29.0	35
$\bar{x}$	57.2	34.7	37.6	43.3	32.7	32.4	29.6	23.2	33.3	17.5	27.0	31.3	32.0	31.0		
C.V. (%)	32	46	46	33	41	30	25	37	61	12	36	62	0	27		

When used as male parents, mean percentage of filled seeds ranged from 17.5 in P_{10} to 57.2 in P_1 and the coefficient of variation ranged from 12 per cent in P_{10} to 62 per cent in P_{12}. Comparatively high variability was observed within crosses when the genotypes were used as both male and female parents.

A comparison of the array means when used as male and female parents revealed P_6 (55.4 %) and P_1 (57.2 %), respectively as the female and the male parents registering the highest values for percentage of filled seeds. Naturally, it followed that the cross P_6 x P_1 registered the highest percentage of filled seeds of 76. The combination P_6 x P_9 also recorded a high percentage of filled seeds (75), although on an average P_9 as the male parent was not a good performer, registering 33.3 per cent filled seeds.

10. Percentage of capsules with germinating seeds

Out of the 81 combinations producing green capsules containing filled seeds, seeds from all capsules harvested from 76 combinations germinated (Table 4.2.1.2). Hundred per cent capsules with germinating seeds was recorded in these 76 combinations. Zero per cent capsules with germinating seeds was registered in five combinations, *viz.*, P_4 x P_8, P_5 x P_4, P_8 x P_3, P_9 x P_3 and P_{10} x P_3 where no seed obtained from green capsules germinated.

4.2.2. Immature embryo (green capsule) culture

Embryo culture studies were conducted on 81 (Table 4.2.1.2) cross combinations, where harvestable green capsules with seeds were

obtained. Investigations were conducted on percentage of filled seeds, seed germination, number of days for germination initiation, number of days taken for the development of protocorm, chlorophyll, first leaf, shoot and root primordia and for planting out (Tables 4.2.2.2.a. to 4.2.2.2.n.; Plate IV).

4.2.2.1. Stages of *in vitro* germination and development

Percentage of filled seeds to total seeds observed per combination varied from 10.79 in $P_{10} \times P_5$ to 75.93 in $P_6 \times P_1$. Seed germination percentage ranged from 8.00 in $P_1 \times P_{12}$ to 70.73 in $P_6 \times P_1$.

During the process of germination, the embryo enlarged in size by imbibing moisture. Visible swelling and glistening of the seed as an indication of germination initiation were observed in 11.50 days in $P_1 \times P_4$, $P_3 \times P_1$ and $P_4 \times P_1$ extending to 40.17 days in $P_8 \times P_{11}$. The embryo eventually enlarged and burst out of the testa as an ovoid, top-shaped, cream coloured protocorm. Time taken for protocorm development ranged from 21.83 days in P_{13} selfed seed to 58.00 days in $P_{11} \times P_7$.

The protocorm increased in size rapidly and differentiated into shoot and root meristems in opposite directions. A pair of leaf primordia (visible as minute, conical points under a 10x magnifying glass) developed from the proximal end of the protocorm. The first signs of chloropyll development were apparent in these leaf initials. The entire protocorm developed chlorophyll in 27.83 days in $P_1 \times P_4$ and $P_7 \times P_1$. $P_5 \times P_7$ needed the longest duration ie., 72.17 days for greening.

After greening of protocorm, the leaf initials resumed growth. A scale leaf developed first, followed by an unequal pair of first embryonic photosynthetic leaves. Simultaneous with the scale leaf development, marginal cells gave rise to unicellular rhizoids. Time taken for the visible elongation of the first photosynthetic leaf ranged from 48.17 days in $P_7 \times P_1$ to 95.33 days in $P_5 \times P_7$.

Along with the development of the second photosynthetic leaf, a distinct shoot region made its appearance. Time required for the development of the first shoot primordium ranged from 76.83 days in $P_3 \times P_1$ to 141.00 days in $P_{12} \times P_{11}$.

After two leaf stage, the differentiating protocorm developed real roots endogenously. Time taken for the development of the first root primordium ranged from 99.67 days in $P_3 \times P_1$ to 169.00 days in $P_5 \times P_7$.

The protocorms were taken through three to four sub-culture passages *in vitro*, prior to final deflasking. Need-based sub-culturing to prevent overcrowding was performed during protocorm stage, one leaf stage, two leaf stage and three leaf stage.

In vitro raised seedlings having 3-4 leaves and 3-5 roots were deflasked and planted out. Time taken for the attainment of planting out stage ranged from 143.50 days in $P_4 \times P_1$ to 279.33 days in $P_{14} \times P_6$.

4.2.2.2 Response of individual parents to hybrid seed culture

The details of embryo culture of hybrid seeds obtained from successful combinations under each of the 14 parental genotypes when

used as the female parent have been analysed. The parameters studied were percentage filled seeds, percentage seed germination, number of days taken for germination initiation, number of days taken for development of protocorms, number of days taken for greening, number of days taken for the development of the first leaf, shoot and root primordia and number of days taken for planting out. Significant differences could be observed between the hybrid combinations involving each female parent with respect to the time taken to attain each of the different *in vitro* developmental stages.

4.2.2.2.a *Dendrobium* Candy Stripe x Tomie Drake (P_1)

Percentage of filled seeds per combination ranged from 12.51 in P_1 x P_{12} to 65.24 in P_1 x P_4 (Table 4.2.2.2.a.). The same trend was observed in percentage seed germination, with P_1 x P_{12} recording 8.00 to P_1 x P_4 registering 58.22.

In P_1 x P_4 the duration for germination initiation (11.50 days), development of protocorm (22.67 days) and development of chlorophyll (27.83 days) were significantly the shortest, while they were significantly the longest in P_1 x P_{12}, requiring 28.83 days, 48.83 days and 65.33 days, respectively to attain each of the above mentioned developmental stages. No further development was observed in P_1 x P_{12} after chlorophyll development and the culture was lost *in vitro*. Rate of development was observed to be the lowest in P_1 x P_{10} with respect to formation of first leaf primordium (78.83 days), first shoot primordium (125.17 days) and first root primordium (158.33 days). Planting out required 268.33 days. The combination P_1 x P_8 recorded the highest rate of development of

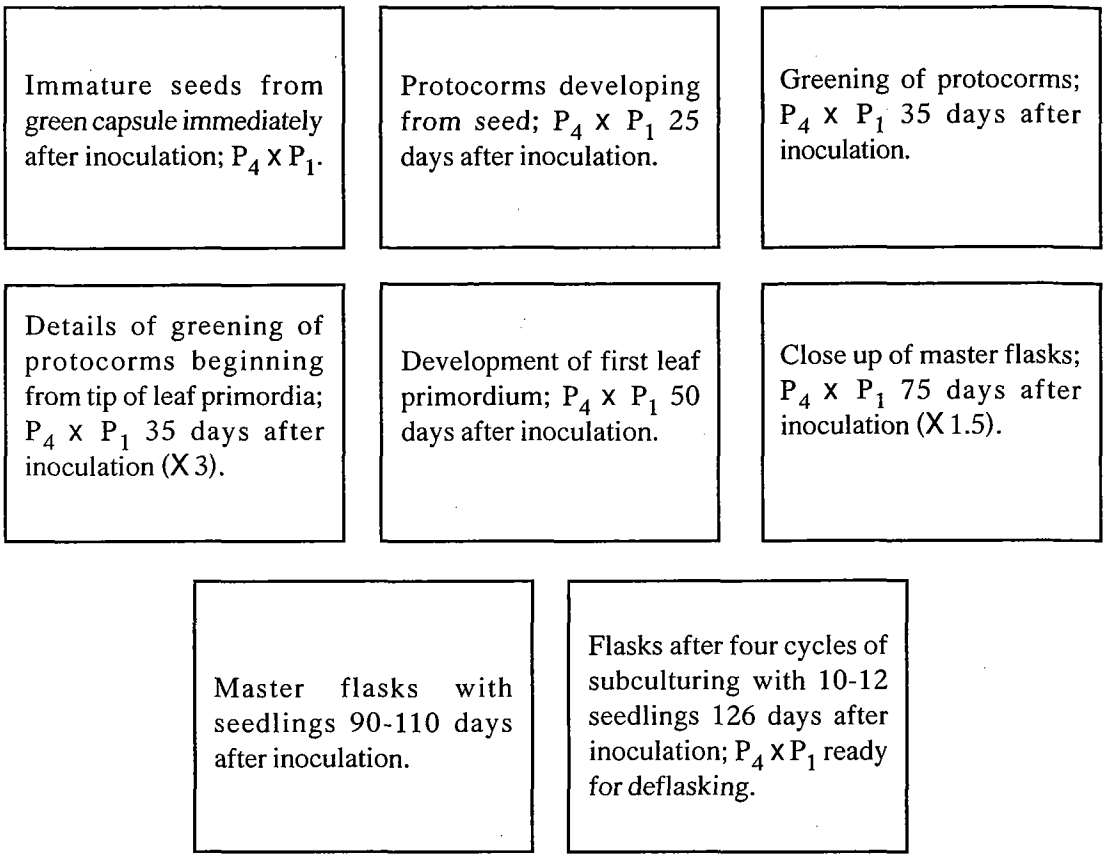
Table 4.2.2.2.a. Details of embryo culture of hybrid seeds obtained from successful combinations using P_1 as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	P_1 selfed	48.60	32.05	18.67	38.83	57.33	74.00	118.67	146.50	237.50
2.	$P_1 \times P_2$	18.37	13.50	15.33	28.67	45.67	70.33	102.00	121.50	209.33
3.	$P_1 \times P_3$	55.90	46.45	12.67	25.17	41.83	62.00	99.50	118.00	193.00
4.	$P_1 \times P_4$	65.24	58.22	11.50	22.67	27.83	55.50	87.67	105.67	176.33
5.	$P_1 \times P_6$	42.43	28.95	19.67	39.83	44.83	62.50	96.83	125.17	218.67
6.	$P_1 \times P_8$	34.30	26.87	13.33	24.00	28.83	51.17	82.50	110.17	180.67
7.	$P_1 \times P_9$	23.73	13.65	22.67	41.83	53.83	72.33	111.67	132.50	226.83
8.	$P_1 \times P_{10}$	16.02	11.57	26.67	43.67	59.17	78.83	125.17	158.33	268.33
9.	$P_1 \times P_{12}$	12.51	8.00	28.83	48.83	65.33	N.f.d.	—	—	—
	SE_m	1.381	0.962	0.300	0.349	0.533	1.363	1.427	1.326	1.991
	CD(0.05)	3.943	2.734	0.841	0.982	1.500	3.238	4.081	3.807	5.690

N.f.d. - No further development

Plate IV. *In vitro* embryo culture and refinement of *in vitro* culture medium

***In vitro* embryo culture**



Immature seeds from green capsule immediately after inoculation; $P_4 \times P_1$.

Protocorms developing from seed; $P_4 \times P_1$ 25 days after inoculation.

Greening of protocorms; $P_4 \times P_1$ 35 days after inoculation.

Details of greening of protocorms beginning from tip of leaf primordia; $P_4 \times P_1$ 35 days after inoculation (X 3).

Development of first leaf primordium; $P_4 \times P_1$ 50 days after inoculation.

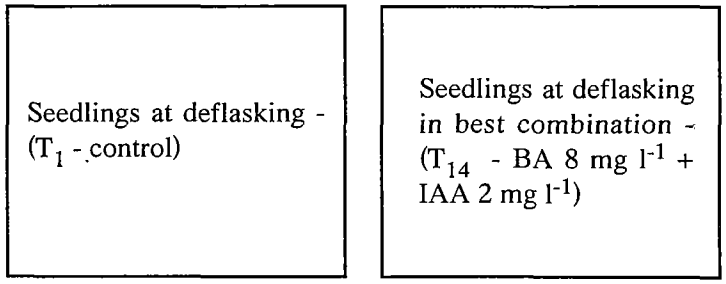
Close up of master flasks; $P_4 \times P_1$ 75 days after inoculation (X 1.5).

Master flasks with seedlings 90-110 days after inoculation.

Flasks after four cycles of subculturing with 10-12 seedlings 126 days after inoculation; $P_4 \times P_1$ ready for deflasking.

Refinement of *in vitro* culture medium

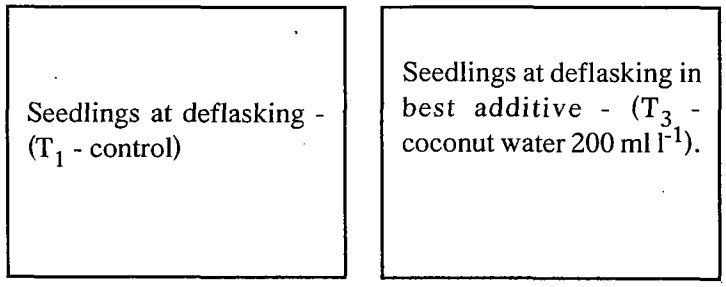
Effect of BA and IAA on growth of seedlings ($P_4 \times P_1$)



Seedlings at deflasking - (T_1 - control)

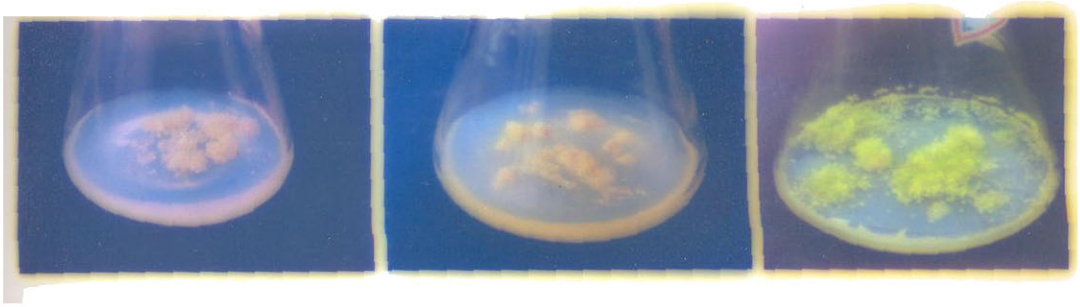
Seedlings at deflasking in best combination - (T_{14} - BA 8 mg l^{-1} + IAA 2 mg l^{-1})

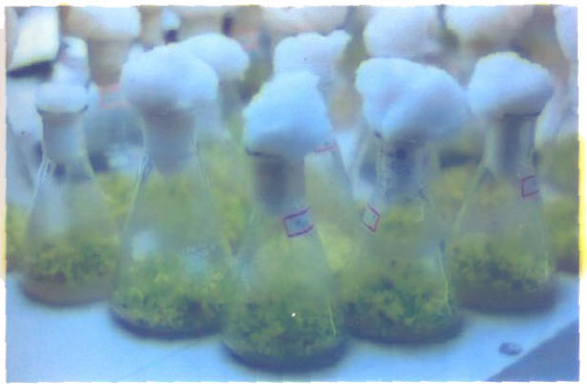
Effect of organic additives on growth of seedlings ($P_4 \times P_1$)

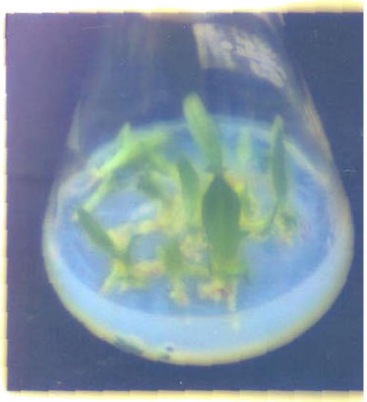


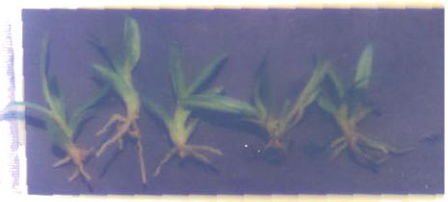
Seedlings at deflasking - (T_1 - control)

Seedlings at deflasking in best additive - (T_3 - coconut water 200 ml l^{-1}).









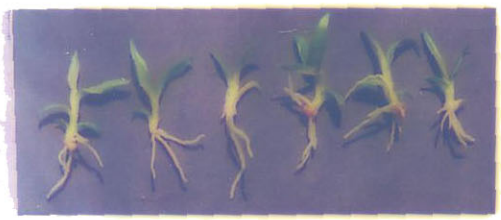


Plate IV

shoot primordia (51.17 days) and root primordia (82.50 days) whereas the early germinating P_1 x P_4 further resumed its rapid rate of *in vitro* development, producing the first root primordium in 105.67 days and presenting mature seedlings for planting out in 176.33 days.

4.2.2.2.b *Dendrobium* Chiangmai Pink (P_2)

The combinations P_2 x P_9 and P_2 x P_{14} registered significantly low values for percentage of filled seeds (21.50 and 24.53, respectively) and percentage seed germination (15.25 and 18.20, respectively) as compared to all the other combinations (Table 4.2.2.2b.). P_2 x P_1 recorded significantly high values for percentage of filled seeds (75.51) and percentage seed germination (60.70).

The same combination *viz.,* P_2 x P_1 further completed all the different growth stages in a significantly short time, becoming ready for deflasking in 159.33 days. The combination P_2 x P_{14} recorded the lowest rate of development till the formation of the first shoot primordium, after which the seedlings perished *in vitro.* Similarly, P_2 x P_5 registered no further development after greening and the protocorms eventually turned brown and dried up in culture. The combination P_2 x P_9 required the highest duration for the development of root primordia (143.00 days) and for planting out (221.50 days).

4.2.2.2.c *Dendrobium* Nagoya Pink (P_3)

With respect to percentage of filled seeds, P_3 x P_1 and P_3 x P_4 recorded significantly high values of 62.91 and 58.73, respectively which

Table 4.2.2.2.b. Details of embryo culture of hybrid seeds obtained from successful combinations using P_2 as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	$P_2 \times P_1$	75.51	60.70	11.67	25.83	31.67	50.00	79.00	101.17	159.33
2.	$P_2 \times P_3$	53.60	47.82	13.67	28.00	37.50	56.17	89.83	110.50	175.67
3.	$P_2 \times P_4$	47.22	36.38	16.83	31.00	42.50	59.33	105.33	121.50	210.83
4.	$P_2 \times P_5$	44.82	38.03	26.33	42.83	69.17	N.f.d.	—	—	—
5.	$P_2 \times P_6$	38.64	30.38	20.00	33.33	47.50	60.50	103.33	119.50	197.83
6.	$P_2 \times P_7$	25.40	18.92	22.67	36.50	52.83	63.83	111.67	127.33	212.67
7.	$P_2 \times P_9$	21.50	15.25	24.33	45.50	60.00	72.83	128.50	143.00	221.50
8.	$P_2 \times P_{14}$	24.53	18.20	28.83	49.00	65.67	75.33	134.83	N.f.d.	—
	SE_m	1.419	1.047	0.283	0.373	0.663	1.234	1.227	1.648	2.351
	CD (0.05)	4.091	3.032	0.807	1.070	1.886	3.602	3.546	4.832	6.937

N.f.d. - No further development

were on par (Table 4.2.2.2.c.). The lowest values for the character were recorded by P_3 x P_8 (18.10 %) and P_3 x P_{10} (18.64 %), the two treatments being statistically on par. Percentage of seed germination followed the same trend with P_3 x P_1 registering 53.30 and P_3 x P_8 and P_3 x P_{10} recording on par values of 13.28 and 11.23, respectively.

Initiation of germination was observed in 11.50 days in P_3 x P_1. The same combination completed the different developmental stages within the lowest time, the seedlings becoming ready for planting out in 157.67 days. The combination P_3 x P_{10} took the highest duration for completing each developmental stage, with the seedlings reaching deflasking stage in 257.50 days. Germination started in 11.50 days in P_3 x P_1 extending to 28.33 days in P_3 x P_{10}. Time taken for protocorm development ranged from 22.33 days (P_3 x P_1) to 47.83 days (P_3 x P_{10}). Duration to greening of protocorms varied from 28.00 days in P_3 x P_1 to 68.83 days in P_3 x P_{10}. The first leaf, shoot and root primordia were initiated within the lowest duration of 52.00, 76.83 and 99.67 days, respectively in P_3 x P_1, whereas the same events required the highest duration of 81.67, 127.00 and 163.50 days, respectively in P_3 x P_{10}.

4.2.2.2.d. *Dendrobium* Pramot 3 (P_4)

Percentage of filled seeds to total number of seeds per combination ranged from 12.80 in P_4 x P_8 to 67.60 in P_4 x P_1 (Table 4.2.2.2.d.). The combination P_4 x P_8 registered no seed germination following inoculation. Seed germination ranged from a significantly low

Table 4.2.2.2.c. Details of embryo culture of hybrid seeds obtained from successful combinations using P_3 as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	P_3 selfed	36.80	30.82	20.83	33.67	44.17	63.67	98.33	120.33	201.00
2.	$P_3 \times P_1$	62.91	53.30	11.50	22.33	28.00	52.00	76.83	99.67	157.67
3.	$P_3 \times P_4$	58.73	48.62	14.50	26.17	36.33	58.50	86.17	108.17	178.17
4.	$P_3 \times P_5$	48.60	37.50	16.80	35.30	51.00	65.50	103.32	128.50	206.67
5.	$P_3 \times P_6$	41.52	38.63	18.33	37.67	53.17	67.83	106.83	133.67	215.50
6.	$P_3 \times P_7$	33.18	24.61	21.70	34.50	46.33	65.83	100.33	125.50	209.67
7.	$P_3 \times P_8$	18.10	13.28	26.67	44.67	62.17	78.00	119.17	146.50	241.50
8.	$P_3 \times P_{10}$	18.64	11.23	28.33	47.83	68.83	81.67	127.00	163.50	257.50
	SE_m	1.710	0.943	0.268	0.317	0.454	1.010	1.292	1.486	2.215
	CD (0.05)	4.932	2.724	0.738	0.891	1.304	2.908	3.732	4.283	6.384

Table 4.2.2.2.d. Details of embryo culture of hybrid seeds obtained from successful combinations using P_4 as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	P_4 selfed	42.82	35.88	19.33	33.33	53.00	71.67	102.17	126.83	201.83
2.	$P_4 \times P_1$	67.60	59.57	11.50	24.83	33.67	49.50	79.17	106.67	143.50
3.	$P_4 \times P_3$	37.83	31.20	15.33	26.50	38.17	54.67	87.00	112.67	155.83
4.	$P_4 \times P_5$	30.31	21.53	21.83	36.83	48.50	65.00	97.17	137.50	215.33
5.	$P_4 \times P_6$	22.41	15.22	27.33	43.83	66.67	81.83	131.00	156.33	250.50
6.	$P_4 \times P_8$	12.80	N.f.d.	—	—	—	—	—	—	—
	SE_m	1.506	1.053	0.247	0.276	0.530	1.043	1.439	1.253	2.267
	CD (0.05)	4.334	3.072	0.693	0.801	1.562	3.024	4.178	3.638	6.572

N.f.d. - No further development

value of 15.22 per cent in P_4 x P_6 to a significantly high value of 59.57 per cent in P_4 x P_1.

The combination P_4 x P_1 exhibited rapid development, germination initiation occurring in 11.50 days. All the different growth stages such as protocorm development (24.83 days), greening (33.67 days), development of first leaf (49.50 days), shoot (79.17 days) and root (106.67 days) primordia were covered within a significantly low time. The seedlings were ready for deflasking in 143.50 days.

The hybrid P_4 x P_6 needed a significantly high number of days for covering each growth stage. In P_4 x P_6, germination was initiated in 27.33 days, protocorm developed in 43.83 days, greening was observed in 66.67 days, first leaf primordium made its appearance in 81.83 days, shoot initial developed in 131.00 days, first root was formed in 156.33 days and the seedlings were ready for planting out in 250.50 days.

4.2.2.2.e *Dendrobium* Renapa Red 3 (P_5)

Filled seeds per combination ranged from a significantly low value of 14.82 per cent in P_5 x P_4 to a significantly high value of 63.61 per cent in P_5 x P_1 (Table 4.2.2.2.e.). Percentage of seed germination ranged from 9.78 in P_5 x P_9 to 55.83 in P_5 x P_1.

Rapid development was observed in P_5 x P_{12}, reaching germination initiation (13.67 days), protocorm development (24.33 days), greening (33.67 days), leaf initiation (50.33 days), shoot initiation (84.33 days),

Table 4.2.2.2.e. Details of embryo culture of hybrid seeds obtained from successful combinations using P_5 as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	$P_5 \times P_1$	63.61	55.83	19.50	35.83	45.17	64.00	105.83	126.00	196.17
2.	$P_5 \times P_4$	14.82	N.f.d.	—	—	—	—	—	—	—
3.	$P_5 \times P_6$	31.50	25.37	21.83	38.67	52.83	68.83	112.17	135.17	218.50
4.	$P_5 \times P_7$	21.10	14.95	30.67	53.50	72.17	95.33	138.50	169.00	273.17
5.	$P_5 \times P_8$	29.81	21.47	29.00	48.67	67.17	86.17	130.33	158.00	265.83
6.	$P_5 \times P_9$	20.03	9.78	26.33	46.33	63.83	N.f.d.	—	—	—
7.	$P_5 \times P_{12}$	56.62	52.37	13.67	24.33	33.67	50.33	84.33	102.00	145.33
	SE_m	1.400	1.583	0.322	0.356	0.497	1.046	1.232	1.660	1.832
	CD (0.05)	4.020	4.557	0.916	1.002	1.424	3.033	3.571	4.843	5.331

N.f.d. - No further development

root initiation (102.00 days) and planting out (145.33 days) stages within the shortest time.

Slow development was recorded in P_5 x P_7, showing germination initiation in 30.67 days and mature seedling formation in 273.17 days.

Two combinations *viz.*, P_5 x P_4 and P_5 x P_9 ceased to develop further while in culture, at different stages. Seeds of P_5 x P_4 failed to germinate while the protocorms of P_5 x P_9 showed no further development after greening.

4.2.2.2.f *Dendrobium* Rinabha (P_6)

Percentage of filled seeds and percentage seed germination ranged from significantly low values of 33.64 and 24.07, respectively in P_6 x P_{12} to 75.93 and 70.73, respectively in P_6 x P_1 (Table 4.2.2.2.f.).

The combination P_6 x P_9 completed the different developmental stages within the lowest duration whereas P_6 x P_4 required the highest duration for completing growth *in vitro*. Germination initiation was observed in 17.50 days in P_6 x P_9 and in 29.67 days in P_6 x P_4. Time taken for protocorm development ranged from 34.17 days in P_6 x P_9 to 48.50 days in P_6 x P_4. Duration to greening of protocorms varied from 45.17 days (P_6 x P_9) to 71.83 days (P_6 x P_4). The first leaf, shoot and root primordia were initiated within the shortest time of 63.00, 97.67 and 121.17 days, respectively in P_6 x P_9 and took the longest time of 83.50, 131.50 and 156.33 days, respectively in P_6 x P_4. The seedlings

Table 4.2.2.2.f. Details of embryo culture of hybrid seeds obtained from successful combinations using P_6 as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	$P_6 \times P_1$	75.93	70.73	22.83	39.50	55.83	70.00	111.33	138.50	230.17
2.	$P_6 \times P_3$	48.50	40.48	25.83	43.67	63.50	75.33	119.5	148.00	239.50
3.	$P_6 \times P_4$	42.52	37.17	29.67	48.50	71.83	83.50	131.5	156.33	262.83
4.	$P_6 \times P_9$	74.81	64.15	17.50	34.17	45.17	63.00	97.67	121.17	191.50
5.	$P_6 \times P_{12}$	33.64	24.07	21.17	36.33	48.17	65.67	100.33	123.67	213.00
	SE_m	1.762	1.721	0.304	0.313	0.640	1.673	1.910	1.484	1.951
	CD (0.05)	5.140	5.003	0.867	0.915	1.862	3.405	3.472	4.314	5.687

were ready for deflasking in 191.50 days in P_6 x P_9 and in 262.83 days in P_6 x P_4.

4.2.2.2.g. *Dendrobium* Sakura (P_7)

Percentage of filled seeds to total number of seeds ranged from 20.30 in P_7 x P_9 to 71.22 in P_7 x P_1 (Table 4.2.2.2.g.). Seed germination was found to vary from 12.05 per cent in P_7 x P_9 to 56.32 per cent in P_7 x P_1.

The combination P_7 x P_1 exhibited rapid development, recording germination initiation within 12.50 days. All the different growth stages such as protocorm development (22.67 days), greening (27.83 days), development of first leaf primordium (48.17 days), first shoot primordium (77.33 days) and first root initial (103.17 days) were completed within a significantly short time. The seedlings attained sufficient growth for planting out in 176.17 days.

The hybrid P_7 x P_4 recorded the lowest rate of development, requiring 28.83 days for germination initiation. In this combination, protocorms developed in 46.00 days and chlorophyll developed in 70.67 days. The first leaf, shoot and root primordia developed in 87.83, 132.00 and 162.50 days, respectively. The seedlings were ready for deflasking in 270.00 days.

The selfed capsule of P_7 recorded 42.51 per cent filled seeds with 33.02 per cent germination. The rate of development was moderate, the seedlings reaching planting out stage in 199.17 days.

Table 4.2.2.2.g. Details of embryo culture of hybrid seeds obtained from successful combinations using P_7 as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	P_7 selfed	42.51	33.02	19.67	34.67	49.17	63.17	106.50	123.17	199.17
2.	$P_7 \times P_1$	71.22	56.32	12.50	22.67	27.83	48.17	77.33	103.17	176.17
3.	$P_7 \times P_2$	30.27	18.30	26.33	43.67	64.50	91.50	122.50	148.00	255.50
4.	$P_7 \times P_3$	53.87	48.63	14.83	24.50	31.83	58.17	90.17	113.67	180.50
5.	$P_7 \times P_4$	31.63	24.28	28.83	46.00	70.67	87.83	132.00	162.50	270.00
6.	$P_7 \times P_9$	20.30	12.05	22.33	36.67	53.50	74.67	112.50	133.17	203.67
	SE_m	1.862	1.779	0.314	0.313	0.502	0.931	1.247	1.581	2.592
	CD (0.05)	5.364	5.132	0.889	0.880	1.446	2.693	3.618	4.573	7.474

4.2.2.2.h. *Dendrobium* Sonia 16 (P_8)

The combinations P_8 x P_3 and P_8 x P_{11} recorded significantly low values for percentage of filled seeds, being 15.23 and 19.50, respectively (Table 4.2.2.2.h.). On inoculation, the seeds of P_8 x P_3 turned brown *in vitro*, manifesting no germination and eventually succumbed to fungal contamination. The combination P_8 x P_{11} registered a low germination of 13.08 per cent. The highest values for both percentage of filled seeds and percentage seed germination were recorded by P_8 x P_1, being 64.90 per cent and 54.82 per cent, respectively.

Number of days taken for germination initiation ranged from 17.33 days in P_8 x P_1 to 40.17 days in P_8 x P_{11}. After initial swelling and glistening, the seeds of P_8 x P_3 showed no further development in terms of protocorm differentiation, perishing *in vitro*. Time taken for the differentiation of protocorms ranged from 27.16 days in P_8 x P_1 to 47.67 days in P_8 x P_7. Protocorms turned green in 33.83 days in P_8 x P_1 whereas they required 58.50 days for greening in P_8 x P_7. In the combination P_8 x P_1, initiation of the first leaf, shoot and root primordia occurred in 62.33, 96.33 and 124.33 days, respectively. In this combination, the seedlings reached planting out stage in 200.50 days. Lowest rate of development was observed in P_8 x P_7, taking 83.67, 130.83 and 164.17 days, respectively for the initiation of the first leaf, shoot and root primordia. The seedlings were ready for deflasking in 269.50 days in P_8 x P_7.

Table 4.2.2.2.h. Details of embryo culture of hybrid seeds obtained from successful combinations using P_8 as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	$P_8 \times P_1$	64.90	54.82	17.33	27.16	33.83	62.33	96.33	124.33	200.50
2.	$P_8 \times P_2$	52.81	42.92	26.83	42.83	57.17	75.67	119.67	146.00	244.17
3.	$P_8 \times P_3$	15.23	N.g.	—	—	—	—	—	—	—
4.	$P_8 \times P_7$	28.52	21.07	31.17	47.67	58.50	83.67	130.83	164.17	269.50
5.	$P_8 \times P_9$	46.28	38.73	23.67	31.00	39.33	66.17	112.50	132.17	223.00
6.	$P_8 \times P_{11}$	19.50	13.08	40.17	N.f.d.	—	—	—	—	—
	SE_m	1.766	1.745	0.654	0.422	0.560	1.061	1.291	1.373	2.094
	CD (0.05)	5.084	5.063	1.891	1.230	1.671	3.132	3.815	4.037	6.170

N.g. - No germination

N.f.d. - No further development

4.2.2.2.i. *Dendrobium* White Fairy (P_9)

Percentage of filled seeds per combination was found to be the lowest in P_9 x P_3 (13.81 %) (Table 4.2.2.2.i.). The seeds showed no germination on *in vitro* inoculation, gradually turning brown and necrotic. P_9 x P_6 showed the highest percentage of filled seeds, being 32.50 per cent. Percentage filled seeds was low in all combinations using P_9 as the female parent, as compared to all the other hybrids. Percentage germination ranged from 10.67 (P_9 x P_2) to 28.15 (P_9 x P_6), even the highest value being comparatively low.

Germination initiation was observed in 14.33 days in P_9 x P_6, being the combination showing the highest rate of development. Protocorm development and greening occurred in 23.33 and 32.67 days, respectively. First leaf, shoot and root primordia developed in 51.00, 87.17 and 107.50 days, respectively in P_9 x P_6. For complete development of seedlings, it took 174.50 days. The combination P_9 x P_2 took the highest duration (28.33 days) for germination initiation. After an initial slow swelling, no further development was observed in these seeds, which perished *in vitro*. The combination P_9 x P_5 took the highest duration of 41.83 days for the development of protocorms. Greening was completed in 55.50 days in P_9 x P_5. The same combination required the highest number of days for the development of the first leaf, shoot and root primordia, being 74.67, 119.50 and 150.50 days, respectively. The seedlings were ready for planting out in 190.33 days.

Table 4.2.2.2.i. Details of embryo culture of hybrid seeds obtained from successful combinations using P_9 as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	$P_9 \times P_1$	20.50	15.87	18.67	29.50	41.50	61.33	96.50	120.67	181.83
2.	$P_9 \times P_2$	16.23	10.67	28.33	N.f.d.	—	—	—	—	—
3.	$P_9 \times P_3$	13.81	N.g.	—	—	—	—	—	—	—
4.	$P_9 \times P_5$	28.92	19.20	25.00	41.83	55.50	74.67	119.50	150.50	190.33
5.	$P_9 \times P_6$	32.50	28.15	14.33	23.33	32.67	51.00	87.17	107.50	174.50
6.	$P_9 \times P_7$	23.33	14.05	23.33	39.17	51.50	70.50	112.67	133.67	215.00
	SE_m	1.572	1.323	0.295	0.364	0.520	1.208	1.403	1.689	2.132
	CD (0.05)	4.520	3.844	0.823	1.052	1.529	3.543	4.076	4.927	6.193

N.g. - No germination N.f.d. - No further development

4.2.2.2.j. *Dendrobium* Caesar Pink (P_{10})

Percentage of filled seeds per combination ranged from 10.79 in $P_{10} \times P_5$, being the lowest among all the different hybrid combinations to 40.48 in $P_{10} \times P_2$ (Table 4.2.2.2.j). The combination $P_{10} \times P_5$ recorded no germination on inoculation. The seeds turned necrotic and the culture was eventually lost due to microbial contamination. $P_{10} \times P_7$ took the highest duration (29.67 days) for initiation of germination. The combination $P_{10} \times P_7$ maintained the same trend throughout the *in vitro* growth period, showing protocorm development in 42.00 days and greening in 53.17 days. Development of the first leaf, shoot and root primordia took 75.33, 124.83 and 151.00 days, respectively in $P_{10} \times P_7$. Complete development of seedlings took 272.33 days.

4.2.2.2.k. *Dendrobium* Uniwai Pink (P_{11})

The combination $P_{11} \times P_{12}$ recorded significantly low values for percentage of filled seeds (20.82) and germination percentage (15.08) (Table 4.2.2.2.k.). The hybrid $P_{11} \times P_2$ recorded the highest values for both the characters, being 56.30 per cent filled seeds and 50.13 per cent germination.

The combination $P_{11} \times P_2$ showed rapid development, passing through the different developmental stages such as germination initiation (14.33 days), protocorm development (26.50 days), greening (39.17 days), leaf initiation (57.83 days), shoot initiation (87.17 days), root initiation (107.67 days) and deflasking (168.33 days) within the lowest time.

Table 4.2.2.2.j. Details of embryo culture of hybrid seeds obtained from successful combinations using P_{10} as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	$P_{10} \times P_1$	35.10	29.50	14.67	24.67	34.83	54.67	92.00	116.83	182.17
2.	$P_{10} \times P_2$	40.48	34.23	23.00	35.83	48.50	67.00	105.33	139.17	225.50
3.	$P_{10} \times P_5$	10.79	N.g.	—	—	—	—	—	—	—
4.	$P_{10} \times P_7$	34.72	27.48	29.67	42.00	53.17	75.33	124.83	151.00	272.33
	SE_m	1.863	0.260	0.342	0.301	0.460	1.177	1.270	1.761	1.626
	CD (0.05)	5.474	N.S.	1.044	0.912	1.389	3.554	3.814	5.310	4.917

N.g. - No germination

Table 4.2.2.2.k. Details of embryo culture of hybrid seeds obtained from successful combinations using P_{11} as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	P_{11} selfed	38.38	27.83	23.67	35.83	50.83	68.67	125.5	151.00	243.00
2.	$P_{11} \times P_2$	56.30	50.13	14.33	26.50	39.17	57.83	87.17	107.67	168.33
3.	$P_{11} \times P_4$	42.51	35.50	19.00	31.67	43.50	58.83	112.00	126.33	201.00
4.	$P_{11} \times P_7$	28.57	21.78	28.83	58.00	N.f.d.	—	—	—	—
5.	$P_{11} \times P_{12}$	20.82	15.08	21.67	33.83	48.83	65.83	118.83	140.50	188.33
	SE_m	1.693	1.527	0.334	0.452	0.427	1.562	1.263	1.605	2.003
	CD (0.05)	4.912	4.473	0.958	1.314	1.259	4.620	3.704	4.734	5.904

N.f.d. - No further development

The hybrid P_{11} x P_7 was late in development, germination initiation taking 28.83 days and protocorm development requiring 58.00 days, being the highest duration for the same, as compared to all the other hybrids cultured *in vitro*. The culture P_{11} x P_7 perished *in vitro*, recording no further development after protocorm formation. Seeds of P_{11} selfed showed the lowest rate of development thereafter, taking 50.83 days for greening. First leaf, shoot and root initials appeared in 68.67, 125.50 and 151.00 days, respectively. The seedlings attained sufficient growth for planting out in 243.00 days.

4.2.2.2.l. *Dendrobium* Walter Oumae (P_{12})

Percentage of filled seeds to total number of seeds per combination ranged from 28.20 in P_{12} x P_{11} to 42.57 in P_{12} x P_4 (Table 4.2.2.2.l.). Seed germination varied from 22.30 per cent in P_{12} x P_{11} to 36.75 per cent in P_{12} x P_4.

The hybrid combination P_{12} x P_5 showed rapid development taking 17.50 days for germination initiation, 34.00 days for protocorm development and 44.33 days for greening. The first leaf, shoot and root primordia were initiated within 70.83, 95.50 and 127.33 days, respectively. The seedlings were ready for deflasking in 173.33 days. The hybrid P_{12} x P_{11} showed the lowest rate of development, needing 25.67 days for germination initiation, 45.00 days for protocorm development and 57.17 days for greening. The first leaf, shoot and root initials developed in 81.67, 141.00 and 158.17 days, respectively. The seedlings attained sufficient growth for *ex vitro* establishment in 261.50 days.

Table 4.2.2.2.1. Details of embryo culture of hybrid seeds obtained from successful combinations using P_{12} as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	$P_{12} \times P_4$	42.57	36.75	22.17	39.17	51.50	74.00	127.83	152.83	238.00
2.	$P_{12} \times P_5$	31.81	26.62	17.50	34.00	44.33	70.83	95.50	127.33	173.33
3.	$P_{12} \times P_{11}$	28.20	22.30	25.67	45.00	57.17	81.67	141.00	158.17	261.50
	SE_m	1.523	1.464	0.339	0.592	0.470	0.861	1.191	1.686	1.823
	CD (0.05)	4.592	4.203	0.986	1.764	1.407	2.582	3.604	5.053	5.472

4.2.2.2.m. *Dendrobium barbatulum* (P_{13})

Percentage of filled seeds and percentage seed germination were the highest in the selfed seeds, being 32.81 and 30.55, respectively (Table 4.2.2.2.m.). The combination P_{13} x P_8 registered the lowest percentage of filled seeds (21.40) and seed germination (16.55). Number of days to germination initiation was the lowest in P_{13} selfed seeds (12.33 days) and the highest in P_{13} x P_9 (23.00 days). Protocorms developed in 21.83 days in P_{13} selfed seeds being the lowest duration as compared to all other *in vitro* hybrid seed cultures. The hybrid P_{13} x P_9 took 35.83 days for protocorm development. Time taken for greening of protocorms was 30.33 days in P_{13} selfed and 46.83 days in P_{13} x P_9. The first leaf, shoot and root primordia developed in 49.17, 82.33 and 108.33 days, respectively in P_{13} selfed and in 70.33, 123.17 and 151.50 days, respectively in P_{13} x P_9. Duration to deflasking varied from 166.00 days in P_{13} selfed to 238.50 days in P_{13} x P_9.

4.2.2.2.n. *Dendrobium philippica* (P_{14})

Filled seeds per combination ranged from a significantly low value of 16.81 per cent in P_{14} x P_5 to 41.12 per cent in P_{14} x P_1 (Table 4.2.2.2.n.). Seed germination ranged from 10.48 per cent in P_{14} x P_5 to 36.72 per cent in P_{14} x P_1.

Number of days taken for germination initiation ranged from 12.33 days in P_{14} selfed to 29.00 days in P_{14} x P_5. Time taken for protocorm development varied from 22.50 days in P_{14} selfed to 42.83

Table 4.2.2.2.m. Details of embryo culture of hybrid seeds obtained from successful combinations using P_{13} as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	P_{13} selfed	32.81	30.55	12.33	21.83	30.33	49.17	82.33	108.83	166.00
2.	$P_{13} \times P_8$	21.40	16.55	18.67	33.83	43.17	65.17	114.67	140.17	220.17
3.	$P_{13} \times P_9$	24.64	18.18	23.00	35.83	46.83	70.33	123.17	151.50	238.50
	SE_m	1.923	1.467	0.274	0.316	0.430	0.942	1.733	1.474	1.635
	CD (0.05)	5.782	4.390	0.816	0.932	1.304	2.834	5.217	4.436	4.900

Table 4.2.2.2.n. Details of embryo culture of hybrid seeds obtained from successful combinations using P_{14} as the female parent

Sl. No.	Cross combination	Filled seeds (%)	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of					No. of days taken for planting out
					Protocorm	Chlorophyll	First leaf primordium	First shoot primordium	First root primordium	
1.	P_{14} selfed	37.40	27.93	12.33	22.50	34.17	54.17	88.00	110.67	178.83
2.	$P_{14} \times P_1$	41.12	36.72	15.67	26.00	37.33	50.83	80.17	100.67	162.50
3.	$P_{14} \times P_2$	28.73	23.25	26.00	40.67	59.33	80.00	110.83	152.83	220.17
4.	$P_{14} \times P_3$	20.50	12.77	22.83	35.50	53.50	66.83	101.50	126.33	228.33
5.	$P_{14} \times P_6$	16.81	10.48	29.00	42.83	61.50	86.00	126.17	159.67	279.33
	SE_m	1.823	1.887	0.345	0.305	0.490	1.176	1.643	1.687	1.929
	CD (0.05)	5.302	5.484	1.000	0.863	1.436	3.417	4.774	4.932	5.614

days in P_{14} x P_5. Greening was observed in 34.17 days in P_{14} selfed, showing the fastest growth and in 61.50 days in P_{14} x P_5 showing the lowest rate of growth. Time taken for initiation of first leaf primordium ranged from 50.83 days in P_{14} x P_1 to 86.00 days in P_{14} x P_5. Time taken for first shoot initial development ranged from 80.17 days in P_{14} x P_1 to 126.17 days in P_{14} x P_5. The first root made its appearance in 100.67 days in P_{14} x P_1 and in 159.67 days in P_{14} x P_5. Time taken for deflasking and planting out ranged from 162.50 days in P_{14} x P_1 to 279.33 days in P_{14} x P_5.

4.2.3. Refinement of *in vitro* culture medium

4.2.3.1. Effect of media on seed germination and protocorm development

Different basal media such as MS full strength, MS half strength, MS quarter strength, VW full strength and KC full strength were evaluated to find out their effects on seed germination and further development of the protocorm (Table 4.2.3.1.).

The basal medium MS half strength recorded the highest seed germination percentage of 59.57, which was on par with MS quarter strength (57.00) and full strength MS (54.50). The basal media KC and VW recorded lower values which were on par with full strength MS.

Number of days taken for germination initiation differed significantly with the media. Seeds germinated faster in quarter strength

Table 4.2.3.1. Effect of media on *in vitro* seed germination and protocorm development in *Dendrobium*

Medium : Basal + sucrose 30 g l^{-1} + agar 5.5 g l^{-1} + CW 200 ml l^{-1} + BA 8 mg l^{-1} + IAA 4 mg l^{-1}

Combination : $P_4 \times P_1$

Sl. No.	Medium	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of			
				Protocorm	Chlorophyll	First leaf primordium	First root primordium
1.	MS (¼ strength)	57.00	10.30	29.50	38.33	56.33	127.83
2.	MS (½ strength)	59.57	11.50	24.83	35.50	51.17	114.50
3.	MS (full strength)	54.50	23.83	38.67	47.83	61.33	122.33
4.	VW (full strength)	50.30	32.83	42.00	50.50	66.00	136.17
5.	KC (full strength)	52.00	25.67	35.67	42.83	58.00	132.33
	SE_m	1.689	0.303	0.374	0.516	0.869	1.326
	CD (0.05)	4.925	0.884	1.090	1.504	2.534	3.863

MS (10.30 days) and half strength MS (11.50 days) as compared to full strength MS (23.83 days). Germination was relatively slow in KC (25.67 days) and VW (32.83 days) media.

Number of days taken for development of protocorms showed significant differences depending on the media. Rapid protocorm development was observed in half strength MS (24.83 days) followed by quarter strength MS (29.50 days). Comparatively slow protocorm development was recorded in full strength MS (38.67 days), KC (35.67 days) and VW (42.00 days).

Number of days taken for development of chlorophyll and first leaf primordium followed the same trend. Significantly early development of chlorophyll (35.50 days) and first leaf (51.17 days) was observed in half strength MS, followed by quarter strength MS, KC, full strength MS and VW, in that order.

Protocorm differentiation was completed with the development of the first root primordium in 114.50 days in half strength MS. Full strength MS came next, taking 122.33 days for root initiation, closely followed by MS quarter strength (127.83 days). Protocorm differentiation was comparatively slow in VW, taking 136.17 days for root initiation.

4.2.3.2. Effect of media on growth of seedlings

Significant difference in the growth of seedlings at six months after inoculation was observed in the different basal media tried such as

MS at full, half and quarter strengths and VW and KC at full strengths (Table 4.2.3.2.).

Half strength MS showed the best response for seedling height, recording a significantly superior height of 4.06 cm. Quarter strength MS came next, expressing a height of 3.77 cm. Full strength MS (3.47 cm) and VW (3.40 cm) were on par with each other. Seedlings were the shortest in KC (3.14 cm).

Half strength MS produced more number of leaves (3.70) with the highest mean leaf length (2.47 cm) per seedling. Quarter strength MS produced 3.20 leaves and full strength MS produced 2.90 leaves per seedling. Leaf length was considerably high in MS full strength (2.18 cm) and VW (1.98 cm).

Quarter strength MS produced the highest number of roots (4.30) per seedling, which was on par with MS half strength (3.90). A significantly high mean root length of 2.16 cm was also recorded in MS half strength. Quarter strength MS and KC were found to be on par, registering mean values of 1.88 cm and 1.77 cm, respectively.

4.2.3.3. Effect of BA and IAA on protocorm differentiation and seedling growth

The effect of BA and IAA on *in vitro* protocorm differentiation and growth of seedlings in terms of leaf and root development have been studied (Table 4.2.3.3.).

Table 4.2.3.2. Effect of media on *in vitro* growth of seedlings in *Dendrobium*

Medium : Basal + sucrose 30 g l^{-1} + agar 5.5 g l^{-1} + CW 200 ml l^{-1} + BA 8 mg l^{-1} + IAA 4 mg l^{-1}

Combination : $P_4 \times P_1$

Sl. No.	Medium	Seedling characters six months after inoculation				
		Height (cm)	No. of leaves	Mean length of leaf (cm)	No. of roots	Mean length of root (cm)
1.	MS (¼ strength)	3.77	3.20	1.84	4.30	1.88
2.	MS (½ strength)	4.06	3.70	2.47	3.90	2.16
3.	MS (full strength)	3.47	2.90	2.18	3.50	1.25
4.	VW (full strength)	3.40	2.40	1.98	2.30	1.59
5.	KC (full strength)	3.14	2.20	1.65	2.60	1.77
	SE_m	0.063	0.224	0.069	0.193	0.058
	CD (0.05)	0.190	0.641	0.214	0.544	0.192

Table 4.2.3.3. Effect of BA and IAA on *in vitro* protocorm differentiation and growth of seedlings in *Dendrobium*

Medium : MS (½ strength) + sucrose 30 g l^{-1} + agar 5.5 g l^{-1} + CW 200 ml l^{-1}

Combination : P_4 x P_1

Treatment	Concentration (mg l^{-1}) of		Days to development of		Seedling characters six months after inoculation			
	BA	IAA	First leaf primordium	First root primordium	No. of leaves	Mean length of leaf (cm)	No. of roots	Mean length of root (cm)
T_1	0	0	57.33	135.83	3.50	1.75	3.50	1.30
T_2	2	2	59.50	141.33	3.17	1.67	3.67	1.28
T_3	2	4	58.33	140.83	3.00	1.50	3.50	1.40
T_4	2	6	60.33	142.33	3.67	1.70	3.33	1.47
T_5	2	8	58.00	145.33	3.33	1.63	3.83	1.58
T_6	4	2	52.67	130.50	4.33	2.50	4.50	2.18
T_7	4	4	58.50	140.50	3.50	1.72	3.67	1.92
T_8	4	6	55.83	140.00	3.50	1.75	3.83	1.93
T_9	4	8	60.67	141.17	3.33	1.70	3.67	1.97
T_{10}	6	2	52.17	127.17	4.67	2.93	4.83	2.05
T_{11}	6	4	50.83	130.00	4.67	2.72	5.00	2.62
T_{12}	6	6	59.33	141.17	3.67	1.78	4.00	1.75
T_{13}	6	8	50.83	129.50	4.50	2.68	4.83	2.08
T_{14}	8	2	45.67	107.67	5.30	3.17	5.67	2.42
T_{15}	8	4	46.50	111.17	5.17	3.20	5.50	2.50
T_{16}	8	6	52.67	128.17	4.50	2.67	4.67	2.15
T_{17}	8	8	59.67	129.33	3.50	1.78	4.00	1.72
SE_m			1.298	1.693	0.222	0.081	0.252	0.069
CD(0.05)			3.654	4.774	0.631	0.234	0.700	0.211

Out of the 17 treatments (T_1 to T_{17}) employed, T_{14} (BA 8 mgl^{-1} + IAA 2 mgl^{-1}) and T_{15} (BA 8 mgl^{-1} + IAA 4 mgl^{-1}) were the best, both in terms of early differentiation of protocorms and development of larger seedlings (Plate IV).

In T_{14} first leaf primordium developed in 45.67 days and first root primordium differentiated in 107.67 days. In T_{15} leaf and root primordia appeared in 46.50 and 111.17 days, respectively. These two significantly superior treatments were on par with each other. Significant difference in duration to the development of first leaf and root primordia could not be detected between several of the other combinations tried.

With respect to seedling characters, the highest number of leaves was observed in T_{14} (BA 8mgl^{-1} + IAA 2mgl^{-1}), producing 5.30 leaves per seedling. Three other treatments *viz.*, T_{15} (BA 8 mgl^{-1} + IAA 4 mgl^{-1}) recording 5.17 leaves and T_{10} (BA 6 mgl^{-1} + IAA 2 mgl^{-1}) and T_{11} (BA 6 mgl^{-1} + IAA 4 mgl^{-1}) registering 4.67 leaves each per seedling were on par with T_{14}. Low leaf numbers were recorded in the lower concentrations of growth regulators and the control.

The highest average leaf length of 3.20 cm was recorded in T_{15} which was on par with the value (3.17 cm) recorded in T_{14}. The treatment T_{10} resulted in a leaf length of 2.93 cm and T_{11} produced a leaf length of 2.72cm. Combinations involving the lower concentrations of BA recorded comparatively lower leaf lengths.

The treatment T_{14} recorded the highest number of roots per seedling (5.67), which was on par with T_{15} (5.50) and T_{11} (5.00). Root production was low in the lower concentrations of growth regulators and the control.

The highest mean root length of 2.62 cm was recorded in T_{11} which was on par with T_{15} (2.50 cm) and T_{14} (2.42 cm). Root length was considerably low in the lower concentrations of growth regulators and the control.

4.2.3.4. Effect of kinetin and IBA on protocorm differentiation and seedling growth

The effect of kinetin and IBA on *in vitro* protocorm differentiation and seedling growth have been analysed (Table 4.2.3.4.).

Significantly early protocorm differentiation and development of significantly larger mature seedlings were observed in T_{16} (kinetin 8 mg l^{-1} + IBA 6 mgl^{-1}) and T_{11} (kinetin 6 mgl^{-1} + IBA 4 mgl^{-1}).

First leaf primordium developed in 52.00 days and first root primordium differentiated in 111.83 days in T_{16}. In T_{11} leaf and root primordia appeared in 55.83 and 116.17 days, respectively. These two superior treatments were on par for both the characters considered. Significant difference could not be observed for both the characters in several treatments, particularly those involving lower concentrations of growth regulators.

With respect to number of leaves, again T_{16} and T_{11} were significantly superior and on par with each other, producing 5.50 and 5.17 leaves, respectively per seedling. The treatment T_{14} (kinetin 8 mgl^{-1} + IBA 2 mgl^{-1}) and T_{15} (kinetin 8 mgl^{-1} + IBA 4 mgl^{-1}) gave rise to 4.67 leaves each and T_{10} (kinetin 6 mgl^{-1} + IBA 2 mgl^{-1}) recorded 4.50 leaves.

Table 4.2.3.4. Effect of kinetin and IBA on *in vitro* protocorm differentiation and growth of seedlings in *Dendrobium*

Medium : MS (½ strength) + sucrose 30 g l^{-1} + agar 5.5 g l^{-1} + CW 200 ml l^{-1}

Combination : $P_4 \times P_1$

Treatment	Concentration (mg l^{-1}) of		Days to development of		Seedling characters six months after inoculation			
	Kinetin	IAA	First leaf primordium	First root primordium	No. of leaves	Mean length of leaf (cm)	No. of roots	Mean length of root (cm)
T_1	0	0	57.33	135.83	3.50	1.72	3.50	1.30
T_2	2	2	73.17	144.17	3.33	1.60	3.67	1.33
T_3	2	4	73.83	147.83	2.83	1.58	3.33	1.38
T_4	2	6	72.00	146.50	3.33	1.58	3.17	1.47
T_5	2	8	73.33	148.50	3.33	1.58	3.67	1.68
T_6	4	2	63.33	134.00	4.17	2.90	4.17	2.15
T_7	4	4	71.67	144.83	3.33	1.67	3.67	1.52
T_8	4	6	72.33	145.17	3.50	1.65	3.50	1.48
T_9	4	8	73.17	148.67	3.17	1.68	3.50	1.50
T_{10}	6	2	62.83	130.67	4.50	2.73	4.67	2.17
T_{11}	6	4	55.83	116.17	5.17	3.23	5.50	2.45
T_{12}	6	6	70.83	143.17	4.00	1.72	3.67	1.58
T_{13}	6	8	69.50	145.00	3.50	1.65	3.50	1.57
T_{14}	8	2	59.50	132.67	4.67	2.52	4.83	2.17
T_{15}	8	4	61.83	133.17	4.67	2.62	4.83	2.20
T_{16}	8	6	52.00	111.83	5.50	3.13	5.33	2.48
T_{17}	8	8	72.17	142.33	3.33	1.68	4.33	2.10
SE_m			1.448	1.637	0.289	0.082	0.287	0.081
CD(0.05)			4.074	4.602	0.774	0.230	0.823	0.217

The treatments T_{11} and T_{16} produced significantly longer leaves measuring 3.23 cm and 3.13 cm, respectively. The treatment T_6 (kinetin 4 mgl^{-1} + IBA 2 mgl^{-1}), recording a leaf length of 2.90 cm, was found to be on par with T_{16}.

The treatment T_{11} recorded the highest number of roots per seedling (5.50) which was on par with T_{16} (5.33), T_{14} and T_{15} (4.83 each). Significantly superior values for root length were recorded by T_{16} (2.48 cm) and T_{11} (2.45 cm). The other treatments expressing high root lengths were T_{15} (kinetin 8 mgl^{-1} + IBA 4 mgl^{-1}), T_{14} (kinetin 8 mgl^{-1} + IBA 2 mgl^{-1}) and T_{10} (kinetin 6 mgl^{-1} + IBA 2 mgl^{-1}) being 2.20 cm for T_{15} and 2.17 cm each for T_{14} and T_{10}.

4.2.3.5. Effect of organic additives on protocorm differentiation and seedling growth

Effect of organic additives, namely coconut water (CW), peptone (P) and banana pulp (BP) on protocorm differentiation and seedling growth under aseptic culture was studied (Table 4.2.3.5.).

Out of the nine treatments tried with organic additives, CW 200 ml l^{-1} proved to be the best, both in terms of early differentiation of protocorms and development of seedlings of a better size (Plate IV). All treatments with organic additives were found to give earlier and better growth compared to the control.

Significant difference could not be observed among the different treatments regarding the time taken for the development of the first leaf primordium.

Table 4.2.3.5. Effect of organic additives on *in vitro* protocorm differentiation and seedling growth in *Dendrobium*

Medium : MS (½ strength) + sucrose 30 g l^{-1} + agar 5.5 g l^{-1} + BA 8 mg l^{-1} + IAA 4 mg l^{-1}

Combination : $P_4 \times P_1$

Sl. No.	Concentration of organic additives	Days to development of		Seedling characters six months after inoculation			
		First leaf primordium	First root primordium	No. of leaves	Mean length of leaf (cm)	No. of roots	Mean length of root (cm)
1.	Nil	57.33	136.00	3.67	1.75	3.50	1.30
2.	Coconut water 100 ml l^{-1}	52.30	113.17	5.33	2.67	4.83	1.98
3.	Coconut water 200 ml l^{-1}	50.67	106.67	5.50	3.20	5.67	2.47
4.	Coconut water 300 ml l^{-1}	58.50	127.17	4.00	1.95	3.83	1.72
5.	Peptone 0.5 g l^{-1}	56.67	119.83	4.67	2.52	4.50	2.02
6.	Peptone 1.0 g l^{-1}	62.17	137.50	3.50	2.97	3.83	2.27
7.	Banana pulp 25 g l^{-1}	58.00	121.00	4.83	1.92	4.50	1.60
8.	Banana pulp 50 g l^{-1}	60.33	128.83	4.00	2.65	3.67	2.03
9.	Banana pulp 75 g l^{-1}	54.67	138.33	3.33	3.03	3.33	2.25
	SE_m	3.841	1.493	0.278	0.082	0.289	0.090
	CD(0.05)	NS	4.231	0.804	0.220	0.831	0.252

In CW 200 ml l^{-1}, root primordia differentiated in 106.67 days, which was significantly early as compared to all the other treatments. The second best treatment was CW 100 ml l^{-1}, leading to differentiation of root primordia in 113.17 days. Peptone 0.5 g l^{-1} resulted in root initiation in 119.83 days.

With respect to the number of leaves, CW 200 ml l^{-1}, CW 100 ml l^{-1} and BP 25 g l^{-1} were found to be significantly superior and on par with each other, recording 5.50, 5.33 and 4.83 leaves, respectively per seedling. Peptone 0.5 g l^{-1} ranked next, producing 4.67 leaves.

The treatment CW 200 ml l^{-1} recorded the highest leaf length of 3.20 cm which was on par with BP 75 gl^{-1}, recording a leaf length of 3.03 cm. Peptone 1.0 gl^{-1} led to the development of leaves of an average length of 2.97 cm.

Number of roots produced was significantly high in CW 200 ml l^{-1} (5.67). The treatment CW 100 ml l^{-1} recorded 4.83 roots and peptone 0.5 gl^{-1} and BP 25 gl^{-1} recorded 4.50 roots each, all the three treatments being on par with each other.

Significantly superior root lengths were recorded in the treatments CW 200 ml l^{-1} (2.47 cm), peptone 1.0 gl^{-1} (2.27 cm) and BP 75 gl^{-1} (2.25 cm) which were on par with each other. Average length of root was next to these three treatments in BP 50 gl^{-1} (2.03 cm) and peptone 0.5 gl^{-1} (2.02 cm).

4.2.3.6. Effect of sucrose on seed germination and protocorm development

The effect of sucrose at different concentrations on seed germination and protocorm development have been analysed (Table 4.2.3.6.).

No significant difference was observed in germination percentage between the different treatments.

Early initiation of germination as well as the development of protocorm and chlorophyll were observed in sucrose 5 gl^{-1} followed by sucrose 10 gl^{-1}. For initiation of germination and the development of protocorm, both the treatments were found to be on par. Sucrose 5 gl^{-1} initiated germination in 10.83 days and developed protocorms in 23.33 days. Sucrose 10 gl^{-1} required 11.83 days for germination initiation and 24.67 days for protocorm development.

Significantly early greening was observed in sucrose 5 gl^{-1}, exhibiting chlorophyll development in 33.83 days whereas sucrose 10 gl^{-1} took 35.50 days for greening.

Sucrose 30 gl^{-1} showed significantly early development of first leaf (51.67 days) and root (115.17 days) primordia. Sucrose 20 gl^{-1} followed the former, completing leaf initiation in 58.83 days and root initiation in 128.17 days.

Table 4.2.3.6. Effect of sucrose on *in vitro* seed germination and protocorm development in *Dendrobium*

Medium : MS (½ strength) + agar 5.5 g l^{-1} + CW 200 ml l^{-1} + BA 8 mg l^{-1} + IAA 4 mg l^{-1}

Combination : $P_4 \times P_1$

Sl. No.	Concentration of sucrose (g l^{-1})	Seed germination (%)	No. of days to germination initiation	No. of days taken for development of			
				Protocorm	Chlorophyll	First leaf primordium	First root primordium
1.	5	58.27	10.83	23.33	33.83	66.17	136.67
2.	10	55.40	11.83	24.67	35.50	63.00	135.33
3.	20	57.50	13.83	27.50	37.50	58.83	128.17
4.	30	57.30	14.67	29.50	38.83	51.67	115.17
	SE_m	1.760	0.389	0.464	0.550	1.109	1.854
	CD(0.05)	NS	1.143	1.362	1.631	3.293	5.457

4.2.3.7. Effect of charcoal on seed germination and protocorm development

There was no significant variation between different concentrations of charcoal regarding percentage of seed germination (Table 4.2.3.7.).

Significant difference could be observed between treatments with respect to the time taken for germination initiation. Early development of protocorm in 20.17 days was observed in charcoal 0.5 gl^{-1}, which was on par with the control (20.83 days).

Number of days taken for the greening of protocorm also followed the same trend as the duration taken for development of protocorm. Significantly early development of chlorophyll in protocorm was observed in charcoal 0.5 gl^{-1} (25.00 days). This was found to be on par with the control, turning green in 25.33 days. Both protocorm development and greening of protocorm were found to slow down with progressive increase in the concentration of charcoal from 1.0 gl^{-1} to 2.0 gl^{-1}.

Charcoal 1.0 gl^{-1} excelled all the other treatments regarding the time taken for the development of first leaf primordium (48.17 days) (Plate V). Charcoal 1.5 g^{-1} and 0.5 gl^{-1} were found to be on par, taking 54.67 and 56.33 days, respectively for leaf initiation.

Significantly early development of first root primordium was observed in charcoal 1.0 gl^{-1} (103.17 days). Charcoal 1.5 gl^{-1} and 0.5 gl^{-1}

Table 4.2.3.7. Effect of charcoal on *in vitro* seed germination and protocorm development in *Dendrobium*

Medium : MS (½ strength) + sucrose 30 g l^{-1} + agar 5.5 g l^{-1} + CW 200 ml l^{-1} + BA 8 mg l^{-1} + IAA 4 mg l^{-1}

Combination : $P_7 \times P_1$

Sl. No.	Concentration of charcoal (g l^{-1})	Seed germination (%)	No. of days taken for development of			
			Protocorm	Chlorophyll	First leaf primordium	First root primordium
1.	0	54.17	20.83	25.33	62.67	129.50
2.	0.5	53.85	20.17	25.00	56.33	113.67
3.	1.0	56.32	22.50	27.83	48.17	103.17
4.	1.5	53.60	26.33	34.33	54.67	109.33
5.	2.0	55.58	28.17	37.50	62.33	114.50
	SE_m	1.630	0.394	0.496	1.058	1.571
	CD(0.05)	NS	1.149	1.446	3.084	4.578

took 109.33 days and 113.67 days, respectively for root initial development and were observed to be on par with each other.

Lower concentration of charcoal (0.5 gl^{-1}) and the control promoted early development of protocorm and chlorophyll, whereas higher concentration of charcoal (2.0 gl^{-1}) promoted early differentiation of leaf and root primordia.

4.2.3.8. Effect of charcoal on *in vitro* growth of seedlings

The effect of charcoal on *in vitro* growth of seedlings were studied (Table 4.2.3.8.).

Significantly superior seedling height was recorded in charcoal 0.5 gl^{-1} (3.83 cm) and charcoal 1.0 gl^{-1} (3.82 cm), which were found to be on par.

Regarding the number of leaves, charcoal 1.0 gl^{-1} and 1.5 gl^{-1} were found to be on par, resulting in 4.00 and 3.67 leaves per seedling, respectively.

Charcoal 1.0 gl^{-1} led to the development of leaves of length 2.50 cm. Leaf length was high in charcoal 1.5 gl^{-1} (2.33 cm) and charcoal 2.0 gl^{-1} (2.21 cm). All the three treatments were on par with each other.

Root production also followed the same trend as leaf growth. The highest number of roots was observed in charcoal 1.0 gl^{-1} (3.83) followed by charcoal 1.5 gl^{-1} (3.67) and charcoal 2.0 gl^{-1} (3.50), all the three treatments being on par.

Table 4.2.3.8. Effect of charcoal on *in vitro* growth of seedlings in *Dendrobium*

Medium : MS (½ strength) + sucrose 30 g l^{-1} + agar 5.5 g l^{-1} + CW 200 ml l^{-1} + BA 8 mg l^{-1} + IAA 4 mg l^{-1}

Combination : P_7 x P_1

Sl. No.	Concentration of charcoal (gl^{-1})	Seedling characters six months after inoculation				
		Height (cm)	No. of leaves	Mean length of leaf (cm)	No. of roots	Mean length of root (cm)
1.	0	2.33	1.67	1.88	1.33	1.48
2.	0.5	3.83	3.00	2.06	2.00	1.63
3.	1.0	3.82	4.00	2.50	3.83	2.30
4.	1.5	3.40	3.67	2.33	3.67	2.12
5.	2.0	3.23	2.67	2.21	3.50	1.90
	SE_m	0.094	0.326	0.102	0.294	0.119
	CD (0.05)	0.283	0.951	0.297	0.861	0.348

Root length was the highest when charcoal 1.0 gl^{-1} (2.30 cm) and charcoal 1.5 gl^{-1} (2.12 cm) were used. The two treatments were on par with each other.

Significant difference in the growth of seedlings at six months after inoculation could be observed in the different concentrations of charcoal. Charcoal at 1.0 gl^{-1} and 1.5 gl^{-1} were found to be the best while the control resulted in comparatively poor seedling growth (Plate V).

4.3. Evaluation of hybrid material

4.3.1. *Ex vitro* establishment of hybrids

4.3.1.1. Standardisation of techniques for *ex vitro* survival and acclimatization

In vitro raised *Dendrobium* hybrid seedlings were deflasked and planted out when they had developed a minimum of 3-4 leaves and 3-5 roots. Trials were conducted for *ex vitro* establishment of deflasked seedlings (Table 4.3.1.1.).

Direct planting out into the green house (T_1) under 50 per cent shade (using black agro shade net) with misting for 15 minutes thrice daily proved to be the most disadvantageous for *ex vitro* establishment. Only eight per cent seedlings could withstand direct planting out at the end of two weeks. Further growth was arrested and seedlings underwent desiccation and wilting, resulting in near complete mortality (2 % survival) after four weeks.

Table 4.3.1.1 Effect of *ex vitro* establishment techniques on survival of seedlings in *Dendrobium*

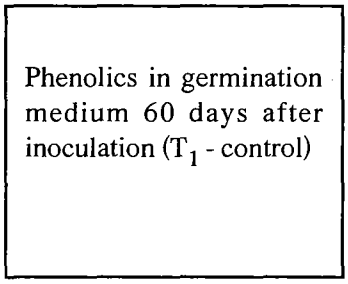
Treatments		Seedling survival (%) after	
		2 weeks	4 weeks
T_1	Direct planting out into green house	8	2
T_2	Planting out into green house under enhanced shade and controlled irrigation	18	10
T_3	Planting out into humidity chamber	98	94

Number of seedlings per treatment - 50

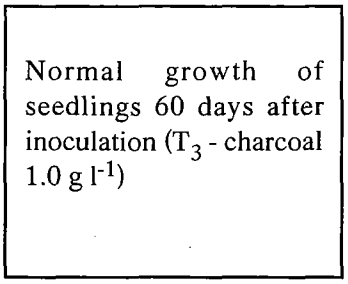
Plate V

Refinement of *in vitro* culture medium (Contd...)

Effect of charcoal on *in vitro* embryo development in P_7 x P_1

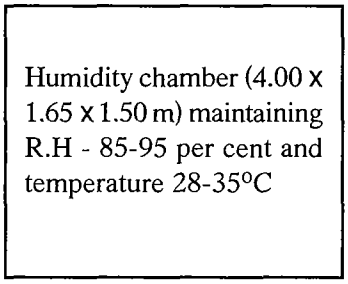


Phenolics in germination medium 60 days after inoculation (T_1 - control)

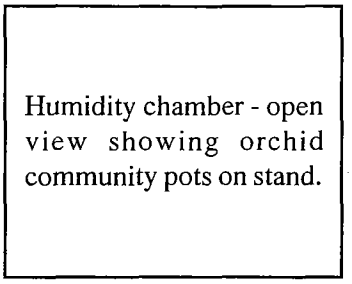


Normal growth of seedlings 60 days after inoculation (T_3 - charcoal 1.0 g l^{-1})

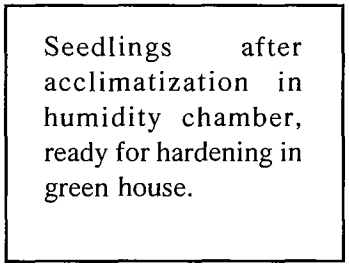
Acclimatization and *ex vitro* establishment of seedlings



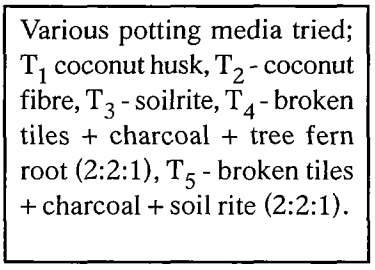
Humidity chamber (4.00 x 1.65 x 1.50 m) maintaining R.H - 85-95 per cent and temperature 28-35°C



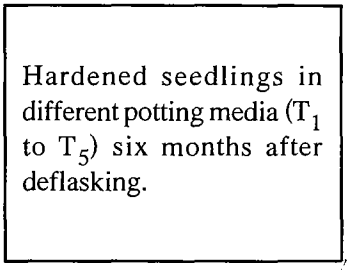
Humidity chamber - open view showing orchid community pots on stand.



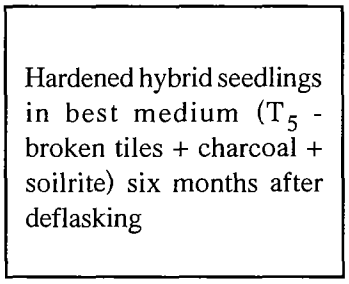
Seedlings after acclimatization in humidity chamber, ready for hardening in green house.



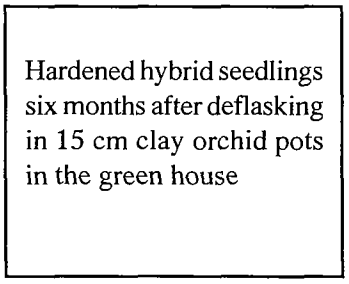
Various potting media tried; T_1 coconut husk, T_2 - coconut fibre, T_3 - soilrite, T_4 - broken tiles + charcoal + tree fern root (2:2:1), T_5 - broken tiles + charcoal + soil rite (2:2:1).



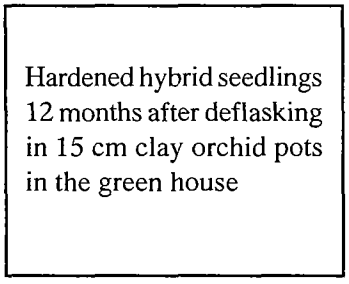
Hardened seedlings in different potting media (T_1 to T_5) six months after deflasking.



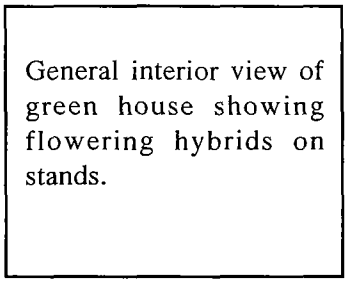
Hardened hybrid seedlings in best medium (T_5 - broken tiles + charcoal + soilrite) six months after deflasking



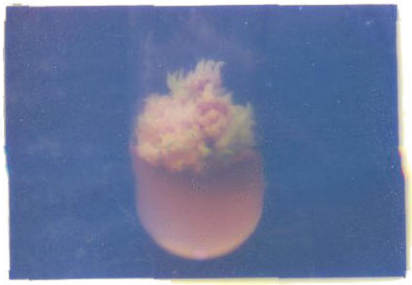
Hardened hybrid seedlings six months after deflasking in 15 cm clay orchid pots in the green house

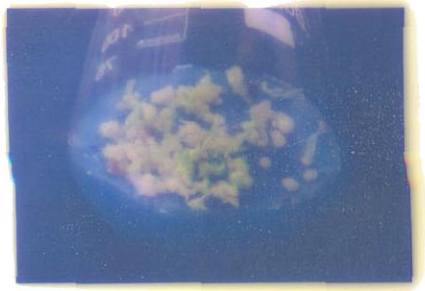


Hardened hybrid seedlings 12 months after deflasking in 15 cm clay orchid pots in the green house



General interior view of green house showing flowering hybrids on stands.





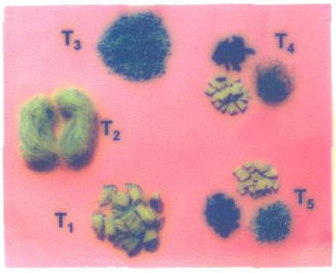


Plate V

Planting out into the green house with 75 per cent shade under black agro shade net (T_2) with controlled irrigation (rain/mist irrigation proofing using clear plastic sheet and artificial irrigation once in three hours using hand sprayer with fine mist nozzle), recorded 18 per cent seedling survival after two weeks which was further reduced to 10 per cent by the end of four weeks.

Out of the three different techniques tried for *ex vitro* establishment of *Dendrobium* hybrid seed progeny, planting out into the humidity chamber (T_3) was found to be the best (Plate V). Under conditions of high relative humidity (85-95 %) and controlled irrigation (every alternate day using a hand sprayer with fine mist nozzle) inside the chamber, the seedlings registered 98 per cent survival after two weeks and 94 per cent survival after four weeks.

Since T_3 was found to be the best treatment, a post transplantation four weeks period of humidity acclimatization in a humidity controlled chamber was followed for all further routine transplanting.

4.3.1.2. Standardisation of potting media

Effects of various potting media on survival and post transplantation growth of *Dendrobium* hybrid seed progeny have been analysed after six months (Table 4.3.1.2.; Plate V).

1. Survival after 6 months

The five different potting media tried were T_1 (coconut husk), T_2 (coconut fibre), T_3 (Soilrite), T_4 (broken tiles + charcoal + Soilrite) and

Table 4.3.1.2. Effect of potting media on survival and *ex vitro* growth of seedlings in *Dendrobium*

Material : $P_3 \times P_5$

Treatments	Potting media	Final survival (%)	Seedling characters at deflasking (initial) and six months after transplanting (final)											
			No. of shoots		No. of leaves		Mean length of leaf (cm)		Mean width of leaf (cm)		No. of roots		Mean length of root (cm)	
			Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
T_1	Coconut husk	66	1	2.03 (103.00)*	3.40	5.37 (57.94)	2.32	3.44 (48.28)	0.52	0.95 (82.69)	4.42	5.79 (31.00)	1.62	6.54 (303.70)
T_2	Coconut fibre	80	1	1.21 (21.00)	3.56	4.47 (25.56)	2.64	3.63 (37.50)	0.53	0.74 (39.62)	4.34	6.30 (45.16)	1.70	8.78 (416.47)
T_3	Soilrite	72	1	1.78 (78.00)	3.42	5.22 (52.63)	2.66	4.58 (72.18)	0.48	0.82 (70.83)	4.46	7.19 (61.21)	1.54	7.23 (369.48)
T_4	Broken tiles + charcoal + Soilrite	88	1	2.30 (130.00)	3.66	6.15 (68.03)	2.45	4.78 (95.10)	0.55	1.24 (125.45)	4.64	11.17 (140.73)	1.60	7.92 (395.00)
T_5	Broken tiles + charcoal + fern root	92	1	2.26 (126.00)	3.64	6.05 (66.21)	2.46	4.43 (80.08)	0.49	1.00 (104.08)	4.58	10.76 (134.93)	1.65	7.88 (377.58)
	SE_m		—	0.071	—	0.104	—	0.083	—	0.062	—	0.768	—	0.188
	CD (0.05)		—	0.218	—	0.325	—	0.268	—	0.195	—	2.314	—	0.548

* Figures in parenthesis indicate percentage increase

T_5 (broken tiles + charcoal + fern root). Out of these, T_1 recorded a survival percentage of 66, T_2 recorded 80 per cent, T_3 showed 72 per cent, T_4 registered 88 per cent and T_5 recorded 92 per cent at the end of six months after transplanting.

Subsequent performance of seedlings with respect to increase in number and size of shoots, leaves and roots also varied with the composition of the medium.

2. Number of shoots per seedling

The treatments T_4 and T_5 resulted in the highest increase in the number of shoots per seedling after six months growth, recording an enhancement of 130 and 126 per cent, respectively. Number of shoots increased from one to 2.30 in T_4 and from one to 2.26 in T_5 at the end of six months from planting out. These two superior treatments were followed by T_1, T_3 and T_2 in the descending order, recording significantly different mean values of 2.03, 1.78 and 1.21, accounting for 103, 78 and 21 per cent enhancement, respectively for the character.

3. Number of leaves per seedling

Increase in the number of leaves per seedling was significantly high in T_4 and T_5, both being on par with each other. The treatment T_4 recorded an increase of 68.03 per cent, *viz.,* from 3.66 to 6.15 and T_5 recorded an enhancement of 66.21 per cent, *viz.,* from 3.64 to 6.05 at the end of six months from transplanting. The treatment T_1 recording

57.94 per cent increase (from 3.40 to 5.37) and T_3 recording 52.63 per cent increase (from 3.42 to 5.22) in the number of leaves per seedling came next. The treatment T_2 appeared to be the least capable of supporting increase in leaf number, the enhancement being 25.56 per cent, *viz.,* from 3.56 to 4.47 in six months.

4. Length of leaf

Increase in leaf length after six months of planting out was significantly high in T_4 (95.10 %) and T_3 (72.18 %), both being on par with each other. Mean leaf length increased from 2.45 to 4.78 cm in T_4 and from 2.66 to 4.58 cm in T_3. The treatment T_5 was on par with T_3, recording an increase of 80.08 per cent (from 2.46 to 4.43 cm) after six months of planting out. Leaf length registered after six months of planting out in T_2 (3.63 cm) and T_1 (3.44 cm) were on par with each other, recording an enhancement of 37.50 and 48.28 per cent, respectively.

5. Width of leaf

Increase in leaf width was significantly high in T_4 (125.45 %), where it increased to 1.24 cm from 0.55 cm in six months. The treatments T_5, T_1 and T_3 were on par with each other for mean leaf width after six months of planting out. Leaf width after six months was 1.00 cm (an increase of 104.08 %) in T_5, 0.95 cm (an increase of 82.69 %) in T_1 and 0.82 cm (an increase of 70.83 %) in T_3. The treatment T_2 turned out to be significantly inferior for enhancement in leaf width, recording the lowest increase of 39.62 per cent *viz.,* from 0.53 cm to 0.74 cm in six months.

6. Number of roots per seedling

Number of roots per seedling recorded in T_4 after six months of planting out showed an increase of 140.73 per cent (from 4.64 to 11.17) and was on par with T_5 registering an increase of 134.93 per cent (from 4.58 to 10.76). The percentage increase in number of roots observed in T_3, T_2 and T_1 after six months from deflasking were on par, being 61.21 per cent, 45.16 per cent and 31.00 per cent, respectively.

7. Length of root

Length of root recorded a phenomenal increase of 416.47 per cent in T_2, *viz.,* from 1.70 to 8.78 cm, being superior to all other treatments. The treatments T_4 and T_5 were on par with each other; the increase was 395.00 per cent (from 1.60 to 7.92 cm) in T_4 and 377.58 per cent (from 1.65 to 7.88 cm) in T_5. The treatment T_3 was on par with T_5, recording a mean root length of 7.23 cm (an increase of 369.48 %) after six months of deflasking. The mean root length recorded in T_1 was significantly low, being 6.54 cm, accounting for an increase of 303.70 per cent at six months after transplanting.

The treatment T_4 was found to favour high survival and well balanced growth of seedlings after planting out, closely followed by T_5 (Plate V). Considering the easy availability of Soilrite, one of the ingredients of T_4 (broken tiles, charcoal and Soilrite) as compared to fern root in T_5 (broken tiles, charcoal and fern root), the former potting medium (T_4) was selected for all further routine transplanting operations.

4.3.2 *Ex vitro* survival during acclimatization

In vitro raised seedlings obtained from the 69 successful hybrid combinations were deflasked and planted out into a potting medium comprising of broken tiles + charcoal + Soilrite when the plantlets had developed a minimum of 3-4 leaves and 3-5 roots. For humidity acclimatization, the seedlings were nursed in a humidity chamber providing high relative humidity (85-95 %) and controlled irrigation (every alternate day using a hand sprayer with fine mist nozzle). Survival during acclimatization at two weeks and four weeks after planting out into the humidity chamber have been recorded (Tables 4.3.2.1. and 4.3.2.2.).

4.3.2.1 Survival at two weeks after transplanting

Survival at two weeks after planting out ranged from 14.2 per cent in P_{13} selfed to 98.6 per cent in $P_3 \times P_5$. Percentage of survival was high in eight combinations *viz.*, $P_5 \times P_{12}$ and $P_{11} \times P_2$ (97.3), $P_1 \times P_8$, $P_3 \times P_6$, $P_7 \times P_2$ and $P_{11} \times P_4$ (96.0) and $P_1 \times P_3$ and $P_6 \times P_{12}$ (95.5), at this stage. Percentage of survival was low (25.2) in P_{14} selfed. Excepting for combinations using the two species (P_{13} and P_{14}) as female parents, all others recorded more than 80.0 per cent survival at two weeks after planting out. At the same time, in the eight combinations where these two species (P_{13} and P_{14}) were used as female parents, survival was less than 40.0 per cent.

The mean percentage of survival at two weeks after planting out among the 14 genotypes when treated as female parents, ranged from 29.0

Table 4.3.2.1. *Ex vitro* survival (per cent) during acclimatization of *Dendrobium* hybrid seed progeny two weeks after transplanting

♀ \ ♂	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_{13}	P_{14}	$\bar{x}$	C.V. (%)
P_1	90.2	93.1	95.5	92.5		94.0		96.0	85.3	83.8					91.0	5
P_2	88.5		90.8	93.8		92.5	89.1		86.4						89.7	3
P_3	93.2		94.5	95.0	98.6	96.0	95.5	94.0		88.9					94.1	3
P_4	94.5		92.0	95.5	90.3	93.2									92.8	2
P_5	94.5					90.5	87.0	94.8				97.3			92.4	4
P_6	95.3		93.5	92.0					87.0			95.5			92.4	4
P_7	95.1	96.0	90.0	90.3			88.5		86.5						90.8	4
P_8	92.3	93.0					84.8		82.0						87.8	6
P_9	82.6				86.5	87.8	91.7								86.5	4
P_{10}	93.5	91.5					95.5								93.0	2
P_{11}		97.3		96.0							90.5	88.0			92.8	5
P_{12}				92.5	93.8						91.3				92.0	1
P_{13}								34.6	39.5				14.2		29.0	46
P_{14}	32.3	31.8	36.3			30.0								25.2	30.8	13
$\bar{x}$	86.2	83.5	84.3	93.1	91.8	83.1	89.9	79.5	77.5	85.5	91.0	93.3	14.2	25.2		
C.V.(%)	21	31	25	2	6	28	5	39	24	4	1	5	0	0		

Table 4.3.2.2. *Ex vitro* survival (per cent) during acclimatization of *Dendrobium* hybrid seed progeny four weeks after transplanting

♀ \ ♂	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_{13}	P_{14}	$\bar{x}$	C.V. (%)
P_1	82.4	85.5	90.0	86.3		88.0		90.0	78.1	76.1					84.4	6
P_2	78.3		82.5	84.5		86.5	77.0		75.4						80.3	5
P_3	82.6		87.0	89.5	94.5	90.0	88.0	84.5		78.2					86.5	6
P_4	85.0		84.0	86.0	82.0	85.0									84.4	2
P_5	87.0					82.0	79.0	88.2				94.5			86.0	7
P_6	88.5		86.5	85.0					79.5			93.8			86.2	6
P_7	89.0	91.5	84.5	82.6			83.0		81.2						85.0	5
P_8	83.7	86.5					76.1		74.5						79.8	7
P_9	74.0				76.5	78.2	80.6								77.0	3
P_{10}	85.5	82.3					88.5								85.0	4
P_{11}		93.8		90.0							86.2	84.0			88.3	5
P_{12}				82.5	84.3						80.0				82.0	2
P_{13}								23.2	26.0				8.1		19.0	51
P_{14}	20.1	18.5	27.1			16.0								12.0	18.6	30
$\bar{x}$	77.5	75.8	77.1	85.5	84.0	75.0	81.6	71.3	68.8	77.0	83.0	90.3	8.1	12.0		
C.V.(%)	25	38	29	3	7	35	6	45	31	2	5	6	0	0		

in P_{13} to 93.0 in P_{10} with a variation of 46.0 per cent and 2.0 per cent, respectively. High survival of above 85.0 per cent was observed in the 12 varieties (P_1 to P_{12}) whereas it was low in the two species, (29.0 % in P_{13} and 30.8 % in P_{14}). Variability for the character was found to be low (less the 10.0 %) in the 12 varieties and high (more than 10.0 %) in the two species.

When the 14 parental genotypes were used as male parents, the mean percentage of survival at two weeks after planting out ranged from 14.2 in P_{13} to 93.3 in P_{12} with a variation of 0.0 per cent (single entry) and 5.0 per cent, respectively. High survival of above 75.0 per cent was observed in all the 12 varieties. High variability of greater than 10.0 per cent was observed in the six varieties P_1, P_2, P_3, P_6, P_8 and P_9 whereas low variability of less than 10.0 per cent was recorded in the remaining six varieties.

4.3.2.2 Survival at four weeks after transplanting

Survival at four weeks after planting out ranged from 8.1 per cent in P_{13} selfed to 94.5 per cent in both P_3 x P_5 and P_5 x P_{12}. Survival percentage was high in seven combinations *viz.*, P_6 x P_{12} and P_{11} x P_2 (93.8), P_7 x P_2 (91.5) and P_1 x P_3, P_1 x P_8, P_3 x P_6 and P_{11} x P_4 (90.0). Percentage of survival was low (12.0) in P_{14} selfed. In the 61 hybrid combinations where a variety (P_1 to P_{12}) was used as the female parent, survival at four weeks after planting out was more than 70.0 per cent. In the eight combinations where a species was used as the female parent, survival was less than 30.0 per cent.

Percentage survival at four weeks after planting out among the 14 genotypes when considered as female parents ranged from 18.6 in P_{14} to 88.3 in P_{11} with a variation of 30.0 per cent and 5.0 per cent, respectively. Percentage survival was high in the 12 varieties (P_1 to P_{12}) recording values above 75.0 per cent, whereas it was low in the two species registering 19.0 per cent in P_{13} and 18.6 per cent in P_{14}. Variability for the character was observed to be low (less than 10.0 %) in the 12 varieties and high (more than 30.0 %) in the two species.

When the 14 parental genotypes were used as male parents, the mean percentage of survival at four weeks after planting out ranged from 8.1 in P_{13} to 90.3 in P_{12} with a variation of 0.0 per cent (single entry) and 6.0 per cent, respectively. High survival of above 70.0 per cent was observed for all the 12 varieties. Low variability of less than 10.0 per cent was observed for the six varieties *viz.*, P_4, P_5, P_7, P_{10}, P_{11} and P_{12} whereas it was high (above 10.0 %) for P_1, P_2, P_3, P_6, P_8 and P_9.

4.3.3 *Ex vitro* survival during hardening

Survival of *Dendrobium* hybrid seed progeny during hardening recorded at one month and three months after acclimatization (viz. after removal from the humidity chamber) have been analysed (Tables 4.3.3.1. and 4.3.3.2.).

4.3.3.1 Survival one month after acclimatization

Survival one month after acclimatization ranged from 5.3 per cent in P_{13} selfed to 94.1 per cent in P_5 x P_{12}. Percentage of survival was

Table 4.3.3.1. *Ex vitro* survival (per cent) during hardening of *Dendrobium* hybrid seed progeny one month after acclimatization

♀ \ ♂	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_{13}	P_{14}	$\bar{x}$	C.V. (%)
P_1	82.2	85.2	89.5	86.8		88.3		90.5	76.5	76.5					84.0	7
P_2	78.5		82.3	84.3		86.8	77.5		75.5						80.3	5
P_3	82.8		86.8	89.5	93.0	90.5	88.5	84.3		78.0					86.3	6
P_4	84.5		83.4	85.0	82.8	84.5									83.6	1
P_5	86.6					82.5	79.5	87.0				94.1			85.6	7
P_6	88.5		85.0	85.8					78.0			93.0			85.8	6
P_7	89.0	90.5	84.5	81.0			82.0		80.5						84.3	5
P_8	82.8	86.3					76.8		73.0						79.3	7
P_9	73.2				75.5	77.0	79.3								76.0	3
P_{10}	85.5	81.5					88.5								84.7	4
P_{11}		93.0		89.5							86.0	84.6			88.0	4
P_{12}				81.7	84.5						80.5				81.7	3
P_{13}								20.0	21.5				5.3		15.3	59
P_{14}	18.2	15.1	26.3			15.5								8.2	16.4	40
$\bar{x}$	70.6	75.0	76.4	85.0	83.5	74.6	81.3	70.3	67.2	77.0	83.0	90.3	5.3	8.2		
C.V.(%)	41	40	29	4	9	36	6	48	34	2	5	6	0	0		

Table 4.3.3.2. *Ex vitro* survival (per cent) during hardening of *Dendrobium* hybrid seed progeny three months after acclimatization

♀ \ ♂	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_{13}	P_{14}	$\bar{x}$	C.V. (%)
P_1	80.0	84.4	88.0	84.2		87.1		88.0	76.0	75.0					83.0	6
P_2	78.2		81.1	83.2		86.5	75.0		73.0						79.3	6
P_3	81.5		85.2	87.5	92.5	88.2	87.0	84.0		75.0					84.9	6
P_4	84.0		82.3	84.0	81.4	84.0									83.0	2
P_5	86.0			-		81.0	78.5	87.0				93.0			85.0	7
P_6	87.5		85.0	84.0					75.0			92.5			84.6	7
P_7	88.0	89.0	83.0	81.0			81.0		79.5						83.5	5
P_8	82.0	86.1					75.5		73.0						79.0	8
P_9	73.0				72.2	76.0	76.0								74.0	2
P_{10}	85.5	80.2					87.0								84.0	4
P_{11}		92.5		89.0							85.0	83.3			87.3	5
P_{12}				78.2	81.2						77.3				78.7	3
P_{13}								17.0	15.0				0.0		10.7	87
P_{14}	16.3	12.0	25.0			11.0								0.0	12.8	71
$\bar{x}$	76.4	73.8	75.6	83.8	81.5	73.1	79.9	69.0	65.2	75.0	81.0	89.3	0.0	0.0		
C.V.(%)	37	41	30	4	10	38	7	50	38	0	7	6	—	—		

found to be high in six combinations *viz.*, P_3 x P_5, P_6 x P_{12} and P_{11} x P_2 (93.0) and P_1 x P_8, P_3 x P_6 and P_7 x P_2 (90.5), at this stage. A low percentage of survival of 8.2 was noted in P_{14} selfed. In all the 61 hybrid combinations where a variety (P_1 to P_{12}) was used as the female parent, survival one month after acclimatization was more than 70.0 per cent. In the eight combinations where a species (P_{13} and P_{14}) was used as the female parent, survival was less than 30.0 per cent.

The mean percentage of survival one month after acclimatization among the 14 genotypes when considered as female parents ranged from 15.3 in P_{13} to 88.0 in P_{11} with a variation coefficient of 59.0 per cent and 4.0 per cent, respectively. High survival of above 75.0 per cent was observed in the 12 varieties (P_1 to P_{12}). The two species registered low survival of 15.3 per cent in P_{13} and 16.4 per cent in P_{14}. Variability was observed to be less than 10.0 per cent for all the 12 varieties and more than 40.0 per cent for the two species.

When the 14 parental genotypes were used as male parents, the mean percentage of survival one month after acclimatization ranged from 5.3 in P_{13} to 90.3 in P_{12} with a variation of 0.0 per cent (single entry) and 6.0 per cent, respectively. High percentage survival of above 65.0 was observed for all the 12 varieties. Low variability of less than 10.0 per cent was observed for the six varieties *viz.*, P_4, P_5, P_7, P_{10}, P_{11} and P_{12} whereas high variability of greater than 20.0 per cent was noted for the six varieties *viz.*, P_1, P_2, P_3, P_6, P_8 and P_9.

4.3.3.2 Survival three months after acclimatization

Survival at three months after acclimatization ranged from zero in P_{13} selfed and P_{14} selfed to 93.0 per cent in $P_5 \times P_{12}$. Percentage of survival was high in $P_3 \times P_5$, $P_6 \times P_{12}$ and $P_{11} \times P_2$ (92.5). In the 61 hybrid combinations where a variety (P_1 to P_{12}) was used as the female parent, survival three months after acclimatization was always more than 70.0 per cent. In the eight combinations where a species was used as the female parent, survival was less than 30.0 per cent.

When the 14 parental genotypes were considered as female parents, percentage of survival ranged from 10.7 in P_{13} to 87.3 in P_{11} with a variation of 87.0 per cent and 5.0 per cent, respectively. All the 12 varieties registered high survival of above 70.0 per cent along with low variability of below 10.0 per cent. The two species registered low survival of 10.7 per cent and 12.8 per cent in P_{13} and P_{14}, respectively. High variability of 87.0 per cent in P_{13} and 71.0 per cent in P_{14} was recorded.

The mean percentage survival at three months after acclimatization among the 14 genotypes when considered as male parents ranged from 0.0 in P_{13} and P_{14} to 89.3 in P_{12} with a variability of 6.0 per cent. All the 12 varieties registered high percentage survival of above 65.0. Low variability of 10.0 per cent or less was noted in P_4, P_5, P_7, P_{10}, P_{11} and P_{12} and high variability of 30.0 per cent or more was recorded in P_1, P_2, P_3, P_6, P_8 and P_9.

4.3.4 Comparison of performance of hybrids six months after transplanting

The mean performance of *Dendrobium* hybrid seed progeny obtained from the 67 successful combinations six months after planting out based on different vegetative characters have been studied (Tables 4.3.4.1. to 4.3.4.6.). Significant differences among the hybrid combinations were observed for four out of the six biometric characters studied.

1. Number of shoots per seedling

The average number of shoots per seedling ranged from 1.2 in the four hybrids *viz.*, $P_5 \times P_8$, $P_8 \times P_2$, $P_{13} \times P_8$ and $P_{13} \times P_9$ to 2.7 in $P_{10} \times P_2$ (Table 4.3.4.1.). Twenty one combinations recorded values on par with the highest. The hybrid $P_9 \times P_1$ recorded 2.5 shoots per seedling and was on par with the highest. The other hybrids recording values on par with the highest were the four combinations *viz.*, $P_1 \times P_2$, $P_3 \times P_5$, $P_9 \times P_5$ and $P_{10} \times P_7$ recording 2.3 shoots per seedling, the 11 combinations *viz.*, P_1 selfed, $P_1 \times P_3$, $P_1 \times P_6$, $P_1 \times P_8$, $P_1 \times P_9$, $P_3 \times P_1$, P_3 selfed, $P_5 \times P_{12}$, $P_9 \times P_7$, $P_{10} \times P_1$ and $P_{14} \times P_1$ registering 2.2 shoots per seedling and the five combinations *viz.*, $P_2 \times P_1$, $P_3 \times P_4$, $P_3 \times P_8$, $P_6 \times P_1$ and $P_6 \times P_4$ recording 2.0 shoots per seedling. The remaining 41 out of the 67 combinations recorded mean values on par with the lowest, eight hybrids recording 1.3, 12 hybrids recording 1.5, 11 hybrids recording 1.7 and ten hybrids recording 1.8 shoots per seedling.

Table 4.3.4.1. Mean number of shoots per seedling of *Dendrobium* hybrid seed progeny six months after transplanting

♀ \ ♂	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_{13}	P_{14}
P_1	2.2	2.3	2.2	1.7		2.2		2.2	2.2	1.3				
P_2	2.0		1.5	1.7		1.5	1.3		1.5					
P_3	2.2		2.2	2.0	2.3	1.8	1.8	2.0		1.7				
P_4	1.5		1.8	1.7	1.3	1.5								
P_5	1.3					1.3	1.5	1.2				2.2		
P_6	2.0		1.7	2.0					1.8			1.3		
P_7	1.5	1.7	1.5	1.7			1.5		1.7					
P_8	1.8	1.2					1.7		1.8					
P_9	2.5				2.3	1.8	2.2							
P_{10}	2.2	2.7					2.3							
P_{11}		1.8		1.8							1.7	1.8		
P_{12}				1.5	1.5						1.3			
P_{13}								1.2	1.2					
P_{14}	2.2	1.5	1.7			1.3								

SE_m 0.282 CD (0.05) 0.783

2. Height of seedling

Height of seedling ranged from 2.78 cm in P_5 x P_8 to 9.18 cm in P_{10} x P_2 (Table 4.3.4.2.). Thirteen hybrids recorded values on par with the highest for height of seedling. These 13 hybrids comprised of P_9 x P_1 registering a height of 8.05 cm, P_3 x P_5 with 7.32 cm, P_{10} x P_7 with 7.10 cm, P_9 x P_5 with 7.03cm, P_3 x P_1 with 6.98 cm, P_3 x P_8 with 6.92 cm, P_1 x P_9 with 6.80 cm, P_3 selfed with 6.77 cm, P_{10} x P_1 with 6.72 cm, P_{14} x P_1 with 6.57 cm, P_1 x P_4 with 6.53 cm, P_5 x P_{12} with 6.32 cm and P_3 x P_4 with 6.28 cm. Values on par with the lowest were recorded by four hybrids *viz.*, P_5 x P_6 (3.03 cm), P_{13} x P_9 and P_8 x P_2 (2.88 cm) and P_{13} x P_8 (2.83 cm). Forty eight hybrids with on par mean values ranging from 3.23 to 6.15 cm fitted into an intermediate class.

3. Number of leaves per seedling

Mean number of leaves per seedling was observed to range from 4.67 in the three combinations P_5 x P_{12}, P_6 x P_{12} and P_{13} x P_9 to 6.83 in the hybrid P_{10} x P_1 (Table 4.3.4.3.). Mean number of leaves per seedling was high in P_8 x P_7, being 6.67. High mean value of 6.50 was observed in the three hybrids P_9 x P_1, P_9 x P_6 and P_{14} x P_1 followed by P_{10} x P_2 registering 6.33 leaves per seedling.

4. Length of leaf

Length of longest leaf ranged from 4.53 cm in P_{13} x P_9 to 9.23 cm in P_{10} x P_2 (Table 4.3.4.4.). Values on par with the highest were

Table 4.3.4.2. Mean height (cm) of seedling of *Dendrobium* hybrid seed progeny six months after transplanting

♀ \ ♂	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_{13}	P_{14}
P_1	5.45	5.27	4.93	6.53		4.57		6.02	6.80	3.82				
P_2	6.02		4.22	4.75		4.03	3.58		4.15					
P_3	6.98		6.77	6.28	7.32	5.50	5.27	6.92		4.77				
P_4	3.92		5.23	3.90	3.23	3.53								
P_5	3.30					3.03	3.67	2.78				6.32		
P_6	5.75		4.40	5.85					5.40			3.32		
P_7	4.35	4.85	3.88	4.20			3.88		4.35					
P_8	5.10	2.88					4.75		5.15					
P_9	8.05				7.03	5.38	6.15							
P_{10}	6.72	9.18					7.10							
P_{11}		5.15		5.13							4.68	5.73		
P_{12}				3.82	3.83						3.47			
P_{13}								2.83	2.88					
P_{14}	6.57	3.78	4.63			3.40								

SE_m 1.070 CD (0.05) 2.966

Table 4.3.4.3. Mean number of leaves per seedling of *Dendrobium* hybrid seed progeny six months after transplanting

♀ \ ♂	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_{13}	P_{14}
P_1	6.17	5.67	5.33	5.83		5.33		4.83	5.50	4.83				
P_2	5.50		5.17	5.17		5.17	5.50		5.67					
P_3	6.00		6.00	5.50	6.17	6.00	5.33	5.67		5.17				
P_4	5.00		5.83	5.33	4.83	5.00								
P_5	5.00					6.00	5.17	5.00				4.67		
P_6	5.50		5.33	6.00					5.83			4.67		
P_7	5.17	5.33	5.17	5.33			5.33		5.33					
P_8	6.00	4.83					6.67		5.77					
P_9	6.50				5.83	6.50	6.17							
P_{10}	6.83	6.33					5.83							
P_{11}		5.83		5.67							5.50	5.17		
P_{12}				5.67	5.50						5.17			
P_{13}								5.00	4.67					
P_{14}	6.50	5.17	5.67			5.33								

SE_m 1.706 N.S

Table 4.3.4.4. Mean length (cm) of longest leaf of *Dendrobium* hybrid seed progeny six months after transplanting

♀ \ ♂	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_{13}	P_{14}
P_1	6.33	7.48	6.05	6.05		5.25		5.83	7.85	6.07				
P_2	7.13		5.67	6.67		6.28	5.28		5.23					
P_3	6.42		6.48	6.35	7.55	6.80	5.97	5.88		5.72				
P_4	5.43		5.43	6.37	5.02	6.22								
P_5	5.82					5.67	6.32	5.50				7.52		
P_6	6.85		5.50	6.02					5.55			4.57		
P_7	4.85	5.05	5.23	5.82			5.92		6.75					
P_8	6.28	4.77					5.52		5.78					
P_9	7.95				8.77	7.08	7.65							
P_{10}	7.62	9.23					7.85							
P_{11}		6.12		6.52							6.90	6.32		
P_{12}				5.28	5.23						4.78			
P_{13}								4.55	4.53					
P_{14}	5.58	4.98	5.40			5.27								

SE_m 0.706 CD (0.05) 1.958

observed in nine combinations *viz.*, 8.77 cm in $P_9 \times P_5$, 7.95 cm in $P_9 \times P_1$, 7.85 cm in $P_1 \times P_9$ and $P_{10} \times P_7$, 7.65 cm in $P_9 \times P_7$, 7.62 cm in $P_{10} \times P_1$, 7.55 cm in $P_3 \times P_5$, 7.52 cm in $P_5 \times P_{12}$ and 7.48 cm in $P_1 \times P_2$. Values on par with the lowest were noted in 48 combinations. Eight combinations recording on par values were observed to fall in a median class between the lowest and the highest, being $P_2 \times P_1$ (7.13 cm), $P_9 \times P_6$ (7.08 cm), P_{11} selfed (6.90 cm), $P_6 \times P_1$ (6.85 cm), $P_3 \times P_6$ (6.80 cm), $P_7 \times P_9$ (6.75 cm), $P_2 \times P_4$ (6.67 cm) and $P_{11} \times P_4$ (6.52 cm).

5. Width of leaf

Width of longest leaf ranged from 1.35 cm in $P_8 \times P_2$ and $P_{12} \times P_{11}$ to 3.03 cm in $P_{10} \times P_1$. Mean values on par with the highest were noted in 23 hybrid combinations. The remaining 41 out of the 67 combinations registered mean values on par with the lowest (Table 4.3.4.5.).

6. Number of roots per seedling

The average number of roots per seedling ranged from 7.33 in $P_{12} \times P_{11}$ to 13.00 in $P_{10} \times P_1$ (Table 4.3.4.6.). High values of 12.83 in $P_{10} \times P_2$, 12.50 in $P_9 \times P_1$, 12.17 in $P_9 \times P_5$, 11.83 in $P_{13} \times P_9$, 11.67 each in $P_1 \times P_3$, $P_9 \times P_6$ and $P_9 \times P_7$ and 11.50 in $P_1 \times P_2$ were observed for the character at six months after transplanting, but statistically all the values were on par.

Table 4.3.4.5. Mean width (cm) of longest leaf of *Dendrobium* hybrid seed progeny six months after transplanting

♀ \ ♂	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_{13}	P_{14}
P_1	1.68	1.73	1.83	2.00		1.78		1.96	2.02	1.83				
P_2	2.13		1.73	1.98		1.85	1.40		1.75					
P_3	2.42		2.57	2.15	2.53	2.15	2.18	2.35		1.95				
P_4	1.78		1.97	2.07	1.55	1.80								
P_5	1.53					1.95	2.15	1.48				2.58		
P_6	1.88		1.85	2.20					2.02			1.68		
P_7	2.50	2.43	2.15	2.20			2.13		2.20					
P_8	1.88	1.35					1.90		2.02					
P_9	2.82				2.23	2.98	2.55							
P_{10}	3.03	2.63					2.18							
P_{11}		2.38		1.97							2.00	1.62		
P_{12}				1.70	1.52						1.35			
P_{13}								1.37	2.48					
P_{14}	1.65	2.00	1.98			1.58								

SE_m 0.321 CD (0.05) 0.890

Table 4.3.4.6. Mean number of roots per seedling of *Dendrobium* hybrid seed progeny six months after transplanting

♀ \ ♂	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_{13}	P_{14}
P_1	11.17	11.50	11.67	10.17		9.67		10.83	11.00	8.17				
P_2	11.33		9.00	10.33		9.17	8.17		9.33					
P_3	10.83		11.33	10.00	11.17	10.33	11.00	10.83		9.67				
P_4	9.50		10.00	10.00	8.33	8.67								
P_5	9.40					9.70	9.53	8.50				11.00		
P_6	9.33		11.17	11.00					10.67			8.67		
P_7	9.17	10.17	9.67	10.33			10.00		10.33					
P_8	10.67	8.33					10.83		10.50					
P_9	12.50				12.17	11.67	11.67							
P_{10}	13.00	12.83					11.17							
P_{11}		10.83		10.17							10.83	9.67		
P_{12}				9.67	8.67						7.33			
P_{13}								7.83	11.83					
P_{14}	9.83	9.67	9.00			9.17								

SE_m 0.203 N.S

4.3.5. Comparison of performance of hybrids 1.5 - 2.0 years after transplanting

4.3.5.1. Comparison based on vegetative characters

The average performance of *Dendrobium* hybrid seed progeny obtained from the 67 successful combinations 1.5 - 2.0 years after planting out based on different vegetative characters have been studied (Tables 4.3.5.1.a to 4.3.5.1.h.). Significant differences among the hybrid combinations were observed with respect to all the biometric characters studied.

1. Number of shoots per clump

Number of shoots per clump ranged from 4.4 in $P_{13} \times P_9$ to 6.3 in $P_{10} \times P_2$ (Table 4.3.5.1.a.). Shoot numbers on par with the highest value recorded were observed in the seven hybrids *viz.*, $P_5 \times P_{12}$ (6.0), $P_{10} \times P_7$ (6.0), $P_{10} \times P_1$ (5.8), $P_1 \times P_9$ (5.7), $P_3 \times P_5$ (5.7), $P_9 \times P_5$ (5.7) and $P_{14} \times P_1$ (5.7). Shoot numbers on par with the lowest were observed in 30 hybrid combinations. These 30 combinations included three out of eight successful combinations using P_1 as the female parent, five out of six successful hybrid combinations using P_2 as the female parent, four out of five using P_4 and P_5, all the six using P_7, two out of four using P_8, two out of three using P_{12}, both the successful combinations using P_{13} and two out of four using P_{14} as the respective female parents. All the remaining 27 hybrid combinations out of the total of 67 fell into the same median parity group.

Table 4.3.5.1.a. Mean number of shoots per clump of *Dendrobium* hybrid seed progeny at 1.5 - 2.0 years after transplanting

♀ \ ♂	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_{13}	P_{14}	$\bar{x}$
P_1	5.2	5.3	4.8	5.1		5.0		5.5	5.7	4.7					5.2
P_2	5.3		4.9	4.9		4.6	4.5		4.6						4.8
P_3	5.5		5.2	5.4	5.7	5.3	5.3	5.5		4.9					5.4
P_4	5.3		5.0	4.9	4.7	4.8									4.9
P_5	4.7					4.6	4.8	4.7				6.0			5.0
P_6	5.8		5.2	5.3					5.4			4.9			5.3
P_7	5.0	4.9	4.9	4.9			5.0		5.0						5.0
P_8	5.1	4.7					5.0		5.1						4.9
P_9	5.6				5.7	5.2	5.5								5.5
P_{10}	5.8	6.3					6.0								6.0
P_{11}		5.6		5.5							5.6	5.2			5.5
P_{12}				4.8	5.1						4.7				4.9
P_{13}								4.6	4.4						4.5
P_{14}	5.7	5.0	5.4			4.9									5.3
$\bar{x}$	5.4	5.3	5.0	5.1	5.3	4.9	5.2	5.1	5.1	4.8	5.2	5.4	–	–	

SE_m 0.231 CD (0.05) 0.634

A comparison of array means when used as male and female parents revealed that P_{10} resulted in the highest mean number of shoots as the female parent (6.0) and the lowest average number of shoots as the male parent (4.8). The parent P_{13} recorded the lowest number of shoots as the female parent (4.5). The highest number of shoots (5.4) resulted when P_1 and P_{12} were used as male parents.

2. Number of leaves per clump

Total number of leaves per clump was observed to be the highest in $P_{14} \times P_1$ (33.0) and the lowest in $P_2 \times P_7$ (20.4) (Table 4.3.5.1.b.). Leaf numbers on par with the highest value recorded were observed in nine combinations *viz.*, $P_3 \times P_6$ (30.9), $P_3 \times P_5$ (30.1), $P_8 \times P_9$ (30.0), $P_{10} \times P_2$ (29.9), $P_9 \times P_7$ (29.8), $P_3 \times P_8$ (29.4), $P_9 \times P_1$ (29.3), $P_6 \times P_9$ (29.2) and $P_3 \times P_7$ (29.1). Leaf numbers on par with the lowest value recorded were observed in 16 combinations *viz.*, $P_5 \times P_6$ (20.9), P_4 selfed (21.3), $P_1 \times P_{10}$ (21.4), $P_5 \times P_1$ (21.4), $P_5 \times P_8$ (21.9), $P_{13} \times P_9$ (22.3), $P_1 \times P_3$ (22.4), $P_7 \times P_2$ (23.1),$P_4 \times P_6$ (23.2), $P_7 \times P_1$ (23.3), $P_{13} \times P_8$ (23.4), $P_4 \times P_1$ (24.0), $P_{14} \times P_6$ (24.0), $P_2 \times P_4$ (24.2), $P_{12} \times P_{11}$ (24.2) and $P_1 \times P_4$ (24.3). The remaining 40 hybrid combinations fell into two main non overlapping intermediate parity groups.

An analysis of the mean performance of the parental genotypes as male and female parents was conducted. The parent P_4 resulted in the lowest number of leaves per clump when used as female (22.6) and male (21.6) parents. The highest number of leaves was recorded by P_{10} (28.6) as the female and P_2 (27.3) as the male parents.

Table 4.3.5.1.b. Mean number of leaves per clump of *Dendrobium* hybrid seed progeny at 1.5 - 2.0 years after transplanting

♀ \ ♂	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_{13}	P_{14}	$\bar{x}$
P_1	27.1	24.4	22.4	24.3		26.8		27.2	26.5	21.4					25.0
P_2	27.8		23.8	24.2		25.0	20.4		20.7						23.7
P_3	25.7		26.2	25.8	30.1	30.9	29.1	29.4		28.6					28.2
P_4	24.0		22.4	21.3	22.1	23.2									22.6
P_5	21.4					20.9	24.6	21.9				27.6			23.3
P_6	27.9		27.8	28.8					29.2			26.7			28.1
P_7	23.3	23.1	23.5	25.8			26.3		27.9						25.0
P_8	28.2	29.0					25.3		30.0						28.1
P_9	29.3				28.2	25.5	29.8								28.2
P_{10}	28.0	29.9					27.8								28.6
P_{11}		27.6		26.4							28.2	24.4			26.7
P_{12}				26.4	27.1						24.2				25.9
P_{13}								23.4	22.3						22.9
P_{14}	33.0	29.0	26.2			24.0									28.1
$\bar{x}$	26.9	27.3	24.6	21.6	26.9	25.2	26.2	25.5	26.1	25.0	26.2	26.2	–	–	

SE_m 1.423 CD (0.05) 3.938

3. Height/length of cane

Mean height of cane ranged from 20.34 cm in P_3 selfed to 36.20 cm in P_7 x P_9 (Table 4.3.5.1.c.). Mean cane heights which were on par with the highest were recorded in six other combinations *viz.*, P_5 x P_{12} (35.91 cm), P_6 x P_9 (35.73 cm), P_9 x P_7 (34.71 cm), P_5 x P_7 (33.70 cm), P_7 x P_4 (33.54 cm) and P_6 x P_4 (33.31 cm). Cane heights on par with the lowest were noted in eight combinations *viz.*, P_3 x P_{10} (20.41 cm), P_2 x P_7 (21.03 cm), P_2 x P_9 (21.63 cm), P_1 x P_3 (21.72 cm), P_3 x P_1 (21.77 cm), P_1 x P_{10} (22.33 cm), P_3 x P_4 (22.55 cm) and P_4 x P_3 (23.00 cm). The remaining 51 hybrid combinations could be accommodated in four main parity groups.

A study of the mean parent performance revealed that P_6 resulted in the highest (32.2 cm) cane height and P_{13} (24.9 cm) resulted in the lowest cane height as the female parent. As the male parent, P_{12} registered the highest (31.2 cm) and P_{10} recorded the lowest (21.4 cm) mean values.

4. Number of nodes per cane

Average number of nodes per cane ranged from 6.8 in P_2 x P_3 to 12.6 in P_9 x P_7 (Table 4.3.5.1.d.). Value on par with the highest was observed in its reciprocal, P_7 x P_9 (12.2). P_2 x P_1 recorded 7.2 nodes per cane which was on par with the lowest. Five main parity groups could be identified among the remaining 63 hybrid combinations. Number of nodes per cane recorded in P_{11} selfed (11.5), P_6 x P_4 (11.0) and P_{11} x P_2 (11.0) were on par, coming next to the best. The third best parity group

Table 4.3.5.1.c. Mean height (cm) of cane of *Dendrobium* hybrid seed progeny at 1.5 - 2.0 years after transplanting

♀ \ ♂	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_{13}	P_{14}	$\bar{x}$
P_1	25.32	27.23	21.72	26.46		27.19		24.20	27.51	22.33					25.2
P_2	28.12		27.57	28.26		31.63	21.03		21.63						26.4
P_3	21.77		20.34	22.55	30.80	29.15	29.31	31.34		20.41					25.7
P_4	24.08		23.00	24.45	25.35	28.70									25.1
P_5	25.34					25.90	33.70	32.38				35.91			30.6
P_6	29.20		30.02	33.31					35.73			32.76			32.2
P_7	26.24	28.85	30.58	33.54			29.05		36.20						30.7
P_8	28.05	30.36					30.15		31.46						30.0
P_9	31.02				30.32	28.01	34.71								30.9
P_{10}	28.80	28.33					29.25								28.8
P_{11}		32.46		30.66							31.34	29.54			31.0
P_{12}				26.24	29.70						26.23				27.4
P_{13}								25.89	23.93						24.9
P_{14}	26.42	29.66	27.91			26.60									27.6
$\bar{x}$	26.8	29.5	25.9	28.2	29.0	28.2	29.6	28.5	29.4	21.4	28.8	31.2	–	–	

SE_m 1.132 CD (0.05) 3.154

Table 4.3.5.1.d. Mean number of nodes per cane of *Dendrobium* hybrid seed progeny at 1.5 - 2.0 years after transplanting

♀ \ ♂	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_{13}	P_{14}	$\bar{x}$
P_1	9.4	8.3	8.6	9.1		9.3		10.2	8.0	10.2					9.1
P_2	7.2		6.8	8.9		10.6	8.9		9.4						8.6
P_3	9.2		9.3	8.5	9.6	9.6	9.8	9.3		9.8					9.4
P_4	8.2		8.5	8.3	9.1	8.9									8.6
P_5	9.8					9.6	10.8	10.6				10.3			10.2
P_6	10.2		10.0	11.0					10.8			9.9			10.4
P_7	9.2	9.8	9.9	10.4			9.9		12.2						10.2
P_8	9.4	9.0					9.4		10.0						9.5
P_9	10.8				10.2	10.2	12.6								11.0
P_{10}	9.0	8.7					9.0								8.9
P_{11}		11.0		10.0							11.5	9.2			10.4
P_{12}				9.8	10.3						9.6				9.9
P_{13}								7.7	7.7						7.7
P_{14}	10.1	10.1	9.9			9.2									9.8
$\bar{x}$	9.3	9.5	9.0	9.5	9.8	9.6	9.9	9.5	9.7	10.0	10.6	9.8	–	–	

SE_m 0.306 CD (0.05) 0.852

for this character consisted of 15 combinations *viz.*, P_5 x P_7 (10.8), P_6 x P_9 (10.8), P_9 x P_1 (10.8), P_2 x P_6 (10.6), P_5 x P_8 (10.6), P_7 x P_4 (10.4), P_5 x P_{12} (10.3), P_{12} x P_5 (10.3) , P_1 x P_{10} (10.2), P_1 x P_8 (10.2), P_6 x P_1 (10.2), P_9 x P_5 (10.2), P_9 x P_6 (10.2), P_{14} x P_1 (10.1) and P_{14} x P_2 (10.1).

An analysis of array means revealed that P_9 with 11.0 nodes resulted in the highest and P_{13} with 7.7 nodes resulted in the lowest mean values as the female parent. When used as male parents, most of the varieties were found to perform well, recording high mean values. The highest mean value was recorded by P_{11} (10.6) and the lowest mean value was registered by P_3 (9.0) as the male parent.

5. Number of leaves per cane

Mean number of leaves per cane in the different hybrid combinations ranged from 4.9 in P_2 x P_3 to 9.5 in P_9 x P_7 (Table 4.3.5.1.e.). Five combinations were found to record leaf numbers on par with the highest. These five hybrids were P_7 x P_9 (9.4), P_6 x P_4 (9.0), P_5 x P_7 (8.8), P_6 x P_9 (8.7) and P_{11} selfed (8.7). Leaf numbers per cane on par with the lowest were observed in three combinations *viz.*, P_2 x P_1 (5.1), P_{13} x P_8 (5.5) and P_{13} x P_9 (5.7). The remaining 57 hybrid combinations fell into three main parity groups between the highest and the lowest groups. The second best parity group for number of leaves per cane was constituted by 24 hybrid combinations. These 24 combinations included the three combinations *viz.*, P_5 x P_{12}, P_9 x P_6 and P_{11} x P_2 recording 8.6 leaves per cane, P_9 x P_1 registering 8.4 leaves, the three hybrids *viz.* P_1 x P_9, P_2 x P_6 and P_6 x P_1 recording 8.3 leaves, the three combinations *viz.*, P_1 x P_8, P_7 x P_4 and P_9 x P_5 registering 8.2 leaves,

Table 4.3.5.1.e. Mean number of leaves per cane of *Dendrobium* hybrid seed progeny at 1.5 - 2.0 years after transplanting

♀ \ ♂	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_{13}	P_{14}	$\bar{x}$
P_1	7.5	6.8	7.0	7.3		7.5		8.2	8.3	6.4					7.4
P_2	5.1		4.9	7.0		8.3	7.0		7.5						6.6
P_3	7.3		7.2	6.9	7.8	7.8	8.0	7.5		7.0					7.4
P_4	6.2		6.9	6.3	7.6	7.3									6.9
P_5	7.6					7.8	8.8	8.0				8.6			8.2
P_6	8.3		8.1	9.0					8.7			7.8			8.4
P_7	7.6	7.4	7.8	8.2			7.9		9.4						8.1
P_8	7.4	7.0					7.4		7.9						7.4
P_9	8.4				8.2	8.6	9.5								8.7
P_{10}	7.0	6.3					7.1								6.8
P_{11}		8.6		7.5							8.7	6.7			7.9
P_{12}				7.8	8.0						7.0				7.6
P_{13}								5.5	5.7						5.6
P_{14}	7.9	7.6	7.9			6.4									7.5
$\bar{x}$	7.3	7.3	7.1	7.5	7.6	7.7	8.0	7.3	7.9	6.7	7.3	7.7	–	–	

SE_m 0.312 CD (0.05) 0.863

P_6 x P_3 recording 8.1 leaves, the three hybrids *viz.*, P_3 x P_7, P_5 x P_8 and P_{12} x P_5 registering 8.0 leaves, the four combinations *viz.*, P_7 selfed, P_8 x P_9, P_{14} x P_1 and P_{14} x P_3 recording 7.9 leaves and the six hybrid combinations *viz.*, P_3 x P_5, P_3 x P_6, P_5 x P_6, P_6 x P_{12}, P_7 x P_3 and P_{12} x P_4 registering 7.8 leaves per cane.

A comparison of parental performance revealed P_9 (8.7) and P_7 (8.0) to be the best female and male parents, respectively for number of leaves per cane. The parent P_{13} with 5.6 and P_{10} with 6.7 leaves per cane recorded the lowest mean values as the female and the male parents, respectively for this character.

6. Leaf area per cane

Mean leaf area per cane was found to range from 159.60 cm^2 in P_1 x P_{10} to 665.90 cm^2 in P_5 x P_{12} (Table 4.3.5.1.f.). Values for leaf area per cane on par with the highest were observed in P_5 x P_7 (627.15 cm^2) and P_6 x P_9 (599.25 cm^2). Twelve other combinations registered low leaf areas which were on par with the lowest. These 12 combinations were P_4 x P_1 (236.75 cm^2), P_{14} x P_6 (234.40 cm^2), P_1 x P_4 (226.80 cm^2), P_1 selfed (213.86 cm^2), P_1 x P_2 (212.35 cm^2), P_2 x P_1 (210.35 cm^2), P_2 x P_3 (207.75 cm^2), P_{10} x P_7 (199.65 cm^2), P_1 x P_3 (198.65 cm^2), P_{13} x P_8 (184.65 cm^2), P_{10} x P_2 (180.35 cm^2) and P_{13} x P_9 (167.15 cm^2). The remaining 53 hybrid combinations fell into 7 main parity groups. The second best parity group for leaf area per cane was constituted by P_7 x P_9 (559.75 cm^2), its reciprocal P_9 x P_7 (558.55 cm^2) and P_{11} x P_2 (540.75 cm^2). The third best parity group consisted of three hybrids *viz.*, P_6 x P_4 (495.53 cm^2), P_7 x P_4 (484.55 cm^2) and P_{12} x P_5 (455.50 cm^2).

Table 4.3.5.1.f. Mean leaf area (cm^2) per cane of *Dendrobium* hybrid seed progeny at 1.5 - 2.0 years after transplanting

♀ \ ♂	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_{13}	P_{14}	$\bar{x}$
P_1	213.86	212.35	198.65	226.80		332.45		263.15	400.80	159.60					250.8
P_2	210.35		207.75	267.05		399.15	272.10		287.00						273.9
P_3	293.93		306.85	312.55	402.15	407.15	436.15	433.70		358.65					368.9
P_4	236.75		265.90	254.80	316.85	339.50									282.8
P_5	323.30					309.70	627.15	449.75				665.90			475.2
P_6	351.70		363.80	495.53					599.25			329.90			428.1
P_7	416.45	430.40	426.35	484.55			410.10		559.75						454.5
P_8	303.45	347.30					312.50		392.45						339.1
P_9	446.40				402.25	397.45	558.55								451.2
P_{10}	166.50	180.35					199.65								182.2
P_{11}		540.75		443.00							361.05	348.75			423.4
P_{12}				349.45	455.50						288.95				364.7
P_{13}								184.65	167.15						175.9
P_{14}	262.70	284.10	261.60			234.40									260.7
$\bar{x}$	293.1	249.2	290.1	354.1	394.2	345.7	402.3	332.9	401.1	259.2	325.0	448.2	–	–	

SE_m 25.411 CD (0.05) 70.474

An analysis of the average performance of the parental genotypes as male and female parents was conducted. As the female parent, P_5 resulted in the highest (475.2 cm^2) and P_{13} resulted in the lowest (175.9 cm^2) leaf areas. As the male parent, P_{12} recorded the highest (448.2 cm^2) and P_2 recorded the lowest leaf areas (249.2 cm^2).

7. Length of leaf

Length of leaf ranged from 11.95 cm in $P_{12} \times P_{11}$ to 17.92 cm in $P_1 \times P_9$ (Table 4.3.5.1.g.). Five other combinations *viz.*, $P_9 \times P_7$ (17.38 cm), $P_5 \times P_{12}$ (17.34 cm), $P_7 \times P_9$ (17.14 cm), $P_6 \times P_9$ (16.88 cm) and $P_7 \times P_4$ (16.47 cm) recorded leaf lengths on par with the highest. Leaf lengths on par with the lowest were recorded by 19 combinations *viz.*, $P_1 \times P_2$ (13.46 cm), $P_9 \times P_5$ (13.42 cm), $P_1 \times P_4$ (13.37 cm), P_7 selfed (13.35 cm), $P_8 \times P_1$ (13.28 cm), P_4 selfed (13.22 cm), P_1 selfed (13.04 cm), $P_{10} \times P_2$ (13.02 cm), $P_8 \times P_2$ (12.91 cm), $P_4 \times P_3$ (12.89 cm), $P_7 \times P_2$ (12.88 cm), $P_8 \times P_7$ (12.83 cm), $P_3 \times P_1$ (12.79 cm), $P_7 \times P_1$ (12.56 cm), $P_1 \times P_{10}$ (12.43 cm), $P_{10} \times P_1$ (12.42 cm), $P_2 \times P_1$ (12.39 cm), $P_2 \times P_3$ (12.27 cm) and $P_{10} \times P_7$ (12.15 cm). The remaining 31 hybrid combinations fell into two parity groups between the highest and the lowest. The second best group of hybrids for leaf length was constituted by 14 combinations *viz.*, $P_3 \times P_8$ (16.05 cm), $P_5 \times P_1$ (16.03 cm), $P_3 \times P_{10}$ (15.88 cm), $P_{12} \times P_5$ (15.74 cm), $P_{11} \times P_2$ (15.65 cm), $P_5 \times P_8$ (15.56 cm), $P_3 \times P_6$ (15.34 cm), $P_2 \times P_7$ (15.28 cm), $P_2 \times P_4$ (15.23 cm), $P_6 \times P_4$ (15.23 cm), $P_{11} \times P_4$ (15.20 cm), $P_3 \times P_5$ (15.10 cm), $P_8 \times P_9$ (15.07 cm) and $P_2 \times P_6$ (14.97 cm).

Table 4.3.5.1.g. Mean length (cm) of leaf of *Dendrobium* hybrid seed progeny at 1.5 - 2.0 years after transplanting

♀ \ ♂	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_{13}	P_{14}	$\bar{x}$
P_1	13.04	13.46	12.90	13.37		14.62		13.66	17.92	12.43					13.9
P_2	12.39		12.27	15.23		14.97	15.28		13.89						14.0
P_3	12.79		13.67	14.17	15.10	15.34	14.56	16.05		15.88					14.7
P_4	13.93		12.89	13.22	13.77	14.24									13.6
P_5	16.03					14.72	16.34	15.56				17.34			16.0
P_6	14.45		13.96	15.23					16.88			14.12			14.9
P_7	12.56	12.88	14.37	16.47			13.35		17.14						14.5
P_8	13.28	12.91					12.83		15.07						14.0
P_9	14.46				13.42	14.39	17.38								14.9
P_{10}	12.42	13.02					12.15								12.5
P_{11}		15.65		15.20							13.69	14.40			14.7
P_{12}				13.54	15.74						11.95				13.7
P_{13}								14.10	12.97						13.5
P_{14}	13.71	13.90	14.72			13.83									14.0
$\bar{x}$	13.6	13.6	13.5	14.6	14.5	14.6	13.9	14.8	15.7	14.2	12.8	15.3	–	–	

SE_m 0.572 CD (0.05) 1.574

A study of the mean parental performance revealed that as female parents, P_5 resulted in the highest (16.0 cm) and P_{10} resulted in the lowest (12.5 cm) leaf lengths. The parent P_9 recorded the highest (15.7 cm) and P_{11} recorded the lowest (12.8 cm) leaf lengths as male parents.

8. Width of leaf

Average width of leaf in the 67 different hybrid combinations was observed to range from 2.79 cm in $P_1 \times P_{10}$ to 6.15 cm in $P_7 \times P_1$ (Table 4.3.5.1.h.). Values for width of leaf on par with the highest were recorded in seven combinations *viz.*, $P_7 \times P_2$ (6.14 cm), $P_5 \times P_{12}$ (6.12 cm), $P_5 \times P_7$ (5.90 cm), $P_9 \times P_7$ (5.85 cm), $P_7 \times P_9$ (5.72 cm), $P_{10} \times P_1$ (5.71 cm) and $P_6 \times P_4$ (5.61 cm). Values for width of leaf on par with the lowest were registered by 10 combinations *viz.*, $P_1 \times P_2$ (2.84 cm), $P_{10} \times P_2$ (2.84 cm), $P_{10} \times P_7$ (2.90 cm), $P_1 \times P_3$ (2.94 cm), $P_{13} \times P_9$ (3.05 cm), $P_2 \times P_1$ (3.06 cm), $P_2 \times P_3$ (3.09 cm), $P_{13} \times P_8$ (3.10 cm), $P_1 \times P_8$ (3.15 cm) and $P_{14} \times P_3$ (3.16 cm). The remaining 48 hybrid combinations fitted into four main parity groups between the lowest and the highest groups. The second best parity group for width of leaf consisted of six combinations *viz.*, $P_{11} \times P_2$ (5.42 cm), $P_6 \times P_9$ (5.39 cm), P_7 selfed (5.37 cm), $P_7 \times P_3$ (5.35 cm), $P_7 \times P_4$ (5.33 cm) and $P_{11} \times P_4$ (5.23 cm).

A comparison of mean parental performance revealed P_7 (5.7 cm) and P_{12} (5.0 cm) as the best female and male parents, respectively for width of leaf. The parent P_{13} with 3.1 cm and P_{10} with 3.7 cm recorded the lowest mean values as the female and the male parents, respectively.

Table 4.3.5.1.h. Mean width (cm) of leaf of *Dendrobium* hybrid seed progeny at 1.5 - 2.0 years after transplanting

♀ \ ♂	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_{13}	P_{14}	$\bar{x}$
P_1	3.33	2.84	2.94	3.29		4.23		3.15	3.61	2.79					3.3
P_2	3.06		3.09	3.60		4.26	3.66		3.56						3.5
P_3	4.15		4.01	4.04	4.48	4.55	4.37	4.84		4.54					4.4
P_4	3.69		3.94	4.09	4.03	4.29									4.0
P_5	4.03					3.97	5.90	4.89				6.12			5.0
P_6	3.95		4.51	5.61					5.39			3.89			4.7
P_7	6.15	6.14	5.35	5.33			5.37		5.72						5.7
P_8	3.92	3.86					4.27		4.42						4.1
P_9	4.96				4.84	4.90	5.85								5.1
P_{10}	5.71	2.84					2.90								3.8
P_{11}		5.42		5.23							4.76	5.04			5.1
P_{12}				4.44	4.79						4.37				4.5
P_{13}								3.10	3.05						3.1
P_{14}	3.41	3.51	3.16			3.32									3.4
$\bar{x}$	4.2	4.1	3.9	4.5	4.5	4.2	3.9	4.0	4.3	3.7	4.6	5.0	–	–	

SE_m 0.182 CD (0.05) 0.546

4.3.5.2. Comparison of performance of hybrids based on quantitative floral characters

Out of the 67 successful hybrid combinations from which hardened plants were established in the green house, 16 combinations flowered during the course of study. The mean performance of *Dendrobium* hybrid seed progeny obtained from these 16 hybrid combinations based on different quantitative floral characters has been analysed (Table 4.3.5.2.; Figs. 8 to 10). Significant differences among the hybrid combinations were observed with respect to all the floral characters studied. Details of inflorescence and single flower of some of the promising hybrids are presented (Plates VI to XIII).

1. Age at first flowering

Age at first flowering was found to range from 16.02 months after transplanting in $P_5 \times P_1$ to 20.78 months in $P_6 \times P_3$.

Very early onset of the reproductive phase was observed in $P_5 \times P_1$ and $P_5 \times P_{12}$ flowering at a mean age of 16.02 months and 16.03 months, respectively. Following these two were the four hybrids *viz.*, $P_4 \times P_1$, $P_6 \times P_1$, $P_2 \times P_3$ and $P_3 \times P_1$, each flowering at 17.12, 17.37, 17.60 and 17.75 months, respectively after transplanting. The six hybrids *viz.*, $P_6 \times P_9$, $P_{11} \times P_2$, $P_3 \times P_6$, $P_2 \times P_1$, $P_1 \times P_4$ and $P_7 \times P_1$ were of medium duration, flowering at the age of 18.18, 18.23, 18.32, 18.48, 18.48 and 18.58 months, respectively. The four hybrids *viz.*, $P_4 \times P_3$, $P_8 \times P_1$, $P_7 \times P_9$ and $P_6 \times P_3$ flowered late, requiring 19.18, 19.28, 19.95 and 20.78 months, respectively from transplanting to flower.

Table 4.3.5.2. Mean performance of *Dendrobium* hybrid seed progeny in 16 flowering combinations for floral characters

Hybrid combination	Age at 1st flowering (months)	Cane to flower first	Days to 1st flower opening	Flowering time (days)	Days for wilting of all flowers	Length of inflorescence (cm)	Length of scape (cm)	No. of flowers/ inflorescence	Length of internode (cm)	Diameter of inflorescence axis (cm)	Length of flower (cm)	Width of flower (cm)
$P_1 \times P_4$	18.48	5.0	41.2	20.3	34.3	40.00	18.67	9.2	2.72	0.493	7.27	8.62
$P_2 \times P_1$	18.48	5.2	35.7	16.0	39.7	26.50	7.35	8.3	2.65	0.525	6.25	6.40
$P_2 \times P_3$	17.60	5.3	44.8	14.7	43.7	41.22	20.08	8.8	2.83	0.508	5.93	6.65
$P_3 \times P_1$	17.75	4.8	41.8	25.5	37.7	41.77	14.63	9.5	3.15	0.350	7.28	7.50
$P_3 \times P_6$	18.32	5.7	34.5	16.2	31.2	38.88	17.80	7.5	3.33	0.522	6.48	7.45
$P_4 \times P_1$	17.12	5.0	36.7	22.8	46.5	41.67	19.47	13.3	2.20	0.355	6.85	7.35
$P_4 \times P_3$	19.18	5.5	38.2	15.2	24.5	35.58	19.42	8.2	2.32	0.533	7.87	8.10
$P_5 \times P_1$	16.02	4.8	38.2	24.7	43.2	39.42	16.58	9.8	2.71	0.532	7.00	7.60
$P_5 \times P_{12}$	16.03	5.0	41.7	23.3	48.2	48.42	15.78	12.7	2.88	0.428	6.35	7.28
$P_6 \times P_1$	17.37	5.8	39.8	26.7	35.5	43.42	15.52	9.5	3.30	0.452	7.29	7.18
$P_6 \times P_3$	20.78	5.3	36.0	20.0	34.0	35.50	15.83	7.0	3.10	0.563	6.67	7.30
$P_6 \times P_9$	18.18	6.0	41.7	24.8	39.5	44.45	15.20	10.5	3.36	0.483	6.00	7.50
$P_7 \times P_1$	18.58	6.5	47.7	23.3	36.5	29.25	11.57	8.8	2.58	0.517	6.20	7.25
$P_7 \times P_9$	19.95	5.2	30.7	21.8	34.7	32.98	11.40	9.2	2.39	0.458	6.48	7.15
$P_8 \times P_1$	19.28	6.3	43.3	13.7	34.5	39.92	18.87	8.0	3.44	0.458	7.12	7.88
$P_{11} \times P_2$	18.23	4.7	38.3	17.8	44.3	42.00	14.83	12.5	2.43	0.392	5.62	6.18
SE_m	0.348	0.353	1.538	1.244	1.583	2.023	1.333	0.767	0.177	0.033	0.195	0.205
CD (0.05)	0.981	0.996	4.467	3.510	4.467	6.138	3.762	2.164	0.501	0.093	0.551	0.579

2. Cane to flower first

Cane to flower first ranged from 4.7 in P_{11} x P_2 to 6.5 in P_7 x P_1, among the 16 flowering hybrid combinations. Nine combinations *viz.*, P_3 x P_1(4.8), P_5 x P_1(4.8), P_5 x P_{12} (5.0), P_1x P_4 (5.0), P_4 x P_1(5.0), P_2 x P_1(5.2), P_7 x P_9 (5.2), P_2 x P_3 (5.3) and P_6 x P_3 (5.3) were found to be on par with P_{11} x P_2 (4.7). Five combinations *viz.*, P_4 x P_3 (5.5), P_3 x P_6 (5.7), P_6 x P_1(5.8), P_6 x P_9 (6.0) and P_8 x P_1(6.3) were on par with P_7 x P_1(6.5).

3. Days to first flower opening

Days to the opening of the first flower from inflorescence emergence ranged from 30.7 days in P_7 x P_9 to 47.7 days in P_7 x P_1. The combination P_3 x P_6 (34.5 days) was on par with P_7 x P_9. These two earliest combinations were followed by six combinations *viz.*, P_2 x P_1(35.7 days), P_6 x P_3 (36.0 days), P_4 x P_1(36.7 days), P_4 x P_3 (38.2 days), P_5 x P_1(38.2 days) and P_{11} x P_2 (38.3 days) which were on par with each other for days to first flower opening. A higher time interval between inflorescence emergence and first flower opening was observed in six hybrids *viz.*, P_6 x P_1(39.8 days), P_1x P_4 (41.2 days), P_6 x P_9 (41.7 days), P_5 x P_{12} (41.7 days), P_3 x P_1(41.8 days) and P_8 x P_1(43.3 days). Maximum time interval from inflorescence emergence to first flower opening was noted in P_2 x P_3 registering 44.8 days and P_7 x P_1recording 47.7 days.

Plate VI. Some of the new, promising *Dendrobium* hybrids

Single Flower Inflorescence

H-151 *Dendrobium* CSTD x *D.* Pramot 3 [P_1 x P_4]

Medium long arching inflorescences with 6-10 flowers per inflorescence. Flowers large, deep magenta pink and faintly striped with stellar appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-93 *Dendrobium* Chiangmai Pink x *D.* CSTD [P_2 x P_1]

Medium long arching inflorescences with 7-12 flowers per inflorescence. Flowers medium large, striped and light magenta with a green tinge, having rounded, full appearance. Sepals and petals medium thick and glossy.

Single Flower Inflorescence

H-223 *D.* Chiangmai Pink x *D.* CSTD [P_2 x P_1]

Long arching inflorescences with 10-14 flowers per inflorescence. Flowers medium large and flat, with a squarish appearance, light pink and greenish white with stripes. Sepals and petals moderately thick and glossy.

Single Flower Inflorescence

H-332 *D.* Chiangmai Pink x *D.* CSTD [P_2 x P_1]

Medium long arching inflorescences with 5-8 flowers per inflorescence. Flowers medium large with rounded appearance, light pink and greenish white with stripes. Sepals and petals moderately thick and glossy.

Single Flower Inflorescence

H-60 *Dendrobium* Nagoya Pink x *D.* CSTD [P_3 x P_1]

Long arching inflorescences with 8-12 flowers per inflorescence. Flowers large, light pink with prominent dark pink stripes and full appearance. Petals very broad, overlapping the sepals. Sepals and petals thick and glossy.

★ Selected promising hybrids

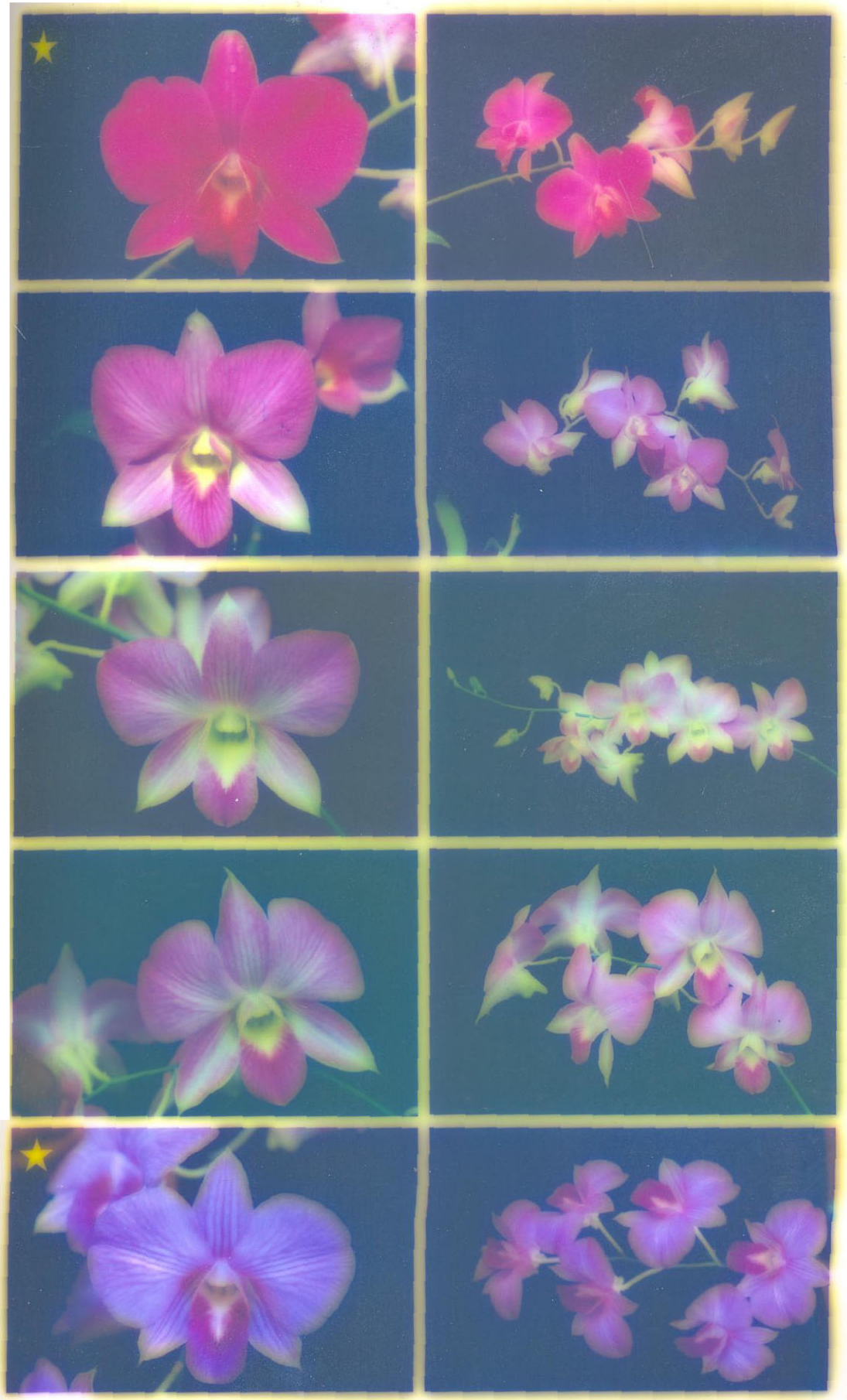


Plate VI

4. Flowering time

Time interval from first flower opening to the opening of the last flower in the inflorescence ranged from 13.7 days in P_8 x P_1 to 26.7 days in P_6 x P_1. A short flowering time on par with the shortest was observed in P_2 x P_3 (14.7 days), P_4 x P_3 (15.2 days), P_2 x P_1(16.0 days) and P_3 x P_6 (16.2 days). A medium time interval was observed between the opening of the first and the last flowers in an inflorescence in P_{11} x P_2 (17.8 days), P_6 x P_3 (20.0 days), P_1x P_4 (20.3 days), P_7 x P_9 (21.8 days) and P_4 x P_1(22.8 days). A long flowering time on par with the highest was observed in P_7 x P_1 (23.3 days), P_5 x P_{12} (23.3 days), P_5 x P_1(24.7 days), P_6 x P_9 (24.8 days) and P_3 x P_1(25.5 days).

5. Days for wilting of all flowers

Days for wilting of all flowers in an inflorescence ranged from 24.5 days in P_4 x P_3 to 48.2 days in P_5 x P_{12}. Values on par with the highest were recorded by the four combinations *viz.*, P_4 x P_1(46.5 days), P_{11} x P_2 (44.3 days), P_2 x P_3 (43.7 days) and P_5 x P_1(43.2 days), for this character. These were followed by the five combinations *viz.*, P_2 x P_1(39.7 days), P_6 x P_9 (39.5 days), P_3 x P_1(37.7 days), P_7 x P_1(36.5 days) and P_6 x P_1(35.5 days) which were on par with each other for days for wilting of all flowers in an inflorescence. The five combinations *viz.*, P_7 x P_9, P_8 x P_1, P_1x P_4, P_6 x P_3 and P_3 x P_6 recording values of 34.7, 34.5, 34.3, 34.0 and 31.2 days, respectively were observed to be significantly superior only to P_4 x P_3 for this character. The value recorded by P_4 x P_3 (24.5 days) was significantly low as compared to the values recorded by all the other 15 combinations.

Plate VII. Some of the new, promising *Dendrobium* hybrids

Single Flower Inflorescence

H-132 *Dendrobium* Nagoya Pink x *D.* CSTD [P_3 x P_1]

Long arching inflorescences with 9-12 flowers per inflorescence. Flowers large, magenta coloured and striped with full appearance. Petals very broad, overlapping sepals. Sepals and petals thick and glossy

Single Flower Inflorescence

H-376 *Dendrobium* Nagoya Pink x *D.* CSTD [P_3 x P_1]

Short arching inflorescences with 4-8 flowers per inflorescence. Flowers large, light pink and striped with full appearance. Petals very broad, overlapping sepals. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-61 *Dendrobium* Nagoya Pink x *D.* CSTD [P_3 x P_1]

Short arching inflorescences with 5-8 flowers per inflorescence. Flowers large, purplish-magenta coloured, striped and with full appearance. Petals very broad, overlapping sepals. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-63 *Dendrobium* Nagoya Pink x *D.* CSTD [P_3 x P_1]

Medium long arching inflorescences with 7-12 flowers per inflorescence. Flowers large, light pink and striped with full appearance. Petals very broad, overlapping sepals. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-64 *Dendrobium* Nagoya Pink x *D.* CSTD [P_3 x P_1]

Short slanting inflorescences with 4-8 flowers per inflorescence. Flowers large, magenta coloured and very lightly striped with very full appearance. Petals extremely broad, almost completely overlapping sepals. Sepals and petals thick and glossy.

★ Selected promising hybrids

Plate VII

6. Length of inflorescence

Length of inflorescence ranged from 26.50 cm in P_2 x $P_1$to 48.42 cm in P_5 x P_{12} (Fig. 8). Considerably long inflorescences on par with the longest were observed in the two combinations *viz.*, P_6 x P_9 and P_6 x P_1recording values of 44.45 and 43.42 cm, respectively. Medium sized inflorescences were noted in the eight combinations *viz.*, P_{11} x P_2 (42.00 cm), P_4 x P_1(41.67 cm), P_3 x P_1(41.77 cm), P_2 x P_3 (41.22 cm), P_1x P_4 (40.00 cm), P_8 x P_1(39.92 cm), P_5 x P_1(39.42 cm) and P_3 x P_6 (38.88 cm). The three combinations *viz.*, P_4 x P_3, P_6 x P_3 and P_7 x P_9 produced short inflorescences of 35.58, 35.50 and 32.98 cm, respectively and were observed to be on par with each other for length of inflorescence. The hybrid P_7 x P_1recorded an inflorescence length of 29.25 cm, being on par with the shortest.

7. Length of scape

Length of scape was found to range from 7.35 cm in P_2 x P_1 to 20.08 cm in P_2 x P_3 (Fig. 8). Scape lengths on par with the highest were observed in six combinations *viz.*, P_4 x P_1(19.47 cm), P_4 x P_3 (19.42 cm), P_8 x P_1(18.87 cm), P_1x P_4 (18.67 cm), P_3 x P_6 (17.80 cm) and P_5 x P_1(16.58 cm). Length of scape was observed to be medium in six hybrids being 15.83 cm in P_6 x P_3, 15.78 cm in P_5 x P_{12}, 15.52 cm in P_6 x P_1, 15.20 cm in P_6 x P_9, 14.83 cm in P_{11} x P_2 and 14.63 cm in P_3 x P_1. The scapes were short in P_7 x $P_1$and P_7 x P_9, being 11.57 and 11.40 cm, respectively. The scape in P_2 x P_1(7.35 cm) was significantly short as compared to the scapes in all the other 15 combinations.

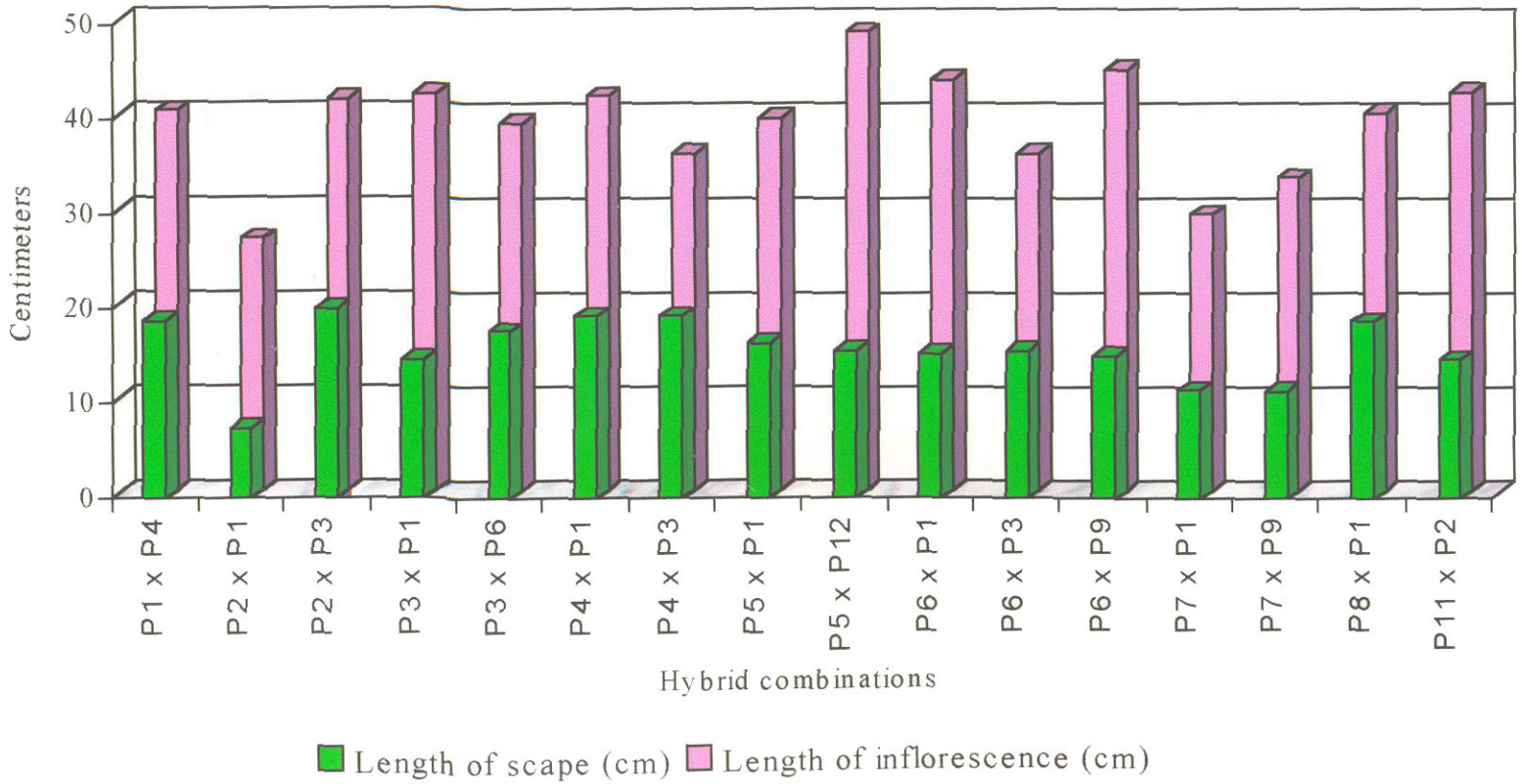


Fig. 8. Length of scape (cm) and length of inflorescence (cm) in 16 hybrid combinations of *Dendrobium*

8. Number of flowers per inflorescence

Number of flowers per inflorescence ranged from 7.0 in P_6 x P_3 to 13.3 in P_4 x P_1. The combinations P_5 x P_{12} and P_{11} x P_2 recorded 12.7 and 12.5 flowers, respectively per inflorescence, being on par with the highest. Mean number of flowers recorded per inflorescence was relatively high in the eight combinations *viz.*, P_6 x P_9 (10.5), P_5 x P_1(9.8), P_6 x P_1(9.5), P_3 x P_1(9.5), P_1x P_4 (9.2), P_7 x P_9 (9.2), P_7 x P_1(8.8) and P_2 x P_3 (8.8). Comparatively low mean number of flowers per inflorescence was observed in the four hybrids *viz.*, P_2 x P_1, P_4 x P_3, P_8 x $P_1$and P_3 x P_6 being 8.3, 8.2, 8.0 and 7.5, respectively, which were on par with the lowest value recorded.

9. Length of internode of inflorescence

The mean internodal length between the flowers in an inflorescence ranged from 2.20 cm in P_4 x $P_1$to 3.44 cm in P_8 x P_1. Values for length of internode on par with the highest were observed in five combinations *viz.*, P_6 x P_9, P_3 x P_6, P_6 x P_1, P_3 x $P_1$and P_6 x P_3 being 3.36, 3.33, 3.30, 3.15 and 3.10 cm, respectively.

Clearance between successive flowers was intermediate in the five hybrids, *viz.*, P_5 x P_{12} (2.88 cm), P_2 x P_3 (2.83 cm), P_1x P_4 (2.72 cm), P_5 x P_1(2.71 cm) and P_2 x P_1(2.65 cm). Shorter internodes on par with the lowest were observed in four combinations being 2.58 cm in P_7 x P_1, 2.43 cm in P_{11} x P_2, 2.39 cm in P_7 x P_9 and 2.32 cm in P_4 x P_3.

10. Diameter of inflorescence axis

Diameter of inflorescence axis ranged from 0.350 cm in P_3 x $P_1$to 0.563 cm in P_6 x P_3. Eight hybrids *viz.*, P_4 x P_3 (0.533 cm), P_5 x P_1(0.532 cm), P_2 x P_1(0.525 cm), P_3 x P_6 (0.522 cm), P_7 x P_1(0.517 cm), P_2 x P_3 (0.508 cm), P_1x P_4 (0.493 cm) and P_6 x P_9 (0.483 cm) registered values on par with the highest. Six combinations *viz.*, P_7 x P_9 (0.458 cm), P_8 x P_1(0.458 cm), P_6 x P_1(0.452 cm), P_5 x P_{12} (0.428 cm), P_{11} x P_2 (0.392 cm) and P_4 x P_1(0.355 cm) recorded values on par with the lowest, for diameter of inflorescence axis.

11. Length of flower

Length of flower ranged from 5.62 cm in P_{11} x P_2 to 7.87 cm in P_4 x P_3. Flower length recorded in P_4 x P_3 (7.87 cm) was significantly superior to the values recorded in all the other combinations (Fig. 9). Following P_4 x P_3 were the six combinations *viz.*, P_6 x P_1, P_3 x P_1, P_1 x P_4, P_8 x P_1, P_5 x $P_1$and P_4 x P_1 recording values of 7.29, 7.28, 7.27, 7.12, 7.00 and 6.85 cm, respectively for length of flower. Flower length was medium in six hybrids *viz.*, P_6 x P_3 (6.67 cm), P_3 x P_6 (6.48 cm), P_7 x P_9 (6.48 cm), P_5 x P_{12} (6.35 cm), P_2 x P_1 (6.25 cm) and P_7 x P_1 (6.20 cm). Comparatively short flowers recording values on par with the lowest (5.62 cm in P_{11} x P_2) were observed in two combinations *viz.*, P_6 x P_9 (6.00 cm) and P_2 x P_3 (5.93 cm).

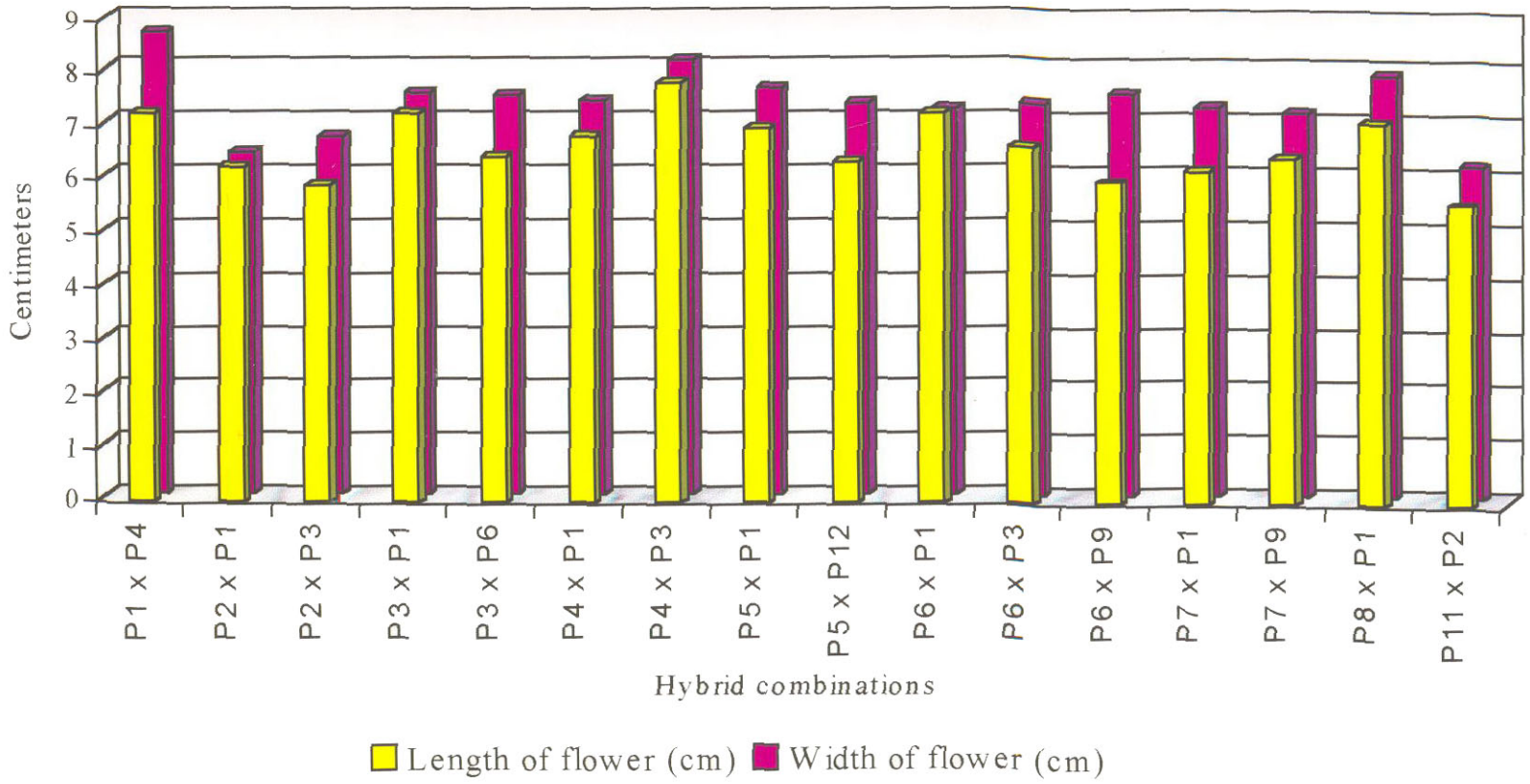


Fig. 9. Length of flower (cm) and width of flower (cm) in 16 hybrid combinations of *Dendrobium*

12. Width of flower

Width of flower ranged from 6.18 cm in P_{11} x P_2 to 8.62 cm in P_1x P_4 (Fig. 9). The combination P_4 x P_3 registered a mean flower width of 8.10 cm which was on par with the highest (8.62 cm in P_1x P_4). Following these two hybrids were the six combinations P_8 x P_1, P_5 x P_1, P_3 x P_1, P_6 x P_9, P_3 x P_6 and P_4 x P_1recording values of 7.88, 7.60, 7.50, 7.50, 7.45 and 7.35 cm, respectively for flower width. Flowers of intermediate width were observed in the five combinations P_6 x P_3, P_5 x P_{12}, P_7 x P_1, P_6 x P_1 and P_7 x P_9 recording on par values of 7.30, 7.28, 7.25, 7.18 and 7.15 cm, respectively for the character. The flowers produced in the three hybrids P_2 x P_3, P_2 x P_1 and P_{11} x P_2 were of the lowest width, recording values of 6.65, 6.40 and 6.18 cm, respectively.

4.3.5.3. Comparison of performance of hybrids based on qualitative floral characters

Details related to flowering and qualitative floral characters in *Dendrobium* hybrid seed progeny in the 16 combinations that flowered during the period of study have been recorded (Table 4.3.5.3.) (Plates VI to XIII).

1. Time taken for single flower opening

Time taken for the opening of one flower ranged from 1.55 days in P_2 x P_1 to 2.38 days in P_6 x P_1. A single flower took 1.84 days

Plate VIII. Some of the new, promising *Dendrobium* hybrids

Single Flower Inflorescence

H-59 *Dendrobium* Nagoya Pink x *D.* CSTD [P_3 x P_1]

Medium long arching inflorescences with 5-8 flowers per inflorescence. Flowers large, deep pink and striped with full appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-377 *Dendrobium* Nagoya Pink x *D.* Rinabha [P_3 x P_6]

Medium long arching inflorescences with 5-10 flowers per inflorescence. Flowers large, pink and white with a full, rounded look. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-253 *Dendrobium* Pramot 3 x *D.* CSTD [P_4 x P_1]

Short arching inflorescences with 5-8 flowers per inflorescence. Flowers large, pink and striped with full appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-111 *Dendrobium* Pramot 3 x *D.* CSTD [P_4 x P_1]

Long arching inflorescences with 8-12 flowers per inflorescence. Flowers large, dark magenta coloured and lightly striped with full appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-383 *Dendrobium* Pramot 3 x *D.* CSTD [P_4 x P_1]

Medium long arching inflorescences with 6-10 flowers per inflorescence. Flowers large, pink and striped with full appearance. Petals broad, overlapping sepals. Sepals and petals thick and glossy.

★ Selected promising hybrids

Plate VIII

Table 4.3.5.3. Performance of *Dendrobium* hybrid seed progeny in 16 combinations for qualitative floral characters

Hybrid combination	Average time taken for single flower opening (days)	Nature of inflorescence axis	Mode of display of flowers	Colour of flower	Texture of flower	Shape of flower	Flower size (circumference) (cm)	Fullness value	Fragrance
$P_1 \times P_4$	1.84	Arching	Alternate and facing opposite sides	Light purple / deep magenta pink and striped; stripes-dark pink, faint or prominent throughout	Moderately thick/thick, substantial looking and glossy	Flat; sepals and petals-broad / narrow and spatulate; labellum-flat/reflexed	24.95	3.17	Nil
$P_2 \times P_1$	1.55	Erect / arching	Whorled/alternate and facing opposite sides	Light pink and striped; center-greenish white; sepals-light pink; petals-deeper pink; stripes-dark pink	Moderately thick and glossy	Flat; squarish/rounded look; sepals and petals – broad; sepal tips-slightly incurved/twisted	19.86	3.19	Nil
$P_2 \times P_3$	2.12	Arching	Alternate and facing opposite sides	Light pink with green tinge towards center; sepals-greenish white	Moderately thick, substantial looking and glossy	Full; sepals and petals – broad and rounded	19.75	2.62	Nil
$P_3 \times P_1$	2.33	Arching / pendulous	Alternate and facing opposite sides / alternate and facing dorsal side	Light pink / deep pink and striped; stripes – faint and along center of petals/prominent and seen throughout	Thick / very thick, substantial looking and glossy	Perfectly shaped with full, rounded sepals and petals; sometimes slight fenestration of petals and labellum	23.20	2.16	Nil

P_3 x P_6	2.11	Arching	Alternate and facing opposite sides	Pink and white; labellum – deep pink, petals – light pink, sepals lighter pink, center-white	Thick, substantial looking and glossy	Perfectly shaped with full rounded sepals and petals, petals - almost fully overlapping lateral sepals	21.87	2.26	Nil
P_4 x P_1	2.24	Arching / erect	Alternate and facing opposite sides / whorled	Deep pink and striped; stripes – faint or prominent; pink and striped or not striped; pink and striped with prominent shading on sepals and petals; purple and white – unstriped; outside – purple, center – white	Very thick / thick, substantial looking and glossy	Ranging from perfectly shaped, full and rounded to squarish, sepals and petals – narrow to broad, lateral sepal tips – close together to wide apart, labellum – broadening to tip to tapering and pointed, labellum – flat to reflexed, edges of labellum smooth to fenestrated	22.29	2.95	Nil
P_4 x P_3	1.98	Arching	Alternate and facing opposite sides	Light pink with deeper pink tinge towards the outside, center – white	Thick, substantial looking and glossy	Full, rounded look; sepals and petals – broad	25.07	2.97	Nil
P_5 x P_1	1.92	Erect	Whorled	Deep pink and white; petals and labellum – deep pink, sepal tips – white	Very thick, substantial looking and glossy	Perfectly shaped, full and flat, sepals and petals – broad and rounded	22.92	2.72	Nil
P_5 x P_{12}	1.85	Arching	Alternate and facing opposite sides	Uniform deep pink/ labellum – deep pink, petals – light pink, sepals – lighter pink/ uniform white with faint pink tinge on labellum	Thick, substantial looking and glossy	Sepals and labellum – narrow, petals – spatulate, slight twist for dorsal sepal or for all sepals	21.40	3.28	Nil

$P_6 \times P_1$	2.38	Arching	Alternate and facing opposite sides	Ranging from solid deep magenta pink to solid deep purple, column - white/ deep magenta pink; operculum – white; central hairy thickening of labellum – white / deep velvety magenta	Very thick, substantial looking and glossy	Perfectly shaped and full, sepals and petals – broad and rounded; stellar appearance with full, flat pointed sepals and labellum; petals – spatulate, sepals – long, narrow	22.72	2.67	Nil
$P_6 \times P_3$	2.17	Arching	Alternate and facing opposite sides	Solid deep magenta pink; central hairy thickening of labellum – white towards center, deep magenta towards periphery	Thick, substantial looking and glossy	Perfectly shaped with full, rounded sepals and petals, petals almost fully overlapping lateral sepals	21.93	2.36	Nil
$P_6 \times P_9$	2.10	Erect / slightly arching	Whorled / alternate and facing opposite sides	Solid deep magenta pink / light or deep purple; operculum – white; central hairy thickening of labellum pink	Very thick, substantial looking and glossy	Full; sepals and petals – broad and rounded, petals – spatulate	21.20	3.21	Nil
$P_7 \times P_1$	2.21	Arching	Alternate and facing opposite sides	Uniform light pink with deep pink stripes throughout	Thick, substantial looking and glossy	Sepals and petals – broad and rounded / broad or narrow, angular and pointed with stellar appearance / flat or connivant with half - open look for flower	21.12	3.16	Nil

P_7 x P_9	2.35	Arching	Alternate and facing opposite sides	Very light pink and white; sepals, petals and labellum-light pink towards the outside, center – white	Very thick, substantial looking and glossy	Petals – spatulate, sepals – narrow, pointed and slightly twisted	21.40	3.27	Nil
P_8 x P_1	2.05	Arching	Alternate and facing opposite sides	Solid deep purple with deep purple stripes – prominent or faint/ pink and striped throughout with labellum and petal tips deeper pink	Very thick, substantial looking and glossy	Flat; pointed sepals and petals with stellar appearance; perfectly shaped with full, flat look; sepals and petals – broad and rounded	23.55	2.60	Nil
P_{11} x P_2	2.14	Erect / slightly arching / fully arching	Whorled / alternate and facing opposite sides	Single solid colour – deep magenta pink or pink/ colour gradation with deep pink labellum, light pink petals, lighter pink sepals; central hairy thicknening of labellum – white with pink stripes/white at center and magenta pink to periphery	Thick, substantial looking and glossy	Petals- spatulate/slightly reflexed; sepals – flat and pointed with a stellar appearance/ reflexed; slight twisting of sepals, petals and labellum	18.53	3.53	Fragrant

to complete opening in $P_1 \times P_4$, 1.85 days in $P_5 \times P_{12}$, 1.92 days in $P_5 \times P_1$and 1.98 days in $P_4 \times P_3$. The other ten combinations required more than two days for single flower opening *viz.*, 2.05 days in $P_8 \times P_1$, 2.10 days in $P_6 \times P_9$, 2.11 days in $P_3 \times P_6$, 2.12 days in $P_2 \times P_3$, 2.14 days in $P_{11} \times P_2$, 2.17 days in $P_6 \times P_3$, 2.21 days in $P_7 \times P_1$, 2.24 days in $P_4 \times P_1$, 2.33 days in $P_3 \times P_1$ and 2.35 days in $P_7 \times P_9$.

2. Nature of inflorescence axis

Nature of inflorescence axis was observed to be erect in $P_5 \times P_1$. In $P_6 \times P_9$, it was usually erect and sometimes slightly arching. In $P_{11} \times P_2$ the inflorescence axis was mostly erect but rarely arching, slightly or fully. The combination $P_2 \times P_1$ produced inflorescences that were either erect or arching. In ten hybrid combinations *viz.*, $P_1 \times P_4$, $P_2 \times P_3$, $P_3 \times P_6$, $P_4 \times P_3$, $P_5 \times P_{12}$, $P_6 \times P_1$, $P_6 \times P_3$, $P_7 \times P_1$, $P_7 \times P_9$ and $P_8 \times P_1$ the inflorescence axis was found to be arching in nature. In $P_4 \times P_1$the inflorescence axis was mostly arching and rarely erect. In $P_3 \times P_1$, it was usually arching, while rarely pendulous.

3. Mode of display of flowers

Mode of display of flowers was found to be whorled in $P_5 \times P_1$. The three combinations *viz.*, $P_6 \times P_9$, $P_{11} \times P_2$ and $P_2 \times P_1$ displayed the flowers either in a whorled manner or in an alternate pattern, facing opposite sides. In ten combinations *viz.*, $P_1 \times P_4$, $P_2 \times P_3$, $P_3 \times P_6$, $P_4 \times P_3$, $P_5 \times P_{12}$, $P_6 \times P_1$, $P_6 \times P_3$, $P_7 \times P_1$, $P_7 \times P_9$ and $P_8 \times P_1$ the mode of display was observed to be alternate and facing opposite sides. In $P_4 \times P_1$ the

Plate IX. Some of the new, promising *Dendrobium* hybrids

Single Flower Inflorescence

H-3 *Dendrobium* Pramot 3 x *D.* CSTD [P_4 x P_1]

Long arching inflorescences with 8-12 flowers per inflorescence. Flowers large, light pink and striped with semi full appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-20 *D.* Pramot 3 x *D.* CSTD [P_4 x P_1]

Long arching inflorescences with 10-14 flowers per inflorescence. Flowers large, deep pink and striped with broad, rounded appearance. Sepals and petals thick and glossy, labellum attractively rounded.

Single Flower Inflorescence

H-122 *D.* Pramot 3 x *D.* CSTD [P_4 x P_1]

Long arching inflorescences with 8-12 flowers per inflorescence. Flowers large, deep pink and striped with a full appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-124 *Dendrobium* Pramot 3 x *D.* CSTD [P_4 x P_1]

Medium long erect inflorescence with 6-8 flowers per inflorescence. Flowers large, pink and striped with novel shading on the prominently spatulate petals. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-231 *Dendrobium* Renapa Red 3 x *D.* CSTD [P_5 x P_1]

Medium long arching inflorescences with 6-10 flowers per inflorescence. Flowers medium large and dark magenta coloured with rounded, full appearance. Sepals and petals thick and glossy.

★ Selected promising hybrids

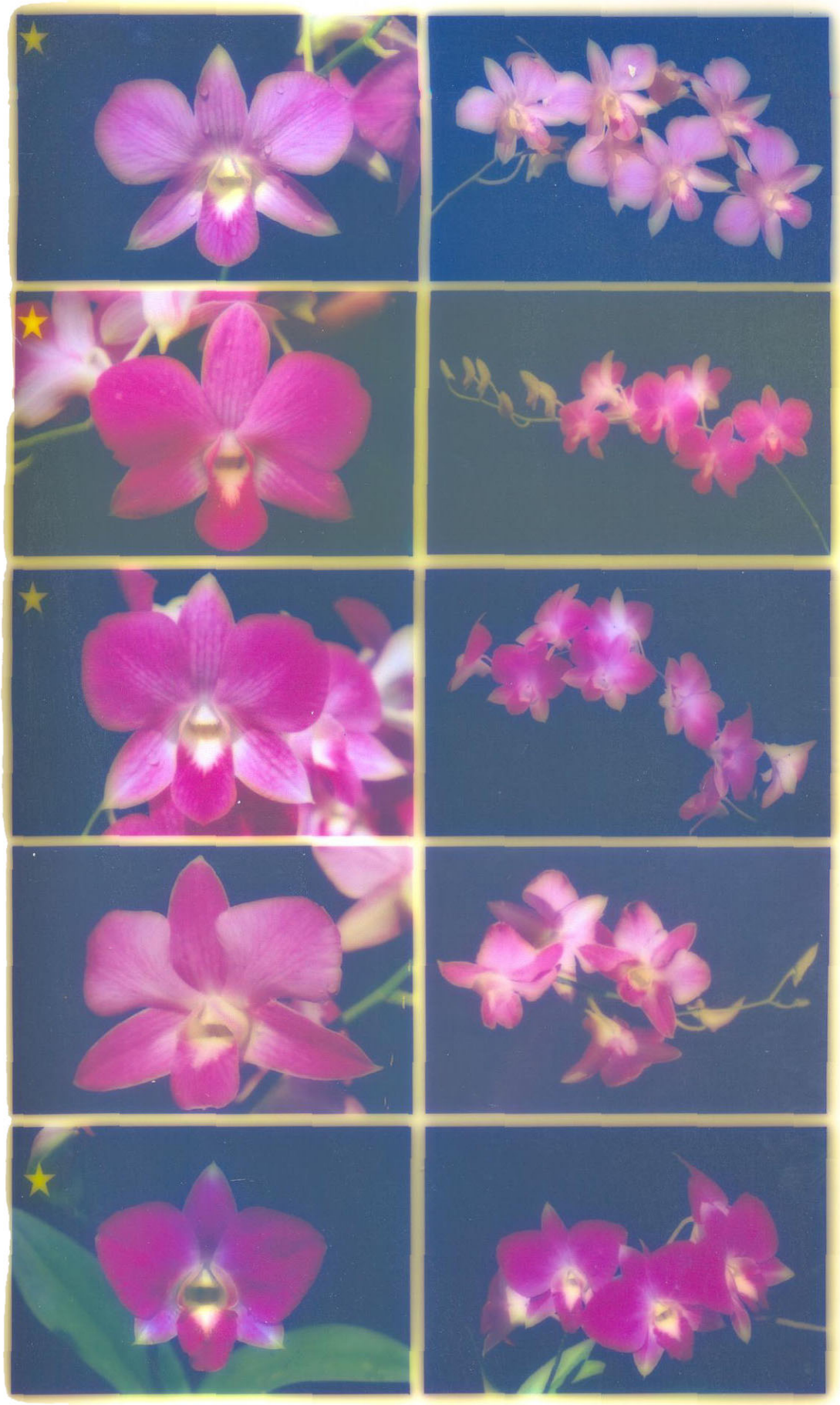


Plate IX

mode of display was mostly alternate and facing opposite sides, and rarely whorled. The combination P_3 x P_1 displayed the flowers in an alternate manner usually facing opposite sides and rarely facing the dorsal side.

4. Colour of flower

The pigmentation of the flowers have been studied in detail (Plates VI to XIII). Floral pigmentation in the hybrids ranged from single solid colour throughout to colour gradation between and within the sepals and petals. Striping of sepals and petals was found to be regular, ranging from very faint stripes along the middle portion of petals to prominent, deep coloured stripes throughout. Pronounced variation in floral pigmentation was observed within specific combinations also.

In P_1 x P_4 deep magenta pink flowers with faint stripes throughout were observed. The labellum was attractively coloured with deeper magenta venation. The central, hairy thickening of labellum was white along the middle and deep magenta pink along the borders. The same combination produced light purple flowers with prominent, deep purple stripes throughout. Central part of the flower and the broad, central hairy thickening of labellum appeared greenish-white.

In P_2 x P_1 the flowers were light pink and striped, the sepals exhibiting a lighter shade compared to the petals and the central portion of the flower including the hairy thickening of labellum appearing greenish-white. The flowers were striped, showing regular, dark pink striations.

Plate X. Some of the new, promising *Dendrobium* hybrids

Single Flower Inflorescence

H-95 *D.* Renapa Red 3 x *D.* CSTD [P_5 x P_1]

Medium long stanting inflorescences with 5-8 flowers per inflorescence. Flowers large, deep pink and striped with a full, rounded look. Sepals and petals very thick and glossy.

Single Flower Inflorescence

H-74 *Dendrobium* Renapa Red 3 x *D.* Walter Oumae [P_5 x P_{12}]

Long arching inflorescences with 12-18 flowers per inflorescence. Flowers medium large and light purple (lilac) coloured with semi full appearance. Sepals and petals medium thick and glossy.

Single Flower Inflorescence

H-73 *Dendrobium* Renapa Red x *D.* Walter Oumae [P_5 x P_{12}]

Long arching inflorescences with 12-15 flowers per inflorescence. Flowers large, magenta coloured and stellar in appearance. Sepals and petals thick and glossy

Single Flower Inflorescence

H-196 *D.* Renapa Red 3 x *D.* Walter Oumae [P_5 x P_{12}]

Long arching inflorescences with 10-14 flowers per inflorescence. Flowers large, uniform white with faint pink tinge on labellum, sepals and petals narrow. Flowers thick and glossy.

Single Flower Inflorescence

H-210 *Dendrobium* Rinabha x *D.* CSTD [P_6 x P_1]

Medium large slightly arching inflorescences with 7-10 flowers per inflorescence. Flowers large and faintly striped, magenta coloured with rounded, full appearance. Sepals and petals thick and glossy.

★ Selected promising hybrids

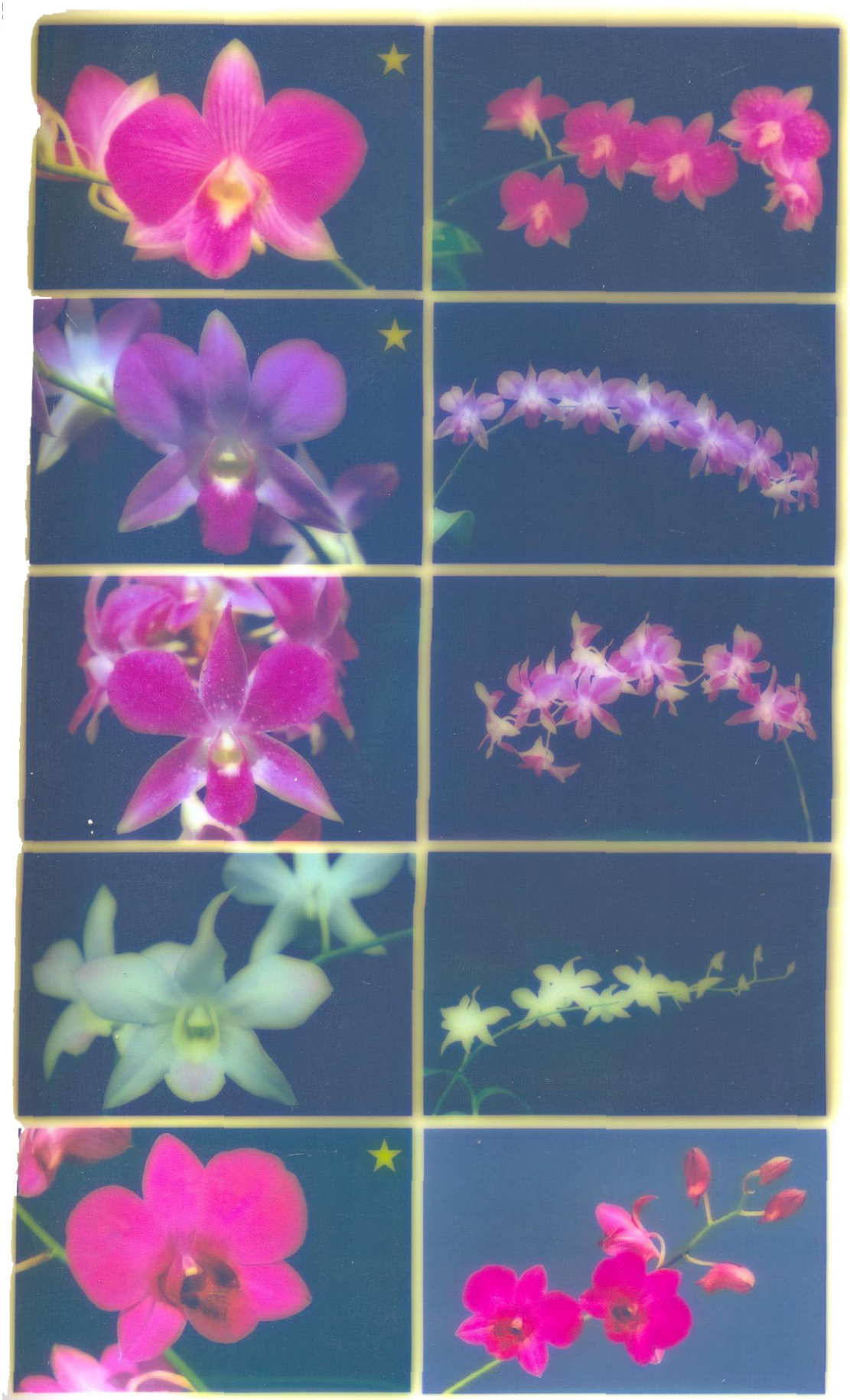


Plate X

In P_2 x P_3 the flowers were light pink in colour with a green tinge at the central part of the flower and on the sepals. The central hairy thickening of labellum appeared as a broad, white band.

In P_3 x P_1 the flowers were light pink to deep pink in colour and striped. Gradation in pigmentation was observed in some cases with the sepals showing light pink, the lateral petals showing a deeper shade and the labellum exhibiting the deepest shade of pink. In most of the cases, the labellum was of a deeper hue compared to the rest of the flower. The central hairy thickening of the labellum was almost completely deep pink in certain cases, had a narrow patch of white along the centre with deep pink margins in certain others and was a comparatively broader patch of white in some other cases. Stripes were deep pink in colour, ranging in clarity from faint to prominent and in distribution from along the centre of petals to throughout the entire flower.

In P_3 x P_6 the flowers were pink, labellum with deep pink, lateral petals with lighter pink and the sepals with a pale pink colour. The central part of the flower was white in colour. The central hairy thickening of labellum had a narrow white patch along the centre with deep pink borders. White colouration was observed at the sepal tips also.

Variation in floral pigmentation was much pronounced within the combination P_4 x P_1. In one type, the flowers were double coloured and unstriped, being purple towards the outside and white towards the central portion, giving a radiant appearance. In another type, the flowers were a uniform medium pink or deep pink and striped, the stripes being

Plate XI. Some of the new, promising *Dendrobium* hybrids

Single Flower Inflorescence

H-298 *Dendrobium* Rinabha x *D.* CSTD [P_6 x P_1]

Long slightly arching inflorescences with 8-12 flowers per inflorescence. Flowers large magenta coloured and striped with full appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-372 *Dendrobium* Rinabha x *D.* CSTD [P_6 x P_1]

Short inflorescences with 5-8 flowers per inflorescence. Flowers large and faintly striped, magenta coloured with rounded full appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-176 *Dendrobium* Rinabha x *D.* CSTD [P_6 x P_1]

Long slightly arching inflorescences with 10-15 flowers per inflorescence. Flowers large, purplish magenta coloured and striped with full appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-316 *Dendrobium* Rinabha x *D.* CSTD [P_6 x P_1]

Long slightly arching inflorescences with 10-12 flowers per inflorescence. Flowers large and dark solid purple coloured with rounded, full appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-178 *D.* Rinabha x *D.* Nagoya Pink [P_6 x P_3]

Medium long arching inflorescences with 6-10 flowers per inflorescence. Flowers large, deep and light pink and perfectly shaped with a full, rounded look, the petals overlapping the sepals. Sepals and petals thick and glossy.

★ Selected promising hybrids

Plate XI

prominent in some cases and faint in certain others. In yet another type, the flowers were pink and striped with prominent colour gradation between and shading on the sepals and petals.

In P_4 x P_3 the flowers appeared light pink, the central portion being white and outer regions being a slightly deeper shade of pink.

In P_5 x P_1 the flowers were deep pink and white, with the petals, labellum and sepals showing a deep pink colour. The sepal tips exhibited a prominent patch of white colour. The labellum showed a broad, tapering, hairy patch of white along the centre.

Much variation in pigmentation was observed within P_5 x P_{12}. The majority exhibited deep pink labellum, light pink petals and lighter pink sepals whereas certain types were a uniform deep pink. Uniform white types with a faint pink tinge along the veins at the tip of the labellum and petals were also observed.

In P_6 x P_1 floral pigmentation ranged from solid deep magenta pink to solid deep purple. The central part of the flower was white in certain cases and deep magenta pink in others. The central hairy thickening of labellum was either white or deep velvety magenta. In several solid deep magenta pink types, the entire flower was pigmented except the operculum (anther cap), which was white in colour.

In P_6 x P_3 the flowers were solid deep magenta pink in colour. Central part of the flower was white. The central hairy thickening of

Plate XII. Some of the new, promising *Dendrobium* hybrids

Single Flower Inflorescence

H-479 *D.* Rinabha x *D.* Nagoya Pink [P_6 x P_3]

Long arching inflorescences with 10-14 flowers per inflorescence. Flowers large, deep and light pink, perfectly shaped and full with the petals overlapping the sepals. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-480 *Dendrobium* Rinabha x *D.* White Fairy [P_6 x P_9]

Long slightly arching inflorescences with 15-20 flowers per inflorescence. Flowers medium sized, solid deep magenta pink and full. Sepals and petals very thick and glossy.

Single Flower Inflorescence

H-291 *D.* Rinabha x *D.* White Fairy [P_6 x P_9]

Long erect inflorescences with 12-18 flowers per inflorescence. Flowers medium large and solid deep magenta pink with narrow sepals, spatulate petals and narrow labellum. Sepals and petals very thick and glossy.

Single Flower Inflorescence

H-368 *D.* Rinabha x *D.* White Fairy [P_6 x P_9]

Long slightly arching inflorescences with 8-12 flowers per inflorescence. Flowers medium sized, medium purple and full. Sepals and petals very thick and glossy.

Single Flower Inflorescence

H-53 *Dendrobium* Sakura x *D.* CSTD [P_7 x P_1]

Long arching inflorescences with 12-15 flowers per inflorescence. Flowers large with bold dark pink stripes and stellar appearance. Sepals and petals thick and glossy.

★ Selected promising hybrids

Plate XII

labellum was white towards the centre and a deeper magenta pink towards the periphery.

The pigmentation in P_6 x P_9 was either solid deep magenta throughout or deep purple in the petals and labellum with lighter purple sepals. Central part of the flower was also pigmented except the operculum, which was white in colour. Central hairy thickening of labellum always exhibited a deeper shade of the same colour as the labellum, which is a rarity.

The combination P_7 x P_1 exhibited uniform light pink flowers with deep pink stripes throughout. The uniformity of the background colour and the clarity of stripes varied in different cases. Central part of the flower and central hairy thickening of labellum appeared white.

In P_7 x P_9 the pigmentation was very light pink and white. Central part of the flower, inner portion of sepals and petals and the central hairy thickening of labellum appeared white. Outer portion of sepals and petals exhibited a faint pink colour.

In P_8 x P_1 the flowers were solid deep purple with faint or prominent deeper coloured stripes. In another type, the flowers were light pink with deeper pink petal tips and labellum and striped throughout.

High variation for floral pigmentation was noticed within the combination P_{11} x P_2. The flowers were either deep magenta pink or medium pink in colour. Some types exhibited single solid colour whereas in others a colour gradation was observed with deep pink labellum, light

pink petals and lighter pink sepals. The central hairy thickening of labellum was white with pink stripes or sometimes white at the centre and magenta pink towards the periphery.

5. Texture of flower

The flowers appeared very thick, substantial looking and glossy in $P_5 \times P_1$, $P_6 \times P_1$, $P_6 \times P_9$, $P_7 \times P_9$ and $P_8 \times P_1$. Thick, substantial looking and glossy flowers were observed in $P_3 \times P_6$, $P_4 \times P_3$, $P_5 \times P_{12}$, $P_6 \times P_3$, $P_7 \times P_1$and $P_{11} \times P_2$. In $P_4 \times P_1$ the flowers were substantial looking and glossy, usually very thick and rarely, thick. In $P_3 \times P_1$ the flowers appeared substantial looking and glossy, usually thick and rarely, very thick. The combination $P_1 \times P_4$ produced flowers that were thick or moderately thick, substantial looking and glossy. In $P_2 \times P_1$ the flowers appeared moderately thick and glossy. The flowers in $P_2 \times P_3$ were moderately thick, substantial looking and glossy.

6. Shape of flower

The shape of flower in the hybrids ranged from flowers with full, broad, rounded sepals and petals to those possessing narrow, spatulate, pointed sepals and petals. The flowers in certain hybrids appeared flat, whereas in certain others they were reflexed. Sepal tips appeared reflexed, straight, incurved or twisted depending on the hybrid. Variation for flower shape within the combination was observed in some cases.

Plate XIII. Some of the new, promising *Dendrobium* hybrids

Single Flower Inflorescence

H-380 *Dendrobium* Sakura x *D.* White Fairy [P_7 x P_9]

Short erect inflorescences with 5-8 flowers per inflorescence. Flowers medium large, white with pink tinge and stellar appearance. Sepals and petals medium thick and glossy.

Single Flower Inflorescence

H-283 *D.* Sakura x *D.* White Fairy [P_7 x P_9]

Short erect inflorescences with 4-8 flowers per inflorescence. Flowers medium large, very light pink and white with narrow, spatulate sepals and petals and a stellar appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-177 - *Dendrobium* Sonia 16 x *Dendrobium* (Candy Stripe x Tomie Drake) (D. CSTD) [P_8 x P_1]

Long arching inflorescences with 8 to 12 flowers per inflorescence. Flowers large and dark purple coloured with stellar appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-147 *Dendrobium* Sonia 16 x *D.* CSTD [P_8 x P_1]

Medium long arching inflorescences with 6-10 flowers per inflorescence. Flowers large and dark magenta coloured with rounded, full appearance. Sepals and petals thick and glossy.

Single Flower Inflorescence

H-183 *Dendrobium* Uniwai Pink x *D.* Chiangmai Pink [P_{11} x P_2]

Short slanting inflorescences with 7-10 flowers per inflorescence having short internodes. Flowers small, purple coloured with semi full appearance. Sepals and petals thick and glossy.

★ Selected promising hybrids

Plate XIII

In P_1x P_4 the flowers appeared flat with the sepals and petals broad or narrow and spatulate. The labellum was either flat or reflexed.

In P_2 x P_1 the flowers were flat, presenting either a squarish or a rounded look. Sepals and petals appeared broad with the sepal tips slightly incurved or twisted.

In P_2 x P_3 the flowers presented a full appearance, with broad and rounded sepals and petals.

The combination P_3 x P_1 presented flowers that were perfectly shaped with full, rounded sepals and petals; the petals sometimes appeared to completely overlap the sepals. The labellum was broad, full and flowing down straight. In certain cases, slight fenestration of the edges of petals and labellum was observed.

The flowers in P_3 x P_6 were perfectly shaped with full, rounded sepals and petals, the petals almost fully overlapping the lateral sepals. The labellum was broad, full and flat.

Much variation in flower shape was evident within P_4 x P_1ranging from full, rounded flowers to squarish flowers. Sepals and petals ranged in shape from broad to narrow. Some flowers presented a compact appearance without much clearance between the lateral sepal tips, whereas in some other flowers they were wide apart. Labellum appeared flat in certain cases and reflexed in certain others. The edges of the labellum mostly appeared smooth and entire, or was rarely fenestrated. The tip of

the labellum presented different shapes such as broad and flat, rounded or pointed.

The flowers in $P_4 \times P_3$ presented a full, rounded look with broad sepals and petals. In $P_5 \times P_1$ the flowers were perfectly shaped, full and flat with broad and rounded sepals and petals. The flowers in $P_5 \times P_{12}$ had narrow sepals, spatulate petals and a narrow labellum. A slight twisting of the sepals was noticed, usually restricted to the dorsal sepal alone, but occasionally evident in all sepals.

Considerable range in flower shape was observed within $P_6 \times P_1$ varying from perfectly shaped flowers with full, broad sepals and petals to flowers with spatulate petals and long, narrow sepals. The sepals and labellum were usually rounded, but sometimes pointed, giving a stellar appearance.

In $P_6 \times P_3$ the flowers were perfectly shaped with full, rounded sepals and petals, the petals almost completely overlapping the lateral sepals.

In $P_6 \times P_9$ the flowers were full with broad, rounded sepals; petals were either broad and rounded or spatulate.

In $P_7 \times P_1$ some flowers presented a half-open look with the labellum tucked up and the dorsal sepal slanting down, giving a squarish appearance. The sepals and petals were either broad or narrow, but usually angular and pointed, giving a stellar appearance. In a few cases, the flowers exhibited broad and rounded sepals and petals.

In P_7 x P_9 the petals were spatulate and the sepals narrow, pointed and slightly twisted.

In P_8 x P_1 the flowers presented a flat look. The sepals and petals were sometimes pointed, giving a stellar appearance. In certain cases, the sepals and petals were broad and rounded giving a full, flat look.

In P_{11} x P_2 the petals appeared spatulate, sometimes flat and in yet other cases slightly reflexed. The sepals were flat and pointed giving a stellar appearance. Slight reflexing of sepals was sometimes noticed. In certain instances, slight twisting of sepals, petals and labellum with or without fenestrations was observed.

7. Flower size

Mean flower size (circumference of flower) in the 16 hybrid combinations ranged from 18.53 cm in P_{11} x P_2 to 25.07 cm in P_4 x P_3. Flowers were comparatively large in size in P_1 x P_4 (24.95 cm), P_8 x P_1 (23.55 cm) and P_3 x P_1 (23.20 cm). Slightly smaller flowers were observed in P_5 x P_1 (22.92 cm), P_6 x P_1 (22.72 cm) and P_4 x P_1(22.29 cm). Coming next were the hybrids P_6 x P_3 with 21.93 cm, P_3 x P_6 with 21.87 cm, P_7 x P_9 and P_5 x P_{12} with 21.40 cm each, P_6 x P_9 with 21.20 cm and P_7 x $P_1$with 21.12 cm flower size. The smallest flowers were produced by P_2 x P_1(19.86 cm) and P_2 x P_3 (19.75 cm).

8. Fullness value

Fullness value was observed to range from 2.16 in P_3 x $P_1$to 3.53 in P_{11} x P_2. The flowers appeared remarkably full with low fullness values in two other combinations also, being 2.26 in P_3 x P_6 and 2.36 in P_6 x P_3. Comparatively low fullness values indicating considerable fullness were noted in P_8 x P_1(2.60), P_2 x P_3 (2.62), P_6 x P_1(2.67), P_5 x P_1(2.72), P_4 x P_1(2.95) and P_4 x P_3 (2.97). Fullness values recorded were slightly higher in P_7 x P_1(3.16), P_1x P_4 (3.17), P_2 x P_1(3.19), P_6 x P_9 (3.21), P_7 x P_9 (3.27) and P_5 x P_{12} (3.28).

9. Fragrance

Fragrance was observed in several hybrids belonging to the combination P_{11} x P_2. The intensity of the pleasant scent ranged from faint to considerably strong and the duration of scent evolution varied from a few days to several days after flower opening.

4.3.6. Combining ability analysis

All possible combinations including reciprocals were tried among the 14 parental genotypes. Out of these, seedlings from 67 combinations were successfully hardened and established in the green house. In the interest of studying gene action in terms of combining ability effects, a partial diallel (Kempthorne and Curnow, 1961) with 18 cross combinations was developed (Table 4.3.6.a). The 18 crosses were raised in a CRD with ten single plant replications. Data generated from these

Table 4.3.6.a. Matrix showing combinations used in partial diallel out of the 67 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}	P_{13}	P_{14}
P_1	x	x	x	x		x		x	x	x				
P_2	⊗		x	x		x	⊗		⊗					
P_3	⊗		x	⊗	⊗	⊗	x	x		x				
P_4	⊗		x	x	⊗	⊗								
P_5	x					⊗	⊗	x				x		
P_6	x		x	x					x			x		
P_7	x	x	x	x			x		x					
P_8	⊗	⊗					⊗		⊗					
P_9	x				x	⊗	⊗							
P_{10}	x	x					x							
P_{11}														
P_{12}														
P_{13}														
P_{14}														

x - Successful combinations ⊗ - Combinations used in partial diallel

Contd....

Table 4.3.6.a. (Contd....)

Crossing scheme in partial diallel mating

	P_8	P_3	P_2	P_4	P_9	P_5	P_7	P_6	P_1
P_8			⊗		⊗		⊗		⊗
P_3				⊗		⊗		⊗	⊗
P_2					⊗		⊗		⊗
P_4						⊗		⊗	⊗
P_9							⊗	⊗	
P_5							⊗	⊗	
P_7									
P_6									
P_1									

18 crosses with respect to the eight vegetative characters *viz.*, number of shoots per clump, number of leaves per clump, height/length of cane, number of nodes per cane, number of leaves per cane, leaf area per cane, length of leaf and width of leaf were subjected to combining ability analysis (Sharma, 1998).

Skeleton of ANOVA for partial diallel model

(Combining ability analysis)

Source	df	MS	Expected MS
Crosses (C)	c-1 = 17	Mc	
gca (n)	n-1 = 8	Mg	$\sigma^2_e + r\,\sigma^2_{sca} + \frac{rs\,(n-2)}{(n-1)}\,\sigma^2_{gca}$
sca (s)	c-n = 9	Ms	$\sigma^2_e + r\sigma^2_{sca}$
Error (e)	c(r-1) = 162	Me	σ^2_e
Total	cr-1 = 179		

The following estimates were computed

Error variance $\hat{\sigma}_e^2 = Me$

sca variance $\hat{\sigma}^2_{sca} = \frac{Ms - Me}{r}$

gca variance $\hat{\sigma}^2_{gca} = \frac{n-1}{rs\,(n-2)}$ (Mg - Ms)

Additive variance $\hat{\sigma}^2_a = 2\hat{\sigma}^2_{gca}$

Dominance variance $\hat{\sigma}^2_d = \hat{\sigma}^2_{sca}$

s = number of crosses per parent = 4, n = 9

gca effects are estimated as

$g_i = \underline{Q}\underset{\sim}{A}^{-1}$

$\underline{Q}$ is the vector of the adjusted array means

$\underset{\sim}{A}$ is the incidence matrix given as

$$\begin{bmatrix} 4 & 0 & 1 & 0 & 1 & 0 & 1 & 0 & 1 \\ 0 & 4 & 0 & 1 & 0 & 1 & 0 & 1 & 1 \\ 1 & 0 & 4 & 0 & 1 & 0 & 1 & 0 & 1 \\ 0 & 1 & 0 & 4 & 0 & 1 & 0 & 1 & 1 \\ 1 & 0 & 1 & 0 & 4 & 0 & 1 & 1 & 0 \\ 0 & 1 & 0 & 1 & 0 & 4 & 1 & 1 & 0 \\ 1 & 0 & 1 & 0 & 1 & 1 & 4 & 0 & 0 \\ 0 & 1 & 0 & 1 & 1 & 1 & 0 & 4 & 0 \\ 1 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 4 \end{bmatrix}$$

$$SE\ (g_i - g_j) = \left\{ 2\left[\frac{na''}{n-1} - \frac{1}{2s(n-1)}\right]\left[\sigma^2_{sca} + \frac{\sigma^2_e}{r}\right]\right\}^{1/2}$$

where a" = diagonal element of A^{-1} matrix

4.3.6.1. Analysis of variance in partial diallel

Analysis of variance in partial diallel has been presented (Table 4.3.6.b.). Both gca and sca variances were significant for all characters

Table 4.3.6.b. Abstract of ANOVA in partial diallel

Source	df	MS							
		No. of shoots per clump	No. of leaves per clump	Height / length of cane (cm)	No. of nodes per cane	No. of leaves per cane	Leaf area per cane (cm^2)	Length of leaf (cm)	Width of leaf (cm)
Replications	9	1.1240	38.8767	18.3385	1.1852	0.9284	775.5556	5.5213	0.1573
Crosses	17	1.2957	119.2886	187.2270	16.2889	14.1673	117519.5	19.2057	5.7273
gca	8	2.2654*	128.4527**	182.3247**	20.8237**	18.6236**	136020**	16.1404**	7.8034**
sca	9	2.4346	111.1427**	191.5847**	12.2580**	10.2061**	101074.6**	21.9303**	3.8819**
Error	153	0.4875	15.5380	10.4082	0.8139	0.8761	756.0915	2.9651	0.1254

** - Significant at 1 per cent level * - Significant at 5 per cent level

except number of shoots per clump where gca variance alone was significant showing that both additive and non additive gene actions were controlling character expression. For the character number of shoots per clump, environmental variance was higher than sca. Mean square sca (Ms) was greater than mean square of gca (Mg) for number of shoots per clump, height of cane and leaf length. Specific combining ability variance was higher than gca variance (Table 4.3.6.c.) for all the five traits *viz.,* number of leaves per clump, number of nodes per cane, number of leaves per cane, leaf area per cane and width of leaf, indicating the predominance of dominance gene action. The ratio of additive variance to dominance variance also indicated the predominance of dominance gene action over additive gene action.

4.3.6.2. gca effects of the nine parents in partial diallel

The gca effects of the nine parents included in the partial diallel analysis have been studied (Table 4.3.6.d.; Fig. 10). The gca effect of P_3 was positive and P_2 was negative and both were significant for number of shoots per clump. Since more number of shoots are preferred, the gca effect of P_3 alone assumed importance and that of P_2 was not desirable. The gca effects of P_2 were significant and negative for number of nodes per cane, number of leaves per cane, length of leaf and width of leaf. Since more number of nodes and more leaves having greater length and width are preferred, these negative effects were not desirable.

Table 4.3.6.c. Variance components in partial diallel

Character	σ^2gca	σ^2sca	σ^2a	σ^2d	σ^2e	$\sigma a^2 / \sigma d^2$
X_1 - Number of shoots per clump	ne	ne	0.1046	ne	0.4875	ne
X_2 - Number of leaves per clump	0.4946	9.5604	0.9892	9.5604	15.5380	< 1
X_3 - Height of cane (cm)	ne	18.1177	ne	18.1177	10.4082	ne
X_4 - Number of nodes per cane	0.2447	1.1444	0.4895	1.1444	0.8139	< 1
X_5 - Number of leaves per cane	0.2405	0.9330	0.4810	0.9330	0.8761	< 1
X_6 - Leaf area per cane (cm^2)	998.4398	10031.85	1996.88	10031.85	756.0915	< 1
X_7 - Length of leaf (cm)	ne	1.8965	ne	1.8965	2.9651	ne
X_8 - Width of leaf (cm)	0.1120	0.3756	0.2241	0.3756	0.1254	< 1

ne - not estimable

Table 4.3.6.d. gca effects of parents in partial diallel

Parental genotypes	No. of shoots per clump	No. of leaves per clump	Height / length of cane (cm)	No. of nodes per cane	No. of leaves per cane	Leaf area per cane (cm^2)	Length of leaf (cm)	Width of leaf (cm)
P_1	0.1855	0.2211	-0.9490	0.2911	0.2322	-30.7578	0.2323	-0.0232
P_2	-0.4588*	-2.0791	-3.5661	-1.8247*	-1.7846*	-111.9199	-1.2451*	-1.0526*
P_3	0.4932*	3.3980	-1.3542	-0.0897	-0.0409	14.3579	-0.0379	-0.0785
P_4	-0.0734	-2.4020	-2.5508	-0.8231	-0.6409	-55.6857	-0.4679	-0.3115
P_5	-0.1516	-1.1673	2.0753	0.4649	0.6063	56.5779	0.6699	0.2359
P_6	-0.1891	-0.8548	0.5515	0.2149	0.3438	-0.1471	0.1837	-0.0872
P_7	-0.0202	-0.8830	2.2425	0.8274	0.4907	82.6516	0.6390	0.6644
P_8	-0.0588	2.7875	2.7272	0.1519	0.1153	-5.8532	-0.8318	-0.1192
P_9	0.2327	0.9795	0.8237	0.7774	0.6782	50.7766	0.8577	0.4406
SEg_i	0.1191	1.9054	2.5017	0.6328	0.5774	57.4606	0.8464	0.3561
$SE(g_i-g_j)$	0.1685	2.6943	3.5374	0.8948	0.8164	81.2492	1.1968	0.5035
CD (0.05)	0.3302	5.2807	6.9332	1.7537	1.6002	159.2485	2.3457	0.9869

* - Significant at 5 per cent level

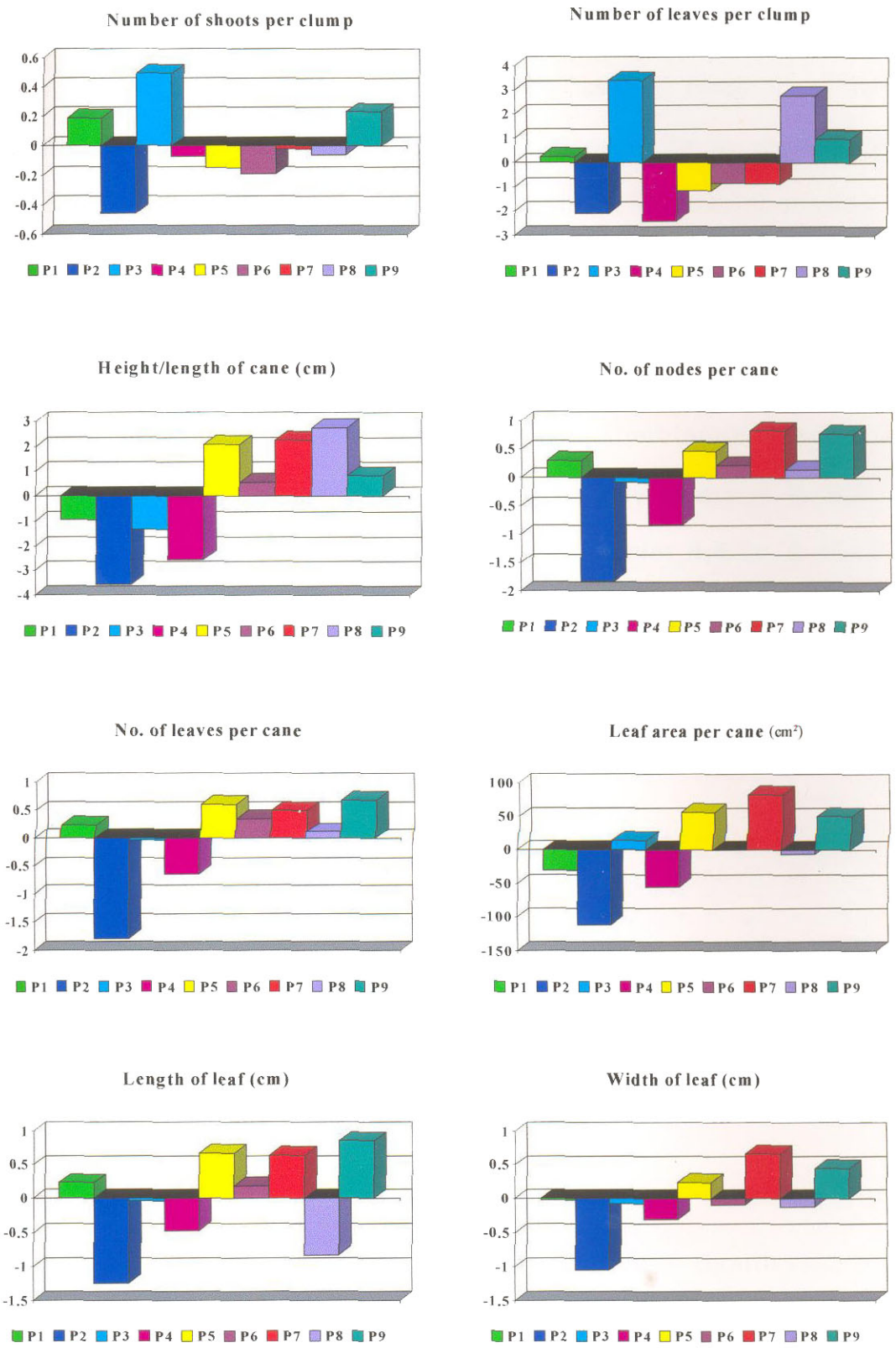


Fig. 10. gca effects of parents in partial diallel

4.4. Heterosis

Heterosis for the 62 (29 direct F_1s and 33 reciprocals) hybrid combinations were estimated with respect to seven vegetative characters at 1.5 - 2.0 years after transplanting. Estimation of economic heterosis (in relation to the economic parent P_8), relative heterosis and heterobeltiosis was done. Percentage estimates of economic heterosis, relative heterosis and heterobeltiosis for each character have been studied (Tables 4.4.1 to 4.4.7.). Hybrid combinations exhibiting significant positive heterosis in each of its three measures for each of the seven characters have been represented (Tables 4.4.1.a to 4.4.7.a.). The mean values for the seven vegetative characters at 1.5 - 2.0 years growth with respect to the 14 parents (Table 4.4) and with respect to the 62 hybrids (Tables 4.3.5.1.b. to 4.3.5.1.h.) have been provided for comparison.

4.4.1. Number of leaves per clump

Percentage estimates of economic heterosis, relative heterosis and heterobeltiosis for number of leaves per clump (Table 4.4.1.) and combinations showing positive significance (Table 4.4.1.a.) have been studied.

Ten hybrids out of the 62 exhibited positive significance for all the three measures of heterosis. These ten hybrids consisted of four F_1s, *viz.*, $P_1 \times P_8$, $P_3 \times P_6$, $P_3 \times P_8$ and $P_3 \times P_{10}$ and six reciprocals *viz.*, $P_2 \times P_1$, $P_8 \times P_1$, $P_8 \times P_2$, $P_{10} \times P_1$, $P_{10} \times P_2$ and $P_{11} \times P_2$.

Table 4.4. Mean performance of parental genotypes of *Dendrobium* at 1.5-2.0 years for vegetative characters

Parental genotypes	No. of shoots per clump	No. of leaves per clump	Height / length of cane (cm)	No. of nodes per cane	No. of leaves per cane (cm)	Leaf area per cane (cm^2)	Length of leaf (cm)	Width of leaf (cm)
P_1	6.2	20.2	17.80	5.8	5.2	170.85	15.10	3.38
P_2	6.1	21.6	17.40	6.2	5.6	184.40	15.05	3.37
P_3	6.0	20.8	18.15	6.4	5.1	180.50	13.95	3.55
P_4	6.9	22.5	22.50	6.3	5.5	187.15	13.35	3.48
P_5	6.7	27.6	27.45	6.8	6.4	254.40	13.10	3.73
P_6	7.8	25.5	24.65	6.4	5.4	227.80	12.90	3.75
P_7	7.9	27.8	26.45	8.2	6.3	281.85	12.55	4.56
P_8	6.8	22.3	22.80	6.4	5.4	208.35	11.75	3.42
P_9	7.8	29.1	30.90	8.5	6.6	316.55	11.70	4.27
P_{10}	7.8	22.0	30.80	8.5	6.5	106.25	11.40	2.07
P_{11}	6.2	22.4	22.40	8.3	6.7	224.15	11.30	3.75
P_{12}	6.0	21.0	16.65	7.5	5.9	164.10	11.15	2.91
P_{13}	6.8	21.9	23.60	15.1	9.3	98.40	6.70	1.86
P_{14}	6.9	33.6	34.93	15.7	8.5	257.45	8.55	2.65
SEm	0.247	0.781	1.181	0.477	0.325	13.595	0.365	0.065
CD (0.05)	0.692	2.188	3.308	1.335	0.910	30.068	1.022	0.182

Table 4.4.1. Heterosis for number of leaves per clump (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}	P_{13}	P_{14}
P_1	EH		9.42	0.45	8.97		20.18*		21.97*	18.83*	-4.04				
	RH		16.75*	9.27	13.62		17.29*		28.00*	7.51	1.42				
	HB		12.96	7.69	8.00		5.1		21.97*	-8.94	-2.73				
P_2	EH	24.66*		6.73	8.52		12.11	-8.52		-7.58					
	RH	33.01*		12.26	9.76		6.16	-17.49*		-16.34*					
	HB	28.70*		10.19	7.56		-1.96	-26.62*		-28.87*					
P_3	EH	15.25			15.70	34.98*	38.57*	30.49*	31.84*		28.28*				
	RH	25.37*			19.17*	24.38*	33.48*	19.75*	36.43*		33.65*				
	HB	23.56*			14.67	9.06	21.18*	4.68	31.84*		30.00*				
P_4	EH	7.62		0.45		-0.90	4.04								
	RH	12.41		3.46		-11.78	-3.33								
	HB	6.67		-0.44		-19.93*	-9.02								
P_5	EH	4.04					-6.28	10.31	-1.79				23.77*		
	RH	-10.46					-21.28*	-11.19	-12.22				13.58		
	HB	-22.46*					-24.27*	-11.51	-20.65*				0.00		
P_6	EH	25.11*		24.66*	29.15*					30.94*			19.73*		
	RH	22.10*		20.09*	20.00*					6.96			14.84		
	HB	9.41		9.02	12.94					0.34			4.71		
P_7	EH	4.48	3.59	5.38	15.7					25.11*					
	RH	-2.92	-6.48	-3.29	2.58					-1.93					
	HB	-16.19*	-16.91*	-15.47*	-7.19					-4.12					

Table 4.4.1. (Contd...)

♀	♂	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_{13}	P_{14}
P_8	EH	26.46*	30.05*					13.45		34.53*					
	RH	32.71*	32.12*					1.00		16.73*					
	HB	26.46*	30.05*					-8.99		3.09					
P_9	EH	31.39*				26.46*	14.35	33.63*							
	RH	18.86*				-0.53	-6.59	4.75							
	HB	0.69				-3.09	-12.37	2.41							
P_{10}	EH	25.56*	34.08*					24.66*							
	RH	32.70*	37.16*					11.65							
	HB	27.27*	33.91*					0.00							
P_{11}	EH		23.77*		18.39*								9.41		
	RH		25.46*		17.60*								12.44		
	HB		23.21*		17.33								8.93		
P_{12}	EH				18.39*	21.52*						8.52			
	RH				21.38*	11.52						11.52			
	HB				17.33	-1.81						8.04			
P_{13}	EH								4.93	0.00					
	RH								5.88	-12.55					
	HB								4.93	-23.37*					
P_{14}	EH	47.98*	30.05*	17.49			7.62								
	RH	22.68*	5.07	-3.68			-18.78*								
	HB	-1.79	-13.69	-22.02*			-28.57*								

CD (0.05) EH - 3.938 RH - 3.410 HB - 3.938

* Significant at 5 per cent level

Table 4.4.1.a. Significant positive heterosis for number of leaves per clump

♀ \ ♂	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_{13}	P_{14}
P_1		R				ER		ERH	E					
P_2	ERH													
P_3	RH			R	ER	ERH	ER	ERH		ERH				
P_4														
P_5												E		
P_6	ER		ER	ER					E			E		
P_7									E					
P_8	ERH	ERH							ER					
P_9	ER				E		E							
P_{10}	ERH	ERH					E							
P_{11}		ERH		ER										
P_{12}				ER	E									
P_{13}														
P_{14}	ER	E												

E - Significant positive economic heterosis R - Significant positive relative heterosis H - Significant positive heterobeltiosis

Significant positive economic heterosis was displayed by 31 hybrids comprising of 13 F_1s and 18 reciprocals. Relative heterosis was significant and positive in 24 combinations constituted by ten F_1s and 14 reciprocals. Heterobeltiosis was found to be significant and positive in four F_1s and seven reciprocals. Ten out of these 11 hybrids recorded positive significance for heterosis in all the three measures and in $P_3 \times P_1$, relative heterosis and heterobeltiosis were positive and significant.

All the hybrids were either on par with or were significantly superior to the economic parent for number of leaves per clump. Significant, but negative relative heterosis was recorded by $P_2 \times P_7$, $P_2 \times P_9$, $P_5 \times P_6$ and $P_{14} \times P_6$. Heterobeltiosis was significant and negative in 12 combinations, *viz.,* five F_1s and seven reciprocals; in addition to the four combinations mentioned above, these included $P_4 \times P_5$, $P_5 \times P_8$, $P_5 \times P_1$, $P_7 \times P_1$, $P_7 \times P_2$, $P_7 \times P_3$, $P_{13} \times P_9$ and $P_{14} \times P_3$.

4.4.2. Height/length of cane

Percentage estimates of economic heterosis, relative heterosis and heterobeltiosis for length of cane (Table 4.4.2.) and the combinations recording positive significance (Table 4.4.2.a.) have been studied.

Thirty hybrids out of the 62 registered positive significance for all the three measures of heterosis for cane height. These 30 hybrids consisted of 16 F_1s and 14 reciprocals.

Significant positive economic heterosis was exhibited by 48 hybrids constituted by 21 F_1s and 27 reciprocals. Significant positive

Table 4.4.2. Heterosis for height of cane (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}	P_{13}	P_{14}
P_1	EH		19.43*	-4.74	16.05*		19.25*		6.14	20.66*	-2.06				
	RH		54.72*	20.83*	31.32*		28.10*		19.21*	12.98*	-8.11				
	HB		52.98*	19.67*	17.60*		10.30		6.14	-10.97*	-27.50*				
P_2	EH	23.33*		20.92*	23.95*		38.73*	-7.76		-5.13					
	RH	59.77*		55.11*	41.65*		50.44*	-4.08		-10.44					
	HB	57.98*		51.90*	25.60*		28.32*	-20.49*		-30.00*					
P_3	EH	-4.52			-1.09	35.09*	27.85*	28.55*	37.46*		-10.48				
	RH	21.11*			10.95	35.09*	36.22*	31.44*	53.07*		-16.61*				
	HB	19.95*			0.22	12.20*	18.25*	10.81	37.46*		-33.73*				
P_4	EH	5.61		0.88		11.18	25.88*								
	RH	19.50*		13.16		1.50	21.74*								
	HB	7.02		2.22		-7.65	16.43*								
P_5	EH	11.14					13.60	56.58*	42.02*				57.50*		
	RH	12.00					-0.58	32.47*	28.88*				62.86*		
	HB	-7.68					-5.65	30.06*	17.96*				30.82*		
P_6	EH	28.07*		31.67*	46.10*					56.71*			43.68*		
	RH	37.57*		40.28*	41.29*					28.64*			58.64*		
	HB	18.46*		21.79*	35.13*					15.63*			32.90*		
P_7	EH	15.09*	26.54*	34.12*	47.10*					58.77*					
	RH	18.60*	31.59*	37.13*	37.03*					26.24*					
	HB	-0.79	9.07	15.61*	26.81*					17.15*					

Table 4.4.2. (Contd...)

♀	♂	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_{13}	P_{14}
P_8	EH	23.03*	33.16*					32.24*		37.98*					
	RH	38.18*	51.05*					22.44*		17.17*					
	HB	23.03*	33.16*					13.99*		1.81					
P_9	EH	36.05*				32.98*	22.85*	52.24*							
	RH	27.39*				3.93	0.85	21.05*							
	HB	0.39				-1.88	-9.35	12.33*							
P_{10}	EH	26.32*	24.25*					28.29*							
	RH	18.52*	17.55*					2.18							
	HB	-6.49	-8.02					-5.03							
P_{11}	EH		42.36*		34.47*								29.56*		
	RH		63.12*		36.57*								51.29*		
	HB		44.91*		36.27*								31.88*		
P_{12}	EH				15.08*	30.26*						15.04*			
	RH				34.05*	34.69*						34.34*			
	HB				16.62*	8.20						17.10*			
P_{13}	EH								13.55	4.96					
	RH								11.60	-12.18					
	HB								9.70	-22.56*					
P_{14}	EH	15.88*	30.09*	22.41*			16.67*								
	RH	0.20	13.36*	5.16			-10.71								
	HB	-24.36*	-15.09*	-20.10*			-23.85*								

CD (0.05) EH - 3.154 RH - 2.731 HB - 3.154

* Significant at 5 per cent level

Table 4.4.2.a. Significant positive heterosis for height of cane

♀ \ ♂	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_{13}	P_{14}
P_1		ERH	ER	ERH		ER		R	ER					
P_2	ERH		ERH	ERH		ERH								
P_3	RH				ERH	ERH	ER	ERH						
P_4	R					ERH								
P_5							ERH	ERH				ERH		
P_6	ERH		ERH	ERH					ERH			ERH		
P_7	ER	ER	ERH	ERH					ERH					
P_8	ERH	ERH					ERH		ER					
P_9	ER				E	E	ERH							
P_{10}	ER	ER					E							
P_{11}		ERH		ERH								ERH		
P_{12}				ERH	ER						ERH			
P_{13}														
P_{14}	E	ER	E			E								

E - Significant positive economic heterosis R - Significant positive relative heterosis H - Significant positive heterobeltiosis

relative heterosis was expressed by 45 hybrids (22 F_1s and 23 reciprocals), while 31 hybrids (16 F_1s and 15 reciprocals) showed significant positive heterobeltiosis.

In addition to the 30 combinations mentioned previously which expressed significant positive heterosis in all three measures, economic heterosis and relative heterosis were significant and positive in the following five F_1s : $P_1 \times P_3$, $P_1 \times P_6$, $P_1 \times P_9$, $P_3 \times P_7$ and $P_8 \times P_9$ and the following seven reciprocals *viz.*, $P_7 \times P_1$, $P_7 \times P_2$, $P_9 \times P_1$, $P_{10} \times P_1$, $P_{10} \times P_2$, $P_{12} \times P_5$ and $P_{14} \times P_2$.

All the hybrids were either on par with or significantly superior to the economic parent for length of cane. Significant negative heterobeltiosis was expressed by ten hybrids.

4.4.3. Number of nodes per cane

Estimates of economic heterosis, relative heterosis and heterobeltiosis for number of nodes per cane (Table 4.4.3.) and the hybrids showing positive significance (Table 4.4.3.a.) have been studied.

Positive significance in all the three measures of heterosis was displayed by 50 hybrids out of the 62, for number of nodes per cane. These 50 hybrids consisted of 26 F_1s and 24 reciprocals. All F_1s except three *viz.*, $P_1 \times P_{10}$, $P_2 \times P_7$ and $P_2 \times P_9$ registered positive significance in all three measures of heterosis. Likewise, all reciprocals except the three combinations with P_{10} as the female parent and P_1, P_2 and P_7 as the

Table 4.4.3. Percentage heterosis for number of nodes per cane

♀	♂	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_{13}	P_{14}
P_1	EH		29.69*	34.38*	42.19*		45.31*		59.38*	59.38*	25.00*				
	RH		38.33*	40.98*	50.41*		52.46*		67.21*	42.66*	11.89*				
	HB		33.87*	34.38*	44.44*		45.31*		59.38*	20.00*	-5.88				
P_2	EH	39.06*		46.88*	39.06*		65.63*	12.50		6.25					
	RH	48.33*		49.21*	42.40*		68.25*	0.00		-7.48					
	HB	43.55*		46.88*	41.27*		65.63*	-12.20*		-20.00*					
P_3	EH	43.75*			32.81*	50.00*	50.00*	53.13*	45.31*		53.13*				
	RH	50.82*			33.86*	45.46*	50.00*	34.25*	45.31*		31.54*				
	HB	43.75*			32.81*	41.18*	50.00*	19.51*	45.31*		15.29*				
P_4	EH	28.13*		32.81*		42.19*	39.06*								
	RH	35.54*		33.85*		38.93*	40.16*								
	HB	30.16*		32.81*		33.82*	39.06*								
P_5	EH	53.13*					50.00*	68.75*	65.62*				60.94*		
	RH	55.56*					45.46*	44.00*	60.61*				44.06*		
	HB	44.12*					41.18*	31.71*	55.88*				37.33*		
P_6	EH	59.38*		56.25*	71.88*					68.75*			54.69*		
	RH	67.21*		56.25*	73.23*					44.97*			42.45*		
	HB	59.38*		56.25*	71.88*					27.06*			32.00*		
P_7	EH	43.75*	53.13*	54.69*	62.50*					90.63*					
	RH	31.43*	36.11*	35.62*	43.45*					46.11*					
	HB	12.20*	19.51*	20.73*	26.83*					43.53*					

Table 4.4.3. (Contd...)

♀	♂	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_{13}	P_{14}
P_8	EH	46.88*	40.63*					46.88*		56.25*					
	RH	54.10*	42.86*					28.77*		34.23*					
	HB	46.88*	40.63*					14.63*		17.65*					
P_9	EH	68.75*				59.38*	59.38*	96.88*							
	RH	51.05*				33.33*	36.91*	50.90*							
	HB	27.06*				20.00*	20.00*	48.24*							
P_{10}	EH	40.63*	35.94*					40.63*							
	RH	25.87*	18.37*					7.78							
	HB	5.88	2.35					5.88							
P_{11}	EH		71.88*		56.25*								43.75*		
	RH		51.72*		36.99*								16.46*		
	HB		32.53*		20.48*								10.84*		
P_{12}	EH				53.13*	60.94*						50.00*			
	RH				42.03*	44.06*						21.52*			
	HB				30.67*	37.33*						15.66*			
P_{13}	EH								20.31*	20.31*					
	RH								-28.37*	-34.75*					
	HB								-49.01*	-49.01*					
P_{14}	EH	57.81*	57.81*	54.69*			43.75*								
	RH	-6.05	-7.76	-10.41*			-16.74*								
	HB	-35.67*	-35.67*	-36.94*			-41.40*								

CD (0.05) EH - 0.852 RH - 0.738 HB - 0.852

* Significant at 5 per cent level

Table 4.4.3.a. Significant positive heterosis for number of nodes per cane

♀ \ ♂	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_{13}	P_{14}
P_1		ERH	ERH	ERH		ERH		ERH	ERH	ER				
P_2	ERH		ERH	ERH		ERH								
P_3	ERH			ERH	ERH	ERH	ERH	ERH		ERH				
P_4	ERH		ERH		ERH	ERH								
P_5	ERH					ERH	ERH	ERH				ERH		
P_6	ERH		ERH	ERH					ERH			ERH		
P_7	ERH	ERH	ERH	ERH					ERH					
P_8	ERH	ERH					ERH		ERH					
P_9	ERH				ERH	ERH	ERH							
P_{10}	ER	ER					E							
P_{11}		ERH		ERH								ERH		
P_{12}				ERH	ERH						ERH			
P_{13}								E	E					
P_{14}	E	E	E			E								

E - Significant positive economic heterosis R - Significant positive relative heterosis H - Significant positive heterobeltiosis

male parents, the two combinations with P_{13} as the female parent and P_8 and P_9 as the male parents and the four combinations with P_{14} as the female parent and P_1, P_2, P_3 and P_6 as the male parents recorded significant positive heterosis at all levels of comparison.

Significant positive economic heterosis was expressed by 60 hybrids *viz.,* all except the two F_1 hybrids with P_2 as the female parent and P_7 and P_9 as the male parents. Relative heterosis was positive and significant in 53 hybrids consisting of 27 F_1s and 26 reciprocals. Significantly higher number of nodes than their corresponding better parents was observed in 50 hybrids including 26 F_1s and 24 reciprocals.

Economic heterosis and relative heterosis were significant and positive in $P_1 \times P_{10}$, its reciprocal $P_{10} \times P_1$ and $P_{10} \times P_2$.

All the hybrids were either on par with or significantly superior to the economic parent for number of nodes per cane. The hybrids $P_{13} \times P_8$, $P_{13} \times P_9$, $P_{14} \times P_3$ and $P_{14} \times P_6$ registered significant negative heterosis in comparison with the mid parental and the better parental values. Significant negative heterobeltiosis was observed in $P_2 \times P_7$, $P_2 \times P_9$, $P_{14} \times P_1$ and $P_{14} \times P_2$.

4.4.4. Number of leaves per cane

Economic heterosis, relative heterosis and heterobeltiosis for number of leaves per cane (Table 4.4.4.) and the combinations registering positive significance (Table 4.4.4.a.) have been studied.

Table 4.4.4. Heterosis for number of leaves per cane (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}	P_{13}	P_{14}
P_1	EH		25.93*	29.63*	35.19*		38.89*		51.85*	55.56*	18.52*				
	RH		25.93*	35.92*	36.45*		41.51*		54.72*	42.37*	9.40				
	HB		21.43*	34.62*	32.73*		38.89*		51.85*	27.27*	-1.54				
P_2	EH	29.63*		38.89*	29.63*		53.70*	-5.56		-9.26*					
	RH	29.63*		40.19*	26.13*		50.91*	-14.29*		-19.67*					
	HB	25.00*		33.93*	25.00*		48.21*	-19.05*		-25.76*					
P_3	EH	35.19*			27.78*	44.44*	44.44*	48.15*	38.89*		29.63*				
	RH	41.18*			30.19*	35.65*	48.57*	40.35*	42.86*		20.69*				
	HB	40.39*			25.46*	21.88*	44.44*	26.98*	38.89*		7.69				
P_4	EH	14.82		27.78*		40.74*	35.19*								
	RH	15.89*		30.19*		27.73*	33.95*								
	HB	12.73		25.46*		18.75*	32.73*								
P_5	EH	40.74*					44.44*	62.96*	48.15*				59.26*		
	RH	31.03*					32.20*	38.58*	35.59*				39.84*		
	HB	18.75*					21.88*	37.50*	25.00*				34.38*		
P_6	EH	53.70*		50.00*	66.67*					61.11*			44.44*		
	RH	56.60*		54.29*	65.14*					45.00*			38.05*		
	HB	53.70*		50.00*	63.64*					31.82*			32.20*		
P_7	EH	40.74*	37.04*	44.44*	51.85*					75.93*					
	RH	32.19*	24.37*	36.84*	38.98*					47.29*					
	HB	20.64*	17.46*	23.81*	30.16*					43.94*					

Table 4.4.4. (Contd...)

♀	♂	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_{13}	P_{14}
P_8	EH	38.89*	25.93*					33.33*		48.15*					
	RH	41.51*	23.64*					23.08*		33.33*					
	HB	38.89*	21.43*					14.29*		21.21*					
P_9	EH	57.41*				50.00*	57.41*	83.33*							
	RH	44.07*				24.62*	41.67*	53.49*							
	HB	28.79*				22.73*	28.79*	50.00*							
P_{10}	EH	27.78*	16.67*					29.63*							
	RH	17.95*	4.13					9.38							
	HB	6.15	-3.08					7.69							
P_{11}	EH		57.41*		40.74*								25.93*		
	RH		38.21*		24.59*								7.94		
	HB		26.87*		13.43								1.49		
P_{12}	EH				42.59*	51.85*						29.63*			
	RH				35.09*	33.33*						11.11			
	HB				30.51*	28.13*						4.48			
P_{13}	EH								1.85	3.70					
	RH								-25.17*	-29.56*					
	HB								-40.86*	-39.79*					
P_{14}	EH	44.44*	42.59*	44.44*			33.33*								
	RH	13.87*	9.22	14.71*			3.60								
	HB	-8.24	-9.41	-8.24			-15.29*								

CD (0.05) EH - 0.863 RH - 0.747 HB - 0.863

* Significant at 5 per cent level

Table 4.4.4.a. Significant positive heterosis for number of leaves per cane

♀ \ ♂	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_{13}	P_{14}
P_1		ERH	ERH	ERH		ERH		ERH	ERH	E				
P_2	ERH		ERH	ERH		ERH								
P_3	ERH			ERH	ERH	ERH	ERH	ERH		ER				
P_4	R		ERH		ERH	ERH								
P_5	ERH					ERH	ERH	ERH				ERH		
P_6	ERH		ERH	ERH					ERH			ERH		
P_7	ERH	ERH	ERH	ERH					ERH					
P_8	ERH	ERH					ERH		ERH					
P_9	ERH				ERH	ERH	ERH							
P_{10}	ER	E					E					E		
P_{11}		ERH		ER										
P_{12}				ERH	ERH						E			
P_{13}														
P_{14}	ER	E	ER			E								

E - Significant positive economic heterosis R - Significant positive relative heterosis H - Significant positive heterobeltiosis

In 45 out of the 62 hybrids, heterosis for number of leaves per cane was observed to be positive and significant in all the three measures tested. Twenty four F_1s and 21 reciprocals constituted these 45 hybrids.

Economic heterosis was significant and positive in 57 hybrids. All hybrids except the F_1s $P_2 \times P_7$ and $P_2 \times P_9$ and the reciprocals $P_4 \times P_1$, $P_{13} \times P_8$ and $P_{13} \times P_9$ recorded significant positive economic heterosis. Fifty one hybrids comprising of 25 F_1s and 26 reciprocals registered positive significance for relative heterosis. All these 51 hybrids except $P_4 \times P_1$ displayed significant positive economic heterosis also. Heterobeltiosis was significant and positive in 45 combinations which registered significance in both the other measures too. All hybrids were either on par with or significantly superior to the economic parent for number of leaves per cane.

The four hybrids $P_2 \times P_7$, $P_2 \times P_9$, $P_{13} \times P_8$ and $P_{13} \times P_9$ recorded significant negative relative heterosis and heterobeltiosis, while $P_{14} \times P_6$ recorded significant negative heterobeltiosis alone.

4.4.5. Leaf area per cane

Economic heterosis, relative heterosis and heterobeltiosis for leaf area per cane (Table 4.4.5.) and the hybrids recording positive significance (Table 4.4.5.a.) have been studied.

Forty eight hybrids out of the 62 displayed significant positive heterosis in all the three estimates, for leaf area per cane. Twenty three

Table 4.4.5. Heterosis for leaf area per cane (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}	P_{13}	P_{14}
P_1	EH		1.92	-4.66	8.86		59.56*		26.30*	92.37*	-23.39*				
	RH		19.55*	13.08*	26.70*		66.79*		38.79*	64.47*	15.19*				
	HB		15.16*	10.06	21.19*		45.94*		26.30*	26.62*	-6.59				
P_2	EH	30.60*		37.75*	28.17*		91.58*	0.96		-0.29					
	RH	53.19*		57.30*	43.75*		93.67*	-9.77*		-17.06*					
	HB	47.56*		55.64*	42.69*		75.22*	-25.37*		-34.37*					
P_3	EH	41.08*			50.01*	93.02*	95.42*	109.34*	108.16*		72.14*				
	RH	67.32*			70.03*	84.94*	99.44*	88.67*	123.07*		150.15*				
	HB	62.84*			67.00*	58.08*	78.73*	54.75*	108.16*		98.70*				
P_4	EH	13.63*		27.62*		52.08*	62.95*								
	RH	32.26*		44.65*		43.52*	63.63*								
	HB	26.50*		42.08*		24.55*	49.03*								
P_5	EH	55.17*					48.64*	201.01*	115.86*				219.61*		
	RH	52.05*					29.45*	133.90*	94.38*				218.23*		
	HB	27.08*					21.74*	122.51*	76.79*				161.75*		
P_6	EH	68.80*		74.61*	137.84*					187.62*			58.34*		
	RH	76.45*		78.20*	138.84*					120.17*			68.36*		
	HB	54.39*		59.70*	117.53*					89.31*			44.82*		
P_7	EH	99.88*	106.58*	104.63*	132.57*					168.66*					
	RH	83.99*	84.62*	84.43*	106.63*					87.08*					
	HB	47.76*	52.71*	51.26*	71.92*					76.83*					

Table 4.4.5. (Contd...)

♀	♂	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_{13}	P_{14}
P_8	EH	45.64*	66.69*					49.99*		88.36*					
	RH	60.05*	78.86*					27.50*		49.50*					
	HB	45.64*	66.69*					10.88*		23.98*					
P_9	EH	114.26*				93.07*	90.76*	168.08*							
	RH	83.18*				40.91*	46.03*	86.68*							
	HB	41.02*				27.07*	25.56*	76.45*							
P_{10}	EH	-20.09*	-13.44*					-4.18							
	RH	20.17*	24.10*					2.89							
	HB	-2.55	-2.20					-29.16*							
P_{11}	EH		159.54*		112.62*								67.39*		
	RH		164.72*		115.42*								79.65*		
	HB		141.25*		97.64*								55.59*		
P_{12}	EH				67.72*	118.62*						38.69*			
	RH				98.98*	117.68*						48.85*			
	HB				86.72*	79.05*						28.91*			
P_{13}	EH								-11.38*	-19.77*					
	RH								20.31*	-19.44*					
	HB								-11.38*	-47.20*					
P_{14}	EH	26.09*	36.36*	25.56*			12.50*								
	RH	22.67*	28.60*	19.47*			-3.39								
	HB	2.04	10.35*	1.61			-8.95								

CD (0.05) EH - 70.474 RH - 61.030 HB - 70.474

* Significant at 5 per cent level

Table 4.4.5.a. Significant positive heterosis for leaf area per cane

♀ \ ♂	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_{13}	P_{14}
P_1		RH	R	RH		ERH		ERH	ERH	R				
P_2	ERH		ERH	ERH		ERH								
P_3	ERH			ERH	ERH	ERH	ERH	ERH		ERH				
P_4	ERH		ERH		ERH	ERH								
P_5	ERH					ERH	ERH	ERH				ERH		
P_6	ERH		ERH	ERH					ERH			ERH		
P_7	ERH	ERH	ERH	ERH					ERH					
P_8	ERH	ERH					ERH		ERH					
P_9	ERH				ERH	ERH	ERH							
P_{10}	R	R												
P_{11}		ERH		ERH								ERH		
P_{12}				ERH	ERH						ERH			
P_{13}								R						
P_{14}	ER	ERH	ER			E								

E - Significant positive economic heterosis R - Significant positive relative heterosis H - Significant positive heterobeltiosis

F_1s and 25 reciprocals constituted these 48 combinations. All F_1s except the four combinations with P_1 as the female parent and P_2, P_3, P_4 and P_{10} as the male parents and the two combinations with P_2 as the female parent and P_7 and P_9 as the male parents recorded positive significance. All reciprocals except the three combinations with P_{10} as the female parent and P_1, P_2 and P_7 as the male parents, the two combinations with P_{13} as the female parent and P_8 and P_9 as the male parents and the three combinations with P_{14} as the female parent and P_1, P_3 and P_6 as the male parents registered positive significance for all the three measures of heterosis.

Economic heterosis was significant and positive in 51 hybrids constituted by 23 F_1s and 28 reciprocals. All hybrids except $P_1 \times P_2$, $P_1 \times P_3$, $P_1 \times P_4$, $P_1 \times P_{10}$, $P_2 \times P_7$ and $P_2 \times P_9$ and the reciprocals except $P_{10} \times P_1$, $P_{10} \times P_2$, $P_{10} \times P_7$, $P_{13} \times P_8$ and $P_{13} \times P_9$ registered positive significance.

Relative heterosis was significant and positive in 57 hybrids consisting of 27 F_1s and 30 reciprocals. All hybrids except the F_1s $P_2 \times P_7$ and $P_2 \times P_9$ and all reciprocals except $P_{10} \times P_7$, $P_{13} \times P_9$ and $P_{14} \times P_6$ recorded significant difference.

Heterobeltiosis was significant and positive in 50 hybrids equally constituted by the F_1s and the reciprocals. Along with the 48 hybrids recording positive heterosis in all the three measures, $P_1 \times P_2$ and $P_1 \times P_4$ also recorded significant positive heterobeltiosis.

The combination P_{13} x P_9 recorded significant negative heterosis in all its three measures whereas P_{13} x P_8 recorded significant negative economic heterosis and heterobeltiosis. In addition to these two combinations, economic heterosis was negative and significant in P_1 x P_{10}, P_{10} x P_1 and P_{10} x P_2.

Significant negative heterobeltiosis was exhibited by P_2 x P_7, P_2 x P_9 and P_{10} x P_7 also, in addition to P_{13} x P_8 and P_{13} x P_9.

4.4.6. Length of leaf

Economic heterosis, relative heterosis and heterobeltiosis for length of leaf (Table 4.4.6.) and the combinations registering positive significance (Table 4.4.6.a.) have been studied.

Nineteen hybrids out of the 62 possessed significantly longer leaves compared to the economic parent, mid parental value and the better parent. These 19 hybrids included 12 F_1s and seven reciprocals.

Significant positive economic heterosis was observed in 45 hybrids comprising of 25 F_1s and 20 reciprocals.

Relative heterosis was significant and positive in 34 hybrids consisting of 15 F_1s and 19 reciprocals. Out of these 34 hybrids, 21 registered positive significance for heterobeltiosis also. Twelve F_1s and nine reciprocals constituted these 21 hybrids.

Table 4.4.6. Heterosis for length of leaf (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}	P_{13}	P_{14}
P_1	EH		10.53*	6.71	9.84*		18.24*		12.86*	40.07*	3.69				
	RH		16.79*	12.06	7.61		13.77		1.94	33.48*	22.46				
	HB		14.55*	9.79	2.06		4.80		-9.24	18.68*	5.79				
P_2	EH	3.53		13.29*	22.20*		20.57*	4.43		2.63					
	RH	32.58*		23.74*	24.84*		18.57	2.40		-7.05					
	HB	30.04*		22.92*	16.26*		7.31	-3.95		-18.74*					
P_3	EH	4.98			15.15*	21.33*	22.93*	18.84*	27.65*		26.52*				
	RH	11.70			16.87*	23.27*	22.23*	21.08*	22.52*		61.22*				
	HB	8.85			8.17	13.11*	9.96	12.87*	6.65		42.42*				
P_4	EH	12.56*		6.65		12.49*	16.38*								
	RH	12.11*		6.31		4.12	5.29								
	HB	6.34		-1.60		3.15	2.08								
P_5	EH	26.51*					19.91*	29.57*	24.39*				36.22*		
	RH	27.73*					7.84	24.50*	9.58				38.44*		
	HB	20.08*					5.52	22.40*	3.39				29.89*		
P_6	EH	16.01*		13.76*	22.20*					33.16*			15.92*		
	RH	12.45*		11.24	12.53*					16.21*			10.10		
	HB	3.58		0.07	9.10					11.79*			1.22		
P_7	EH	3.45	6.68	16.48*	30.44*					34.88*					
	RH	1.91	6.45	19.50*	26.69*					22.43*					
	HB	-2.64	-0.16	11.40	22.73*					13.51*					

Table 4.4.6. (Contd...)

♀	♂	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_{13}	P_{14}
P_8	EH	8.24	6.88					6.25		21.13*					
	RH	-0.90	-2.01					-8.19		-0.03					
	HB	-11.76*	-14.22*					-14.75*		-0.20					
P_9	EH	17.08*				11.27*	16.71*	36.48*							
	RH	7.71				-5.66	-0.93	24.14*							
	HB	-4.24				-11.13	-4.70	15.10*							
P_{10}	EH	2.52	7.51					1.83							
	RH	22.37*	31.18*					13.29*							
	HB	5.70	15.22*					-5.81							
P_{11}	EH		24.99*		21.99*								16.79*		
	RH		37.89*		24.08*								24.68*		
	HB		37.28*		16.03*								23.08*		
P_{12}	EH				11.03*	24.59*						0.86			
	RH				9.19	25.67*						3.46			
	HB				3.36	17.90*						2.14			
P_{13}	EH								15.79*	8.82					
	RH								29.66*	18.99*					
	HB								-6.31	-14.11*					
P_{14}	EH	12.90*	13.46*	18.81*			13.91*								
	RH	12.84*	16.56*	24.22*			4.38								
	HB	-9.21	10.76	17.29*			-0.86								

CD (0.05) EH - 1.574 RH - 1.363 HB - 1.574

* Significant at 5 per cent level

Table 4.4.6.a. Significant positive heterosis for length of leaf

♀ \ ♂	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_{13}	P_{14}
P_1		ERH		E		E		E	ERH					
P_2	RH		ERH	ERH		E								
P_3				ER	ERH	ER	ERH	ER		ERH				
P_4	ER				E	E								
P_5	ERH					E	ERH	E				ERH		
P_6	ER		E	ER					ERH			E		
P_7			ER	ERH					ERH					
P_8									E					
P_9	E				E	E	ERH							
P_{10}	R	RH					R							
P_{11}		ERH		ERH								ERH		
P_{12}				E	ERH									
P_{13}								ER	R					
P_{14}	ER	ER	ERH			E								

E - Significant positive economic heterosis R - Significant positive relative heterosis H - Significant positive heterobeltiosis

Heterobeltiosis was significant and negative in the five hybrids $P_2 \times P_9$, $P_8 \times P_1$, $P_8 \times P_2$, $P_8 \times P_7$ and $P_{13} \times P_9$. None of the hybrids displayed significant negative economic heterosis or relative heterosis.

4.4.7. Width of leaf

Economic heterosis, relative heterosis and heterobeltiosis for width of leaf (Table 4.4.7.) and the hybrids recording positive significance (Table 4.4.7.a.) have been studied.

Thirty three hybrids out of the 62 recorded positive significance for all the three estimates of heterosis. These 33 hybrids comprised of 14 F_1s and 19 reciprocals.

Significant positive economic heterosis was displayed by 41 hybrids constituted by 19 F_1s and 22 reciprocals. Relative heterosis was significant and positive in 41 hybrids including 17 F_1s and 24 reciprocals. Significance for economic heterosis and relative heterosis alone was exhibited by five hybrids. Heterobeltiosis was significant and positive only in the 33 hybrids referred to previously.

Significant negative economic heterosis for leaf breadth was observed in eight hybrids constituted by four F_1s and four reciprocals. The F_1s were $P_1 \times P_2$, $P_1 \times P_3$, $P_1 \times P_{10}$ and $P_2 \times P_7$ and the reciprocals were $P_{10} \times P_1$, $P_{10} \times P_2$, $P_{10} \times P_7$ and $P_{13} \times P_9$. Significant negative relative heterosis was exhibited by four F_1s and one reciprocal. The F_1s were $P_1 \times P_2$, $P_1 \times P_3$, $P_2 \times P_7$ and $P_2 \times P_9$ and the reciprocal was $P_{10} \times P_7$.

Table 4.4.7. Heterosis for width of leaf (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}	P_{13}	P_{14}
P_1	EH		-16.96*	-14.04*	-3.80		23.68*		-7.90	5.56	-18.42*				
	RH		-15.85*	-15.15*	-4.08		18.64*		-7.35	-5.62	2.39				
	HB		-15.98*	-17.18*	-5.46		12.80*		-7.90	-15.46*	-17.46*				
P_2	EH	7.02		4.09	5.26		24.56*	-10.53*		-9.65					
	RH	8.44		2.89	5.11		19.66*	-22.83*		-19.11*					
	HB	8.28		0.28	3.45		13.60*	-32.90*		-27.64*					
P_3	EH	21.35*			18.13*	30.99*	33.04*	27.78*	41.52*		32.75*				
	RH	19.97*			14.94*	23.08*	24.66*	7.77	38.88*		61.57*				
	HB	16.90*			13.80*	20.11*	21.33*	-4.17	36.34*		27.89*				
P_4	EH	7.90		15.21*		17.84*	25.44*								
	RH	7.58		12.09*		11.79*	18.67*								
	HB	6.03		10.99*		8.04	14.40*								
P_5	EH	17.84*					16.08*	72.52*	42.98*				78.95*		
	RH	13.36*					6.15	42.34*	36.78*				84.34*		
	HB	8.04					5.87	29.39*	31.10*				64.08*		
P_6	EH	15.50*		31.87*	64.04*					57.60*			13.74*		
	RH	10.80*		23.56*	55.19*					34.41*			16.82*		
	HB	5.33		20.27*	49.60*					26.23*			3.73		
P_7	EH	79.83*	79.53*	56.43*	55.85*					67.25*					
	RH	54.91*	54.86*	31.94*	32.59*					29.56*					
	HB	34.87*	34.65*	17.33*	16.89*					25.44*					

Table 4.4.7. (Contd...)

♀	♂	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_{13}	P_{14}
P_8	EH	14.62*	12.87*					24.85*		29.24*					
	RH	15.29*	13.70*					7.02		14.95*					
	HB	14.62*	12.87*					-6.36		3.51					
P_9	EH	45.03*				41.52*	43.28*	71.05*							
	RH	29.67*				21.00*	22.20*	32.50*							
	HB	16.16*				13.35*	14.75*	28.29*							
P_{10}	EH	-20.76*	-16.96*					-15.21*							
	RH	-0.55	4.41					-12.52*							
	HB	-19.82*	-15.73*					-36.40*							
P_{11}	EH		58.92*		52.92*								47.37*		
	RH		52.25*		44.68*								51.35*		
	HB		44.53*		39.47*								34.40*		
P_{12}	EH				29.83*	40.06*						27.78*			
	RH				38.97*	44.28*						31.23*			
	HB				27.59*	28.42*						16.53*			
P_{13}	EH								-9.36	-10.82*					
	RH								17.42*	-0.49					
	HB								-9.36	-28.57*					
P_{14}	EH	-0.29	2.63	-7.60			-2.92								
	RH	13.10*	16.61*	1.94			3.75								
	HB	0.89	4.15	-10.99*			-11.47*								

CD (0.05) EH - 0.546 RH - 0.473 HB - 0.546

* Significant at 5 per cent level

Table 4.4.7.a. Significant positive heterosis for width of leaf

♀ \ ♂	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_{13}	P_{14}
P_1						ERH								
P_2						ERH								
P_3	ERH			ERH	ERH	ERH	E	ERH		ERH				
P_4			ERH		ER	ERH								
P_5	ER					E	ERH	ERH				ERH		
P_6	ER		ERH	ERH						ERH		ER		
P_7	ERH	ERH	ERH	ERH					ERH					
P_8	ERH	ERH					E		ER					
P_9	ERH				ERH	ERH	ERH							
P_{10}														
P_{11}		ERH		ERH								ERH		
P_{12}				ERH	ERH						ERH			
P_{13}								R						
P_{14}	R	R												

E - Significant positive economic heterosis R - Significant positive relative heterosis H - Significant positive heterobeltiosis

Out of these five hybrids, economic heterosis was significant and negative in all the four hybrids except $P_2 \times P_9$. Significant negative heterobeltiosis was recorded by 12 combinations, comprising of six F_1s and six reciprocals. In addition to the eight combinations recording significant negative economic heterosis, these 12 combinations were constituted by $P_1 \times P_9$, $P_2 \times P_9$, $P_{14} \times P_3$ and $P_{14} \times P_6$.

Heterosis for the 16 hybrid combinations that reached flowering stage during the period under study was estimated with respect to 12 floral characters. Percentage estimates of economic heterosis, relative heterosis and heterobeltiosis for each character along with the corresponding parental and hybrid means have been recorded (Tables 4.4.8 to 4.4.19.).

4.4.8 Age at first flowering

With respect to age at first flowering, eleven hybrids out of the 16 exhibited significance in the desired direction for all the three measures of heterosis (Table 4.4.8.). These 11 hybrids which flowered early as compared to the economic parent, the early flowering parent and the mid parental value were $P_2 \times P_3$, $P_3 \times P_1$, $P_3 \times P_6$, $P_4 \times P_1$, $P_4 \times P_3$, $P_5 \times P_1$, $P_5 \times P_{12}$, $P_6 \times P_1$, $P_6 \times P_3$, $P_6 \times P_9$ and $P_7 \times P_9$.

Significant negative economic heterosis was displayed by all the 16 hybrid combinations, denoting that all hybrids flowered at a significantly early age as compared to the economic parent. Same was the case with relative heterosis, where all combinations except $P_1 \times P_4$

Table 4.4.8. Heterosis for age at first flowering

Parents/ Hybrids	Mean (months)	Economic heterosis (%) (against P_8)	Relative heterosis (%)	Heterobeltiosis (%)
P_1	18.71	-	-	-
P_2	21.00	-	-	-
P_3	21.90	-	-	-
P_4	21.00	-	-	-
P_5	19.85	-	-	-
P_6	24.10	-	-	-
P_7	22.95	-	-	-
P_8	23.95	-	-	-
P_9	24.55	-	-	-
P_{10}	22.85	-	-	-
P_{11}	18.90	-	-	-
P_{12}	18.85	-	-	-
P_{13}	19.05	-	-	-
P_{14}	19.10	-	-	-
$P_1 \times P_4$	18.48	-19.46*	-7.00	-1.42
$P_2 \times P_1$	18.48	-19.46*	-17.00*	-1.42
$P_2 \times P_3$	17.60	-23.31*	-27.95*	-26.19*
$P_3 \times P_1$	17.75	-22.66*	-22.67*	-15.33*
$P_3 \times P_6$	18.32	-19.90*	-21.94*	-17.39*
$P_4 \times P_1$	17.12	-25.53*	-24.01*	-18.85*
$P_4 \times P_3$	19.18	-16.41*	-20.57*	-18.65*
$P_5 \times P_1$	16.02	-30.21*	-27.01*	-24.58*
$P_5 \times P_{12}$	16.03	-29.24*	-26.90*	-24.70*
$P_6 \times P_1$	17.37	-24.33*	-28.94*	-27.38*
$P_6 \times P_3$	20.78	-19.44*	-24.56*	-23.76*
$P_6 \times P_9$	18.18	-20.77*	-22.79*	-23.02*
$P_7 \times P_1$	18.58	-19.03*	-27.13*	-5.15
$P_7 \times P_9$	19.95	-13.07*	-26.00*	-23.07*
$P_8 \times P_1$	19.28	-15.98*	-19.68*	2.84
$P_{11} \times P_2$	18.23	-20.57*	-18.62*	-3.55
CD (0.05)		0.981	0.850	0.981

* Significant at 5 per cent level

flowered significantly earlier. Significant negative heterobeltiosis for age at first flowering was observed in 11 out of the 16 combinations, these combinations being the same as the 11 listed out earlier. It is notable that all hybrids flowered at an early age as compared to the parents.

4.4.9 Cane to flower first

Flowering occurred on an earlier cane as compared to the economic parent in 13 out of the 16 combinations which showed significant negative economic heterosis (Table 4.4.9.). These 13 combinations were $P_1 \times P_4$, $P_2 \times P_1$, $P_2 \times P_3$, $P_3 \times P_1$, $P_3 \times P_6$, $P_4 \times P_1$, $P_4 \times P_3$, $P_5 \times P_1$, $P_5 \times P_{12}$, $P_6 \times P_1$, $P_6 \times P_3$, $P_7 \times P_9$ and $P_{11} \times P_2$. Significant relative heterosis in the desired direction was noted in 13 combinations. These 13 hybrids comprised of the 12 hybrids except $P_2 \times P_3$ listed earlier, in addition to $P_6 \times P_9$. Significant negative heterobeltiosis was observed in eight combinations *viz.*, $P_1 \times P_4$, $P_3 \times P_1$, $P_4 \times P_1$, $P_5 \times P_1$, $P_5 \times P_{12}$, $P_6 \times P_9$, $P_7 \times P_9$ and $P_{11} \times P_2$. Wherever significance was noted, flowering occurred on an early cane in the hybrid as compared to the parents.

4.2.10 Days to first flower opening

Significant positive economic heterosis for days to first flower opening was exhibited by eleven combinations *viz.*, $P_1 \times P_4$, $P_2 \times P_3$, $P_3 \times P_1$, $P_4 \times P_3$, $P_5 \times P_1$, $P_5 \times P_{12}$, $P_6 \times P_1$, $P_6 \times P_9$, $P_7 \times P_1$, $P_8 \times P_1$ and $P_{11} \times P_2$ (Table 4.4.10.). Relative heterosis was significant and positive in 12 combinations *viz.*, $P_1 \times P_4$, $P_2 \times P_1$, $P_2 \times P_3$, $P_3 \times P_1$,

Table 4.4.9. Heterosis for cane to flower first

Parents/ Hybrids	Mean	Economic heterosis (%) (against P_8)	Relative heterosis (%)	Heterobeltiosis (%)
P_1	6.2	-	-	-
P_2	6.1	-	-	-
P_3	6.0	-	-	-
P_4	6.9	-	-	-
P_5	6.7	-	-	-
P_6	7.8	-	-	-
P_7	7.9	-	-	-
P_8	6.8	-	-	-
P_9	7.8	-	-	-
P_{10}	7.8	-	-	-
P_{11}	6.2	-	-	-
P_{12}	6.0	-	-	-
P_{13}	6.8	-	-	-
P_{14}	6.9	-	-	-
$P_1 \times P_4$	5.0	-36.71*	-23.66*	-19.36*
$P_2 \times P_1$	5.2	-34.60*	-15.99*	-15.30
$P_2 \times P_3$	5.3	-32.49*	-11.85	-11.11
$P_3 \times P_1$	4.8	-38.82*	-20.77*	-19.44*
$P_3 \times P_6$	5.7	-28.27*	-14.76*	-5.56
$P_4 \times P_1$	5.0	-32.91*	-19.08*	-19.52*
$P_4 \times P_3$	5.5	-30.38*	-14.73*	-13.33
$P_5 \times P_1$	4.8	-38.82*	-25.07*	-22.04*
$P_5 \times P_{12}$	5.0	-35.44*	-19.69*	-19.00*
$P_6 \times P_1$	5.8	-26.16*	-16.68*	-5.91
$P_6 \times P_3$	5.3	-32.49*	-31.62*	-3.62
$P_6 \times P_9$	6.0	-24.05	-14.89*	-18.23*
$P_7 \times P_1$	6.5	-17.72	-6.48	8.33
$P_7 \times P_9$	5.2	-34.60*	-34.18*	-33.76*
$P_8 \times P_1$	6.3	-19.83	-2.56	2.15
$P_{11} \times P_2$	4.7	-41.77*	-25.20*	-24.59*
CD (0.05)		0.996	0.863	0.996

* Significant at 5 per cent level

Table 4.4.10. Heterosis for days to first flower opening

Parents/ Hybrids	Mean	Economic heterosis (%) (against P_8)	Relative heterosis (%)	Heterobeltiosis (%)
P_1	26.0	-	-	-
P_2	32.2	-	-	-
P_3	29.0	-	-	-
P_4	32.2	-	-	-
P_5	45.4	-	-	-
P_6	35.6	-	-	-
P_7	47.2	-	-	-
P_8	33.3	-	-	-
P_9	39.5	-	-	-
P_{10}	30.4	-	-	-
P_{11}	31.9	-	-	-
P_{12}	27.3	-	-	-
P_{13}	29.4	-	-	-
P_{14}	32.5	-	-	-
$P_1 \times P_4$	41.2	38.42*	38.03*	54.49*
$P_2 \times P_1$	35.7	24.36	22.57*	17.18
$P_2 \times P_3$	44.8	43.05*	46.51*	54.60*
$P_3 \times P_1$	41.8	39.57*	52.12*	60.90*
$P_3 \times P_6$	34.5	14.21	7.26	-10.35
$P_4 \times P_1$	36.7	28.92	30.92*	36.54*
$P_4 \times P_3$	38.2	32.05*	24.73*	31.61*
$P_5 \times P_1$	38.2	32.05*	6.91	46.80*
$P_5 \times P_{12}$	41.7	39.22*	20.50*	60.44*
$P_6 \times P_1$	39.8	34.38*	29.33*	53.21*
$P_6 \times P_3$	36.0	28.11	-14.13*	1.12
$P_6 \times P_9$	41.7	39.22*	13.84*	10.26
$P_7 \times P_1$	47.7	46.51*	25.11*	64.37*
$P_7 \times P_9$	30.7	-5.03	-29.26*	-22.36*
$P_8 \times P_1$	43.3	40.59*	46.15*	66.67*
$P_{11} \times P_2$	38.3	33.67*	21.99*	22.57*
CD (0.05)		4.467	3.869	4.467

* Significant at 5 per cent level

$P_4 \times P_1$, $P_4 \times P_3$, $P_5 \times P_{12}$, $P_6 \times P_1$, $P_6 \times P_9$, $P_7 \times P_1$, $P_8 \times P_1$ and $P_{11} \times P_2$. Significant negative relative heterosis was exhibited by $P_6 \times P_3$ and $P_7 \times P_9$. Significant heterobeltiosis was observed in 12 combinations out of the 16, except $P_2 \times P_1$, $P_3 \times P_6$, $P_6 \times P_3$ and $P_6 \times P_9$; it was significant and positive in all 11 combinations except $P_7 \times P_9$, where it was negative.

4.4.11 Flowering time

Significant positive economic heterosis for flowering time was noted in ten hybrids *viz.*, $P_1 \times P_4$, $P_3 \times P_1$, $P_4 \times P_1$, $P_5 \times P_1$, $P_5 \times P_{12}$, $P_6 \times P_1$, $P_6 \times P_3$, $P_6 \times P_9$, $P_7 \times P_1$ and $P_7 \times P_9$ (Table 4.4.11.). Relative heterosis and heterobeltiosis were significant and positive in eleven hybrids *viz.*, $P_1 \times P_4$, $P_3 \times P_1$, $P_4 \times P_1$, $P_5 \times P_1$, $P_5 \times P_{12}$, $P_6 \times P_1$, $P_6 \times P_3$, $P_6 \times P_9$, $P_7 \times P_1$, $P_7 \times P_9$ and $P_{11} \times P_2$.

4.4.12 Days for wilting of all flowers

Significant positive economic heterosis for days for wilting of all flowers was observed in five hybrids *viz.*, $P_2 \times P_3$, $P_4 \times P_1$, $P_5 \times P_1$, $P_5 \times P_{12}$ and $P_{11} \times P_{12}$ (Table 4.4.12.). Significant negative economic heterosis was noted in two hybrids *viz.*, $P_3 \times P_6$ and $P_4 \times P_3$. Significant positive relative heterosis was observed in seven hybrids *viz.*, $P_2 \times P_1$, $P_2 \times P_3$, $P_3 \times P_1$, $P_4 \times P_1$, $P_5 \times P_1$, $P_5 \times P_{12}$ and $P_{11} \times P_2$. Significant negative relative heterosis was expressed by five combinations *viz.*, $P_3 \times P_6$, $P_4 \times P_3$, $P_6 \times P_3$, $P_6 \times P_9$ and $P_7 \times P_9$. Heterobeltiosis for days for wilting of all flowers was significant and positive in five hybrids *viz.*, $P_2 \times P_1$, $P_2 \times P_3$, $P_3 \times P_1$, $P_5 \times P_{12}$ and $P_{11} \times P_2$. Significant

Table 4.4.11. Heterosis for flowering time

Parents/ Hybrids	Mean (days)	Economic heterosis (%) (against P_8)	Relative heterosis (%)	Heterobeltiosis (%)
P_1	13.7	-	-	-
P_2	12.9	-	-	-
P_3	14.8	-	-	-
P_4	15.7	-	-	-
P_5	15.3	-	-	-
P_6	15.2	-	-	-
P_7	15.9	-	-	-
P_8	13.7	-	-	-
P_9	15.2	-	-	-
P_{10}	9.5	-	-	-
P_{11}	10.9	-	-	-
P_{12}	6.1	-	-	-
P_{13}	6.6	-	-	-
P_{14}	14.2	-	-	-
$P_1 \times P_4$	20.3	25.92*	38.32*	48.42*
$P_2 \times P_1$	16.0	10.53	5.26	18.53
$P_2 \times P_3$	14.7	5.32	5.90	-1.70
$P_3 \times P_1$	25.5	44.76*	45.26*	61.46*
$P_3 \times P_6$	16.2	11.02	3.16	7.79
$P_4 \times P_1$	22.8	34.36*	42.72*	54.38*
$P_4 \times P_3$	15.2	8.40	3.66	-1.94
$P_5 \times P_1$	24.7	43.09*	48.74*	58.25*
$P_5 \times P_{12}$	23.3	39.26*	109.35*	267.21*
$P_6 \times P_1$	26.7	48.22*	53.86*	151.34*
$P_6 \times P_3$	20.0	25.74*	59.46*	56.20*
$P_6 \times P_9$	24.8	43.22*	37.90*	37.90*
$P_7 \times P_1$	23.3	39.26*	52.01*	57.66*
$P_7 \times P_9$	21.8	30.01*	28.19*	26.54*
$P_8 \times P_1$	13.7	0.00	0.00	0.00
$P_{11} \times P_2$	17.8	17.19	52.10*	66.06*
CD (0.05)		3.510	3.040	3.510

* Significant at 5 per cent level

Table 4.4.12. Heterosis for days for wilting of all flowers

Parents/ Hybrids	Mean	Economic heterosis (%) (against P_8)	Relative heterosis (%)	Heterobeltiosis (%)
P_1	32.1	-	-	-
P_2	34.3	-	-	-
P_3	31.0	-	-	-
P_4	43.3	-	-	-
P_5	42.3	-	-	-
P_6	45.2	-	-	-
P_7	38.3	-	-	-
P_8	38.3	-	-	-
P_9	41.8	-	-	-
P_{10}	27.8	-	-	-
P_{11}	34.7	-	-	-
P_{12}	25.2	-	-	-
P_{13}	27.7	-	-	-
P_{14}	61.8	-	-	-
$P_1 \times P_4$	34.3	-8.70	-4.33	-19.16*
$P_2 \times P_1$	39.7	3.57	22.48*	15.65*
$P_2 \times P_3$	43.7	16.01*	33.74*	27.31*
$P_3 \times P_1$	37.7	-1.65	23.39*	17.34*
$P_3 \times P_6$	31.2	-18.63*	-19.96*	-26.32*
$P_4 \times P_1$	46.5	27.15*	29.18*	12.47
$P_4 \times P_3$	24.5	-36.03*	-34.05*	-43.42*
$P_5 \times P_1$	43.2	15.71*	19.04*	2.05
$P_5 \times P_{12}$	48.2	27.15*	44.30*	15.13*
$P_6 \times P_1$	35.5	-7.13	-8.15	-21.46*
$P_6 \times P_3$	34.0	-11.67	-26.03*	-28.63*
$P_6 \times P_9$	39.5	4.48	-21.76*	-16.48*
$P_7 \times P_1$	36.5	-4.70	5.34	-4.70
$P_7 \times P_9$	34.7	-9.48	-18.44*	-17.07*
$P_8 \times P_1$	34.5	-9.92	0.44	-11.74
$P_{11} \times P_2$	44.3	15.93*	28.70*	27.95*
CD (0.05)		4.467	3.869	4.467

* Significant at 5 per cent level

negative heterobeltiosis was observed in seven combinations *viz.*, $P_1 \times P_4$, $P_3 \times P_6$, $P_4 \times P_3$, $P_6 \times P_1$, $P_6 \times P_3$, $P_6 \times P_9$ and $P_7 \times P_9$.

Days for wilting of all flowers is an indication of vase life. Vase life could not be estimated as the hybrids have just entered the flowering phase producing the first inflorescence which had to be set apart for *in situ* observations.

4.4.13 Length of inflorescence

Significant positive economic heterosis for length of inflorescence was exhibited by seven hybrids *viz.*, $P_2 \times P_3$, $P_3 \times P_1$, $P_4 \times P_1$, $P_5 \times P_{12}$, $P_6 \times P_1$, $P_6 \times P_9$ and $P_{11} \times P_2$ (Table 4.4.13.). One hybrid *viz.*, $P_2 \times P_1$ exhibited significant negative economic heterosis for length of inflorescence. Significant positive relative heterosis for this character was recorded by ten hybrids *viz.* $P_2 \times P_3$, $P_3 \times P_1$, $P_3 \times P_6$, $P_5 \times P_1$, $P_5 \times P_{12}$, $P_6 \times P_1$, $P_6 \times P_3$, $P_6 \times P_9$, $P_8 \times P_1$ and $P_{11} \times P_2$. Two hybrids *viz.*, $P_7 \times P_1$ and $P_7 \times P_9$ showed significant negative relative heterosis for length of inflorescence. Heterobeltiosis was significant and positive in six combinations *viz.*, $P_2 \times P_3$, $P_3 \times P_1$, $P_3 \times P_6$, $P_5 \times P_{12}$, $P_6 \times P_1$ and $P_{11} \times P_2$. Heterobeltiosis for length of inflorescence was significant and negative in three combinations *viz.*, $P_2 \times P_1$, $P_7 \times P_1$ and $P_7 \times P_9$.

4.4.14 Length of scape

Significant positive economic heterosis for scape length was reported in six combinations *viz.*, $P_1 \times P_4$, $P_2 \times P_3$, $P_3 \times P_6$, $P_4 \times P_1$,

Table 4.4.13. Heterosis for length of inflorescence

Parents/ Hybrids	Mean (cm)	Economic heterosis (%) (against P_8)	Relative heterosis (%)	Heterobeltiosis (%)
P_1	34.65	-	-	-
P_2	25.05	-	-	-
P_3	25.55	-	-	-
P_4	38.60	-	-	-
P_5	31.00	-	-	-
P_6	31.75	-	-	-
P_7	41.60	-	-	-
P_8	34.85	-	-	-
P_9	38.80	-	-	-
P_{10}	19.45	-	-	-
P_{11}	25.85	-	-	-
P_{12}	12.50	-	-	-
P_{13}	9.00	-	-	-
P_{14}	52.66	-	-	-
$P_1 \times P_4$	40.00	17.05	9.22	3.63
$P_2 \times P_1$	26.50	-25.91*	-8.62	-25.07*
$P_2 \times P_3$	41.22	18.33*	58.96*	57.40*
$P_3 \times P_1$	41.77	18.98*	21.58*	41.45*
$P_3 \times P_6$	38.88	13.53	37.52*	24.43*
$P_4 \times P_1$	41.67	18.89*	17.69	17.36
$P_4 \times P_3$	35.58	13.89	9.77	-8.36
$P_5 \times P_1$	39.42	16.27	19.85*	9.67
$P_5 \times P_{12}$	48.42	38.94*	121.75*	55.58*
$P_6 \times P_1$	43.42	20.31*	46.46*	32.53*
$P_6 \times P_3$	35.50	13.66	19.64*	8.50
$P_6 \times P_9$	44.45	26.53*	31.12*	17.03
$P_7 \times P_1$	29.25	-16.69	-32.88*	-39.69*
$P_7 \times P_9$	32.98	-8.03	-35.02*	-36.53*
$P_8 \times P_1$	39.92	16.85	18.92*	16.85
$P_{11} \times P_2$	42.00	19.72*	54.11*	51.72*
CD (0.05)		6.138	5.048	6.138

* Significant at 5 per cent level

P_4 x P_3 and P_8 x P_1 while significant negative economic heterosis for the character was observed in one combination *viz.*, P_2 x P_1 (Table 4.4.14.). Relative heterosis was significant and positive in nine combinations *viz.*, P_1 x P_4, P_2 x P_3, P_3 x P_6, P_4 x P_1, P_4 x P_3, P_5 x P_1, P_5 x P_{12}, P_8 x P_1 and P_{11} x P_2. In two combinations *viz.*, P_2 x P_1 and P_7 x P_9 relative heterosis was significant and negative. Significant positive heterobeltiosis was noted in three hybrids *viz.*, P_2 x P_3, P_5 x P_1 and P_8 x P_1. Significant negative heterobeltiosis was observed in three hybrids *viz.*, P_2 x P_1, P_7 x P_1 and P_7 x P_9.

4.4.15 Number of flowers per inflorescence

There was significant positive economic heterosis for number of flowers per inflorescence in seven combinations *viz.*, P_3 x P_1, P_4 x P_1, P_5 x P_1, P_5 x P_{12}, P_6 x P_1, P_6 x P_9 and P_{11} x P_2 (Table 4.4.15.). Significant positive relative heterosis was exhibited by nine hybrids *viz.*, P_2 x P_3, P_3 x P_1, P_3 x P_6, P_4 x P_1, P_5 x P_1, P_5 x P_{12}, P_6 x P_1, P_6 x P_9 and P_{11} x P_2. Significant positive heterobeltiosis for number of flowers per inflorescence was observed in six hybrids *viz.*, P_3 x P_1, P_4 x P_1, P_5 x P_1, P_5 x P_{12}, P_6 x P_1 and P_{11} x P_2. The remaining ten hybrids recorded values on par with the better parent for number of flowers per inflorescence.

4.4.16 Length of internode

Significant positive economic heterosis for length of internode was observed in one combination *viz.*, P_8 x P_1 (Table 4.4.16.). The hybrid P_6 x P_9 registered positive significance in comparison with the mid

Table 4.4.14. Heterosis for length of scape

Parents/ Hybrids	Mean (cm)	Economic heterosis (%) (against P_8)	Relative heterosis (%)	Heterobeltiosis (%)
P_1	12.65	-	-	-
P_2	8.66	-	-	-
P_3	10.25	-	-	-
P_4	17.50	-	-	-
P_5	12.80	-	-	-
P_6	15.15	-	-	-
P_7	15.70	-	-	-
P_8	13.40	-	-	-
P_9	14.75	-	-	-
P_{10}	10.05	-	-	-
P_{11}	12.10	-	-	-
P_{12}	4.99	-	-	-
P_{13}	3.54	-	-	-
P_{14}	17.75	-	-	-
$P_1 \times P_4$	18.67	28.90*	23.83*	6.67
$P_2 \times P_1$	7.35	-53.19*	-31.02*	-41.90*
$P_2 \times P_3$	20.08	41.92*	112.41*	95.94*
$P_3 \times P_1$	14.63	3.79	17.80	15.68
$P_3 \times P_6$	17.80	23.38*	54.45*	9.06
$P_4 \times P_1$	19.47	37.71*	43.42*	23.54
$P_4 \times P_3$	19.42	36.67*	39.94*	20.95
$P_5 \times P_1$	16.58	18.62	30.32*	29.56*
$P_5 \times P_{12}$	15.78	13.69	83.02*	17.19
$P_6 \times P_1$	15.52	11.17	11.63	2.42
$P_6 \times P_3$	15.83	16.85	5.91	4.51
$P_6 \times P_9$	15.20	10.87	3.10	1.40
$P_7 \times P_1$	11.57	-16.33	-10.85	-26.33*
$P_7 \times P_9$	11.40	-17.39	-25.12*	-27.39*
$P_8 \times P_1$	18.87	29.17*	44.85*	40.80*
$P_{11} \times P_2$	14.83	5.61	27.65*	9.50
CD (0.05)		3.762	3.258	3.762

* - Significant at 5 per cent level

Table 4.4.15. Heterosis for number of flowers per inflorescence

Parents/ Hybrids	Mean	Economic heterosis (%) (against P_8)	Relative heterosis (%)	Heterobeltiosis (%)
P_1	6.6	-	-	-
P_2	8.5	-	-	-
P_3	5.8	-	-	-
P_4	9.1	-	-	-
P_5	6.1	-	-	-
P_6	4.6	-	-	-
P_7	9.5	-	-	-
P_8	7.3	-	-	-
P_9	10.8	-	-	-
P_{10}	5.1	-	-	-
P_{11}	7.1	-	-	-
P_{12}	4.6	-	-	-
P_{13}	12.1	-	-	-
P_{14}	20.1	-	-	-
$P_1 \times P_4$	9.2	16.61	14.03	4.26
$P_2 \times P_1$	8.3	8.33	16.12	-1.49
$P_2 \times P_3$	8.8	11.02	23.54*	3.92
$P_3 \times P_1$	9.5	18.58*	24.84*	31.52*
$P_3 \times P_6$	7.5	14.58	29.24*	16.56
$P_4 \times P_1$	13.3	58.95*	68.15*	45.06*
$P_4 \times P_3$	8.2	7.61	10.65	-8.22
$P_5 \times P_1$	9.8	24.64*	23.36*	28.69*
$P_5 \times P_{12}$	12.7	42.53*	131.78*	103.28*
$P_6 \times P_1$	9.5	18.58*	39.64*	31.82*
$P_6 \times P_3$	7.0	-6.32	4.09	4.19
$P_6 \times P_9$	10.5	28.16*	56.52*	-8.96
$P_7 \times P_1$	8.8	11.02	15.47	-7.02
$P_7 \times P_9$	9.2	16.61	-14.10	-11.16
$P_8 \times P_1$	8.0	5.61	16.53	14.58
$P_{11} \times P_2$	12.5	40.42*	56.41*	43.53*
CD (0.05)		2.164	1.874	2.164

* Significant at 5 per cent level

Table 4.4.16. Heterosis for length of internode

Parents/ Hybrids	Mean (cm)	Economic heterosis (%) (against P_8)	Relative heterosis (%)	Heterobeltiosis (%)
P_1	3.29	-	-	-
P_2	2.26	-	-	-
P_3	3.21	-	-	-
P_4	2.65	-	-	-
P_5	3.00	-	-	-
P_6	3.41	-	-	-
P_7	2.59	-	-	-
P_8	2.90	-	-	-
P_9	2.43	-	-	-
P_{10}	2.20	-	-	-
P_{11}	2.21	-	-	-
P_{12}	2.10	-	-	-
P_{13}	1.44	-	-	-
P_{14}	1.91	-	-	-
$P_1 \times P_4$	2.72	-4.83	-8.59	-17.48*
$P_2 \times P_1$	2.65	-2.14	-4.69	-19.61*
$P_2 \times P_3$	2.83	-9.33	3.53	-11.79
$P_3 \times P_1$	3.15	11.43	-3.23	-4.41
$P_3 \times P_6$	3.33	16.51	7.19	-3.69
$P_4 \times P_1$	2.20	-19.72*	-27.37*	-34.44*
$P_4 \times P_3$	2.32	-18.55*	-20.93*	-27.83*
$P_5 \times P_1$	2.71	-4.76	-13.73	-17.53*
$P_5 \times P_{12}$	2.88	-0.69	11.41	-5.30
$P_6 \times P_1$	3.30	27.22	-1.64	-3.37
$P_6 \times P_3$	3.10	19.69	-6.16	-9.09
$P_6 \times P_9$	3.36	29.60	14.17*	-2.03
$P_7 \times P_1$	2.58	-11.58	-11.21	-19.78*
$P_7 \times P_9$	2.39	-16.96*	-4.71	-7.66
$P_8 \times P_1$	3.44	32.82*	11.42	4.56
$P_{11} \times P_2$	2.43	-11.72	6.94	5.75
CD (0.05)		0.501	0.434	0.501

* Significant at 5 per cent level

parental value. Two combinations *viz.*, $P_4 \times P_1$ and $P_4 \times P_3$ registered negative significance for length of internode when compared to the economic parent and the mid parental value. The combination $P_7 \times P_9$ registered significant negative economic heterosis. Six combinations *viz.*, $P_1 \times P_4$, $P_2 \times P_1$, $P_4 \times P_1$, $P_4 \times P_3$, $P_5 \times P_1$ and $P_7 \times P_1$ registered negative significance for the character when compared to the better parent.

4.4.17 Diameter of inflorescence axis

Significant positive economic heterosis for diameter of inflorescence axis was observed in nine hybrids, *viz.*, $P_1 \times P_4$, $P_2 \times P_1$, $P_2 \times P_3$, $P_3 \times P_6$, $P_4 \times P_3$, $P_5 \times P_1$, $P_6 \times P_3$, $P_6 \times P_9$ and $P_7 \times P_1$ (Table 4.4.17.). Relative heterosis was significant and positive in ten combinations including the nine combinations mentioned above and the hybrid, $P_8 \times P_1$. Significant positive heterobeltiosis was noted in $P_6 \times P_3$ and $P_7 \times P_1$ whereas significant negative heterobeltiosis was exhibited by $P_4 \times P_1$.

4.4.18 Length of flower

Economic heterosis was significant and positive in four combinations *viz.*, $P_1 \times P_4$, $P_3 \times P_1$, $P_4 \times P_3$ and $P_6 \times P_1$ (Table 4.4.18.). Significant negative economic heterosis for length of flower was observed in three combinations *viz.*, $P_2 \times P_3$, $P_6 \times P_9$ and $P_{11} \times P_2$. In 11 hybrids *viz.*, $P_1 \times P_4$, $P_3 \times P_1$, $P_3 \times P_6$, $P_4 \times P_1$, $P_4 \times P_3$, $P_5 \times P_1$, $P_5 \times P_{12}$, $P_6 \times P_1$, $P_6 \times P_3$, $P_6 \times P_9$ and $P_{11} \times P_2$ significant positive relative heterosis was noted. One hybrid *viz.*, $P_7 \times P_1$ recorded significant negative relative

Table 4.4.17. Heterosis for diameter of inflorescence axis

Parents/ Hybrids	Mean (cm)	Economic heterosis (%) (against P_8)	Relative heterosis (%)	Heterobeltiosis (%)
P_1	0.336	-	-	-
P_2	0.461	-	-	-
P_3	0.351	-	-	-
P_4	0.480	-	-	-
P_5	0.440	-	-	-
P_6	0.467	-	-	-
P_7	0.404	-	-	-
P_8	0.387	-	-	-
P_9	0.380	-	-	-
P_{10}	0.377	-	-	-
P_{11}	0.361	-	-	-
P_{12}	0.395	-	-	-
P_{13}	0.412	-	-	-
P_{14}	0.446	-	-	-
$P_1 \times P_4$	0.493	22.11*	20.92*	2.78
$P_2 \times P_1$	0.525	29.95*	31.74*	13.88
$P_2 \times P_3$	0.508	25.83*	25.21*	10.27
$P_3 \times P_1$	0.350	13.37	1.89	-0.29
$P_3 \times P_6$	0.522	29.13*	31.90*	18.56
$P_4 \times P_1$	0.355	13.12	-13.97	-26.88*
$P_4 \times P_3$	0.533	32.01*	28.36*	11.11
$P_5 \times P_1$	0.532	31.60*	37.03*	20.83
$P_5 \times P_{12}$	0.428	10.15	6.59	-11.14
$P_6 \times P_1$	0.452	11.80	12.50	-3.28
$P_6 \times P_3$	0.563	39.44*	33.02*	20.63*
$P_6 \times P_9$	0.483	19.64*	30.63*	19.64
$P_7 \times P_1$	0.517	27.89*	36.87*	27.89*
$P_7 \times P_9$	0.458	13.45	16.92	13.45
$P_8 \times P_1$	0.458	13.45	26.79*	18.43
$P_{11} \times P_2$	0.392	4.21	-5.84	-16.05
CD (0.05)		0.093	0.081	0.093

* Significant at 5 per cent level

Table 4.4.18. Heterosis for length of flower

Parents/ Hybrids	Mean (cm)	Economic heterosis (%) (against P_8)	Relative heterosis (%)	Heterobeltiosis (%)
P_1	6.63	-	-	-
P_2	5.39	-	-	-
P_3	5.86	-	-	-
P_4	5.46	-	-	-
P_5	6.05	-	-	-
P_6	5.87	-	-	-
P_7	7.27	-	-	-
P_8	6.71	-	-	-
P_9	4.87	-	-	-
P_{10}	4.18	-	-	-
P_{11}	4.12	-	-	-
P_{12}	3.58	-	-	-
P_{13}	2.93	-	-	-
P_{14}	3.19	-	-	-
$P_1 \times P_4$	7.27	24.15*	26.66*	16.81*
$P_2 \times P_1$	6.25	-14.22	-8.67	-2.73
$P_2 \times P_3$	5.93	-22.18*	7.29	11.97
$P_3 \times P_1$	7.28	26.60*	35.35*	20.48*
$P_3 \times P_6$	6.48	-10.22	24.48*	16.10*
$P_4 \times P_1$	6.85	11.90	25.05*	11.22
$P_4 \times P_3$	7.87	54.36*	60.15*	40.71*
$P_5 \times P_1$	7.00	14.09	22.52*	14.04
$P_5 \times P_{12}$	6.35	-12.33	82.16*	13.27
$P_6 \times P_1$	7.29	28.27*	40.40*	21.54*
$P_6 \times P_3$	6.67	-6.35	34.84*	20.05*
$P_6 \times P_9$	6.00	-18.37*	23.86*	7.35
$P_7 \times P_1$	6.20	-15.28	-33.18*	-21.60*
$P_7 \times P_9$	6.48	-10.22	-11.43	-17.70*
$P_8 \times P_1$	7.12	18.99	9.80	14.39
$P_{11} \times P_2$	5.62	-27.80*	38.28*	11.28
CD (0.05)		0.551	0.477	0.551

* Significant at 5 per cent level

Table 4.4.19. Heterosis for width of flower

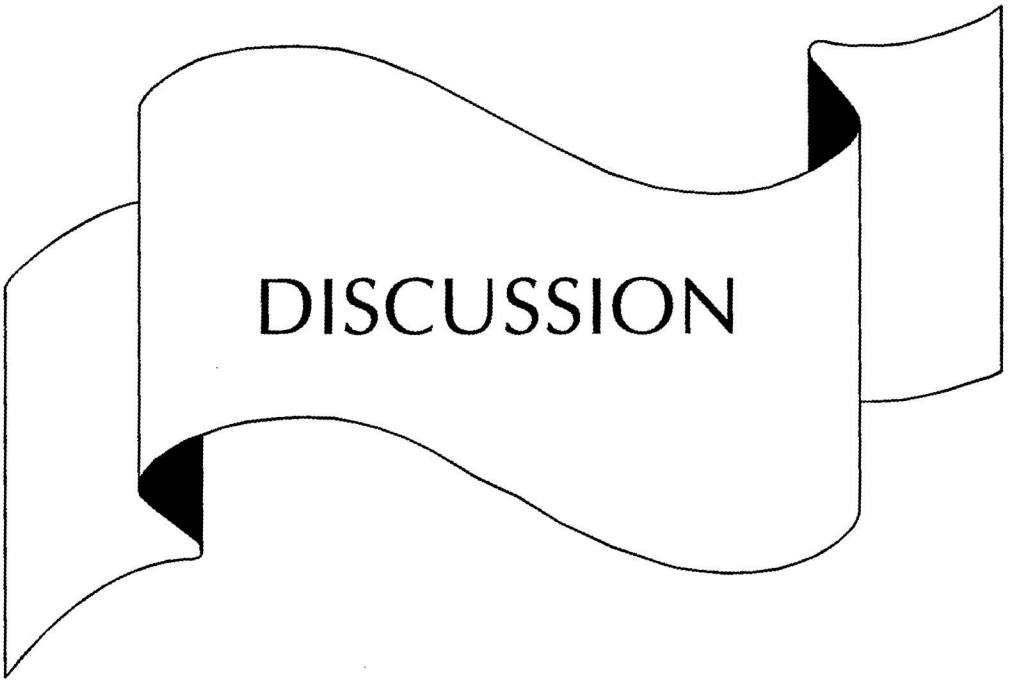
Parents/ Hybrids	Mean (cm)	Economic heterosis (%) (against P_8)	Relative heterosis (%)	Heterobeltiosis (%)
P_1	6.50	-	-	-
P_2	6.02	-	-	-
P_3	6.27	-	-	-
P_4	5.76	-	-	-
P_5	6.26	-	-	-
P_6	6.78	-	-	-
P_7	7.95	-	-	-
P_8	6.25	-	-	-
P_9	5.91	-	-	-
P_{10}	5.14	-	-	-
P_{11}	5.06	-	-	-
P_{12}	4.98	-	-	-
P_{13}	3.04	-	-	-
P_{14}	4.00	-	-	-
$P_1 \times P_4$	8.62	52.64*	60.87*	54.87*
$P_2 \times P_1$	6.40	9.23	5.06	-10.88
$P_2 \times P_3$	6.65	14.32	21.58*	17.10
$P_3 \times P_1$	7.50	26.63*	44.63*	32.69*
$P_3 \times P_6$	7.45	24.10*	35.93*	20.85*
$P_4 \times P_1$	7.35	22.95*	32.64*	24.46*
$P_4 \times P_3$	8.10	43.21*	68.88*	51.21*
$P_5 \times P_1$	7.60	28.03*	36.29*	29.23*
$P_5 \times P_{12}$	7.28	27.85*	45.61*	28.28*
$P_6 \times P_1$	7.18	17.29*	25.65*	5.43
$P_6 \times P_3$	7.30	18.61*	28.17*	16.30
$P_6 \times P_9$	7.50	26.63*	42.11*	21.95*
$P_7 \times P_1$	7.25	20.63*	0.06	-25.09*
$P_7 \times P_9$	7.15	16.06*	14.04	-26.35*
$P_8 \times P_1$	7.88	37.47*	33.14*	35.03*
$P_{11} \times P_2$	6.18	-7.64	21.28*	-14.96
CD (0.05)		0.579	0.501	0.579

* Significant at 5 per cent level

heterosis for length of flower. Heterobeltiosis was significant and positive in six combinations, *viz.*, $P_1 \times P_4$, $P_3 \times P_1$, $P_3 \times P_6$, $P_4 \times P_3$, $P_6 \times P_1$ and $P_6 \times P_3$. Two combinations *viz.*, $P_7 \times P_1$ and $P_7 \times P_9$ registered significant negative heterobeltiosis.

4.4.19 Width of flower

Significant positive economic heterosis for width of flower was observed in 13 out of the 16 combinations *viz.*, all except $P_2 \times P_1$, $P_2 \times P_3$ and $P_{11} \times P_2$ (Table 4.4.19.). Relative heterosis was significant and positive in 13 combinations *viz.*, $P_1 \times P_4$, $P_2 \times P_3$, $P_3 \times P_1$, $P_3 \times P_6$, $P_4 \times P_1$, $P_4 \times P_3$, $P_5 \times P_1$, $P_5 \times P_{12}$, $P_6 \times P_1$, $P_6 \times P_3$, $P_6 \times P_9$, $P_8 \times P_1$ and $P_{11} \times P_2$. Significant positive heterobeltiosis for width of flower was noted in nine combinations *viz.*, $P_1 \times P_4$, $P_3 \times P_1$, $P_3 \times P_6$, $P_4 \times P_1$, $P_4 \times P_3$, $P_5 \times P_1$, $P_5 \times P_{12}$, $P_6 \times P_9$ and $P_8 \times P_1$. Two combinations *viz.*, $P_7 \times P_1$ and $P_7 \times P_9$ exhibited significant negative heterobeltiosis.


DISCUSSION

5. DISCUSSION

Orchids form the royalty among flowering plants. 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 initiated for developing new hybrids of *Dendrobium* with commercial cutflower qualities for the export market has spelled success, resulting in forty novel, promising hybrids. Salient findings in the course of their development are discussed below.

Comparison between parents and hybrids for 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. 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 utilised and the hybrids generated in the present study is presented.

Parent material was evaluated with respect to vegetative characters at two stages, *viz.*, 1.5-2.0 years after transplanting and three years after transplanting and they were found to follow the same trend at both stages. Hybrid material was evaluated at two stages, *viz.*, six months after transplanting and 1.5-2.0 years after transplanting. Evaluation at six months after transplanting served to give a concise account about survival and establishment of hybrid seedlings following hardening. Heterosis was estimated by comparing the performance of the hybrids with that of the parents at 1.5-2.0 years after transplanting.

In the present study, a far wider range of variation for vegetative biometric characters was observed in the hybrids generated, as compared to the parents. This range was more prominent for the five characters, *viz.*, leaf area per cane (98.40 to 316.55 cm^2 in parents vs. 116.50 to 665.90 cm^2 in hybrids), length of leaf (6.70 to 11.75 cm in parents vs. 11.95 to 17.92 cm in hybrids), width of leaf (1.86 to 4.56 cm in parents vs. 2.79 to 6.15 cm in hybrids), number of leaves per cane (5.1 to 9.3 in parents vs. 4.9 to 9.5 in hybrids) and number of shoots per cane (6.0 to 7.9 in parents vs. 4.4 to 6.3 in hybrids). This finding is in line with the reports of Hurst (1898) that in orchids, secondary hybrids showed a far wider range of character variation as compared to primary hybrids. Majority of the parental genotypes employed in the present study are themselves higher order hybrids. Similar results have been observed in *Dendrobium canaliculatum* by McConnel and Kamemoto (1983). They found that even reciprocal crossings yielded offspring differing in cane height, pseudobulb production and flower yield, bearing evidence to their

highly heterozygous nature. Sobhana (2000) in a similar hybrid evaluation study in *Dendrobium* has reported marked variation for vegetative characters between hybrids under field conditions.

With respect to the character number of nodes per cane, the parents exhibited a wider range of variation, *viz.* 5.8 - 15.7 compared to the hybrids *viz.*, 6.8 - 12.6. The reason is the inclusion of two species with comparatively higher number of nodes per cane as parents. These species, *viz.*, P_{13} and P_{14} recorded 15.1 and 15.7 nodes per cane, respectively compared to 5.8 to 8.5, recorded by the other parental genotypes. Since these species showed leaf shedding at maturity, the mean number of leaves at maximum leaf-stand did not differ considerably from the other parental varieties. Another feature noted with respect to number of nodes per cane was that although this character had a bearing on number of leaves per cane, the two were found to differ. The reason was that several lower nodes of mature canes usually produced scale leaves. This was more evident in the first flowering canes of a clump which shot up rapidly, giving rise to scale leaves in the lower two to four nodes and regular laminate leaves in the upper nodes.

Heterosis in orchids has been reported by several workers *viz.,* Atwood (1989), Porter (1989) and McDonald (1991). The results of the present investigation fully support their views, presenting several hybrid combinations manifesting significant positive economic heterosis, relative heterosis and heterobeltiosis. The combinations showing significant positive economic heterosis for each of the seven different

vegetative characters have been studied (Table 5.1). Significant positive economic heterosis for five or more (out of the seven) vegetative characters studied was shown by 77.42 per cent (48 out of 62) hybrid combinations. Out of these, 19 hybrid combinations showed significant positive economic heterosis for all the characters evaluated. These 19 combinations included four out of the six with *D.* Nagoya Pink as the female parent and all five combinations with *D.* Rinabha as the female parent. The fact that 60 out of the 62 hybrid combinations (excluding the five selfs) generated in the present study showed significant positive economic heterosis for at least one vegetative character bears evidence to the strong heterotic tendency manifested in *Dendrobium*.

Comparison between parents and hybrids for floral characters

Sixteen out of the total 67 combinations flowered during the course of study, presenting a vast array of variations, even exceeding the limits prescribed by the parents. Transgressive seggregants of this pattern have been observed by McConnel and Kamemoto (1983) in *Dendrobium canaliculatum*. But since only 23.88 per cent of the hybrid combinations generated in this study have flowered and a vast majority is yet to flower, nothing can be stated conclusively regarding the range of variability in the hybrid population at this stage. In comparison with the other hybrids, all these 16 flowering combinations (Table 5.2) can be considered as early flowering. While evaluating the performance of the 14 parental genotypes as female parents, considerable earliness was exhibited by P_6 with three hybrids flowering during the course of study, closely followed

List of seven characters mentioned

1 - Number of leaves per clump
2 - Height/length of cane
3 - Number of nodes per cane
4 - Number of leaves per cane
5 - Leaf area per cane
6 - Length of leaf
7 - Width of leaf

Table 5.1. Significant positive economic heterosis for vegetative characters among hybrid 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}	P_{13}	P_{14}
P_1		234 6	34	234 6		1234 567		134 56	1234 56	34				
P_2	1234 5		234 56	234 56		234 567								
P_3	34 57			34 567	1234 567	1234 567	1234 567	1234 567		134 567				
P_4	3 56		34 57		34 567	234 567								
P_5	34 567					34 567	234 567	234 567				1234 567		
P_6	1234 567		1234 567	1234 567					1234 567			1234 567		
P_7	234 57	234 57	234 567	234 567					1234 567					
P_8	1234 57	1234 57					234 567		1234 567					
P_9	1234 567				1234 567	234 567	1234 57							
P_{10}	1234	1234					1234							
P_{11}		1234 567		1234 567								234 567		
P_{12}				1234 567	1234 567						234 57			
P_{13}								3 6	3					
P_{14}	1234 56	1234 56	234 56			234 56								

Table 5.2. Hybrid combinations that flowered

♀ \ ♂	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_{13}	P_{14}
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_{13}									⊗	⊗				
P_{14}	⊗	⊗	⊗			⊗								

⊗ - Combinations that have not flowered

✿ - Combinations that flowered

by P_2, P_3, P_4, P_5 and P_7 with two hybrids each coming to flower. In P_1, P_8 and P_{11} one hybrid combination each flowered. In the semi-commercial hybrids P_{10} and P_{12} and in the species P_{13} and P_{14} no hybrid combination flowered during the course of study. While evaluating the performance of the 14 parental genotypes as male parents, P_1 was observed to excel by far all the other genotypes. Seven hybrids with P_1 as the male parent flowered during the course of study. Three combinations with P_3, two each with P_9 and P_{12} and one each with P_4 and P_6 as male parents flowered during the course of study. The species P_{13} and P_{14} gave no successful combinations as male parent. The semi-commercial hybrids P_{10} and P_{11} produced two successful combinations each as the male parent, but none flowered.

In the present investigation, all the 16 flowering hybrid combinations exhibited significant economic heterosis for earliness, with 15 exhibiting significant relative heterosis and 11 showing significant heterobeltiosis. Since 76.12 per cent of the hybrid combinations including the five selfings of P_1, P_3, P_4, P_7 and P_{11} are yet to flower, no conclusions can be drawn regarding the relative earliness of the hybrids at this stage.

For the character cane to flower first, significant economic heterosis in the desired direction was observed in 13 out of the 16 combinations. In all these 13 combinations, a significantly lower number of unproductive backbulbs were produced before the first flowering cane emerged.

Days to first flower opening from inflorescence emergence is primarily decided by the length of inflorescence and its rate of growth. Significant positive economic heterosis for this character was exhibited by eleven combinations all of which produced inflorescences that were significantly longer or on par with the economic parent. Hence the rate of inflorescence growth in the hybrids appears in no way to be inferior to the parents at the stage of first flowering. Flowering time is decided by the number of flowers per inflorescence and the rate of flower opening. Ten hybrids showed significant positive economic heterosis for this character; all these hybrids produced decidedly more number of flowers, compared to the economic parent. This denotes that the rate of flower opening in the hybrids was comparable to that of the parents. Days for wilting of all flowers in an inflorescence gives an indication of vase life. Vase life could not be estimated in the hybrids as they have just entered the flowering phase producing the first inflorescence, which were used for *in situ* observations. Significant positive economic heterosis for this character was depicted by five hybrid combinations and significant negative economic heterosis was shown by two hybrid combinations. However, further studies are essential to draw conclusions on these characters.

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 diameter of inflorescence axis. Seven hybrid combinations showed significant positive economic heterosis for length of inflorescence whereas one hybrid showed significant negative economic heterosis.

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 arching inflorescence. Diameter of inflorescence axis also has a major say in deciding the shape and nature of inflorescence axis. In nine hybrid combinations significant positive economic heterosis was observed for diameter of 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. Majority of the parents used in the present study including the economic parent P_8 are popular commercial hybrids where this optimum internodal length of inflorescence was expressed. More than 80 per cent of the hybrid combinations were on par with the economic parent for this character, denoting that the optimum balance has not been disrupted.

Number of flowers per inflorescence is a character of prime importance in *Dendrobium* breeding, as has been pointed out by Kamemoto (1983), McConnel and Kamemoto (1983), Singh (1986) and McDonald (1991). Significant positive economic heterosis for this character was shown by seven hybrid combinations; the remaining nine combinations were on par with the economic parent for this character. Relative heterosis was significant and positive in nine hybrid combinations and heterobeltiosis was significant and positive in six combinations. As all the parents of the hybrid combinations that flowered in the present

study are themselves higher order hybrids, this increase in number is in accordance with the observations of Singh (1982) that in orchids, the farther from the species, the better becomes the flower shape, flower size and number of flowers per spike. In the present study, a genotype dependent variation in flower number was noticed in the hybrids. 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 (1977) 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.

Observations on the manifestation of hybrid vigour in flower size has been reported by Porter (1989) and McDonald (1991) in orchids. Economic heterosis was significant and positive in four combinations for length of flower and in 13 combinations for width of flower. Although these two characters followed the same trend in the hybrids, such a variation in the number of combinations expressing significance for length and width was manifested due to the shape of the economic parent P_8. Among the 14 parental genotypes, flower length was found to be greater than flower width only in P_1 and P_8. The hybrids also followed the same trend with only one combination *viz.*, $P_6 \times P_1$ recording higher flower length, compared to width. In the present study, relative heterosis was significant and positive in 11 hybrids for length of flower and in 13 hybrids for width of flower. Atwood (1989) and Porter (1989)

encountered similar situations where flowers in *Paphiopedilum* hybrids were found to be generally longer and wider as compared to parents, probably due to hybrid vigour. Oakeley (1991) in *Lycaste* species has described in detail the advantages of breeding for reduced size. In the present breeding programme also, parents with small flowers *viz.*, P_{10}, P_{11}, P_{12}, P_{13} and P_{14} were included in order to investigate the prospects of breeding for small size. Two combinations *viz.*, $P_5 \times P_{12}$ and $P_{11} \times P_2$ that flowered during the period of study, produced flowers recording reduced length, compared to the economic parent, P_8. In $P_{11} \times P_2$, the flowers, in addition to being of significantly lower length than P_8, recorded reduced width also.

In *Dendrobium*, flowering is either throughout the year or seasonal. In the present study, out of the 14 parental genotypes, five were free-flowering and nine were seasonal, flowering from April-June to October-December. Sobhana (2000) conducted similar parental evaluation in *Dendrobium* prior to hybridization. She studied ten commercial hybrids other than those under the present study 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. 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 those varieties such as P_1 and P_3 to certain periods/months of the year, limiting their market share, while the flowers of those varieties such as P_4, P_8 and P_9 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 as observed in the case of P_8 which is always under constant demand in the market and is free-flowering. 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.

In the present study, time taken for single flower opening among the parental genotypes exhibited a wide range of variation from 0.39 to 2.24 days. Comparatively more time (1.27 - 2.24 days) was required for single flower opening in the nine commercial varieties where the flowers opened one at a time. This finding is in accordance with the reports of Varghese (1995) who stated that another set of ten commercial hybrids of *Dendrobium* she studied, completed single flower opening in one to three days. In the present study, the remaining parentals, especially the species, required lesser time for single flower opening and three to four flowers opened simultaneously. These traits were found to have a bearing on the longevity of the inflorescence and wilting of flowers also. In all cases where the flowers opened slowly and one at a time, they remained fresh for a comparatively longer period of time and wilted off one by one. In situations where the flowers opened in quick succession, vase life was low, the flowers wilting off simultaneously. Time taken for single flower opening was comparatively high in the flowering hybrids, ranging from 1.84 to 2.38 days indicating the possibility for a longer vase life.

Nature of inflorescence axis in *Dendrobium* may be erect, arching or pendulous and the mode of display of flowers, whorled or alternate. In the present study, these two characters were observed to be interlinked such that erect inflorescence axis was related to whorled arrangement, arching axis was related to alternate flowers facing opposite directions and pendulous axis was related to alternate flowers facing the dorsal side. Arching inflorescence axis with the flowers alternate and facing opposite sides is the best mode of display in *Dendrobium* as it 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. Davidson (1994) reported that while selecting parents for hybridization programmes in *Dendrobium*, the distinctive shape of the inflorescence axis and attractive mode of display are important. He obtained intermediate inflorescence characters in the hybrids. Catling and Brownell (1999) observed intermediate inflorescence characters in the natural hybrids of *Platanthera*. In the present study also, most of the hybrids displayed the arching inflorescence character exhibited by majority of the parents. When an erect inflorescence was crossed with a pendulous one, as in $P_2 \times P_3$, the hybrid was intermediate, *viz.*, arching. When erect inflorescences were crossed with arching ones as in $P_2 \times P_1$, $P_2 \times P_{11}$ and $P_6 \times P_9$, both the parental characters were found distributed among the hybrid progeny. On the other hand, in the cross $P_3 \times P_1$, the attractive pendulous display of the parent P_3 could be reproduced as such. This is in tune with the reports of Behar (1993) who could transfer the distinctive inflorescence shape of *Lepanthopsis floripecten* as such to an interspecific

hybrid. A trait not found in the parents was observed to be introduced in some of the progeny in the combination $P_4 \times P_1$. Both the parents and most of the progeny had arching inflorescences while some hybrid progeny had erect inflorescence. An extreme case of this type was exhibited by the combination $P_5 \times P_1$ where the inflorescence was arching in both the parents while it was erect in all their hybrids.

While breeding dendrobiums, including more colours is important as colour has always occupied a prime position in this genus and hybridization has yielded remarkable colour variations (Abraham and Vatsala, 1981). Hence, in our present investigation also, parents exhibiting much variability in flower colour were selected. This was done with a view to develop novel hybrids with new colour combinations and/or blending of colours, demarkating them from the already existing hybrids. Wallbrunn (1989) and Tippit (1997) obtained all sorts of colour variations in the progeny while hybridizing with orchids. Coleman and Glicenstein (1977) in *Platanthera* and Davidson (1994) in *Dendrobium* found the hybrids to be intermediate in colour between the parental species. The present hybridization programme also has resulted in surprising variations in colour with all sorts of intermediate colours and attractive novel shades arising from new gene combinations. Discussing colour variations obtained through intra and interspecific hybridizations is quite complex as colour variations have been observed within particular combinations also, making generalisations meaningless. This fantastic variation gives a clue to the extent of variability contained within the clone in this highly heterozygous family. Alcorn (1990) arrived at the same conclusion when

he obtained novel shades and new colours by selfing *Lycaste* Macama Jocelyn. The ready inheritance of attractive stripes has been reported in *Phalaenopsis* by Takasaki (1989) and in *Paphiopedilum* by Porter (1989). In the present study also, parents with regular stripes (P_1) and criss-cross stripes (P_{14}) were included, yielding astonishing results. The beautiful, regular striping of P_1 was inherited by the progeny in all cross combinations where it was involved. In the combinations $P_5 \times P_1$ and $P_6 \times P_1$ it appeared as if the solid deep purple of P_5 and the solid deep magenta of P_6 had masked the stripes of P_1. A few attractively striped hybrids in the combination $P_4 \times P_1$ showed fading of colours on standing. This phenomenon was quoted as a problem by Oakeley (1991) in *Lycaste* breeding also.

Texture of flower is important as it reflects the substance of the flower which in turn decides vase life (McDonald, 1991). The flowers of the commercial parental hybrids used in the present study were glossy or velvety as observed by Sobhana (2000) in her parental material. The flower texture was moderately thick, thick or very thick and slightly glossy or glossy. The hybrids usually appeared to be intermediate between the parents. A variation noticed was in the hybrid $P_3 \times P_1$ which was thick/ very thick, substantial looking and glossy whereas both the parents were moderately thick with P_3 alone possessing a substantial look. Since other excellent floral qualities have also been expressed by all hybrids that flowered in $P_3 \times P_1$, this heavy texture, indicative of longer vase life is of added importance.

Flower size (circumference) indicates the general spread of a flower whereas 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, the varieties were ranked considering size and fullness together, by assigning place values (Table 5.3). The genotypes P_1 and P_3 ranked first, P_6 ranked second and P_8 ranked third. The variety P_7, although producing the largest flowers, ranked fourth when fullness was also considered. The semi-commercial hybrids P_{11} and P_{12} ranked ninth and P_{10} ranked tenth. The two species ranked eighth (P_{13}) and eleventh (P_{14}). In general, the shape and fullness of the hybrids in the present study were found to be intermediate between the parents. Similar results were observed by Stewart (1986) in the hybrid progeny, while hybridizing orchids. In the present investigation, P_3 proved to be one of the most versatile varieties in the collection for breeding purposes. In all its combinations, the variety succeeded in transferring its perfect shape with full, rounded sepals and petals to the progeny, while allowing the best of the other parent to be expressed. Several scientists have come across similar situations of the apparent 'pre-potency' of particular species/ varieties in orchid breeding. Philips (1986) found that the species *Paphiopedilum rothschildianum* could always greatly improve the desirable floral qualities of the hybrid. Behar (1993) identified *Lepenthes cochlearifolia* as a superior parent capable of transferring its distinctive

Table 5.3. Ranking of parental genotypes of *Dendrobium* based on circumference and fullness of flowers

Parental genotypes	Place value based on Circumference	Fullness	Place value total	Rank assigned
P_1	2	3	5	1
P_2	7	4	11	4
P_3	4	1	5	1
P_4	8	9	17	7
P_5	6	6	12	5
P_6	5	2	7	2
P_7	1	10	11	4
P_8	3	7	10	3
P_9	9	5	14	6
P_{10}	10	14	24	10
P_{11}	11	12	23	9
P_{12}	12	11	23	9
P_{13}	14	8	22	8
P_{14}	13	13	26	11

flower shape to the hybrids. Similarly, Motes (2001) commented on the propensity of *Vanda denisoniana* to confer full form on its progeny. Oakeley (1991) observed that slight reflexing and overlapping of petals are attractive attributes in orchids. In the present breeding programme, the reflexing of petals observed in the semi-commercial parent P_{11} was inherited as an eye-catching attribute by its progeny, $P_{11} \times P_2$. In the combination $P_3 \times P_6$ (Plate VIII) and its reciprocal $P_6 \times P_3$ (Plates XI and XII), the petals sometimes gracefully overlapped the lateral sepals almost completely. In $P_3 \times P_1$ (Plates VI to VIII), hybrid progeny with the petals completely overlapping the dorsal odd sepal were observed. Another novel variation in shape observed was the squarish appearance in some hybrids of $P_2 \times P_1$ (Plate VI).

Fragrance is a cherished additional attribute in *Dendrobium* (Singh, 1984). McDonald (1991) observed that scent may be transmitted to the progeny by the careful selection of perfumed pod parents. In the present investigation, a perfumed parent, P_{11} was included resulting in several fragrant hybrids in the combination $P_{11} \times P_2$. The observation that majority of the scented hybrids were more fragrant than the parent itself bears testimony to the high rate of success obtained with regard to this character in our study. Oakeley (1991) has pointed out that transmission of fragrance is often unpredictable, as in the case of *Lycaste cruenta*. This species was found not to transmit its cinnamon fragrance to its hybrid *L.* Imshootiana although it transmitted the fragrance to its hybrid with *L.* Brugensin. Whether the present study will exhibit such selective transmission of fragrance will be evident only when the other combinations with P_{11} in their parentage flower.

Floral morphology of parents

A clear background knowledge on flower opening, anthesis and stigma receptivity is of special significance in the breeding of orchids due to the structural and functional peculiarities of their flowers. In all the parental genotypes as well as the hybrids studied, flowers opened during the day-time in acropetal succession, flower opening commencing one to one and a half months after inflorescence emergence and concluding one to three weeks later. These findings are in conformity with the reports of Varghese (1995) and Sobhana (2000) in two different sets of *Dendrobium* varieties. In the present study, flower opening commenced from 7.30 a.m. but was delayed till 12.30 p.m. depending on genotype and weather conditions. Christenson (1992) has also reported such a weather-dependent variation in the time of opening of flowers in orchids.

In the present investigation, a concise study of the maximum stigma receptivity period revealed that any hybrid variety could be pollinated with maximum success between the fourth and sixth days after anthesis which is in high accordance with the findings of Varghese (1995). Time of anthesis in the parental genotypes generally coincided with the onset of maximum stigma receptivity.

In order to minimise 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, a gradual decrease in size of pollen from the commercial hybrids (29.60 - 48.64 μ) to the semi-commercial hybrids (25.92 - 31.02 μ) to the species (19.84 - 21.44 μ) was observed. Sobhana (2000), studying another set of commercial hybrids and wild species, reported the same trend. The present investigation revealed that pollen size was related to pollen fertility and pollen germination; the species P_{13} and P_{14} recorded a lower pollen fertility (46.2 and 52.4 %) and germination (16.1 and 21.0 %) as compared to the hybrids (55.5 - 80.8 % and 42.5 - 77.1 %, respectively). Low pollen fertility and germination were found to reflect to some extent on the low percentage of hybrid seed set in our studies.

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 *Dendrobium*. 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 systems 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 incompatibilty are manifested externally as initial flower drop following pollination. In the present investigation, very high (87.14%) ovary drop 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) and Sobhana (2000) have also reported very high initial ovary drop in *Dendrobium*. Post pollination phenomena have to be viewed critically in Orchidaceae as the development of the female gametophyte takes place only after pollination. 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 *Cymbidium*, Leonhardt (1977) encountered 89.30 per cent initial ovary drop. He further observed that in analysing inter and intra group 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 with *Dendrobium*, 1696

pollinations in 84 combinations were made with 218 (12.85%) green capsules harvested, of which 211 (96.78%) in 81 combinations contained an average of 33.54 per cent seeds with apparently viable embryos (filled seeds). 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 several cross combinations in *Dendrobium* by Devi and Deka (1992) and Varghese (1995) also. Some pre- or post-fertilization barriers must be operational in such cases. The production of non-germinating, yet apparently viable seeds (containing embryos when viewed under the microscope) is another incompatibility system. Such embryos may be dormant or non-functional due to various reasons such as chemical inhibitors, structural hybridity, genic and chromosomal imbalances etc. However, additional research would be required to determine with certainty the exact reason. In the present investigation, incompatibility was found to strike at later stages also. Out of the 76 germinating combinations, 69 combinations provided mature seedlings for deflasking. In seven combinations incompatibility was found to strike at different stages in the post zygotic phase, ranging from protocorm development ($P_8 \times P_{11}$) to the development of the first shoot primordium ($P_5 \times P_9$). In these combinations, growth rate was found to slow down considerably prior to degeneration *in vitro*, suggesting perhaps the involvement of genic and chromosomal incompatibility mechanisms. Out of these 69 combinations seedlings from 67 combinations could be successfully hardened in the green house. In the two combinations that died during

hardening *viz.,* the selfings of P_{13} and P_{14}, survival after acclimatization was less than 10 per cent. These seedlings gradually perished in the sporophytic stage during hardening such that no seedling remained at the end of three months after the acclimatization period.

In the present study, failure of fruit development has been observed in many reciprocal crosses especially when the species (P_{13} and P_{14}) were used as male parents. The same problem had been reported by Devi and Deka (1992) and Varghese (1995) in *Dendrobium*. This bears evidence to the operation of a unidirectional incompatibility system within the genus. Leonhardt (1977) has reported a similar incompatibility system in hybridizations between *Cymbidium* and *Ansellia* whereby seedlings are easily produced when *Cymbidium* is used as the female parent and rarely produced when *Ansellia* is 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. However, further investigations would be necessary to determine the basis for this unidirectional phenomenon.

Ranking of the 14 parental genotypes was done based on compatibility, *viz.,* the number of successful combinations providing hardened seedlings in the green house (Table 5.4). The parent P_1 ranked first, providing eight and 11 successful combinations as female and male parents, respectively; P_3 came second with eight combinations as female

Table 5.4. Ranking of parental genotypes of *Dendrobium* based on compatible crosses

Parental genotypes	Number of combinations providing hardened seedlings		Rank assigned
	as female parent	as male parent	
P_1	8	11	1
P_2	6	6	5
P_3	8	7	2
P_4	5	8	4
P_5	5	4	7
P_6	5	7	5
P_7	6	7	3
P_8	4	4	8
P_9	4	6	6
P_{10}	3	2	10
P_{11}	4	2	9
P_{12}	3	3	9
P_{13}	2	-	12
P_{14}	4	-	11

and seven combinations as male parents; P_7 ranked third with six and seven successful combinations as the female and the male parents, respectively to its credit. Next was P_4 with 13 (five as female and eight as male) successful combinations; P_2 and P_6 came fifth with 12 successful combinations each to their credit, P_2 with six each as female and male parents and P_6 with five as female and seven as male parents. The two species P_{13} and P_{14} were the poorest combiners, ranking last; P_{14} had four and P_{13} has two successful combinations as the female parent. No combination turned out to be successful when the two species were used as male parents. Self compatibility was noticed in five genotypes while the remaining nine were self incompatible.

The present study was primarily concerned with achieving the chief objective, *viz.,* development of novel hybrids of *Dendrobium.* Hence the main focus was on the hybrid combinations that passed through the incompatibility sieve at each stage. All attention was concentrated on giving the best of care and maintenance to these hybrids for rapid further development. Consequently, specific studies on incompatibility could not be undertaken although an account of the extent and strength of incompatibility at each stage is presented. Further trials and experiments are essential before conclusive results on incompatibility are drawn.

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. In the present study also, a female parent dependent variation was noticed for harvest time. Capsules were harvested at 62-130 days after pollination with very high success in terms of *in vitro* germination. Harvesting before 75 per cent maturity could not ensure successful fertilization and harvesting after 90 per cent maturity resulted in the bursting of the over-ripe capsule during flaming prior to inoculation.

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. Although the present study revealed a wide range from 11.50 to 40.17 days for *in vitro* germination, the two species *viz.,* P_{13} and P_{14} germinated early, taking 12.33 days for the same, which is in conformity with the above mentioned reports. On the other hand, the highly bred hybrids included in the parentage exhibited a range extending upto 40.17 days. The same general trend was followed by the species and the complex hybrids throughout *in vitro* growth, till deflasking.

Although several media have been employed for *in vitro* orchid seed germination, none of these is universal, the preferences varying with

the species. The relative efficiency of MS medium in supporting better germination and rapid morphogenesis in *Dendrobium* has been underlined by many workers *viz.,* Sangama (1986), Reddy *et al.* (1992), Nagashima (1993) and Hazarika and Sarma (1995). Since MS medium contained high ionic concentration of nutrient salts, the medium at half (Zhang *et al.*, 1993) and even quarter (Bhasker, 1996) strengths were found to promote rapid *in vitro* growth in orchids. In the present study MS half strength was found to be superior to MS quarter strength and full strength MS, KC and VW media for better germination and seedling growth. Larger and more robust seedlings resulted at the end of six months of *in vitro* growth in half strength MS.

Growth regulators gave inconsistent and therefore inconclusive results, varying with the species (Withner, 1959; Arditti, 1979). Rapid protocorm proliferation on medium supplemented with BA/kinetin/IAA has been reported by Fonnesbech (1972a), Prasad and Mitra (1975) and Muralidhar and Mehta (1986). A combination of kinetin and IBA was found to enhance seedling growth in orchids (Lim *et al.*, 1993; Hazarika and Sarma, 1995; Suner, 1995). Hadley and Harvais (1968) emphasized the importance of the ratio rather than the absolute concentration of auxin to cytokinin in maintaining proper shoot:root balance in orchids. The present study showed that BA 8 mg l^{-1} + IAA 2 mg l^{-1} and BA 8 mg l^{-1} + IAA 4 mg l^{-1} were the best combinations for early differentiation of protocorms and development of larger seedlings. Trials with kinetin and IBA revealed the relative superiority of kinetin 8 mg l^{-1} + IBA 6 mg l^{-1} and kinetin 6 mg l^{-1} + IBA 4 mg l^{-1} in bringing about rapid *in vitro* growth. Among the complex additives employed in the present investigation,

coconut water 200 ml l^{-1} proved to be the best. The beneficial effects of coconut water have been pointed out by many workers *viz.,* Intuwong and Sagawa (1973), Morel (1974), Arditti (1979) and Bhasker (1996), . The kinetin content in coconut water may be the probable cause of growth enhancement. The present study further revealed that all the different organic additives employed, *viz.,* coconut water, peptone and banana pulp gave earlier and better growth in comparison with the control. Arditti (1979) made similar conclusions, pointing out the beneficial effects of coconut water, banana pulp and peptone. From the present studies and previous works on similar lines, it may be concluded that no single ingredient as such stimulated germination or plant growth; it was rather an interaction between the additives, components of media and the genotypes involved.

The present investigation showed that sucrose 5 g l^{-1} was the best for initiation of germination as well as the development of protocorm and chlorophyll whereas the development of first leaf and first root primordia was faster in sucrose 30 gl^{-1}. Thus a growth-stage dependent variation in sucrose requirement was evinced. Similar conclusions were arrived at by Pierik *et al.* (1988) in *Paphiopedilum ciliolare* and Rubulo *et al.* (1989) in *Bletia urbana*. In this study, exudation of phenolic compounds into the culture medium during germination was noticed in the combination P_7 x P_1, leading to eventual tissue necrosis. Trials conducted using activated charcoal in the culture medium for the control of phenolic compounds revealed that early development of chlorophyll and protocorm was observed in charcoal 0.5 g l^{-1} whereas charcoal 1.0 g l^{-1} was the best for the growth of seedlings. Several workers *viz.,* Fridborg

et al. (1978), Pierik *et al.* (1988) and Yam and Weatherhead (1988) described in detail the beneficial effects of activated charcoal in the control of phenolics. Yam *et al.* (1990), however, observed that activated charcoal should be used with caution in culture media since they could remove additives such as auxins and cytokinins. Hence need-based use of charcoal was resorted to in this study. Phenolic exudation was observed in no hybrid combination other than $P_7 \times P_1$, and therefore the addition of charcoal in culture medium was restricted to this particular combination.

Ex vitro establishment

Post deflasking survival of plantlets depended greatly on the number of leaves present at the time of planting out and the rate of leaf growth. Sutter *et al.* (1985) expressed the view point that the stage at which deflasking was 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 of 3-4 leaves and 3-5 roots. The role of a thorough post deflasking wash in reducing infection and improving survival has been stressed upon by Kim *et al.* (1988) and Singh (1993). The importance of using sterilized potting media and of soaking the deflasked seedlings for 30 minutes in a disinfectant solution for increased post transplantation survival has also been described in detail by the same workers. In the present study, a post deflasking wash in running tap water and a soaking in the fungicide Dithane M_{45} (0.5%) for 10 minutes were found to give

the best survival. Post deflasking mortality was reported to be mainly caused by desiccation (Dunstan and Turner, 1984). Vij and Pathak (1989) further stressed the necessity of a period of humidity acclimatization to enable the seedlings to undergo morphological and physiological adaptations prior to exposure to the harsh external conditions. The results of the present study fully endorsed the above mentioned views. Planting out into a humidity chamber under conditions of high relative humidity (85-95%) was found to be the best for acclimatization. In the present study, the best potting medium for survival and post transplantation growth was observed to be broken tiles + charcoal + Soilrite (2:2:1) closely followed by broken tiles + charcoal + fern root (2:2:1). Gangaprasad (1996) obtained the best survival of *Dendrobium* species in brick + charcoal medium while Sharma and Tandon (1992) observed good response in brick + charcoal + coconut fibre medium. Considering the easy availability of Soilrite in comparison with fern root, the medium widely used in the present study was brick + charcoal + Soilrite. In this treatment, tiles and charcoal provide the necessary anchorage, while Soilrite supplies the required moisture and aeration to the roots. The emphasis is on an ideal balance between the supporting and the supplying systems in the rooting medium, whereby survival is maximised and growth is optimised.

Partial diallel analysis

A partial diallel had been established with 18 cross combinations. The data generated from these 18 crosses at 1.5 to 2.0 years growth had

been subjected to combining ability analysis. The gca effect of P_3 was found to be positive and P_2 negative for number of shoots per clump and both were observed to be significant. The gca effects of P_2 had been significant and negative for number of nodes per cane, number of leaves per cane, length of leaf and width of leaf. The gca effect of P_3 for number of shoots per clump alone had assumed importance, since more number of shoots are preferred. Estimation of variance components in partial diallel had also been performed. None of the characters exhibited additive gene action for their inheritance. Non-additive variance, especially dominance gene action was responsible for character expression. Another point to be noted is that combining ability analysis was performed on data generated from hybrids at half growth, *viz.,* 1.5 to 2.0 years growth. A conclusive account on gca effects and components of variance can be made only after the hybrids have completed their growth cycle, attaining stability in vegetative growth and inflorescence production.

Some of the promising *Dendrobium* hybrids developed

Floral details of forty new, promising hybrids of *Dendrobium* were presented (Plates VI to XIII). The breeder is concerned with distinctiveness, uniformity and stability while considering a culture for release, while the horticulturist stresses on novelty in a commercial cutflower variety. A comparison of the floral details of the new hybrids with the parentals revealed the novelty and distinctiveness of the new hybrids. Comparisons among hybrids within and between combinations further confirmed their distinctiveness. All hybrids that have produced

the second and the third inflorescences have shown reliable uniformity in floral characters. In floriculture, a market oriented approach is important, satisfying the buyers' expectation in novelty, attractiveness and quality.

In the combination P_1 x P_4 one hybrid *viz.,* H.151 (Plate VI) was identified as promising. It had inherited the elegant posture of the female parent, P_1. The hybrid flower was solid deep purple, having inherited the rich, deep purple hue observed along the border of the sepals and petals of the male parent, P_4. The stripes, characteristic of the female parent appeared in the hybrid also, although the feature was not as distinct because of the deep base colour in the hybrid. However, on the labellum, criss-cross striations of a deeper purple colour were evident, presenting an etched appearance. The reflexing of the tip of the long labellum was observed as a characteristic feature in all flowers of the hybrid inflorescence.

Three hybrids *viz.,* H.93, H.223 and H.332 (Plate VI) in the combination P_2 x P_1 were selected as promising. All the hybrids had inherited the attractive greenish white colour observed at the centre of the flower in P_2 coupled with the clear stripes of P_1, making them enchantingly beautiful. The hybrid H.223 particularly displayed the excellent arching inflorescence of P_1 along with the smooth blending of pastel shades characteristic of P_2. The hybrid H.332 exhibited slight reflexing of petals inherited from P_2 and H.93 showed an angular appearance characteristic of P_1.

All the seven hybrids *viz.,* H.60, H.132, H.376, H.61, H.63, H.64, and H.59 (Plates VI, VII & VIII) identified in P_3 x P_1 showed excellent inflorescence and flower characters, comparable with the best commercial hybrids. All flowers presented considerable fullness. The petals almost completely overlapped the sepals in H.64. The hybrids were more colourful compared to either parent with smooth colour blending. Novel shades, not observed in either parent were introduced in the hybrids. Regular stripes (inherited from P_1) with varying intensity, always enhancing the attractiveness of the flower, were observed in all the hybrids.

In the combination P_3 x P_6 one hybrid *viz.,* H.377 (Plate VIII) was selected as promising. The hybrid had inherited the excellent flower shape and elegant posture of the female parent, P_3. At the same time, it was more colourful than P_3 owing to the magenta colour of the male parent, P_6. The deeper colour of the labellum and the arching display were also inherited from the male parent.

Seven promising and distinctly different hybrids *viz.,* H.253, H.111, H.383, H.3, H.20, H.122 and H.124 (Plates VIII & IX) were selected from the combination P_4 x P_1. Of these, H.20 was notable for its deep magenta colour, elegant natural spread and fan-shaped labellum. The hybrid H.111 also had the deep, rich magenta colour and elegant natural spread. The blending of colours observed in H.122 and H.383 was commendable. The clear, regular stripes on the pink base colour in H3 combined with its elegant posture made it very attractive.

From the combination P_5 x P_1 two hybrids *viz.,* H.231 and H.95 (Plates IX & X) were presented. Both hybrids had inherited the deep magenta colour of the female parent P_5. The hybrid H.231 had inherited the perfect, full shape and elegant stand of P_1 coupled with the attractive, white shading observed on the sepal tips of P_5. The hybrid H.95 had inherited the shading on sepal tips from P_5 along with the prominent stripes from P_1. The labellum was deep coloured and slightly twisted in H.231 resembling P_5, whereas it was full and perfectly shaped in H.95, resembling P_1.

Three hybrids *viz.,* H.74, H.73 and H.196 (Plate X) were selected from P_5 x P_{12}. All the three hybrids were intermediate between parents for most of the characters, still exhibiting admirable variation among themselves. The hybrid H.74 presented medium sized, light purple flowers in long, arching inflorescences. In the hybrid H.73, the flowers were large, magenta coloured and borne on long inflorescences. The flowers were white with very faint pink tinge in H.196, also borne on long, arching inflorescences. All the three hybrids clearly exhibited significant hybrid vigour, producing inflorescences with almost double the number of flowers (12.7) as compared to P_5 (8.2) and P_{12} (6.0). The hybrid H.74 presented a semi-full, flat appearance, in between the two parents. The hybrids H.73 and H.196 exhibited the narrow, slightly twisted sepals and spatulate petals characteristic of the semi-commercial parent P_{12}.

Five hybrids *viz.,* H.210, H.298, H.372, H.176 and H.316 (Plates X & XI) were selected as promising from the combination P_6 x P_1, all showing excellent inflorescence and floral characters. The deep

magenta pink velvety hue of the female parent was inherited by all the hybrids, with slight variations. The striations in P_1 were inherited by all the four hybrids except H.316. The characters of labellum also showed variations. The labellum was narrow and long in H.316, short and broad in H.210, rounded in H.298, pointed in H.176 and slightly twisted in H.372. The fullness of petals and the novel, velvety magenta shade of flowers observed in H.372 greatly enhanced the beauty of the hybrid.

Two hybrids *viz.,* H.178 and H.479 (Plates XI & XII) were identified as promising in P_6 x P_3, both equally attractive. Both the hybrids had very broad petals, exhibiting a prominent backward folding along the outer border (as observed in the petals of *Rosa* spp.), not seen in either parent. The beautiful, light pink shading along the two inner margins of lateral petals and along the margins and tips of sepals appeared slightly more prominent in H.479 than in H. 178. The veins were observed to stand out prominently along the central part of the broad petals in both the hybrids.

The combination P_6 x P_9 produced three promising hybrids *viz.,* H.480, H.368 and H.291 (Plate XII). Out of these, H.291 and H.480 had inherited the solid deep magenta colour of the female parent (P_6) without a trace of the white colour of the male parent (P_9). The hybrid H.480 produced a huge, arching inflorescence with a slightly whorled arrangement as in P_9. The flowers were attractive and medium sized, with rounded sepals and petals. The hybrid H.291 produced a long, erect inflorescence with flowers in whorled arrangement as in P_9. The flowers were medium sized in H.291 with narrow sepals and petals and a narrow, pointed labellum. The main attractions of the hybrid were its deep

magenta colour and floriferous nature. The hybrid H.368 exhibited a blending of maternal and paternal characters with medium sized, medium purple, full flowers. White shading was more prominent on the sepals. In all the three hybrids, the labellum including the central hairy thickening was uniformly dark magenta in colour, as in the female parent, P_6.

One hybrid *viz.,* H.53 (Plate XII) was identified as promising in $P_7 \times P_1$. The flowers presented the angular shape and stellar appearance of P_7 coupled with the elegant posture in P_1. The bold, dark pink stripes inherited from P_1 and seen prominently throughout the flower on a light pink base colour was the chief attraction.

Two hybrids *viz.,* H.380 and H.283 (Plate XIII) were recorded as promising in $P_7 \times P_9$. Both the parents were light coloured, P_7 being white with faint lilac tinge and P_9 being solid white. The hybrids were also light coloured. The hybrid H.380 resembled P_7 closely but had better fullness than P_7. The hybrid H.283 also exhibited the angular nature of P_7, but the slightly more intense pink shading coupled with the gracefully twisted shape rendered an unusual charm to these slender flowers.

Two hybrids *viz.,* H.177 and H.147 (Plate XIII) were selected as promising in $P_8 \times P_1$. Both had inherited the dark purple shade from P_8 and none had inherited the stripes of P_1. In the hybrid H.177, flower shape was stellar, similar to P_8. In the hybrid H.147, the flowers were perfectly shaped and full, closely resembling the male parent P_1 in posture. An attractive white shading as in P_8 was present along the sepal tips of both hybrids.

The combination P_{11} x P_2 had presented one promising hybrid *viz.*, H.183 (Plate XIII). The flowers exhibited fullness intermediate between the semi-commercial hybrid P_{11} and the commercial hybrid P_2. The slight reflexing of petals observed in P_2 was inherited by the hybrid. The flowers were much superior in shape to the female parent P_{11}, presenting a rounded, stellar appearance. The inflorescence in H.183 was compact and of a miniature size with short scape and short internodes, probably due to the influence of the female parent. However, the inflorescence was erect in posture as in P_2.

The present study was undertaken with the objective of developing new hybrids of *Dendrobium* with novel, commercial cut flower qualities for the export market. 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 established *ex vitro*. The seedlings belonging to different successful cross combinations were nurtured in the green house, giving optimum conditions for rapid, balanced growth. Out of these, 223 hybrids belonging to 16 combinations flowered, exhibiting a wide range of variation. Considerable novelty, distinctiveness and uniformity have been exhibited by these hybrids. As such, 40 hybrids have been selected as promising from these 16 combinations. Out of these, at least 25 hybrids have the potential for development into new commercial hybrids after micropropagation and further agronomic trials.

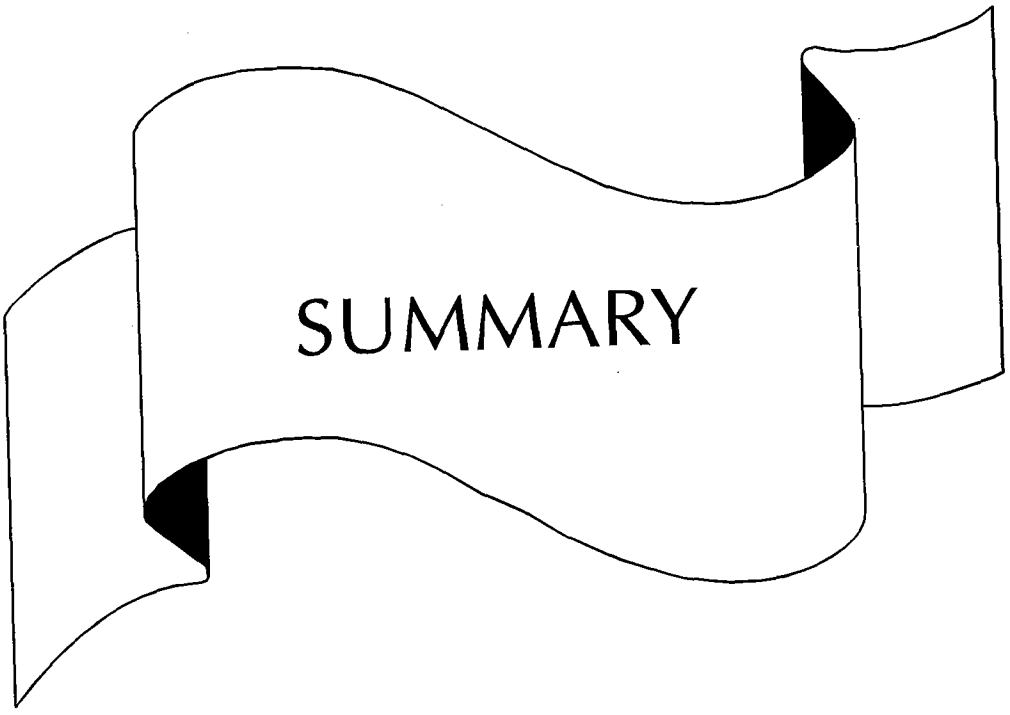
Based on information gathered from the present investigation, the following future lines of work have been suggested :

a) Micropropagation of the new hybrids for further evaluation of stability through generations and adaptability trials for varietal release.

b) Detailed morphological and molecular characterisation of the newly developed hybrids.

c) Cytological, genetic and pollen studies of the parents and hybrids.

d) Analysing the pre and post zygotic incompatibility systems encountered at various stages in the present investigation.


SUMMARY

SUMMARY

A study on "Intra and interspecific hybridization in *Dendrobium* spp." was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 1998-2001.

- Fourteen genotypes of *Dendrobium* comprising of nine leading commercial hybrid varieites (P_1 to P_9), three semi-commercial hybrid varieties (P_{10} to P_{12}) and two species (P_{13} and P_{14}) were evaluated in the green house in a CRD with ten replications.

- Analysis of variance revealed significant differences among the parental genotypes with respect to all the 21 (eight vegetative + 13 floral) biometric characters studied.

- 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.

- High estimates of heritability (> 70 %) were recorded for floral characters such as length and width of flower, length of internode, diameter of inflorescence axis, length of inflorescence, length of scape, number of flowers per inflorescence, days to first flower opening, vase life and days for wilting of all flowers.

- 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 length of inflorescence, length of scape, number of flowers per inflorescence and number of nodes per cane.

- The genotypic, phenotypic and environmental correlations of the 14 parents were studied for 12 biometric characters. High positive correlation at genotypic and phenotypic levels were observed between most of the vegetative and floral characters considered.

- 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.

- 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 ten characters.

- Flower opening commenced early in the morning (7.30 - 9.00 am) in the species (P_{13} to P_{14}) and the semi-commercial hybrids (P_{10} to P_{12}) evaluated, with weather - dependent variations. In all the commercial parental varieties (P_1 to P_9), flower opening

commenced from 9.00 am to 12.30 pm, the only exception being the white-flowered P_9 where it was from 7.30 am to 11.00 am.

- Mean anthesis time based on the capacity of pollinia to effect successful capsule set after pollination ranged from 2.0 days after flower opening in P_{13} to 3.8 days after flower opening in P_1.

- Maximum stigma receptivity period ranged from first to third day after flower opening in P_{13} to fourth to tenth day after flower opening in P_5 and P_6.

- Pollen was found to exist as tetrads which were spherical to rectangular in shape and agglutinated in masses called pollinia. Although similar in shape, significant differences in pollen diameter were observed among the different *Dendrobium* genotypes. Pollen diameter ranged from 19.84 μ in P_{13} to 48.64 μ in P_1. Significantly large pollen tetrads were found in P_1 and P_6 (44.64 μ). Pollen size was comparatively high in the commercial hybrids, medium in the semi-commercial hybrids and low in the species.

- Pollen fertility was found to vary significantly among the genotypes tested. The highest pollen fertility of 80.8 per cent was recorded by P_1 which was on par with P_3 producing 76.1 per cent fertile pollen.

- Pollen germination differed significantly among the varieties tested, with P_1 recording the highest germination of 77.1 per cent. Percentage pollen germination was the lowest in the species P_{13} (16.1).

- Intercrossing in all possible combinations involving the 14 parental genotypes of *Dendrobium* was done, depending on the availability of receptive stigma and fresh pollen. A total of 1696 pollinations were done, covering 190 out of the 196 (n^2) possible combinations. These 190 combinations included 88 crosses and their reciprocals (88) and 14 selfs. Three cross combinations involving the species P_{13}, *viz.*, $P_5 \times P_{13}$, $P_7 \times P_{13}$ and $P_{12} \times P_{13}$ and their reciprocals could not be attempted as their flowering seasons did not synchronise.

- Out of the 190 self and cross combinations attempted, 84 succeeded in producing harvestable green capsules. Out of these, no seeds were obtained from the capsules of three combinations. Seeds from the remaining 81 combinations were cultured axenically.

- The ideal stage of harvest of green capsules for *in vitro* culture was observed to be at 75-90 per cent maturity.

- Percentage capsule yield ranged from 8 to 33 in the various hybrid combinations.

- Percentage filled seeds ranged from 10.79 to 75.93 in the different combinations.

- Seed germination percentage ranged from 8.00 to 70.73. No germination was obtained with seeds from five out of the 81 combinations inoculated *in vitro*. Successful seed germination was observed in 76 combinations.

- Further development was found to be arrested in seven combinations at various stages of *in vitro* development. Mature seedlings were obtained from 69 combinations.

- Selfed seedlings obtained from the two species *viz.*, P_{13} and P_{14} failed to get acclimatized. Progeny from 67 combinations (62 crosses and five selfs) were established successfully in the green house.

- The levels of incompatibility reactions were grouped under nine heads ranging from flower abscission before the onset of any visible post pollination change to failure of hybrid seedlings to get acclimatized. A total of 123 combinations attempted succumbed to incompatibility at these different stages.

- Immature embryos from 81 cross combinations were cultured *in vitro* and taken through three to four subculture passages. Seedlings having 3-4 leaves and 3-5 roots were deflasked and transplanted. Time taken for attaining this stage varied from 143.50 days to 279.33 days. Significant differences among the combinations were observed with respect to seed germination percentage, number of days taken for germination initiation, number of days for development of protocorms, chlorophyll, first leaf, and first root primordia and for deflasking.

- 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.

- Studies on the effect of growth hormones on *in vitro* protocorm differentiation and growth of seedlings were conducted. Out of the 17 treatments tried, T_{14} (BA 8 mgl^{-1} + IAA 2mgl^{-1}) and T_{15} (BA 8 mgl^{-1} + IAA 4mgl^{-1}) were the best for early protocorm differentiation and better seedling development. Out of the 17 treatments using kinetin and IBA, T_{16} (kinetin 8 mgl^{-1} + IBA 6mgl^{-1}) and T_{11} (kinetin 6 mgl^{-1} + IBA 4mgl^{-1}) were the best.

- Effect of organic additives on *in vitro* protocorm differentiation and seedling growth was studied. Coconut water 200 ml l^{-1} was found to be the best for early protocorm differentiation and rapid seedling growth.

- Transplanting into the humidity chamber under conditions of high relative humidity (85-95 %) and controlled irrigation resulted in 94 per cent survival after four weeks.

- The best potting medium was observed to be broken tiles + charcoal + Soilrite (2:2:1), favouring high survival and well balanced growth of seedlings along with the easy availability of all ingredients.

- Significant differences were observed among the 67 hybrid combinations at 1.5-2.0 years after transplanting with respect to all the eight vegetative characters studied.

- A partial diallel was established with 18 cross combinations and the data with respect to the eight vegetative characters

mentioned above were subjected to combining ability analysis. The gca effect of P_3 was found to be beneficial for number of shoots per clump, as it was positive and significant. Non additive gene action was chiefly found to be responsible for character expression, as dominance variance was higher than additive variance.

- All the 62 hybrid combinations showed considerable hybrid vigour. Significant positive economic heterosis for all the seven vegetative characters evaluated was shown by 19 (30.65 %) hybrids. Significant positive economic heterosis for five out of the seven vegetative characters studied was shown by 48 (77.42 %) hybrid combinations.

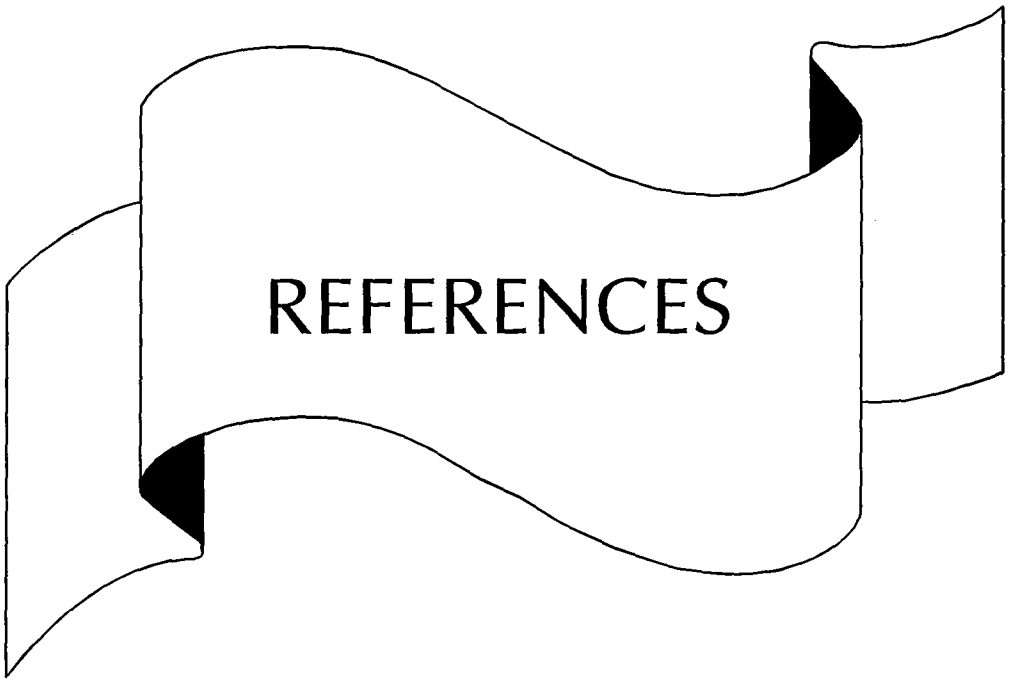
- Sixteen hybrid combinations flowered during the course of study. Significant differences were observed among the hybrid combinations with respect to all the 13 floral characters studied. Significant negative heterosis noted for days to first flower opening and cane to flower first denoted the earliness of the hybrids.

- Significant positive heterosis for all the important biometric characters deciding the size of flower, size and nature of inflorescence and longevity of inflorescence was shown by the hybrid combinations.

- From the 16 flowering combinations, about 40 promising hybrids were identified.

- The floral details of these 40 hybrids presented in Plates VI to XIII bear evidence to the high variability that can be brought about in *Dendrobium* through segregation and recombination. Manifestations of vegetative vigour was also observed in the hybrids.

- Based on three important criteria *viz.,* novelty, distinctiveness and uniformity of the floral characters, 25 out of the 40 new hybrids were selected. These hybrids show the potential for development into new, indigenous commercial varieties.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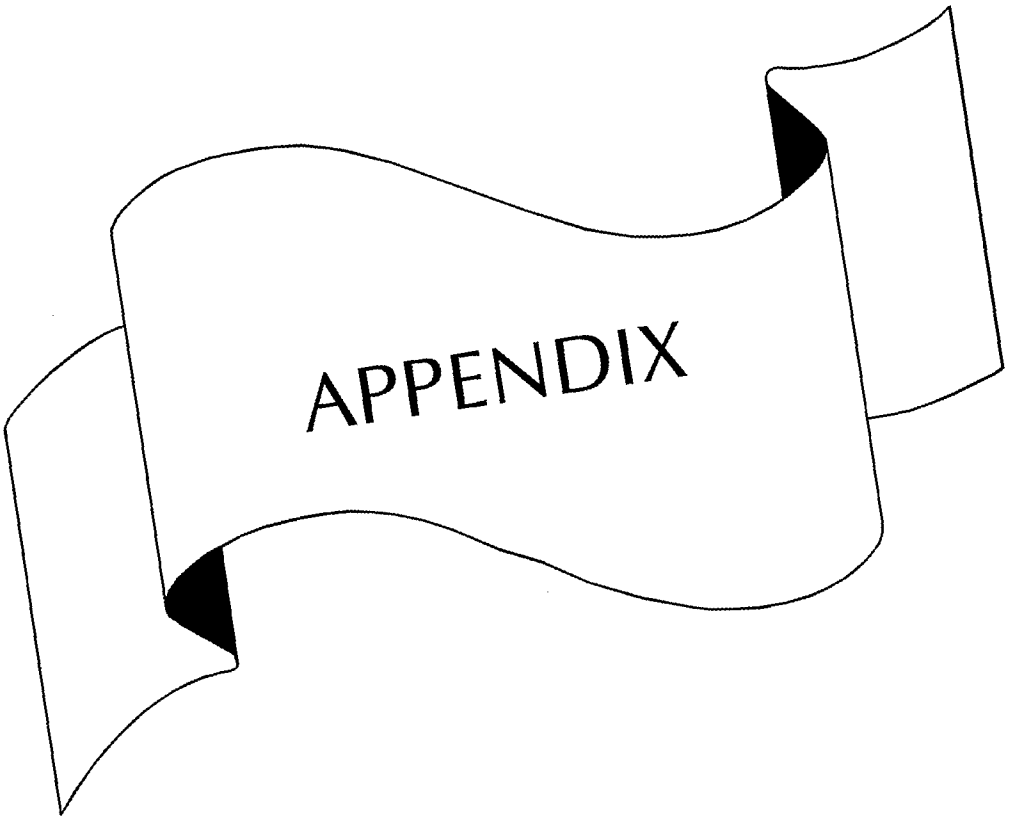
* Orginals not seen


APPENDIX

Appendix I

Basic chemical composition of the media employed for *in vitro* hybrid seed culture

Chemical	Quantity (mg/l)		
	KC	MS	VW
Major Elements			
$Ca(PO_4)_2$	—	—	200.0
$CaCl_2\ 2H_2O$	—	440.000	—
$Ca(NO_3)_2\ 4H_2O$	1000.0	—	—
Ferric citrate	—	—	28.0
$FeSO_4\ H_2O$	25.0	27.800	—
KNO_3	—	1900.000	525.0
KH_2PO_4	250.0	170.000	250.0
$MgSO_4\ 7H_2O$	250.0	370.000	250.0
$(NH_4)2SO_4$	250.0	—	500.0
NH_4NO_3	—	1650.000	—
Na_2EDTA	—	37.300	—
Minor Elements			
$CoCl_2\ 6H_2O$	—	0.025	—
$CuSO_4\ 5H_2O$	—	0.025	—
H_3Bo_3	—	6.200	—
KI	—	0.830	—
$MnSO_4$	7.5	22.300	7.0
$Na_2MoO_4\ 2H_2O$	—	0.250	—
$ZnSO_4$	—	8.600	—
Organic Constituents			
Glycine	—	2.000	—
Myo-inositol	—	100.000	—
Nicotinic acid	—	0.500	—
Pyridoxine HCl	—	0.100	—
Thiamine HCl	—	0.100	—

KC Knudson C medium

MS Murashige and Skoog medium

VW Vacin and Went medium

Intra and interspecific hybridization in *Dendrobium* spp.

By

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ABSTRACT OF THESIS
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ABSTRACT

An investigation on "Intra and interspecific hybridization in *Dendrobium* spp." was carried out in the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 1998-2001 with the objective of developing new hybrids of *Dendrobium* with novel, commercial cutflower qualities for the export market. Fourteen genotypes of *Dendrobium* comprising of nine commercial varieties, three semi-commercial varieties and two species were selected as parents after initial evaluation. The parental genotypes differed significantly with respect to all the 21 characters studied. High heritability combined with high GCV and genetic advance were observed for length of inflorescence, length of scape, number of flowers per inflorescence and number of nodes per cane. Significant positive inter-correlation in all pair-wise combinations at genotypic and phenotypic levels was observed between 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.

The 14 parental genotypes were crossed in all possible combinations (196) after preliminary studies on floral biology, anthesis and pollination. Six combinations could not be attempted as the flowering seasons of the genotypes concerned did not synchronise. A total of 1696

pollinations were done covering 190 (88 crosses + 88 reciprocals + 14 selfs) combinations. Pollinated flowers/immature capsules from 106 combinations abscised at different stages. Green capsules were harvested from 84 combinations. Capsules from three combinations did not contain seeds and seeds from five combinations did not germinate on inoculation. Protocorms/developing seedlings from seven combinations aborted at various stages of *in vitro* development. Seedlings from 69 combinations were deflasked and transplanted. Two combinations failed to get acclimatized. Progeny from 67 (62 crosses and five selfs) combinations were established successfully in the green house. The levels of incompatibility reactions were grouped under nine heads ranging from flower abscission before the onset of any visible post pollination change to failure of hybrid seedlings to get acclimatized to the *ex vitro* green house conditions. A total of 123 combinations attempted succumbed to incompatibility at these different stages. Percentage capsule yield ranged from 8 to 33 in the various hybrid combinations. Percentage filled seeds ranged from 10.79 to 75.93 and percentage seed germination ranged from 8.00 to 70.73. The basal medium MS half strength was the best for early germination and rapid *in vitro* development as compared to MS quarter strength and MS, KC and VW full strengths. The effects of growth hormones, organic additives, sucrose and charcoal on *in vitro* seed germination and seedling growth were studied and the best concentrations were identified.

Transplanting into the humidity chamber under conditions of high relative humidity (85-95%) and controlled irrigation resulted in 94 per cent survival after four weeks. The best among the potting media tried

was broken tiles + charcoal + Soilrite (2:2:1), favouring high survival and well balanced post transplantation seedling growth. The 67 hybrid combinations differed significantly with respect to all the eight vegetative characters studied at 1.5 to 2.0 years after transplanting. A partial diallel established with 18 cross combinations revealed the gca effect of P_3 to be beneficial for number of shoots per clump, as it was positive and significant. Non additive gene action was responsible for character expression, as dominance variance was higher than additive variance. Sixteen hybrid combinations flowered, recording significant differences for all the 12 floral characters studied. Economic heterosis, relative heterosis and heterobeltiosis of the hybrid combinations were studied for all vegetative and floral characters recorded. Considerable vegetative vigour, earliness and increase in the size of flowers and inflorescences were registered by the hybrid combinations.

From the 16 flowering combinations, about 40 promising hybrids were identified. Based on three important criteria *viz.,* novelty, distinctiveness and uniformity of the floral characters, 25 out of these 40 new hybrids were selected. These hybrids show the potential for development into new, indigenous commerical varieties.