FLORAL BIOLOGY AND COMPATIBILITY STUDIES IN *Dendrobium*

By

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THESIS

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DECLARATION

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

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Certified that the thesis entitled '**Floral biology and compatibility studies in *Dendrobium***' is a record of research work done independently by **Ms.Susan Varghese** 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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We, the undersigned members of the Advisory Committee of **Ms.Susan Varghese** a candidate for the degree of **Master of Science in Horticulture**, agree that the thesis entitled **"Floral biology and compatibility studies in *Dendrobium*"** may be submitted by Ms.Susan Varghese in partial fulfilment for the degree.

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ABBREVIATIONS

BA	-	Benzyladenine
cv	-	cultivar
CW	-	Coconut water
2,4-D	-	2,4-dichlorophenoxy acetic acid
KC	-	Knudson C medium
MS	-	Murashige and Skoog's medium
NAA	-	α-naphthalene acetic acid
ppm	-	parts per million; mg/l
var	-	variety
VW	-	Vacin and Went's medium

Introduction

INTRODUCTION

Orchids, the doyen among ornamentals, constitute a biologically interesting and commercially significant group of plants, which, today occupies a prime position in commercial floriculture. The biological adaptability of orchids to different habitats, their amenability to an aerial mode of life, wide range of variation in floral architecture and the specialised mechanism that they have developed for cross pollination, enable a highly successful rate of evolution.

The orchids comprise of nearly 35,000 species and 75,000 hybrids in about 800 different genera and account for about seven per cent of the total species of flowering plants.They bear bewitchingly beautiful, intricately fabricated and long lasting flowers of myriad shapes, sizes and colours and have contributed immensely to the international trade in cut flower and potted plants.

Majority of the cultivated orchids are native of the tropical countries and occur in their greatest diversity in the humid tropical forests of South and Central America, Mexico, India, Ceylon, Burma, South China, Thailand, Malaysia, Philippines, New Guinea and Australia.

India is an orchid rich country with orchids constituting nine per cent of its flora represented by nearly 1300 species in 140 genera, with the Himalayas and the Western Ghats as their natural homes. The diverse climatic belts in India are highly suitable for growing orchids of various types. Inspite of such conducive atmosphere, the orchid industry has remained in its infancy. Due to the lack of organised efforts, the industry is sadly neglected and orchid growing has essentially

remained a hobbyist oriented activity confined to private homes. Orchid research in India has not sufficiently supported commercial ventures. Moreover, a sizeable number of Indian orchids are endangered of survival; several have already succumbed to unregulated pressures of commercial collections and habitat destruction.

At this juncture, it is worth noting the expert opinion that the development and establishment of an indigenous orchid industry depends greatly on large scale and rapid multiplication and cultivation of superior hybrids which are floriferous round the year, and this can be made possible only by the development of novel breeding and biotechnological methods.

There is vast scope in establishing an orchid industry in Kerala with special emphasis on Dendrobiums since the agroclimate prevalent greately favours the popularisation of this genus. Kerala can rightly be called an open green house for orchid cultivation where orchids are found from sea level to the high altitudes upto 2400 m. *Acampe premorsa* is the most common and widely distributed species in the plains. Among the cultivated epiphytes, Dendrobiums and Vandas are the most popular. Majority of orchids are found in the forests and each forest type has its own composition of diverse orchid flora.

Dendrobium is one of the largest and most diverse genera of orchids and thus finds a prominent place in the cut flower industry. *Dendrobium* hybrids are highly floriferous and occur in all possible shape, size and colour. Being sympodial in growth, they are easy to house and maintain. But the various species and hybrids do not have synchronous flowering habit. Many of them do not have year round flowering.

In this context, the present investigation was undertaken with the following objectives:

1. To study the floral biology of *Dendrobium* hybrids.
2. To study the pollen production, fertility, germination and storage in *Dendrobium* hybrids.
3. To assess the extent of compatibility between different hybrids of *Dendrobium*.
4. Embryoculture of the various crosses.

Review of Literature

REVIEW OF LITERATURE

1 Floral biology

1.1 Flower structure

The superior design and very complicated floral machinery of orchids have placed them in the foremost rank in the plant kingdom. Orchids are the highest evolved among the monocotyledons. The floral morphology and pollination mechanisms have coevolved as a result of a continued evolutionary adjustment of flowers and their pollen vectors. In Orchidaceae this has directed to the evolution of numerous complex and often bizarre reproductive systems (Adams, 1975).

The orchid flower is zygomorphic with seven floral parts - three sepals, three petals and the column. Of the three petals, third is highly modified and is called as the lip or labellum. A waxy structure called the gynandrium or column is found in the centre of the orchid flower, either exposed or enclosed by the labellum. The column is the reproductive part of the orchid blossom 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 to form the column. The pistillate part consists of a sticky surfaced area called stigma. In the staminate element the pollen forms compact, waxy masses termed pollinia. Rostellum is a partition wall between the stamen and the stigma. The floral architecture of orchids is thus highly complex showing an extreme specialisation for cross pollination to effect fertilization (Tom and Sheeham, 1979; Mukerjee, 1990).

The flower of orchid is said to be resupinated; the flower as it appears to us, is in an upside down position having twisted through 180° on its pedicel (Abraham and Vatsala, 1981). Nyman *et al.* (1984) reported that flowers, of a *Dendrobium* hybrid flowering for the first time, were borne with the labellum uppermost, 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 relative to the ground and the position of the pedicel. Individual flowers at successive nodes along the infloresence, alternated in twisting clockwise and counter clockwise.

1.2 Anthesis

Modes of flowering behaviour probably have a direct influence on pollination biology and evolution (Croat, 1980). He reported that in *Anthurium* species the maturation of flowers initiated from the basal portion and development proceeded regularly in the direction of the apex. The pistillate phase of flowering could be distinguished by stigmatic droplets or glistening stigmas. The duration of the pistillate phase (female phase) was quite variable, ranging from only a few hours in *A. ravenii* to 21-28 days in *A. luteynii*.

Yeung (1988) observed that the mature stigma of *Epidendrum ibaguense* was covered by a lipid layer at anthesis. Cells within the stigma separate from one another and this is concomittant with a large accumulation of mucilaginous material.

According to Singh (1990) the process of emergence of anthers, their dehiscence and distribution of pollen is termed anthesis. The details of anthesis vary from one crop species to another. They are also greatly affected by environmental

factors such as humidity and temperature. In a study on the reproductive biology of *Stelis argentata*, Christensen (1992) noted that new flowers opened primarily in the mornings, in the late afternoon and during rainy weather. Flowers lasted upto nine days but most pollinia were removed during the first two days of anthesis.

1.3 Orchid hybrids and the Dendrobiums

Orchidaceae represents a peak in the evolution of monocots and is one of the most successful families of flowering plants (Abraham and Vatsala, 1981).

Primary crosses, crosses between two species, are likely to yield plants that are intermediate between the parents and are more or less uniform. But crosses between two hybrids are more complicated and show great variety among the off-spring. The offspring will show various combinations of characteristics of the parent plant as well as combinations reminiscent of many of the types found in their ancestry. Similarly, when a hybrid is self-pollinated, its inherited characteristics also recombine to give a number of different kinds of offspring (Northern, 1970).

The genetic plasticity inherent in Orchidaceae, which permits an uninhibited intermingling of genomes, not only at the species level but also at the generic level, combined with their ability to produce numerous, small and wind dispersed seeds provides with an unrestrained potential for scattering new recombinant forms throughout the habitats in which they are found (Adams, 1975).

Dendrobium is one of the largest genera in the family Orchidaceae. It comprises over 1500 species distributed from the slopes of mountains in Japan that are often snow covered, to the warm tropics. *Dendrobium* is a genus of sympodial epiphytic plants varying immensely in floral and vegetative characteristics (Tom and

Sheeham, 1979). The individual flowers of hybrids are fuller and larger and the flowering season is much broader. The flower sprays keep well after the cut. They are vigorous growers and can even be grown without protective covers. They are attractive, available round the year and have relatively long shelf life (Kamemoto, 1980).

Johansen (1990) demonstrated a unique self-incompatibility system in *Dendrobium*. The majority (72%) of the 61 species that were self pollinated showed self-sterility. In contrast with many other orchid genera, *Dendrobium* showed high incompatibility in interspecific pollinations.

Since 1856, when the first orchid hybrid flowered, a very large number of artificial hybrids have been produced, both at intergeneric and interspecific levels. Most of the orchid genera and species have no genetic barriers and they cross freely with each other. Polyploidy and introgressive hybridisation have also played a major role in the development of orchid hybrids (Chadha and Choudhary, 1992). Kleir *et al.* (1991) opined that introgression increases genetic variability through the production of recombinant genotypes, which provides populations capable of coping with environmental changes or evolving novel adaptation.

Nicolle (1994) suggested that for an orchid to be a good stud, it must be able to produce progeny superior to itself. The varieties can be combined with other studs so that the characteristics are complementary.

2 Pollen studies

The term Palynology refers to pollen and spore science. The importance of palynology in plant taxonomy was emphasized by Wodehouse (1935), Erdtman

(1952) and Stanley and Linskens(1974). Bhojwani and Bhatnagar (1974) referred to the study of external morphological features of mature pollen grains as palynology. Palynology deals with the pre-tetrad and post-tetrad stages, the latter including pollen production, pollen morphology, anthesis, pollen dissemination, pollination, pollen germination and fertilization (Srivastava, 1982).

2.1 Pollen morphology

The form of pollen grains served best in distinguishing between and showing relationships among the higher groups of plants such as families, tribes, genera and some species (Wodehouse, 1935). Erdtman (1952), who reviewed the pollen analysis done in several crop species, concluded that pollen morphology was a useful means in the classification of plants. The analysis of pollen morphology has been used as an effective aid to throw light on taxonomy, phylogeny and evolution of angiosperms (Nair, 1970).

In Orchidaceae, pollen grains are found in Polyads. Individual pollen grains of the group are not regularly arranged and so pressed together that the outline of the individual grains become angular (Moore and Webb, 1978). The pollen of orchid flowers is not powdery as it is in most flowers, but rather agglutinated in masses called pollinia (singular, pollinium). Each orchid flower has from two to eight pollinia under the anther cap, depending on the genus. In *Dendrobium*, pollinia are in two pairs, each tightly compressed (Tom and Sheeham, 1979).

According to Abraham and Vasala (1981) in most members of Orchidaceae, pollen are in tetrads. They are held together by means of elastic threads of tapetal origin. Fitzgerald *et al.* (1994) reported that in *Pterostylis concinna*,

individual pollen grains were all found to possess an outer exine wall, pollinia were mealy and they fell apart easily. While *Dendrobium gouldii* had hard pollinia and only pollen grains on the outer surface had an exine, the inner types were bound by tapetally derived pollen-coat material.

2.2 Pollen production

The relative quantity of pollen produced per blossom or per anther varies from variety to variety within a species (Nair *et al.*, 1964). A precise measure of the amount of pollen produced by individual anthers, flowers or the plant itself is essential to evaluate the worth of a variety as a pollinator more accurately .

Different techniques have been adopted by different workers for the estimation of pollen production. Pohl (1937) computed the pollen output of some plants by emptying the thecae and suspending the grains in a fixed portion of suspension. Oberle and Georgtzen (1952) demonstrated a method of determining the number of pollen grains per anther in grape vines, with the aid of haemocytometer. A marked variation was observed in the number of pollen grains produced by different species and different varieties of the same species. The accuracy of haemocytometer in estimating the pollen production was further confirmed and modified by the the work of Rao and Khader (1962) in fruit crops like papaya, pomegranate and sapota.

Lobanov (1950) observed that pollination of fruit plants with large amount of pollen resulted in the greatest fertilization in intra-varietal and inter-varietal crossings and in hybridisation of more distantly related forms. Thacenko (1960) reported that, in vines, the highest level of pollination gave the greatest fruit set.

Brooks and Puri (1963) and Sharma and Singh (1970) reported variation in atmospheric conditions affected pollen production. Higher temperature and dry climate increased pollen production in mango (Sharma and Singh, 1970).

Srivastava (1982) observed certain members of the Malvaceae family to have higher pollen production in the middle period and the least in the late periods. Pollen production per flower depended on the number of anthers in individual flowers. The number of pollen grains per anther ranged from 87 to 500 in the thirty five different types, varieties and species of *Hibiscus* studied (Markose, 1984).

2.3 Pollen viability

Appearance of the pollen alone, even at collection time, is not always a good index of viability (Stanley and Linskens, 1974).. So pollen fertility is to be tested either by using specific stains or by *in vitro* growth studies.

2.3.1 Pollen staining method

Staining the pollen with different chemicals and dyes has been adopted to assess the viability of the pollen grains.

Zirkle (1937) described a method for mounting pollen grains in aceto-carmine. The grains which stained well and looked plump and normal were taken as viable and the unstained, shrivelled ones as non-viable. Singh *et al.* (1978) used methyl green, glycerine jelly, aldin oil, gelatine violet and acetocarmine as stains to assess the viability of pollen grains. However, Randhawa and Nair (1960) and Singh (1961a) suggested that the stainability test was undependable for the purpose

of assessing pollen viability because the staining reaction depends primarily on the pollen contents and not on their viability.

A positive and significant correlation was found to exist between pollen staining and germination in rose by Pearson and Harney (1984). Staining with aceto-carmine was regarded as the more suitable and objective test in determining pollen viability as it gave better results compared to germination test (Micic *et al.*, 1987).

2.3.2 Pollen germination method

Germination of pollen *in vitro* is considered to be a better means of assessing pollen fertility. An artificial medium supplemented with the required nutrients, especially sugars, have proved successful to germinate pollen grains in a large number of plants.

Brink (1924), O'Kelly (1955) and Vasil (1958) suggested that apart from having an osmotic role, the externally supplied sugars in the medium definitely served as a nutrient material for the growing tubes.

Additives like agar and gelatine can promote pollen germination. Singh (1961b) found that papaya pollen gave 62.9 per cent germination in five per cent sucrose solution. When one per cent agar was added to the sucrose solution, higher germination of 67.6 per cent was obtained. In grapes good germination was obtained with a medium containing five per cent sucrose and two per cent agar (Singh, 1959)

The stimulating effect of boron on pollen germination and tube growth was discovered by Schumucker (1935). He found that 1 to 10 ppm boric acid

stimulated pollen germination and tube growth. Thompson and Batjer (1950) reported that boron in low concentrations of 2.5-40.0 ppm had stimulative effects whereas at higher concentrations, boron inhibited pollen germination and tube growth.

Munzer (1960) found that 0.001 to 0.010 per cent of boric acid had a stimulating effect on pollen germination and tube growth in more than 60 angiosperm species. Rao and Khader (1960) reported that the germination of sapota pollen was enhanced by the addition of 100 ppm boric acid to sucrose-agar media. Johri and Vasil (1961) found that the effect of boron was far better than the effect of any known hormones, vitamins or other chemical substances.

Gausch and Dugger (1953) explained that borate ions reacted with sugar molecules to form an ionisable sucrose-borax complex which moved through the cell readily than non-borated and non-ionisable sucrose molecules.

Rao and Chin (1973) reported that the pollen culture media, sucrose and stigmatic extract were more effective in promoting pollen germination of orchid hybrids than were inorganic salts and growth substances. A medium containing 20 per cent sucrose, one per cent agar and 100 ppm boric acid was the best for pollen germination of shoe flower (Markose, 1984).

Parfitt and Ganeshan (1989), while comparing different procedures for estimating viability of *Prunus* pollen reported that hanging drop slide and agar plate germination procedures were more effective than different staining methods.

2.4 Pollen storage

When planning a cross, one is occasionally thwarted in one's efforts because the prospective parents do not bloom simultaneously, and in other instances, the plants may be self-sterile. However, where attainable, cross-pollination is to be preferred to self-pollination in as much as it is more likely to lead to the production of vigorous seedlings. Consequently, it is often necessary to store pollen for a number of weeks, months or, in certain instances, years, to make the desired cross. A proper combination of factors such as low temperature, relative humidity and light has great bearing on pollen storage (Seaton, 1994).

Pollen of orchid species can be classified as "orthodox" ie., for maximum shelf-life, it is important that pollen be stored at relatively low temperatures and pollen moisture contents (Pritchard, 1985; Seaton, 1985; Seaton and Hailes, 1989). On examining a range of terrestrial species, Pritchard and Prendergast (1989) observed that drying orchid pollen over silica gel normally reduces its viability. This lends support to earlier finding by Meeyot and Kamemoto (1969). They reported that pollen of *Dendrobium phalaenopsis* remained viable for 4 to 6 months and that of *D. sterbloceras* and *D. undulatum* for more than 12 months at 7°C.

A great deal of variability was observed in the viability period of fresh and stored pollen. Furthermore, the pollens stored at 4°C remained viable for a longer period than when stored at room temperature, probably due to their reduced metabolic activities at lower temperatures. In *Dendrobium amoenum* only 25.4 per cent pollen grains remained viable after one week of storage at room temperature. However, when stored at 4°C, 50 per cent remained viable (Devi and Deka, 1992).

Pollinia for storage should not be left on the parent plant for too long or the pollen may succumb to fungal infection, yet they should not be removed until the pollen has had sufficient time to mature. Pollen should be transferred soon after collection to sealed vials, and then stored either in a refrigerator at 40° F or if preferred in a freezer at -4° F after drying to predetermined moisture contents over anhydrous calcium chloride (Seaton, 1994).

3 Compatibility studies

3.1 Pollination biology

Pollination is a simple process involving transfer of pollinia from one flower to the stigma of another flower. In Orchidaceae, to avoid unnecessary wastage of pollen, which is required in large quantities in order to fertilize the incredibly large number of ovules in each flower, they are kept as neat little packets called pollinia. These are placed on a raised platform called column in such a way that an insect can easily locate it and carry it away (Abraham and Vatsala, 1981).

Studies on *Dendrobium speciosum* var. *speciosum* and var. *hillii* showed that pollination was effected by the deposit of the pollinia from one flower into the stigmatic cup of a flower on another plant. The pollinia were submerged into the viscous liquid of the stigmatic cup. This liquid contained the detached stigmatic cells and mucilage (Slater, 1991). It was earlier reported by Woittiez (1979) that the following factors were found to be involved in the sticking of pollen to stigmas: the surface tension force, the wind force, the electrostatic force, the electrodynamic force, the gravity and the essential force. Sticking is mainly determined by the wetness of the pollen or stigma.

3.1.1 Post pollination phenomena

Pollination not only shortens the life of flowers, but also induces numerous and remarkable changes in its morphology and colouration.

Post pollination phenomenon in orchid flowers are regulated by the participation of additional substance produced by pollinated flowers or from pollen (Strauss and Arditti, 1980). Ethylene, produced in abundance after pollination and emasculation also regulates the post pollination phenomena including its own production and the senescence of some floral segments (Chadwick *et al.*, 1986).

The activity of ACC oxidase, which catalyses the conversion of ACC to ethylene, is increased in the stigma after pollination. This increase was due to *de novo* synthesis of mRNA and presumably protein which was induced after pollination (Nadeau *et al.*, 1993).

Post pollination phenomena also include stigmatic closure, increase in fresh and dry weight of ovaries and gynostemia, hormone production, synthesis and/or destruction of pigments, deresupination, nastic movements, new biochemical pathways, cessation of scent evolution, swelling of the column and ovary, cell division in the ovary, breaking apart of pollinia due to tetrads dissociation, progressive dehydration of pollen grains and germination of pollens from the outside of pollinium to the inside (Bose and Yadav, 1989; Slater, 1991).

3.1.2 Incompatibility

Many of the cases of apparent self-incompatibility and cross-sterility commonly encountered among the cultivated orchid hybrids can be attributed to one

of the two causes: hybrid sterility or polyploidy (Lenz and Wimbler, 1959). Hybrid sterility is often the result of non-homologies of the genome complements. Linskens (1975) reported that the interspecific incompatibility was heterogenic, ie., controlled by more than one gene at different loci on the chromosome. Infertility in polyploids often results from pairing abnormalities during meiosis where there has been an addition of one or more complete or incomplete chromosome sets. Triploidy is commonly encountered in many of the cultivated orchids and is one of the most frequent causes of sterility (Abraham and Vatsala, 1981).

Dadlani *et al.* (1988) reported that lack of seed setting was due to the absence of anthers and/or pollen, or pollen sterility, non-viability or failure to germinate, depending upon the genotype involved.

In the 29 interspecific and 47 intergeneric crossing performed to determine compatibility between species belonging to the same genus and to different genera for production of hybrids through embryo/seed culture, ovaries swelled in many crosses but did not develop pods. Percentage of fruit set varied from 0 to 100 in the case of interspecific crosses while it ranged from 0 to 75 in intergeneric crosses. Although pods were formed, effective fertilization leading to embryo formation did not take place in many cases indicating that only the ovary was induced to develop parthenocarpically by the introduction of foreign pollen. Out of 13 hybrid seed capsules formed, embryos of only three crosses germinated (Devi and Deka, 1994).

3.2 Fertilization and maturity

Time taken for maturity of pods depends on the habit of the species

crossed. According to Hegde (1984) pods of *Dendrobium* sp. mature in 9 to 17 months. Generally, it takes 4 to 10 months for a capsule to mature and ripen. The capsules becoming brownish or yellowish is a sign of maturity (Bose and Yadev, 1989).

Blettilla striata and *Calanthe discolor* ovaries reached their final size in 50 to 60 and 30 to 50 days, respectively, after pollination. Embryos and seeds of both species developed in 80 to 110 days after fertilization. The seeds were capable of germinating before embryological development was complete, 45 to 50 days after pollination. In *Cymbidium goeringii* and *Paphiopedilum insignae* var. *sanderae*, ovule formation occurred 43 to 45 and 58 to 60 days after pollinations, respectively. Maturation of embryos took 115 to 120 and 195 to 200 days and the seeds germinated in 80 and 165 days after pollination, respectively. Highest germination was obtained in each of the species when seeds were harvested with the embryos almost mature (Nagashima, 1982).

4 Embryo culture

The orchid plant with its wide variations in growth, flowering, seed production and germination has got many adaptive features. The most suitable example for the adaptive feature is the physiology of orchid seed germination (Nair, 1982). Orchid seeds are unique in many ways. Being very small (dust-like), they are produced in large numbers, ie., 2-3 millions per capsule but these lack metabolic machinery and functional endosperm, with the result that only 0.2-0.3 per cent of them germinate in nature. Earlier, most botanists thought these to be sterile. In 1909, Hans Burgeff established a specific relationship between orchid and fungus and stressed the need for symbiotic germination. However, the propagation and

cultivation of orchids was revolutionised by the discovery, in 1921-22, by Knudson, that orchid seeds can be germinated on a sugar enriched medium (Arditti, 1979; Singh, 1993).

4.1 Nature of seed

Orchid seeds are very minute, weighing 0.3 to 14.0 μg (Harley, 1951) and measure from 0.25 to 1.20 mm in length (Hoene, 1949) and 0.090 to 0.270 mm in width (Arditti, 1967). Each capsule produces 1,300 to 40,00,000 seeds (Arditti, 1961). Two major groups of orchid seeds are usually distinguished. One group has relatively differentiated embryos, including rudimentary cotyledons as in *Bletilla hydracinthia* (Harley, 1951). However, majority of the species have relatively undifferentiated embryos and no endosperm (Maheshwari and Narayana Swami, 1952).

The development of seedling from a seed is not like in any other angiosperms. The rudimentary embryo enclosed in the seeds swell in size and burst out of the seed coat. A cone shaped spherical seedling is formed and this is called protocorm stage (Bernad, 1909). The first leaf primordium is formed as a bulge and the protocorm increases in size and subsequently rhizoids and leaf primordia are formed (Arditti and Bill, 1965; Nair *et al.*, 1986).

4.1.1 Symbiotic seed germination

Prior to the discovery of asymbiotic seed germination, the germination of orchid seed was possible only by means of a specific fungus, generally referred to as mycorrhiza. Bernard (1899), a French botanist, successfully isolated a number of fungi and found that fungal infection was necessary for germination of orchid

seeds. Later, a German botanist, Burgeff, carried out the work and demonstrated the association of various fungal mycelia with the orchid root structure and their role in seed germination. The main fungi associated with orchids are: *Rhizoctonia repens, R. lanuginosa, R. mucoioides* and *Corticium catonii* (Singh, 1993).

4.1.2 Asymbiotic germination

Knudson (1946) demonstrated that orchid seeds germinate freely on a medium containing sugar, mineral nutrients and agar without the help of any fungus or mycorrhiza. This revolutionised the basic approach of orchid cultivation and started a new era of asymbiotic seed germination. Now asymbiotic method of orchid seed germination is widely used in commercial orchid growing (Nair, 1982). It is also on record that 90 to 95 per cent of the orchid hybrids registered so far have been raised on nutrient media (Singh and Prakash, 1984).

4.2 Green pod culture

A major advancement in increasing the germination of orchid seeds and reducing flowering time was the development of the green pod culture (Tsuchiya, 1954). The technique reduces the time gap between pollination and seed sowing besides enhancing the germination frequency; it has now replaced the dry seed culture procedure in most of the commercial laboratories.

In the green pod culture technique, the seed capsule is removed from the plant after fertilization but prior to dehiscence. The surface of the capsule is cleaned with a sterilizing agent and flamed after dipping in alcohol. The seed capsule is opened with a sharp blade and the seeds are sown directly into the medium under aseptic conditions. During this process, the seeds are saved of air contamination and thus do not require treatment with sterilization agents.

The difference in harvesting time between the dry seed culture process and the green pod culture process may be as much as 6-8 months in some genera. This reduction in harvesting time decreases the time required for flowering. The optimum harvesting time for *Dendrobium* species is reported to be 160 days and that for hybrids 175 days (Singh, 1992, 1993).

4.2.1 Embryogenesis

Germination was considered to have occurred when protocorms, either green or white, were observed in the cultures (Yam and Weatherhead, 1988). According of Rubluo *et al.* (1989), germination may be defined as the presence of protocorms with one leaf primordium one month after culture and the adult stage as plantlets with leaves at least 30 mm long, pseudobulbs and roots.

Findings seem to confirm that, for many orchid species, the use of immature seeds taken from green capsules is preferable as starting material for germination because the seeds are viable, or not dormant and germination is faster. Immature embryos from a three month old capsule of *Acampe rigida* germinated within four weeks. Seed capsules of this species reach maturity approximately one year after pollination and mature seeds fail to germinate (Yam and Weatherhead, 1988).

Nagashima (1993) studied seeds of 47 orchid species and reported that germination was poor and took longer in seeds collected when the embryos were at pre-tetrad or intermediary stages. Highest percentage germination was obtained in seeds in which embryo was between the octant stage and completion of embryogenesis. Germination rate among species ranged from 0.8 to 100.0 per cent

and the number of days between sowing and germination ranged from 3 to 305, depending on stage of embryogenesis and the medium used. The *in vitro* germination rate of *Dendrobium candidum* was 95 per cent. Protocormarose from the embryos and could form calluses or plantlets, the calluses sometime later formed plantlets. During germination, starch reduction was observed in the cells at the top of each embryo; it may have been associated with cotyledon formation (Ye *et al.*, 1990).

Singh (1993) reported that when the seeds are planted on a nutrient medium in *in vitro* conditions, not only does the percentage of germination improve to 100 per cent in some cases, but it also takes less time for differentiating of orchid seeds biochemically and morphologically. The *in vitro* germination of orchid seeds proceeds in the sequence that embryo imbibes water and swells, embryo then emerges from the testa and forms a protocorm; after which the protocorm differentiates into shoot meristem and rhizoids in opposite direction. The protocorm becomes green, leaves are produced and it becomes autotrophic in nature. After the two leaf stage, the protocorm and rhizoid lose the nutritive function and real roots are formed endogenously.

4.2.2 Culture media

Sahid (1980) reported that the percentage seed germination of a *Dendrobium* hybrid was higher in agar medium containing potato extract than on Knudson C, but seedling growth was slow. Growth rate could be improved by adding potato and pea extract to Knudson C media. Seeds of *Bletilla hyacintha* took only seven days for greening and germination on Vacin and Went medium (Nair *et al.*, 1986). Vacin and Went medium with 15 per cent coconut water plus 10 ppm NAA led to the rapid proliferation of protocorm like bodies and plantlet formation

and growth in *Dendrobium* cv. Jacqueline Thomas. The addition of vitamins or 2,4-D was found deliterious (Soedjono, 1988).

Rubluo *et al.* (1989) reported that the best germination occurred on Knudson C medium with three per cent sucrose and 10 per cent coconut water at 25°C ± 2 and 16 h photoperiod (1200 lx). All seedlings developed to the adult stage, forming leaves, pseudobulbs and roots after 90 days of *in vitro* culture. Kumaria and Tandon (1991) concluded that *Dendrobium fimbriatum* var. *oculam* seeds require a medium containing high concentrations of nutrient salts and vitamins for germination and development. Highest percentage germination (91%) with the four month old seeds was obtained on Nitsch medium followed by MS (85%). Protocorm stage was reached in 4 to 5 weeks on MS, Nitsch and VW media.

The immature seeds (embryos) of *Rhychostylis retusa* and *Vanda coerulea* germinated better (50 to 60%) in Vacin and Went medium due probably to their specific nutrient requirements. An increased germination frequency (70 to 80%) in medium supplemented with coconut water, banana extract, pineapple extract and vitamin stock of Nitsch media would suggest that the growth adjuncts probably invoke germination in the relatively younger embryos by satisfying their nutritional complexities. The embryo developed into dark green and profusely hairy protocorms in four weeks and proliferated rapidly. First leaf development was observed in 9 to 10 weeks in culture whereas root appeared 5 to 6 weeks later; the seedlings were well formed in about 20 week cultures (Nath *et al.*, 1991).

Nitsch media supplemented with either, 0.5 per cent peptone, 400 mg/l casein hydrolysate and 25 per cent yeast extract, when used for subculturing young seedlings of *Dendrobium* hybrids, it was observed that the average length of leaves

and roots and average fresh weight was maximum in peptone supplemented medium. Rate of growth was slow in medium supplemented with yeast extract. In Knudson C medium supplemented with either of CW (20%), IAA (1 mg/l), NAA (1 mg/l) and 2,4-D (1 mg/l), increase in the rate of growth of the seedlings was observed when IAA and NAA were added but not when supplemented with CW (Devi and Deka, 1994).

Improvement in growth of orchid seedlings after addition of peptone has been earlier reported in Vanda (Mathews and Rao, 1980), *D. monik* (Chung *et al.*, 1981) and *Bletilla striata* (Chung *et al.*, 1983). Yeast extract has been reported to have inhibitory effects on germinating seeds and developing seedlings of *Dendrobium* and Brassolaeliocattleya (Kanu, 1965).

Growth inhibitory effect of coconut water on *Dendrobium* has been reported (Kotomori and Murashige, 1965). Boesman (1962) reported that IAA at 1 mg/l or 2 mg/l was effective on seedling growth of cattleya. NAA stimulated germination and seedling growth in several species like Cattleya (Withner, 1951), *Cymbidium mastersii* (Prasad amd Mitra, 1975) and Vanda (Mathews and Rao, 1980).

5 Planting out and hardening

Tissue culture plants are very tender and their transfer from the artificial environment of the culture vessel to the free living existence of a green house or similar environment and their establishment outside culture conditions is of great importance. Light, temperature and relative humidity are the major factors to be controlled for the successful establishment of the *in vitro* grown plantlet.

Anderson (1980) reported that thorough washing of the plantlets to remove traces of nutrient media and sterilizing the potting mixture eliminated serious problems of fungal infection. Increase in plant survival and adjustment to green house conditions was noticed in *Cymbidium* hybrids from tissue culture when sprayed with 0.15 to 0.25 per cent previcur-N (Propamocarb) (Zimmer *et al.*, 1981). Ziu (1986) reported that the success in acclimatization of *in vitro* cultured plants is dependent not only on the post-transfer growth condition but also on the pre-transfer culture condition.

Wainwright (1988) observed that the environment in a tissue culture container is that of very high humidity, low light levels and usually of a constant temperature. Leaves or shoots or plantlets leaving the environment are as a result very poorly adapted to resist the low relative humidity, high light levels and more variable temperature found *in vivo*.

Kumar (1991) reported that maximum survival and optimal growth of shoots and roots of *Dendrobium* hybrid seedlings occurred in media comprising rubber seed husks, coconut shell pieces and gravel. Thomas and Thomas (1992) transplanted odontoglossums and their hybrids into perlite and rockwool media and found that orchids in perlite made slower growth initially, but after a year started to grow more rapidly with extensive root system.

Sudeep (1994) reported that the *in vitro* rooted *Dendrobium* plantlets exhibited maximum survival rate when planted out in open in a potting media consisting of coconut husk alone, followed by that kept in a mist chamber covered with ventilated plastic sheets.

Materials and Methods

MATERIALS AND METHODS

The study entitled 'Floral biology and compatibility studies in *Dendrobium*' was carried out in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, during the period from April 1994 to August 1995. The investigations on floral biology, pollen morphology, pollen production, pollen fertility, pollen storage, compatibility among the different hybrids and embryoculture were done in the different *Dendrobium* hybrids maintained in the Orchidarium of the AICRP on Floriculture.

The hybrids used in the study included New Pink, Hieng Beauty, Emma White, Kasem White, Sonia 28 Mutant B, (Kiomi Beauty x Banyat Pink), (Hawaiian Beauty x Kasem Pink), Sonia, Sonia # 28 and White Nern.

1 Floral biology

1.1 Floral characteristics

The *Dendrobium* hybrids used in the study were morphologically described. Each inflorescence of the hybrids under study was tagged on the day of emergence and observations were recorded on the following characteristics..tb0.99"

(a) Number of flowers/inflorescence

(b) Days for inflorescence emergence to first flower opening

(c) Days for first flower opening to the opening of last flower in an inflorescence

(d) Size of flower

(e) Length of inflorescence

(f) Internodal length

1.2 Anthesis

Mature buds in each of the hybrids selected for the study was tagged at full bud stage for observing the time of flower opening. The flower buds were observed at hourly intervals to record their time of opening.

1.3 Stigma receptivity

The flowers of the hybrids were pollinated from the day of anthesis in order to study the stigma receptivity period. The pollinations were done three times during the day viz., morning, noon and evening.

2 Pollen studies

Five hybrids of *Dendrobium*, namely, New Pink, Hieng beauty, Emma white, Kasem white and Sonia 28 Mutant B were used for pollen studies. Pollen morphology, pollen production per pollinium, pollen fertility and pollen germination were studied in these hybrids. *Dendrobium* hybrid New Pink alone was used in the studies on pollen storage.

2.1 Pollen morphology

Pollinia were collected from fully opened flowers, 4 to 5 days after anthesis. Each pollinium was placed in a drop of water to enable proper dispersion of pollen grains. Pollen morphology was studied by staining with acetocarmine glycerine mixture.

2.2 Pollen size and shape

Pollen grains dispersed in a drop of acetocarmine glycerine medium was mounted on a clean microscopic slide. This was covered with a zero cover glass and kept for 30 minutes. Diameter of ten normal well shaped, plumpy and well stained pollen grains from each hybrid was measured at random using a standardised ocular micrometer under low power of a microscope. The mean diameter was expressed in microns.

The shape of pollen grains was studied under high power magnification.

Colour of unstained fresh pollen grains was studied under low power of a microscope.

2.3 Pollen production

The number of pollen grains per pollinium was estimated with a haemocytometer. Mature pollinia were gathered from fully opened flowers. Pollen counts were taken as suggested by Rao and Khader (1962).

To the vials containing the pollinia, 1.25 ml of water containing 1.0 per cent extran was added and the contents were shaken thoroughly. To this 1.25 ml of glycerine was added. The pollinia were crushed with the edge of a glass rod in order to suspend all the pollen grains properly. A drop of the above suspension drawn in a fine pipette was transferred to each of the two counting chambers of a haemocytometer. Pollen grains in each of the four corner squares in both the counting chambers were counted and the mean number in eight corner squares were calculated. For each hybrid three such estimates were made.

The number of pollen per pollinium was calculated using the following formula.

If N = Average number of pollen grains counted per corner square and

X = number of pollen per pollinia

then $N : X = 0.1 : 2500$

$0.1\ X = 2500\ N$

$X = 25000\ N$

2.4 Pollen fertility

Fertility of pollen grains was estimated by acetocarmine staining technique.

Pollen grains were dispersed in a drop of acetocarmine-glycerine medium on a clean microscopic slide for 30 minutes, for proper staining and examined under low power of a microscope. Pollen fertility was estimated by counting fertile and sterile pollen grains separately. Pollen grains which stained well, looked plumpy and well shaped were considered as fertile and those unstained, small or shrivelled as sterile or non-viable (Zirkle, 1937). The observations were made in five different microscopic fields. Three such estimates (slides) were prepared. The mean percentage of viable pollen grains was thus arrived at.

2.5 Pollen germination

In vitro germination of pollen grains was studied in artificial media.

2.5.1 Standardisation of media for pollen germination

Normally pod setting hybrid New Pink was used for standardising the medium for germination. The following media were used.

2.5.1.1 Sucrose medium

Germination was studied in the medium consisting of sucrose at concentrations of 0, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 per cent. Pollinia were collected on the fifth day after anthesis and the fresh pollen grains were placed on a drop of the solution in cavity slides and these were allowed to rest as hanging drops. A humid environment was provided for germination by placing the slides in a desiccator containing water. Germinated and non-germinated pollen grains were counted and their tube length was measured after 24 hours, in five different microscopic fields under low power magnification. Viability was expressed as percentage and the tube length was measured in microns (μ).

2.5.1.2 Sucrose with agar medium

Sucrose at concentrations of 1 per cent to 5 per cent along with agar at concentrations of 0.5, 1.0 and 1.5 per cent was tried for *in vitro* pollen germination. Each concentration of sucrose in combination with three different concentration of agar was tried. Pollen germination and tube growth was studied by hanging drop technique.

2.5.1.3 Sucrose agar boric acid medium

From the previous studies, the suitable concentration of sucrose-agar medium was fixed (2 per cent sucrose and 1 per cent agar). Sucrose-agar in the

above concentrations and each in combination with four different concentrations of boric acid, viz., 25, 50, 75 and 100 ppm, were prepared. The percentage of pollen germination and tube growth was assessed by hanging drop technique.

The experiment was repeated thrice.

2.5.2 Assessment of pollen germination in the different hybrids

The germination studies of different hybrids, viz., Hieng Beauty, Emma White, Kasem White and Sonia 28 Mutant B were done using the media standardised in the above experiment.

The length of pollen tubes of ten grains selected at random from each slide was measured and the mean tube length was estimated.

2.6 Pollen storage

The pollinia of *Dendrobium* hybrid New Pink was used for the study. Pollinia were kept in polythene covers of 750 gauge and stored under the following conditions.

(a) At room temperature

(b) Over calcium chloride at room temperature in a desiccator

(c) At 4°C

(d) Over calcium chloride at 4°C in a desiccator

(e) At 0°C

Viability of the stored pollen grains was studied at weekly intervals by acetocarmine glycerine staining technique.

3. Compatibility studies in the different *Dendrobium* hybrids

Ten different hybrids of *Dendrobium*, viz., New Pink, Hieng Beauty, Emma White, Kasem White, Sonia 28 Mutant B, Kiomi Beauty x Banyat Pink, Hawaiian Beauty x Kasem Pink, Sonia # 28, Sonia and White Nern were selected for the study.

Self compatibility was assessed in the hybrids New Pink, Hieng Beauty, Emma White, Kiomi Beauty x Banyat Pink and White Nern.

The following cross combinations were attempted in order to study the cross compatibility between the hybrids selected.

New Pink x Hieng Beauty

New Pink x Emma White

New Pink x White Nern

Hieng Beauty x New Pink

Hieng Beauty x (Hawaiian Beauty x Kasem Pink)

Hieng Beauty x (Kiomi Beauty x Banyat Pink)

Hieng Beauty x Sonia # 28

Hieng Beauty x Sonia

Hieng Beauty x Emma White

Emma White x Sonia

Emma White x New Pink

Emma White x Hieng Beauty

Emma White x White Nern

Emma White x (Kiomi Beauty x Banyat Pink)

Kasem White x (Kiomi Beauty x Banyat Pink)

Sonia 28 Mutant B x White Nern

Sonia 28 Mutant B x Hieng Beauty

(Kiomi Beauty x Banyat Pink) x Hieng Beauty

(Kiomi Beauty x Banyat Pink) x White Nern

(Kiomi Beauty x Banyat Pink) x Kasem White

(Kiomi Beauty x Banyat Pink) x Emma White

Sonia # 28 x Hieng Beauty

White Nern x (Kiomi Beauty x Banyat Pink)

White Nern x Sonia

White Nern x New Pink

(Hawaiian Beauty x Kasem Pink) x Hieng Beauty

(Hawaiian Beauty x Kasem pink) x Emma White

Sonia x Emma White

Sonia x White Nern

Sonia x Hieng Beauty

3.1 Technique of artificial pollination

Hand pollination was carried out by using a pointed toothpick, needle or the tip of ball point pen. The pollinia were collected on a clean paper and then transferred to the stigmatic surface of the female parent.

The post pollination changes were observed in each of the pollinated flowers. Observations were recorded on the following:

(i) Percentage of pod set

(ii) Number of days taken for maturity of pod

4. Embryo culture

The pods were harvested at the green pod stage (58 to 120 days after pollination) and cultured under asceptic conditions. The pods were washed thoroughly, dipped in or wiped with 0.1 per cent alcohol and flamed carefully so as to avoid any injury to the embryo within and also to prevent bursting of the pod. The two ends of the pods were cut using a sharp sterile scalpel. The pod was longitudinally split open. The immature seeds were slowly scraped and carefully dusted on the surface of the media.

The *in vitro* culture of the embryos were done in the following media.

(a) VW (Vacin and Went, 1949)

(b) MS (Murashige and Skoog, 1962)

The germinating embryos were subcultured in the media containing the following additives (per litre) with a view to augment growth:

(i) NAA 2 ppm + 2,4-D 2 ppm + BA 5 ppm

(ii) CW 150 ml + Peptone 500 mg + adenine 20 mg

(iii) 2,4-D 2 ppm + NAA 2 ppm + BA 5 ppm + Peptone 1 g

(iv) Peptone 1 g

The following observations were recorded

(i) Extent of germination (%)

(ii) Days to greening

(iii) Days to rooting

(iv) Days to planting out

5 Planting out and hardening

The seedlings which attained 7-8 cm height with 8-12 leaves and sufficient root growth were taken out from culture tubes and were thoroughly washed in running water to remove the agar and nutrient medium. The plantlets were dipped in one per cent Indofil-M-45 and dried. The seedlings were then planted in coconut husk pieces.

The coconut husk was cut into v shaped pieces. This was autoclaved and sterilized by drenching in two per cent solution of Indofil-M-45. In the transverse area of the husk pieces small vertical cuts were made with a knife and the plantlets were inserted in it and tied with copper wire pieces. The plantlets were left hanging and covered with ventilated polythene sheet. They were sprayed with water twice a day with a sprayer. Observations on plant height, number of leaves and number of roots were recorded at the time of planting out.

Statistical analysis

The data collected on different aspects of floral characters and pollen studies were statistically analysed. Transformations were made wherever necessary and the data analysed by analysis of variance technique. Significant results were compared after finding the critical difference, following Panse and Sukhatme (1985).

Results

RESULTS

Results of the present investigation on 'Floral biology and compatibility studies in *Dendrobium*' are presented under the following heads:

1. Floral biology
2. Pollen studies
3. Compatibility studies in the different *Dendrobium* hybrids
4. Embryoculture
5. Planting out and hardening

1 Floral biology

1.1 Floral characteristics

The *Dendrobium* flower is built on a very simple pattern of three outer sepals and three inner petals. The three sepals are alike in shape, size and colour. Within the sepals are the petals of which, the lateral two are similar and little broader than the sepals. The third petal is highly modified in appearance and is called as the 'lip' or 'labellum'. The lip is entirely different from the other two petals. It is three lobed and is embellished with its own markings of colour and is fantastically decorated with crests and fringed margins. The two lateral lobes are smaller than the terminal lobe and partially encircle the column. The column or gynandrium is a fleshy structure and consists of the fused reproductive parts. At the tip of the column is the anther which bears the pollen. The pollen grains are not powdery but is a sticky mass called pollinia. The pollinia lies in a cavity covered by a hinged cap. Just below the anther, separated by a partition called the rostellum, is the female receptive organ, the stigma. It is a shiny depression filled with extremely

sticky fluid. Floral biology of a typical *Dendrobium* flower is represented in Fig.1 and 2.

Morphological description of the *Dendrobium* hybrids used in the study are presented in Table 1 (Plate 1-6).

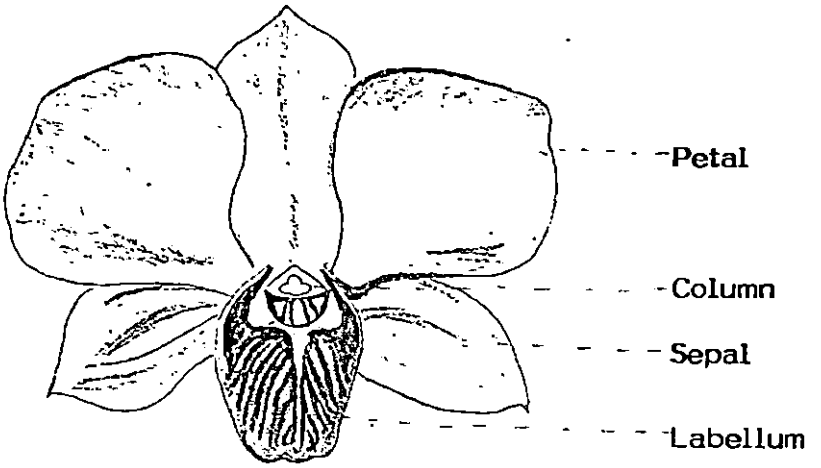
Studies on the floral characters of the hybrids (Table 2 and Appendix-I) revealed that the flowers opened acropetally after the emergence of the inflorescence. The time taken for flower opening from the time of inflorescence emergence varied from 27 days in Emma White to 42 days in New Pink. Days taken for the opening of all the flowers in an inflorescence varied from 8 in Emma White to 20 in White Nern. Among the hybrids the maximum number of flowers per inflorescence (11) was produced by White Nern and the minimum (3) by Kasem White, Sonia 28 Mutant B and (Hawaiian Beauty x Kasem Pink). The length of inflorescence ranged from 16.74 cm in (Hawaiian Beauty x Kasem Pink) to 57.24 cm in White Nern. The internodal length was wider between the basal flowers than the terminal flowers in all the hybrids. Larger sized flowers were borne on Sonia 28 Mutant B (8.3 x 9.1) and smaller ones on Emma white (4.9 x 5.1). The analysis of variance showed that there is significant difference in the floral characters.

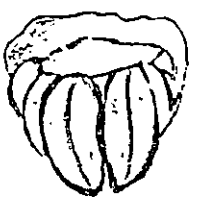
1.2 Anthesis

In all the hybrids of *Dendrobium*, flower opening occurred during the day time. The time of opening was from 8.30 am to 5.30 pm with a peak in anthesis between 9.00 to 10.00 am and 3.00 to 4.00 pm. All the flowers in a particular inflorescence opened during the same time of the day at a uniform time interval. The flowers in an inflorescence of Hieng Beauty, White Nern and Emma White opened

Fig.1. A typical Dendrobium plant

Flower





Anther cap with pollinia

Column

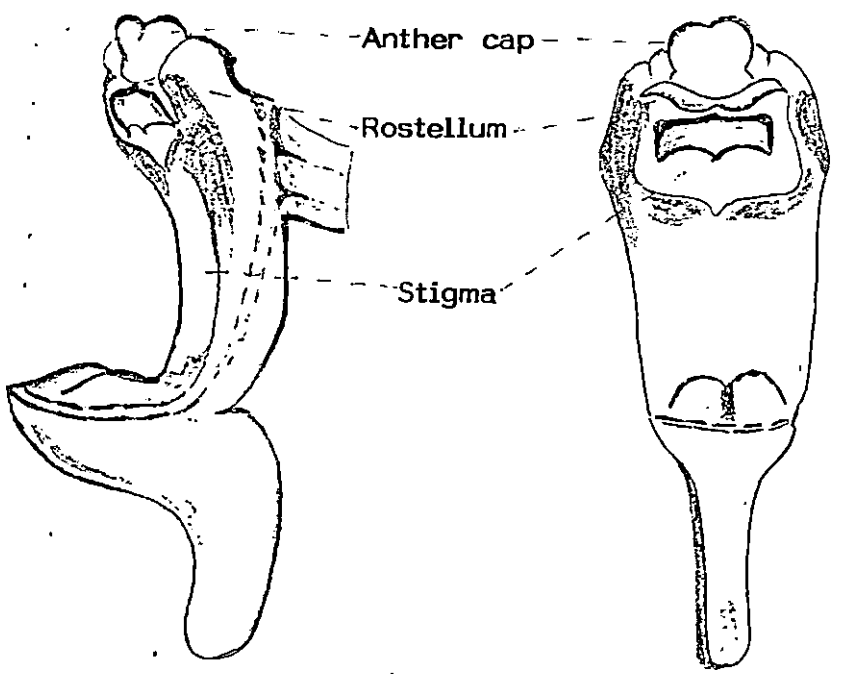


Fig.2. Floral parts of a Dendrobium flower

Plate 1-6. Different *Dendrobium* hybrids

Plate 1. Hieng Beauty

Plate 2. Sonia 28 Mutant B

Plate 3. Emma White

Plate 4. Kiomi Beauty x Banyat Pink

Plate 5. Kasem White

Plate 6. Hawaiian Beauty x Kasem Pink

Table 1. Morphological description of *Dendrobium* hybrids

Name of hybrid	Source	Period of blooming	Time required for single flower opening (days)	Colour of flowers	Lip colour and shape
New Pink	Kerala State Horticultural Products Development Corporation, Panavila, Trivandrum-14	Round the year*	2-3	Purple centrally cream	Purple with feathery out growth longitudinally in centre
Hieng Beauty	Fresh Cut, Angamaly	,,	1	Creamy green, deep purple towards centre	Creamy green margin. Centre intense purple with feathery outgrowth
Emma White	KSHPDC	,,	1	Creamy white	Creamy white with feathery longi-tudinal striations
Kasem White	KSHPDC	,,	1	Creamy green	,,
Sonia 28 Mutant B	KSHPDC	August-October	1	Purple, centrally white. Sepals white with a purple tinge	Deep purple with feathery striations centrally, white towards edge
Kiomi Beauty x Banyat Pink	KSHPDC	September-October	2-3	Deep purple centrally light colour	Intense purple with outgrowth in centre and creamy green towards base
Hawaiian Beauty x Kasem Pink	KSHPDC	September-October	2-3	Deep purple	Intense purple with longitudinal feathery outgrowth centrally. Cream towards base
Sonia	Fresh Cut, Angamaly	,,	2	Purple, centrally cream. Sepals creamy white with purple marking	Light purple centrally feathery outgrowth in lines
Sonia # 28	Fresh Cut, Angamaly	Round the year	1	Deep purple centrally creamy green	,,
White Nern	KSHPDC	August-October	1	Creamy white	Creamy green with feathery outgrowth in the centre

*With exception during the heavy rains

Dendrobiums are sympodial epiphytes. Flowers are borne on recemose inflorescence and have no fragrance.

Table 2. Floral characters of *Dendrobium* hybrids

Hybrid	Days for inflorescence appearance to 1st flower opening	Days for 1st flower appearance to last flower opening	No. of flowers/ infloresence	Length of inflorescence (cm)	Internodal length (cm)		Size of flower (cm)	
					Basal internode	Apical internode	Length	Width
New Pink	42 (6.48)	10 (3.07)	4 (1.91)	23.60	3.2	2.2	8.0	8.6
Hieng Beauty	34 (5.79)	12 (3.45)	7 (2.62)	19.13	3.2	2.3	6.1	6.5
Emma White	27 (5.19)	8 (2.70)	5 (2.09)	18.66	3.4	2.1	4.9	5.5
Kasem White	31 (5.57)	9 (3.02)	3 (1.80)	17.01	3.1	2.3	5.6	5.8
Sonia 28 Mutant B	37 (6.09)	9 (2.92)	3 (1.75)	32.11	3.8	2.8	8.3	9.1
Kiomi Beauty x Banyat Pink	33 (5.74)	11 (3.34)	7 (2.64)	39.56	3.9	2.7	6.3	5.9
Hawaiian Beauty x Kasem Pink	30 (5.47)	12 (3.40)	3 (1.75)	16.74	4.1	3.7	6.9	6.1
Sonia	38 (6.16)	10 (3.06)	4 (2.05)	34.56	3.9	2.6	7.8	8.0
Sonia # 28	36 (6.02)	10 (3.18)	5 (2.19)	35.86	4.2	2.4	8.3	7.8
White Nem	40 (6.39)	20 (4.47)	11 (3.24)	57.24	4.6	2.0	6.8	7.1
CD	0.34	0.51	0.37	4.16	0.46	0.41	0.40	0.43

Figures in parantheses indicate transformed values

almost uniformly at an interval of 24 hrs while those of New Pink, (Hawaiian Beauty x Kasem Pink) and (Kiomi Beauty x Banyat Pink) took 2 to 3 days to complete anthesis of each flower bud.

The longevity of the flowers on the plant was for 45-50 days.

1.3 Stigma receptivity

Data on the time of pollination and the day of stigma receptivity after anthesis, which resulted in successful pod set, are presented in Table 3. Maximum stigma receptivity as observed by pod set was obtained when the flowers were pollinated between four to six days after anthesis. Stigmas remained receptive upto 10th day in White Nern and 26th day in New Pink, although the flowers retained freshness for a longer period. Pollinations resulted in successful pod set when pollinated irrespective of the time of the day.

2 Pollen studies

All the hybrids differed significantly with respect to pollen size, pollen production and pollen fertility (Table 4, Plate 7-12 and Appendix-II).

2.1 Pollen morphology

The pollen grains were found agglutinated in masses called pollinia. Each flower consisted of two pollinia with two lobes each. The pollinia appeared creamy white in case of Hieng Beauty and yellow in colour in rest of the hybrids. Pollinia were oval in shape. Pollen grains appeared as creamy white to white sticky mass. The pollen grains were spherical to rectangular in shape and were found as tetrads. The pollen grains of different hybrids were almost similar in shape.

Table 3. Fertility pattern in *Dendrobium* hybrids

Cross	Time of pollination	Stigma receptivity (Days after anthesis)
1. Hieng Beauty x (Hawaiian Beauty x Kasem Pink)	4.00 pm	4
2. Hieng Beauty x (Kiomi Beauty x Banyat Pink)	11.00 am	6
3. Hieng Beauty x (Hawaiian Beauty x Kasem Pink)	11 am	4
4. Hieng Beauty x New Pink	10.00 am	6
5. Hieng Beauty selfed	5.00 pm	7
6. Hieng Beauty selfed	11.30 am	8
7. Hieng Beauty x (Kiomi Beauty x Banyat Pink)	12.00 noon	4
8. (Hawaiian Beauty x Kasem Pink) x Hieng Beauty	4.00 pm	4
9. (Kiomi Beauty x Banyat Pink) x Hieng Beauty	11.00 am	4
10. (Kiomi Beauty x Banyat Pink) x Kasem White	11.00 am	6
11. Banyat Pink selfed	12.00 noon	5
12. New Pink x Hieng Beauty	2.30 pm	7
13. New Pink selfed	11.00 am	8
14. ,,	11.00 am	5
15. ,,	11.00 am	1
16. ,,	12.00 noon	20
17. ,,	12.00 noon	24
18. ,,	12.00 noon	26
19. ,,	12.00 noon	2
20. Sonia 28 x Hieng Beauty	12.30 pm	5
21. Emma White x New Pink	10.00 am	5
22. Emma White x Hieng Beauty	10.00 am	6
23. Emma White x New Pink	10.00 am	1
24. Emma White x White Nern	5.00 pm	6
25. Emma White x (Kiomi Beauty x Banyat Pink)	10.30 am	7
26. Emma White selfed	10.30 am	6
27. White Nern x New Pink	10.00 am	10
28. White Nern selfed	11.00 am	8
29. White Nern x Kiomi Beauty x Banyat Pink	11.30 am	6
30. ,,	10.30 am	4
31. Kasem White x (Kiomi Beauty x Banyat Pink)	11.00 am	3

Plate 7-11 Pollen grains of the hybrids stained in Acetocarmine-glycerine medium (× 400)

Plate 7. New Pink

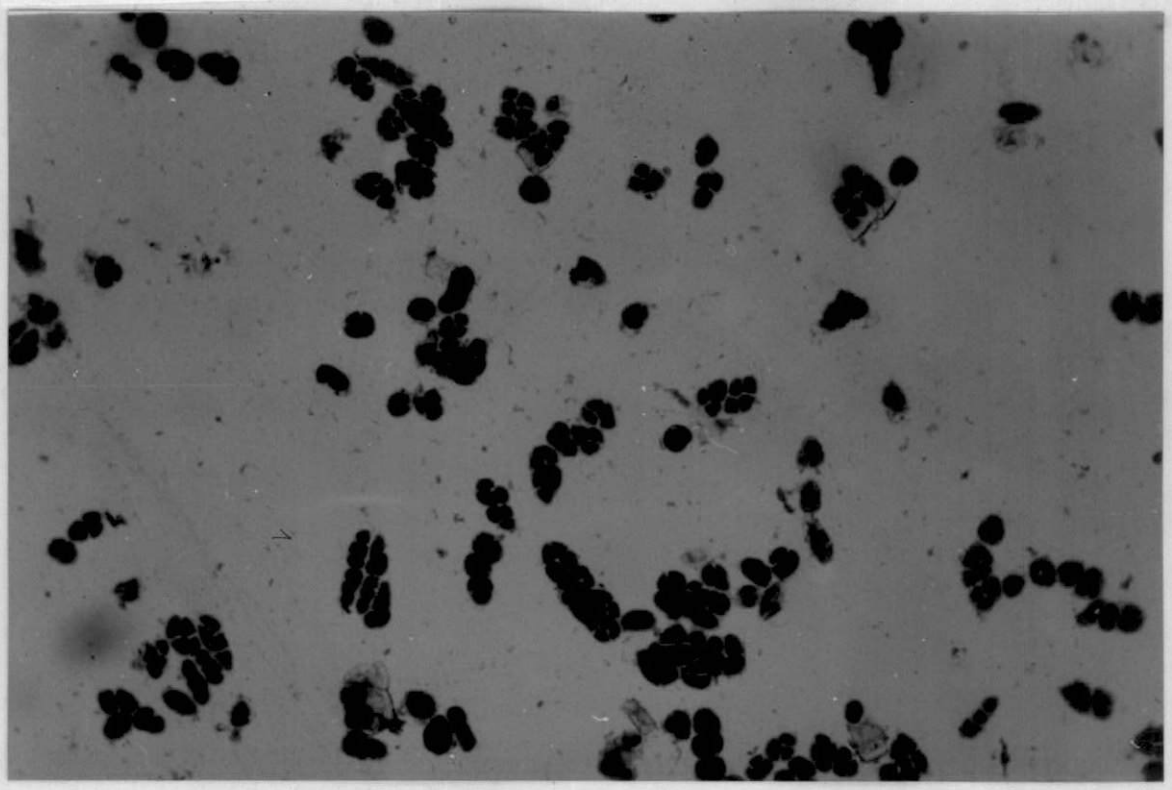


Plate 8. Hieng Beauty

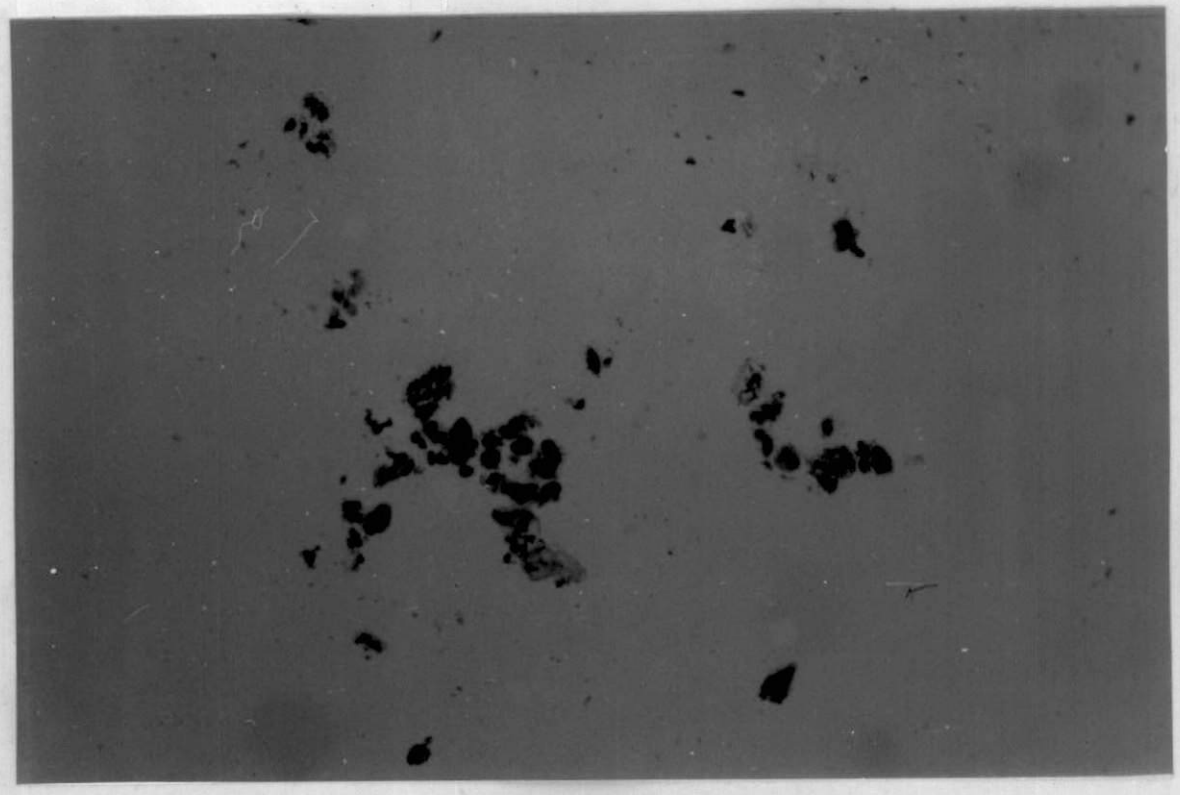


Plate 9. Emma White

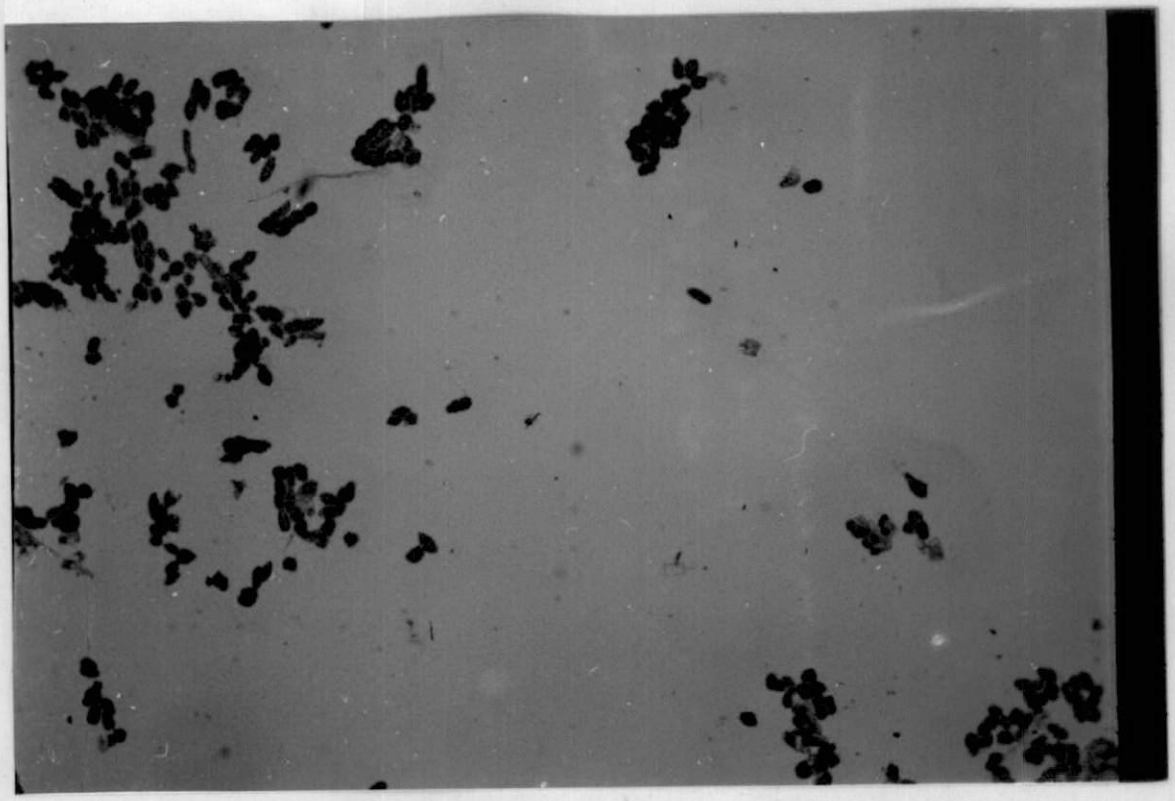


Plate 10. Sonia 28 Mutant B

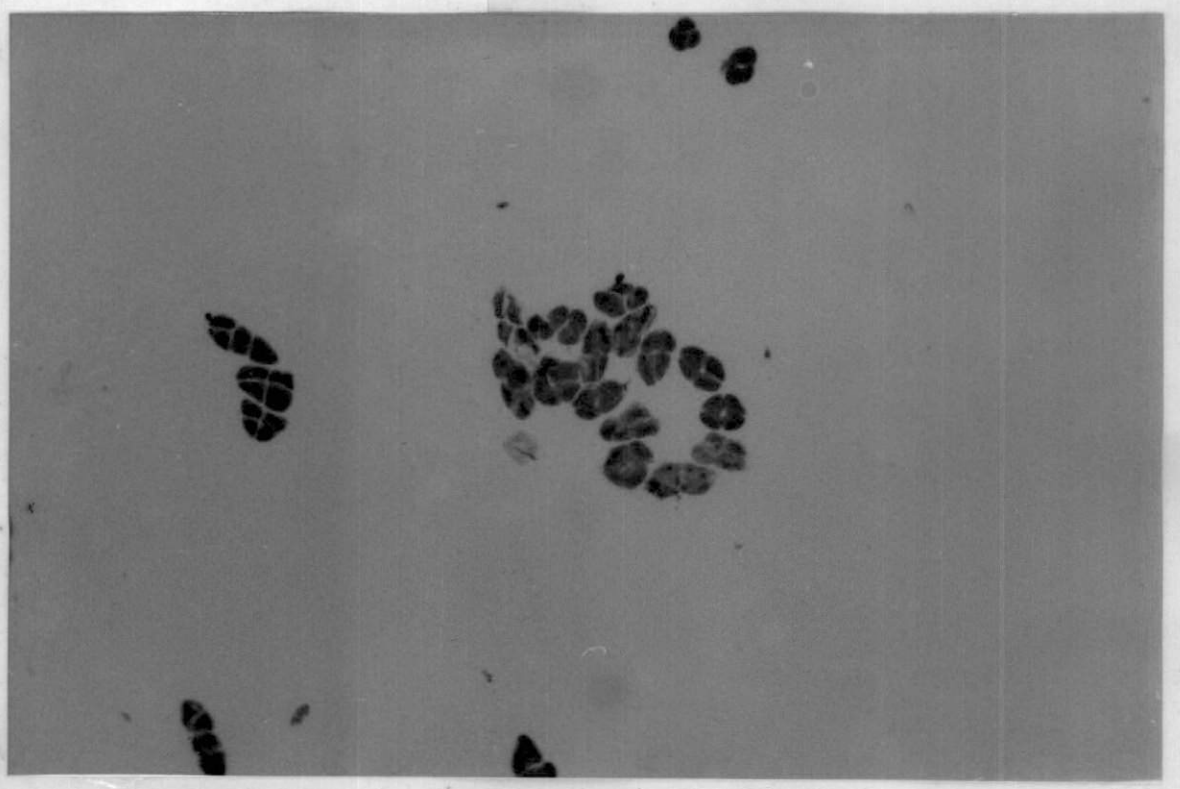


Plate 11. Kasem White

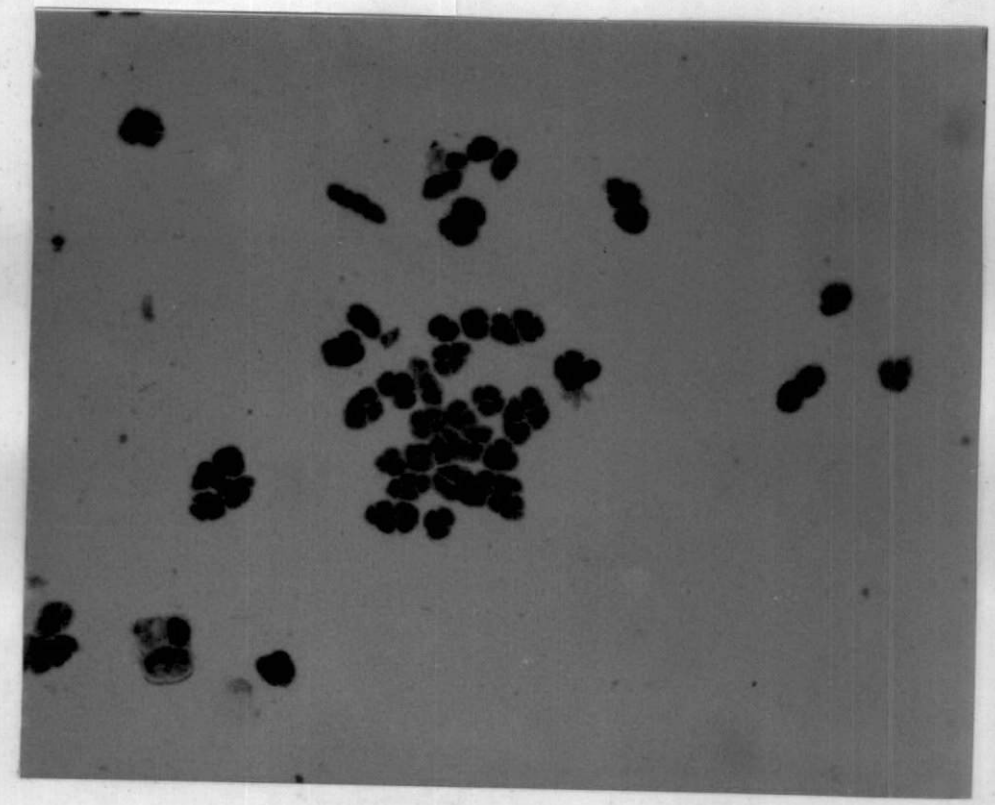


Table 4. Pollen characters of the *Dendrobium* hybrids

Hybrids	Pollen diameter (μ)	Pollen production	Pollen fertility (%)
New Pink	40.40	92188 (296)	73.95 (1.05)
Hieng Beauty	27.91	83888 (292)	4.49 (0.21)
Emma White	48.52	42154 (261)	23.40 (0.50)
Kasem White	39.99	193750 (326)	73.98 (1.04)
Sonia 28 MB	30.61	38282 (258)	4.42 (0.21)
CD (0.05)		25.2	0.158
CD (0.01)	12.326	35.8	0.224

Figures in parantheses indicate transformed values

2.2 Pollen size

There was significant variation for pollen size among the different hybrids. Size of the pollen grains varied from 27.91 μ in Hieng Beauty to 48.52 μ in Emma White.

2.3 Pollen production

Significant difference was observed in the pollen output of the different *Dendrobium* hybrids. Number of pollen grains per pollinium ranged from 38282 (Sonia 28 Mutant B) to 193750 (Kasem White).

2.4 Pollen fertility

The hybrids Kasem White and New Pink showed significantly higher percentage of pollen fertility (73.98% and 73.45%, respectively) compared to the other hybrids. The minimum pollen fertility (4.42%) was observed in Sonia 28 Mutant B.

2.5 Pollen germination

2.5.1 Standardisation of media for pollen germination

2.5.1.1 Sucrose medium

Pollen grains failed to germinate in the different concentrations of sucrose solution tried.

2.5.1.2 Sucrose with agar medium

Pollen germination in sucrose solution at two per cent level along with

agar at various concentrations gave satisfactory pollen germination (Table 5 and Plate 13). Agar concentration of the medium significantly influenced the pollen germination and tube growth. A concentration of one per cent agar was found to be significantly superior to other concentrations for pollen germination as well as tube growth. Agar concentrations at 0.5 per cent and 1.5 per cent were statistically on par, and gave lower germination when compared to one per cent.

2.5.2 Sucrose agar boric acid medium

Addition of boric acid to sucrose-agar medium influenced the percentage of germination and tube growth. Among the four doses of boric acid tried, 75 ppm was found to be significantly superior to others (Table 6).

From the results it was found that a medium consisting of 2 per cent sucrose and 1 per cent agar gave best germination percentage (86.57) while the medium consisting of 2 per cent sucrose, 1 per cent agar and 75 ppm boric acid was the best for pollen tube growth (258.23 μ).

Observations on the germination of pollen grains in the medium consisting of 2 per cent sucrose, 1 per cent agar and 75 ppm boric acid showed that the growth of pollen tubes was initiated after 12 hours of incubation. The germination frequency of the pollen was significantly enhanced between 24 and 48 hours of incubation.

2.5.2 Assessment of pollen germination in the different *Dendrobium* hybrids

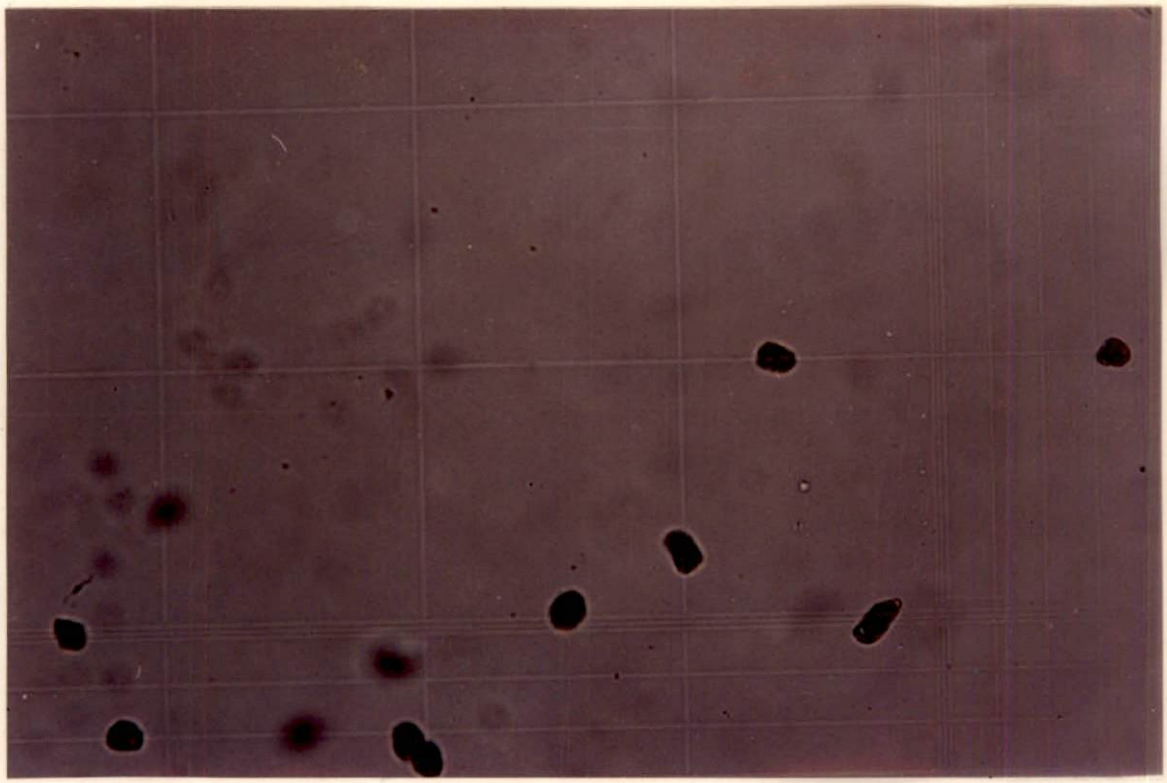
Results of the studies on pollen germination and tube growth showed significant variation among the five different hybrids (Table 7). Pollen grains of New Pink gave the highest percentage of germination (86.57) and tube length

Table 5. Standardisation of media for pollen germination

Concentration of medium	Pollen germination (%)	Tube length (μ)
1% Sucrose + 0.5% Agar	46.20	161.03
1% " + 1.0% "	10.41	16.66
1% " + 1.5% "	19.20	8.33
2% " + 0.5% "	37.93	14.99
2% " + 1.0% "	86.57	166.50
2% " + 1.5% "	13.66	99.96
3% " + 0.5% "	7.68	16.66
3% " + 1.0% "	10.43	16.66
3% " + 1.5% "	5.23	8.33
4% " + 0.5% "	3.52	3.32
4% " + 1.0% "	4.17	3.32
4% " + 1.5% "	2.92	8.33
5% " + 0.5% "	1.05	0.16
5% " + 1.0% "	2.76	0.16
5% " + 1.5% "	0.92	0.16

Plate 12 Pollen count in haemocytometer (× 400)

Plate 13 Pollen grains germinated in sucrose-agar-boric acid medium (× 400)



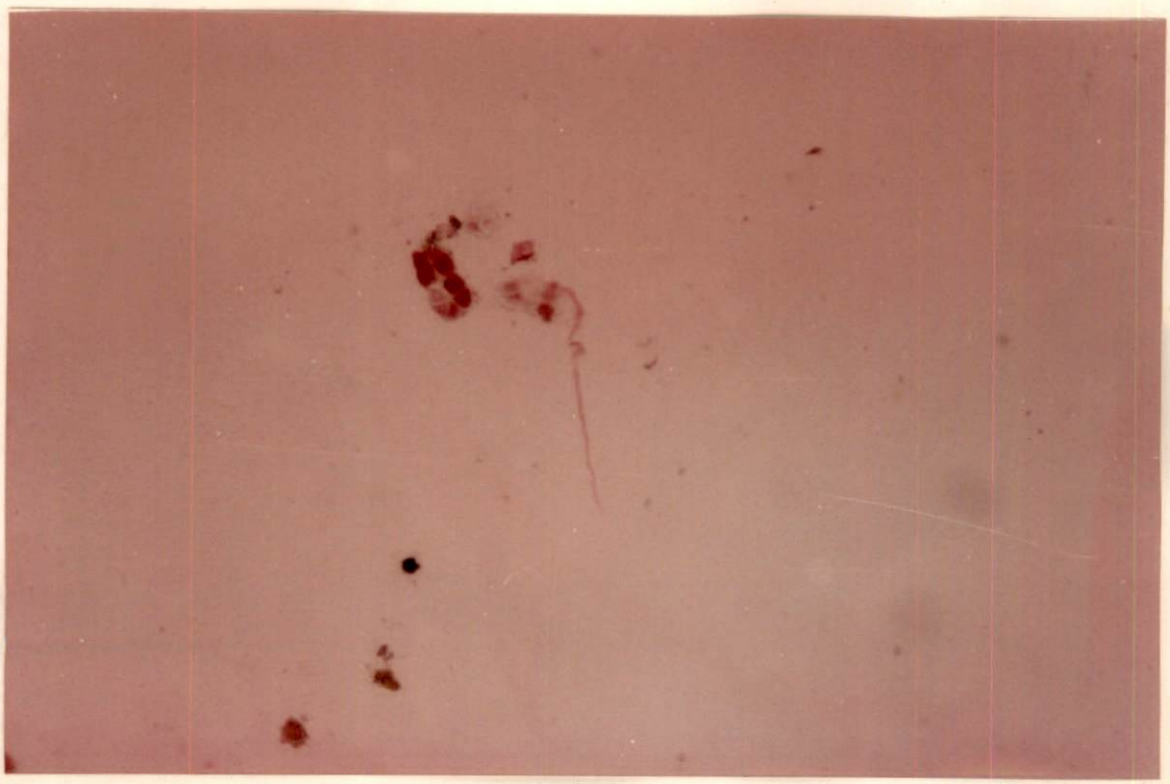


Table 6. Effect of boric acid on germination of pollen grains

Treatment	Germination (%)	Average length of tube (μ)
2 Sucrose + .5 Agar	37.93	14.99 (3.51)
2 Sucrose + .5 Agar + 25 Boric acid	20.63	11.66 (3.32)
2 ,, + .5 ,, + 50 ,,	11.25	9.99 (2.54)
2 ,, + .5 ,, + 75 ,,	22.13	22.91 (4.53)
2 ,, + .5 ,, + 100 ,,	7.98	5.83 (2.12)
2 ,, + 1 ,,	86.54	166.50 (12.80)
2 ,, + 1 ,, + 25 ,,	23.22	95.80 (9.45)
2 ,, + 1 ,, + 50 ,,	27.45	141.49 (11.83)
2 ,, + 1 ,, + 75 ,,	41.96	258.23 (15.97)
2 ,, + 1 ,, + 100 ,,	21.25	33.32 (5.67)
2 ,, + 1.5 ,,	13.66	99.96 (9.95)
2 ,, + 1.5 ,, + 25 ,,	21.40	166.60 (12.01)
2 ,, + 1.5 ,, + 50 ,,	5.30	112.46 (10.28)
2 ,, + 1.5 ,, + 75 ,,	4.82	95.80 (9.72)
2 ,, + 1.5 ,, + 100 ,,	23.68	133.28 (11.43)
CD (0.05)	0.029	3.253

Figures in parantheses indicate transformed values

Table 7. Germination of different *Dendrobium* hybrids in the best medium (2 per cent sucrose + 1 per cent agar + 75 ppm boric acid)

Sl.No.	Hybrid	Germination (%)	Tube length (μ)
1	New Pink	86.57	258.23
2	Hieng Beauty	Nil	Nil
3	Emma White	Nil	Nil
4	Kasem White	14.85	33.32
5	Sonia 28 Mutant B	Nil	Nil

(258.23 μ) followed by Kasem White. The hybrids Hieng Beauty, Emma White and Sonia 28 Mutant B did not respond to *in vitro* germination.

2.6 Pollen storage

The storage life of pollen grains was significantly influenced by the different conditions of storage (Table 8). Among the different storage conditions, that at 4°C (T_3) was found to be the best. The storage life was least when the pollen grains were stored at room temperature in a desiccator over calcium chloride. After three weeks of storage, the viability was almost nil in case of storage at room temperature while viability of pollen at 4°C and 0°C was found to be higher (22.32% and 19.47%, respectively). Even after three months of storage, viability was retained in case of the latter (12.02%).

3 Compatibility studies in the different *Dendrobium* hybrids

Self pollination was attempted in five *Dendrobium* hybrids and cross pollination among 43 crosses involving 10 hybrids. Three hybrids set pods on selfing. Among the 43 crosses attempted, though pod set was initiated in 30, only 14 crosses retained pods upto maturity.

Observations relating to the post pollination phenomenon indicated a variety of changes (Table 9). Following pollination, in unsuccessful crosses the flower was either left intact retaining its freshness or the flowers faded and abscised one or two days after pollination. In case of successful crosses, the ovary remained a part of the receme and gradually enlarged into a pod (Plate 14). The ovaries swelled in many of the cross pollinated flowers, but all the swollen ovaries did not develop into fruits. Only 32.5 per cent of the pods reached maturity.

Table 8. Viability of pollen grains at different storage conditions

	Ist day	After 1 week	2 weeks	3 weeks	4 weeks	6 weeks
T_1	79.07(1.10)	30.06 (0.58)	10.86(0.34)	1.26(0.11)	0.00(0.05)	0.00(0.05)
T_2	79.07(1.02)	12.57(0.33)	2.29(0.15)	0.31(0.07)	0.00(0.05)	0.00(0.05)
T_3	74.87(1.05)	30.92(0.58)	32.10(0.60)	28.40(0.56)	26.14(0.54)	22.32(0.49)
T_4	74.87(1.05)	35.31(0.640	23.37(0.50)	19.05(0.45)	14.95(0.40)	11.55(0.35)
T_5	78.30(1.09)	38.83(0.67)	29.14(0.57)	25.62(0.53)	22.88(0.50)	19.43(0.45)
CD (0.05)	NS	NS	0.095	0.063	0.095	0.095
CD (0.01)	NS	0.314	0.134	0.090	0.134	0.134

T_1 - Storage of pollinia at room temperature

T_2 - Storage of pollinia at room temperature in desiccator

T_3 - Storage of pollinia at 4°C

T_4 - Storage of pollinia at 4°C in desiccator

T_5 - Storage of pollinia at 0°C

Figures in parentheses indicate transformed values

Table 9. Post pollination changes in the different hybrids

Name of hybrid cross	Post pollination changes
1	2
1. New Pink x Hieng Beauty	Flower droop and abscise
2. Hieng Beauty x New Pink	Flower droop and abscise
3. Hieng Beauty x (Hawaiian Beauty x Kasem Pink)	Pedicel of the flower bulges and develops into pod
4. (Hawaiian Beauty x Kasem Pink) x Hieng Beauty	Pedicel slightly bulges initially but flower falls
5. Hieng Beauty x (Kiomi Beauty x Banyat Pink)	Pedicel enlarges and pod develops
6. (Kiomi Beauty x Banyat Pink) x Hieng Beauty	Pedicel enlarges and develops into pod
7. New Pink x Hieng Beauty	Pedicel bulges slightly initially but flower falls
8. Sonia # 28 x Hieng Beauty	Pedicel bulges slightly but flower falls
9. Hieng Beauty x Sonia # 28	No change to the flower
10. Hieng Beauty x (Hawaiian Beauty x Kasem Pink)	Pedicel enlarges and develop into pod
11. Emma White x Sonia	No change to the flower
12. Sonia x Emma White	Flower droop and abscise
13. White Nern x (Kiomi Beauty x Banyat Pink)	No change to the flower
14. (Hawaiian Beauty x Kasem Pink) x Emma White	Flower droop and abscise
15. White Nern x Sonia	No change to the flower
16. Sonia 28 Mutant B x White Nern	Flower droop and abscise
17. Hieng Beauty x Sonia # 28	No change to the flower

Contd.

Table 9. Continued

1	2
18. Sonia x Hieng Beauty	Flower droop and abscise
19. Sonia x Hieng Beauty	Flower droop and abscise
20. Sonia x White Nern	No change to flower
21. Hieng Beauty x Sonia	No change to flower
22. Hieng Beauty x New Pink	Pedicel bulges and develop into pod
23. Emma White x New Pink	Pedicel enlarges and develops into pod
24. New Pink x Emma White	Flower droop and abscise
25. Hieng Beauty x Emma White	Flower droop and abscise
26. Sonia 28 Mutant B x Hieng Beauty	Flower droop and abscise
27. Hieng Beauty x Emma White	No change to the flower
28. Emma White x Hieng Beauty	Pedicel enlarge initially but flower fall
29. White Nern x New Pink	Pedicel enlarges and develop into pod
30. New Pink x Emma White	Flower droop and abscise
31. Emma White x New Pink	Pedicel enlarges and develop into pod
32. New Pink x White Nern	Flower droop and abscise
33. Emma White x White Nern	Pedicel bulges initially but later falls
34. White Nern x (Kiomi Beauty x Banyat Pink)	Pedicel enlarge and develop into pod
35. (Kiomi Beauty x Banyat Pink) x White Nern	Flower droop and abscise
36. (Kiomi Beauty x Banyat Pink) x Emma White	Flower droop and abscise
37. Emma White x (Kiomi Beauty x Banyat Pink)	Pedicel enlarge and develop into pod

Contd.

Table 9. Continued

1	2
38. White Nern x (Kiomi Beauty x Banyat Pink)	Pedicel enlarge and develop into pod
39. New Pink x Hieng Beauty	Flower droop and abscise
40. Heieng Beauty x (Kiomi Beauty x Banyat Pink)	Pedicel enlarges and develop into pod
41. Kasem White x (Kiomi Beauty x Banyat Pink)	Pedicel enlarges and develop into pod
42. (Kiomi Beauty x Banyat Pink) x Hieng Beauty	Flower droop and abscise
43. (Kiomi Beauty x Banyat Pink) x Kasem White	Pedicel enlarges and develop into pod

Plate 14, 15 Pod set in interhybrid crosses

Of the five hybrids self pollinated, only New Pink, Emma White and (Kiomi Beauty x Banyat Pink) set pods which were retained upto maturity (Table 10). Among the interhybrid crosses in which pod set was obtained, the percentage of fruit set varied from zero to 100 in the different crosses (Table 11, Fig. 3). In the interhybrid crosses (1) Hieng Beauty x New Pink (2) Hieng Beauty x (Hawaiian Beauty x Kasem Pink) (3) White Nern x (Kiomi beauty x Banyat Pink) (4) Emma White x New Pink (5) White Nern x New Pink (6) Emma White x (Kiomi Beauty x Banyat Pink), a high frequency of 50 to 100 per cent fruit set was observed. However, fruits failed to set in the reciprocal crosses. A high percentage of fruit set was obtained in the direct as well as reciprocal crosses of Hieng Beauty x (Kiomi Beauty x Banyat Pink) and Kasem White x (Kiomi Beauty x Banyat Pink).

4 Embryo culture

The embryos from 14 hybrid pods were cultured *in vitro*, but germination was observed only in five. The germinated embryos took 3 to 8 weeks for greening. The protocorms differentiated into the first leaf after 7 to 10 weeks in culture. The roots were observed at the two leaf stage of the culture (Plate 15-21).

The mature embryos responded to the basal media, viz., ½ MS and Vacin and Went. Their germination frequency and the subsequent morphogenetic changes leading to seedling development, varied with the hybrids, the media used and the age of the pod (Table 12).

Immature hybrid seeds of the cross, Hieng Beauty x (Hawaiian Beauty x Kasem Pink) germinated in ½ MS and VW media when pods were 91 days old but

Table 10. Self compatibility in *Dendrobium* hybrids

Hybrid	No. of flowers pollinated	No. of flowers setting pods	Pod set (%)	Days to maturity of hyrbid pod
New Pink	9	8	88.9	108
Hieng Beauty	9	0	0.0	-
Emma White	4	1	25.0	97
Kiomi Beauty x Banyat Pink	2	2	100.0	98
White Nern	3	0	0.0	-

Table 11. Cross compatibility in *Dendrobium* hybrids

Cross	No. of flowers pollinated	No. of pod set	Pod set (%)	Days of maturity of hybrid pod
1	2	3	4	5
1. New Pink x Hieng Beauty	3	0	0.0	--
2. Hieng Beauty x New Pink	2	1	50.0	88
3. Hieng Beauty x (Hawaiian Beauty x Kasem Pink)	2	2	100.0	91
4. (Hawaiian Beauty x Kasem Pink) x Hieng Beauty	1	0	0.0	--
5. Hieng Beauty x (Kiomi Beauty x Banyat Pink)	2	2	100.0	89
6. (Kiomi Beauty x Banyat Pink) x Hieng Beauty	2	1	50.0	58
7. Sonia # 28 x Hieng Beauty	1	0	0.0	--
8. Hieng Beauty x Sonia # 28	2	0	0.0	--
9. Emma White x Sonia	1	0	0.0	--
10. Sonia x Emma White	1	0	0.0	--
11. White Nern x (Kiomi Beauty x Banyat Pink)	3	2	66.6	95
12. (Kiomi Beauty x Banyat Pink) x White Nern	1	0	0.0	--
13. (Hawaiian Beauty x Kasem Pink) x Emma White	1	0	0.0	--
14. Sonia 28 Mutant B x White Nern	1	0	0.0	--
15. White Nern x Sonia	1	0	0.0	--
16. Sonia x Hieng Beauty	2	0	0.0	--

Contd.

Table 11. Continued

1	2	3	4	5
17. Sonia x White Nern	1	0	0.0	--
18. Hieng Beauty x Sonia	1	0	0.0	--
19. Emma White x New Pink	2	2	100.0	101
20. New Pink x Emma White	1	0	0.0	--
21. Hieng Beauty x Emma White	1	0	0.0	--
22. Sonia 28 Mutant B x Hieng Beauty	1	0	0.0	--
23. Emma White x Hieng Beauty	1	0	0.0	--
24. White Nern x New Pink	1	1	100.0	97
25. New Pink x White Nern	1	0	0.0	--
26. Emma White x White Nern	1	0	0.0	--
27. (Kiomi Beauty x Banyat Pink) x Emma White	1	0	0.0	--
28. Emma White x (Kiomi Beauty x Banyat Pink)	1	0	100.0	--
29. Kasem White x (Kiomi Beauty x Banyat Pink)	1	1	100.0	120
30. (Kiomi Beauty x Banyat Pink) x Kasem White	1	1	100.0	120

Fig.3. Compatibility among *Dendrobium* hybrids

♀ \ ♂	New Pink	Hieng Beauty	Emma White	Kasem White	Sonia 28 Mutant B	(Kiomi Beauty x Banyat Pink)	(Hawaiian Beauty x Kasem Pink)	Sonia # 28	Sonia	White Nern
New Pink	S	O	O	..	..	..	..	..	..	O
Hieng Beauty	C	x	O	..	..	C	C	O	O	..
Emma White	C	O	S	..	..	C	..	..	O	O
Kasem White	..	..	..	..	..	C	..	..	..	..
Sonia 28 Mutant B	..	O	..	..	..	..	..	..	..	O
(Kiomi Beauty x Banyat Pink)	..	C	O	C	..	S	..	..	..	O
(Hawaiin Beauty x Kasem Pink)	..	O	O	..	..	..	..	..	..	..
Sonia # 28	O	..	..	..	..	..	..	..	..	..
Sonia	O	O	..	..	..	..	..	..	..	O
White Nern	C	..	..	..	..	C	..	..	O	x

C - Cross compatible

S - Self compatible

O - Cross incompatible

X - Self incompatible

.. - Not tried

Plate 16-21 Response of cultured embryos

Plate 16. At the time of innoculation

Plate 17. Four weeks after innoculation

Plate 18. Eight weeks after innoculation

Plate 19. Twenty weeks after innoculation

Plate 20. Well developed seedling (36 weeks)

Plate 21. Proliferation of side shoots

failed to germinate at a maturity of 63 days. The germinated embryos differentiated into protocorms after four weeks in culture. The cross between Emma White and New Pink established cultures in 3-4 days and protocorms differentiated after 3 weeks. Vigorous growth was observed among the seedlings of this cross, when cultured at a pod maturity of 101 days. However, culture of another cross of the same parentage did not develop beyond the green stage. The hybrid embryos of the crosses, Hieng Beauty x New Pink and White Nern x (Kiomi Beauty x Banyat Pink) formed callus tissue after about one week in culture. The callus became green and protocorms were observed after 6 to 8 weeks. The seeds of the cross, White Nern x (Kiomi Beauty x Banyat Pink) when harvested at 95 days maturity gave only 33 per cent germination and the cultures took 8 weeks to differentiate into protocorms. However, 101 days old capsule of this cross showed a higher germination frequency (58%) in a shorter period of time. Protocorms were observed after 6 weeks.

The mature embryos of the cross Emma White x New Pink and Hieng Beauty x (Hawaiian Beauty x Kasem Pink) showed rapid rate of growth and developed into healthy seedlings while seeds of the remaining crosses exhibited poor rate of growth. The seeds failed to differentiate beyond the 2 leaf stage even after 32 weeks in culture (Table 13).

With a view to augment growth, the differentiating protocorms were subcultured in the basal medium (VW and ½ MS) supplemented with either : (1) NAA 2 ppm + BA 5 ppm + 2,4-D 2 ppm; (2) CW 150 ml + Peptone 500 mg + adenine 20 mg; (3) 2,4-D 2 ppm + NAA 2 ppm + BA 5 ppm + Peptone 1 g or (4) Peptone 1 g. The first two media were used for initial subculturing. The growth of seedlings was satisfactory. Final subculturing was done in the media (3) and (4). The

Table 12. Influence of pod maturity and culture media on *in vitro* germination of *Dendrobium* hybrid embryos

Cross	Age of hybrid capsule (days)	Nutrient media	Germination response
(Kiomi Beauty x Banyat Pink) x Hieng Beauty	58	KC VW MS	No response
Hieng Beauty x (Kiomi Beauty x Banyat Pink)	89	MS	No response
Hieng Beauty x (Hawaiian Beauty x Kasem Pink)	91	½MS VW	59% germination. Protocorm observed after 4 weeks
,,	63	KC	No response
Hieng Beauty x New Pink	88	VW	Embryos swelled, 25% germination, protocorms observed after 6 weeks
Emma White x New Pink	100	MS	Greening of embryos observed, but no further development
White Nern x New Pink	97	VW KC	,,
Emma White x New Pink	101	KC VW	92% germination, protocorms observed after 3 weeks
White Nern x (Kiomi Beauty x Banyat Pink)	95	KC	33.3% germination. Protocorms observed after 8 weeks
White Nern x (Kiomi Beauty x Banyat Pink)	101	KC	58% germination. Protocorms observed after 6 weeks
Hieng Beauty x (Kiomi Beauty x Banyat Pink)	113	MS	No response
(Kiomi Beauty x Banyat Pink) x Kasem White	120	VW	No response
Kasem White x (Kiomi Beauty x Banyat Pink)	120	VW	Embryos swelled, but no further development
Emma White x (Kiomi Beauty x Banyat Pink)	103	VW	No response

Table 13. Response of *Dendrobium* hybrids at different stages of culture

Name of the cross	Age of the pod (days)	Medium	Days to greening	Days to protocorm development	Days to shoot development	Days to root development
Hieng Beauty x (Hawaiian Beauty x Kasem Pink)	91	½MS + 2ppm NAA + 5 ml BA + 2 ml 2,4-D VW	12	28	52	100
Hieng Beauty x New Pink	83	½ MS	28	42	71	178
Emma White x New Pink	101	VW ½ MS	10	24	56	108
White Nern x (Kiomi Beauty x Banyat Pink)	95	VW ½ MS	14	56	No further development	
,,	101		10	42	,,	

MS - Murashige-Skoog medium
VW - Vacin and Went medium
KC - Knudson-C medium

½ MS - MS media with half the quantity of inorganic salts

best response was obtained in the medium supplemented with NAA, BA, 2,4-D and Peptone. The medium supplemented with Peptone alone also gave good response (Table 14).

In the crosses Emma White x New Pink and Hieng Beauty x (Hawaiian Beauty x Kasem Pink), first leaf development was observed after 7 to 8 weeks in the culture. Roots appeared 6 to 8 weeks later. The seedlings were well formed in 22 to 24 weeks.

5 Planting out and hardening

Seedlings of 270 days maturity were transplanted to coconut husk pieces (sterilized in two per cent Indofil-M-45) (Plate 22, 23). Observations on plant height number of leaves and roots (Table 15) were recorded at the time of planting out. There was 100 per cent survival of the plantlets.

Table 14. Influence of growth substances and medium supplements on *in vitro* seedling development

Basal media	Growth supplements	After 32 weeks in culture					Remarks
		Length of leaf (cm)	Length of root (cm)	No. of leaves	No. of roots	Height of seedlings (cm)	
VW	1 g peptone	2.5	5.5	7	9	4.5	Proliferation of numerous side shoots
	2 ml 2,4-D + 2 ml NAA + 5 ml BA + 1 g peptone	2.5	4.6	9	5	4.2	Sturdy growth
½ MS	1 g peptone + 4 g charcoal	2.3	2.8	6	8	3.7	Proliferation of few side shoots
	2 ml 2,4-D + 2 ml NAA + 5 ml BA + 1 g peptone + 4 g charcoal	3.6	5.3	15	8	5.1	Vigorous and sturdy growth

Table 15. Growth parameters of *Dendrobium* seedlings

Seedling No.		Height of plant (cm)	No. of leaves	No. of roots
Hieng Beauty x (Kiomi Beauty x Banyat Pink)	1	4.3	14	6
Emma White x New Pink	1	5.2	8	4
,,	2	1.0	5	6
,,	3	2.0	7	4
,,	4	1.5	8	4
,,	5	2.3	7	10
,,	6	2.5	5	7
,,	7	1.1	8	6

Plate 22-23 Planted out *in vitro* plantlets

DISCUSSION

The orchids reign supreme among the floricultural plants due to the beauty and diversity of their long lasting and colourful flowers (Kumar and Sharma, 1992). *Dendrobium* is a very complex and extremely large genus of sympodial epiphytic plants.

1 Floral biology

The flowers of hybrid Dendrobiums were highly variable. They occur in an array of colours ranging from creamy white to deep purple. The hybrids were all similar in shape with significant variations in the size. The flowers are zygomorphic. It comprises of three sepals and three petals of which the third petal is highly modified, called the lip or labellum. The male and female reproductive organs are fused to form the column.

The flowers retained freshness for 45 to 50 days on the inflorescence. The flowers were borne with the labellum uppermost. The buds became resupinate just before or during opening by a twisting of the pedicel in confirmity with the reports of Nyman *et al.* (1984).

The flowers opened acropetally in an inflorescence. The maturation of flowers initiated from the basal portion and development proceeded regularly in the direction of the apex.

Anthesis occurred in the day time with peaks between 9.00 and 10.00 am and also 3.00 and 4.00 pm. Similar report was made by Christensen (1992). He

reported that the new flowers of *Stelis argentia* opened primarily in the mornings, in the late afternoon and during rainy weather.

Stigma receptivity was found to be maximum between four to six days after anthesis although the stigma remained receptive from the day of anthesis to almost 9th day. Devi and Deka (1992) also observed that the stigmas remained receptive upto five days after anthesis in *Dendrobium amoenum*, although the flowers retained freshness for a longer period.

2 Pollen studies

Unlike in other angiosperms, the pollen grains in the family Orchidaceae are found agglutinated in masses called pollinia. In each flower of *Dendrobium*, pollinia are found tightly compressed in two pairs. It is yellow in colour and ovoid in shape as reported by Tom and Sheeham (1979). The pollen grains were found in tetrads in confirmity with the reports of Abraham and Vatsala (1981) that the pollen are in tetrads held together by elastic threads of tapetal origin.

Significant variation was observed among the different hybrids for pollen output per pollinium. It ranged from 38,282 to 1,93,750. Such variation in pollen production has been reported in different varieties of Hibiscus (80 to 500) by Markose (1984), in fruit crops like sapota (682 to 3297), papaya (8950 to 12,465) and pomegranate (15,982 to 23,170) by Rao and Khader (1962). Markose (1984) suggested that the large size of the pollen grains might be the reason for fewer number of pollen grains per anther in hibiscus. The hybrid Kasem White recorded the highest number (1,93,750) of pollen grains per pollinia while Sonia 28 Mutant B had the lowest (38,282).

There existed a direct relationship between pollen size, pollen fertility and pollen production. The pollen grains of hybrids Kasem White and New Pink had a larger diameter of 39.99 μ and 40.4 μ, respectively. These pollen grains also recorded a high pollen fertility of 73.98 and 73.95 percentage and pollen production of 1,93,750 and 92,188 pollen grains/pollinium, respectively. In contrast to this the pollen grains which were smaller in size showed a low fertility and low rate of pollen number per pollinium. The size of pollen grains of Sonia 28 Mutant B was only 30.61 μ and these had a very low fertility (4.42%) and pollen production rate (38,282). Though the pollen grains of Emma White were larger in size (48.52 μ), the low rate of pollen production (42,154) might be due to bigger size of pollen and vice versa in case of Hieng Beauty (27.91 μ and 83,888 respectively).

Pollen failed to germinate in any of the sucrose concentrations, though pollen germination in two per cent sucrose solution has been mentioned for orchid species by Devi and Deka (1992). However, two per cent sucrose along with one per cent agar proved to be a suitable medium for the germination of *Dendrobium* pollen. The influence of agar in culture medium on germination was reported by Vilasini *et al.* (1966) in *Hibiscus rosa-sinensis* and Nair (1982) in pineapple, thus supporting the present findings.

The effect of sucrose on pollen germination could be attributed to factors like nutrition and osmotic or turgour phenomena (Visser, 1955; Johri and Vasil, 1961). The beneficial effects of agar might be attributed to the supply of moisture, carbohydrate and other nutrients as suggested by Stanley and Linskens (1974).

Pollen germination and tube growth were further increased by the addition of 75 ppm boric acid in the medium containing two per cent sucrose and one per cent agar. The stimulatory effect of boric acid on pollen germination and tube growth has been reported by several workers in a wide range of plants like sapota (Rao and Khader, 1960); cocoa (Ravindran, 1977) and Hibiscus (Markose, 1984).

Pollen is generally considered to be deficient in boron and hence its addition could increase pollen germination and tube growth (O'Kelley, 1955). It has been suggested that boron, helps in oxygen uptake, in addition to synthesis of pectic substances required for the formation of germination tube walls (Vasil, 1960). The beneficial effect of boron is also attributed to the promotion of sugar absorption, translocation and/or its metabolism (Gausch and Dugger, 1958; Linskens and Kroh, 1970).

The reason why the pollen grains failed to germinate in a liquid medium consisting of sucrose alone could be attributed to the fact that a solid surface resembling the viscous stigmatic fluid is required for the germination of orchid pollen, which is in accordance with the report of Rao and Chin (1973).

Successful pollen germination was observed 12 to 22 hours after incubation, indicating probably the time lapse after anthesis when the pollen can be successfully used in pollinations.

There was considerable difference among the hybrids with respect to pollen germination and tube growth. Among the five hybrids three failed to germinate. Hybrids New Pink and Kasem White gave satisfactory *in vitro* pollen germination. The results indicate that though certain varieties recorded fertility in

acetocarmine test, they failed to germinate *in vitro*. Thus the need for *in vitro* culture of pollen grains to assess the viability was emphasised by Stanley and Linskens (1974), Markose (1984).

A great deal of variability was noted in the viability of fresh and pollen stored under different conditions. The pollen grains stored at 4°C remained viable for a longer period than when stored at room temperature, probably due to their reduced metabolic activities at lower temperatures, which is in confirmity with the report of Devi and Deka (1992). Loss of viability in pollen kept over calcium chloride in a desiccator could be due to excessive dehydration as suggested by Meeyot and Kamemoto (1969).

3 Compatibility studies in the different *Dendrobium* hybrids

In the present study, eventhough fruit set was observed in many crosses as evidenced by its ovary swelling, further development was not seen. This may be due to some post fertilization barriers or the ovaries might have swelled due to stimulatory parthenocarpy. This aspect needs further study.

Unilateral incompatibility between species of the genus *Dendrobium* and between species of different genera, viz., *Dendrobium, Spathoglottis, Phaius, Coelogyne* and *Rhynchostylis* have been reported by Devi and Deka (1994). The failure of pod development in many reciprocal crosses point at the operation of a unidirectional incompatibility in line with similar earlier findings in several taxa including *Nicotiana, Petunia, Lycopersicon* and *Antirrhinum* (Abdalla, 1974). Failure of the reciprocal cross to set seeds may also be due to physical barriers, antagonism of the maternal cytoplasm to the almost naked sperm nucleus or

sensitivity of one of the plants to the environment which could block the development or functionality of one of the gametes, while it would not affect the other (Lenz and Wimbler, 1959).

Although pod formation took place in many of the crosses, production of seeds with embryos varied from zero to 35.7 per cent, indicating that effective fertilization did not take place. Devi and Deka (1994) also found that the average size and weight of all hybrid pods have been found to be 2 to 4 times less than the normal pods, indicating that the number of embryos/seeds formed in the hybrids were much less.

Of the eight successful crosses only two were reciprocally compatible. This suggests that the hybrids Hieng Beauty, White Nern and Emma White are good as female parent and not suitable as male parent. Further, the hybrid pods in certain cases turned yellow and abscised or splitted before the normal pods burst. Thus it seems that ovaries swelled parthenocarpically due to the pollination stimulus by the introduction of foreign pollen but fertilization elude the ovules due probably to incompatibility of the parents (Devi and Deka, 1992). Laibach and Maschmann (1933) demonstrated that the auxin content of the pollinia was high enough to bring about parthenocarpic development of the ovary.

Among the crosses, only 31 per cent of the cross pollinated flowers and 36 per cent of self pollinated flowers set pods. Lenz and Wimbler (1959) reported that the apparent self incompatibility and cross sterility commonly encountered among orchid hybrids could be either of the two causes; hybrid sterility or polyploidy. Abraham and Vatsala (1981) attributed inferility in polyploids to pairing abnormalities during meiosis where there has been an addition of one or more

complete or incomplete chromosome sets. The incompatibility shown among the intergeneric and interspecific orchid crosses may be due to genetic imbalances (Devi and Deka, 1994).

4 Embryoculture

Orchid seeds are unique in having an unorganised embryo and no functional endosperm. Germination of seeds from unripe capsule indicates that orchid embryos are capable of normal development prior to being fully ripe. In the *Dendrobium* hybrids, the use of immature seeds taken from green capsules is preferable as starting material for germination because the seeds are viable or not dormant and germination is faster.

During seed germination, the embryo first forms a tuberous structure called a protocorm from which the complete plant develops. The embryos/seeds sown on nutrient media start turning green in 10 to 20 days, depending on the genus and develop chlorophyll for photosynthesis in confirmity with the reports of Singh (1993).

In the present study the embryos of *Dendrobium* hybrid pods matured in 85 to 110 days after pollination. Hegde (1984) observed that the time taken for maturity of pods depends on the habit of the species crossed. Generally it takes 4 to 10 months for a capsule to mature and ripen. The capsules becoming brownish or yellowish is a sign of maturity, according to Bose and Yadav (1989). Highest germination was obtained in the seeds of crosses, when seeds were harvested with the embryos almost mature.

The technique reduces the time lapse between pollination and sowing of seeds, saves them from exposure to sterilizing agents and favours production of large number of seedlings (Arditti, 1979).

The mature embryos of the *Dendrobium* hybrids responded in the two basal media, indicating their wider nutritional amplitudes. Nath *et al.* (1991) mentioned that the embryos in an orchid ovary, are generally at different stages of development. Thus an increased germination frequency in medium supplemented with growth adjuncts would suggest that, these probably invoke germination by satisfying their nutritional complexities.

In the present study, the seedlings in the medium supplemented with peptone, NAA, BA and 2,4-D showed best response. Promotory effect of complex additives, on the germination frequency is on record in orchid cultures. From embryoculture studies of *Dendrobium, Spathoglottis* and *Phaius* hybrids, Devi and Deka (1991) reported that the average fresh weight and also the average length of leaves and roots were maximum in peptone supplemented medium, followed by that in coconut water supplemented medium. Increase in rate of growth of seedlings was also observed when IAA and NAA were supplemented.

Improvement in the growth of orchid seedlings after addition of peptone has been reported in Vanda (Mathews and Rao, 1980), *Dendrobium monile* (Chung *et al.*, 1981) and *Blettila striata* (Chung *et al.*, 1983). Growth inhibiting effect of coconut water on *Dendrobium* has been reported by Kotomori and Murashige, 1965. NAA stimulated germination and seedling growth in several genera like Cattleya (Withner, 1951) and Vanda (Mathews and Rao, 1980).

5 Planting out and hardening

Satisfactory *ex vitro* establishment was exhibited by seedlings of nine months maturity planted out in coconut husk bits. This is an ideal potting medium for *Dendrobium* in confirmity with the reports of Sudeep (1994).

Summary

SUMMARY

The present investigations were carried out in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, during the years 1993 to 1995. The summary of the work done and results obtained are presented below.

Floral description of 10 hybrids of *Dendrobium* was made.

In all the hybrids of *Dendrobium* anthesis occurred during the day time. The time of flower opening ranged from 8.30 am to 5.30 pm with peaks between 9.00 and 10.00 am and also 3.00 and 4.00 pm.

Stigma receptivity was maximum between four to six days after anthesis.

Studies on pollen morphology showed that the pollen grains were agglutinated in masses called pollinia. Pollen grains appeared creamy white to white in colour. They were spherical to rectangular in shape and were found as tetrads. The size of the pollen grains varied from 27.91 μ in Hieng Beauty to 48.52 μ in Emma White. The size was found to differ not only between the hybrids but also within themselves.

There was significant difference in the pollen output per pollinia among the different *Dendrobium* hybrids and it ranged from 38,282 (Sonia 28 Mutant B) to 1,93,750 (Kasem White).

Pollen fertility ranged from 4.42 per cent in Sonia 28 Mutant B to 73.98 per cent in Kasem White.

Media for pollen germination was standardised. A medium consisting of two per cent sucrose and one per cent agar was the best for pollen germination while for pollen tube elongation, medium comprising two per cent sucrose, one per cent agar and 75 ppm boric acid was found to be the best.

Significant variation was found in the pollen germination and tube growth among the different hybrids. The pollen grains of New Pink and Kasem White responded to *in vitro* germination (86.57 and 14.85% respectively), while those of Hieng Beauty, Emma White and Sonia 28 Mutant B failed to respond.

Pollen storage capacity was significantly influenced by the storage condition. Maximum pollen viability was obtained when stored at 4°C even after three months. Storage life was least when stored at room temperature in a desiccator over calcium chloride. Low temperature significantly increased the longevity of pollen grains.

Dendrobiums are highly self incompatible. Only the hybrids New Pink, Emma White and (Kiomi Beauty x Banyat Pink) was found to be self compatible.

In the compatible crosses, ovary remained a part of the receme and gradually enlarged into a pod. The hybrids, Hieng Beauty, Emma White and White Nern were found to be good female parents but not very suitable as male parents. The hybrids New Pink and (Hawaiian Beauty x Kasem Pink) were better male parents. However, the hybrids Kasem White and (Kiomi Beauty x Banyat Pink) could be successfully used as both male and female parents. Of the 43 crosses attempted, 14 set pod.

The pods of *Dendrobium* hybrids matured in 85-110 days after pollination. The mature embryos responded in the two basal media, viz., ½ MS and VW. The germinated embryos took 3-8 weeks for greening. The protocorms differentiated into the first leaf after 7-10 weeks in culture. The roots developed at the two leaf stage. The best seedling growth was observed in ½ MS medium, supplemented with 2 ppm NAA + 2 ppm 2,4-D + 5 ppm BA + 1 g peptone.

The seedlings were planted out after nine weeks in culture. Potting medium consisted of coconut husk pieces and the plantlets exhibited 100 per cent *ex vitro* survival.

References

REFERENCES

Abdalla, M.M.F. 1974. Unilateral incompatibility in plant species : Analysis and implications. *Egypt. J. Genet. Cytol.* **3**:133-154

Abraham, A. and Vatsala, P. 1981. *Introduction to Orchids*. Tropical Botanic Garden and Research Institute, Trivandrum. pp.1-128

Adams, R.M. 1975. The reproductive biology of the epiphytic orchids of Florida-1. A statement of the problem. *Am. Orchid Soc. Bull.* 44:699-703

Ahmedullah, M. 1983. Pollen morphology of selected *Vitis* cultivars. *J. Am. Soc. Hort. Sci.* **108**(1):155-160

* Anderson, W.C. 1980. Mass propagation by tissue culture : Principles and techniques. *Proc. Conf. Nursery Production of Fruit Plants through Tissue Culture: Applications and feasibility*. U.S.D.A., Manylana. pp.1-10

* Arditti, J. 1961. *Cycaoches ventricocum* Batea. var. Chlorochilon (Klotzsch) P.H. *Aleen Comb. Rev. Ceibe*. **9**:11-22

Arditti, J. 1967. Factors affecting germination of orchid seeds. *Bot. Rev.* **33**:1-97

Arditti, J. 1979. *Advances in Botanical Research* (Vol. 7). Academic Press, New York. pp.422-438

* Arditti, J. and Bills, R.F. 1965. The germination of an orchid seed. *Orch. Soc. Southern. California, Rev.* 7(5):5-6

* Bernard, N. 1899. Sur la germination du *Neottia nidus* - avis. *Compt. Rent. Acad. Sci.* Paris. **128**:1253-1255

* Bernard, N. 1909. L' evolution dans la symbiose, less orchideas at lur champignous commen - *Sauz. Ann. Sci. Botany*. pp.181-196

Bhojwani, S.S. and Bhatnagar, S.P. 1974. *The Embriology of Angiosperms*. Vikas Publishing House, New Delhi. pp.43-46

Boesman, G. 1962. Experimental results on orchids (seeds and young plants). *Proc. 15th. Int. Hort. Cong. Nice.* 2:368-373

Bose and Yadav. 1989. *Commercial Flowers.* Naya Prokash, Calcutta, India. pp.151-195

Brink, J. 1924. Germination of pollen grains of Apple and other fruit trees. *Bot. Gaz.* 6:131-147

Brooks, S.N. and Puri, Y.P. 1963. Atmospheric conditions influencing pollen shedding in hops (*Humulus lupulus*). *Crop Sci.* 3(6):56-57

Chadha, K.L. and Choudhary. 1992. *Ornamental Horticulture in India.* ICAR. New Delhi. pp.127-253

Chadwick, A.V., Nyman, L.P. and Arditti, J. 1986. Sites of ethylene evolution in orchid flowers. *Lindleyana.* 1:164-168

Christensen, D.E. 1992. Notes on the reproductive biology of *Stelis argentata* lindl. (Orchidaceae : Pleurothallidinae) in eastern Ecuador. *Lindleyana* 7(1):28-33

Chung, J.D., Chun, C.K., Sul, Y.K. and Byun, S.Y. 1981. Studies on aseptic culture of *Dendrobium monile* seeds. IV. Effect of light and/or dark treatment and various media on seed germination and seedling growth. *J. Kor. Soc. Hort. Sci.* 22:139-145

Chung, J.P., Chun, C.K. and Suh, J.H. 1983. Asymbiotic germination of seeds of *Bletilla* Striata II. Effect of peptone, sucrose and agar concentration and pH value on seedling growth. *J. Kor. Soc. Hort. Sci.* 24:243-248

Croat, T.B. 1980. Flowering behaviour of the neotropical genus *Anthurium* (Araceae). *Am. J. Bot.* 67(6):888-904

Dadlani, N.K., More, T.A. and Singh, B. 1988. Studies on pollen fertility and viability in *Jasminum. Ann of Agric. Res.* 9(2):247-251

Devi, J. and Deka, P.C. 1992. Pollen viability, stigma receptivity, and cross compatibility of some Indian Orchids. *J. Orchid. Soc. India.* 6(1,2):79-84

Devi, J. and Deka, P.C. 1994. Embryoculture of orchid hybrids. In *Advances in Plant Tissue Culture in India.* Proceedings of XII Plant Tissue Culture Conference. Pramod Tandon (ed.). Pragathi Prakashan. Meerut. pp.51-59

Erdtman, G. 1952. *Pollen Morphology and Plant Taxonomy.* (Angiosperm). Almquist and Wiksell, stockholm and Waltham Mass, U.S.A. pp.539

Erdtman, G. 1954. *An Introduction to Pollen Analysis.* The chronica Botanica Co., Waltham. pp.239

Fitzgerald, M.A., Barnes, S.H., Blackmore, S., Calder, D.H. and Knox, R.B. 1994. Exine formation in the pollinium of *Dendrobium. Protoplasma.* 179(3-4):121-130

Gausch, H.G. and Dugger, W.M. 1953. The role of boron in the translocation of sucrose. *Pl. Physiol.* 28:457-466

Harley, J.L. 1951. Recent progress in the study of endotrophic mycorrhiza. *Am. Orchid Soc. Bull.* 20(5):23-25

Hegde, S.N. 1984. *Orchids of Arunachal Pradesh.* Forest Department, Itanagar, Arunachal Pradesh, India.

*Hoene, F.C. 1949. Iconographia de orchidaceas do Brazil. *Secretaria de Agricultura.* Sao Paulo, pp.301

Johansen, B. 1990. Incompatibility in *Dendrobium* (Orchidaceae). *Botanical J. of the Linnean Society.* 103(2):165-196

Johri, B.M. and Vasil, I.K. 1961. Physiology of pollen. *Bot. Rev.* 27(3):325-381

Kamemoto, H. 1980. Breeding Dendrobiums for commercial cut flower production. In: Chadha.K.L. and Singh,F. (ed.). *Progress in Orchid Research.* IIHR, Bangalore. p.123-135

* Kanu, K. 1965. Studies on the media for orchid seed germination. *Mem. Fac. Agr. Kagawa Univ.* 20:1-68

Klier, K., Leoschike, M.J., Wendel, J.F. 1991. Hybridisation and introgression in white and yellow lady slipper orchids (*Cypripedium candidum* and *C. pubescens*. *J. Hered.* 82(4):302-318

Knudson, L. 1946. A new nutrient solution for germination of orchid seed. *Am. Orchid Soc. Bull.* 15:214-217

Kotomori, S. and Murashige, T. 1965. Some aspects of aseptic propagation of orchids. *Am. Orchid Soc. Bull.* 34:484-489

Kumar, P.K.S. 1991. Post-transplantation growth of *Dendrobium* hybrid seedlings in community pots. Paper presented for National Seminar on Biology, Improvement, Propagation and Commercialization of Indian Orchids at IIHR, Bangalore.

Kumar, Y. and Sharma, J. 1992. The Orchids of North-East India : towards 'A Rainbow Revolution'. *J. Orchid Soc. India.* 6(1,2):21-25

Kumaria, S. and Tandon, P. 1991. Asymbiotic germination of *Dendrobium* fimbriatum var. oculatum Hk.f. seeds on different media. *Proc. Indian National Sci. Acad. Part B, Biological Sciences.* 57(3-4):277-279

* Laibach, F. and Maschmann, E. 1933. Uber den wuchstoff der orchideen-pollinien. *Jahrb. wiss. Bot.* 78:399-430

Lenz, L.W. and Wimbkr, D.E. 1959. Hybridisation and inheritance in orchids. In: Withner, C.L.(ed): The Orchids: A scientific survey, Ronald Press, New York. pp.261-314

Linskens, H.F. 1975. The physiological basis of incompatibility in angiosperms. In: Duckett, J.G. and Rocey, P.A. (eds.). The Biology of the Male Gamete. *Biological J. Linnean Society*. Suppl. 6 pp.143-152

Linskens, H.F. and Kroh, M. 1970. Regulation of pollen tube growth. In: Moscana, A.A. and Monroy, A. (eds.) *Current Topics in Developmental Biology*. (Vol. 5) Academic Press, New York, U.S.A. pp.89-113

*Lobanov, G.A. 1950. The effect of different quantities of pollen on fertility. *Agrobiologija*. 3:78-86

*Maheshwari, P. and Narayanaswami, S. 1952. Embryological studies on *Spiranthes australis Lindl. J. Linn. Soc. London* (Bot.). 53:474-486

Markose, B.L. 1984. Pollen production, fertility and compatibility studies in shoe flower (*Hibiscus rosa-sinensis*) M.Sc. (Hort.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur.

Mathews, V.H. and Rao, P.S. 1980. *In vitro* multiplication of vanda hybrids through tissue culture technique. *Plant Sci. Lett.* 17:383-389

Meeyot, M. and Kamemoto, H. 1969. Studies on storage of orchid pollen. *Am. Orchid Soc. Bull.* 38:388-393

*Micic, N., Kurtovic, M., Jarebica, D. and Rados, L. 1987. Comparative study of the reliability of germination and staining methods in determining the viability of hazel pollen. *Jugoslovensko Vocarstvo.* 21(3):41-49

Moore, P.D. and Webb, J.A. 1978. *An Illustrated Guide to Pollen Analysis.* Hooder and Stoughton, London. pp.36-50

Mukherjee, S.K. 1990. *Orchids*. Indian Council of Agricultural Research, New Delhi. pp.1-28

Munzer, R. 1960. Investigations on the physiology of pollen germination with special reference to the effect of boric acid. *Biological Series.* 79:59-84

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plantarum.* 15:473-497

*Nadeau, J.A., Zhang, X.S., Nair, H. and O'Neill, S.D. 1993. Temporal and spatial regulation of 1-aminocyclopropane-1-carboxylate oxydase in the pollination induced senescence of orchid flowers. *Pl. physiol.* **103**(1):31-39

Nagashima, T. 1982. Studies on embryogenesis and seed germination in *Cymbidium goeringii* and *Paphiopedilum* insignae var sanderae. *J.Jap. Soc. Hort. Sci.* 51(1):82-93

Nagashima, T. 1993. Studies on the relationship between embryogenesis and germination in Orchidaceae. *J. Jap. Soc. Hort. Sci.* 62(3):581-594

Nair, C.S.J. 1982. Floral Biology, pollination and fruit development in pineapple (*Ananas comosus* L.) M.Sc.(Hort.) thesis, Kerala Agricultural University, Thrissur.

Nair, P.K.K. 1970. *Pollen Morphology of Angiosperms - A Historical and Phylogenetic study.* Scholar publishing House, Lucknow.

Nair, P.K.K., Balasubramanyan, V.R. and Khan, H.H. 1964. Palynological investigations of some guava varieties. *Indian J. Hort.* 21:79-84

Nair, S.R. 1982. Seed germination and tissue culture studies in orchids. Ph.D.(Hort.) thesis, University of Agricultural Sciences, Bangalore.

Nair, S.R., Singh, F. and Joshi, S.S. 1986. Orchid seed germination and carbohydrate mobilization pattern. *Agric. Res. J. Kerala.* 24(12):128-131

Nath, M., Devi, J., Borthakur, B., Sharma, J. and Deka, P.C. 1991. Embryoculture of *Rhyncostylis retusa* and *Vanda coerulea. J. Orchid Soc. India* 5(1,2):97-101

Nicolle, R.W. 1994. What makes a good stud? *Australian Orchid Review* 52(3):26-29

Northern, R.T. 1970. *Home Orchid growing.* Van Nostrand Reinhold Company. New York. pp.12-17, 71-94

Nyman, L.P., Soediono, N. and Arditti, J. 1984. Opening and resupination in buds and flowers of *Dendrobium* (Orchidaceae) hybrids. *Bot. gaz.* 145(2):215-221

Oberle, G.D. and Geortzen, K.L. 1952. In: Stanley, R.G. and Linskens, H.F. (eds.). *Pollen Biology Biochemistry Management*. Springer - Verlag Berlin Heidelberg, New York. pp.31

O'Kelly, 1955. Physiology of pollen. *Bot. Rev.* 27(3):325-381

Panse, V.G. and Sukhatme, P.V. 1985. *Statistical Methods for Agricultural Workers*. 4th ed. ICAR, New Delhi.

Parfit, D.E. and Ganeshan, S. 1989. Comparison of procedures for estimating viability of *Prunus* pollen. *Hort. Sci.* 24(2):354-356

Pearson, H.M. and Harney, P.M. 1984. Pollen viability in Rosa. *Hort. Sci.* 19(5):710-711

Pohl, F. 1937. In: Stanley, R.G. and Linskens, H.F. (eds.): Pollen Biology Biochemistry Management. Springer - Verlag Berlin Heidelberg. New York. pp.28

Prasad, R.N. and Mitra, G.C. 1975. Nutrient requirements for germination of seeds and development of protocorms and seedlings of *Cymbidium* in aseptic cultures. *Indian J. Exp. Biol.* 13:123-126

Pritchard, H.W. 1985. Growth and storage of orchid seeds. In: Tan, K.W. (ed.) *Proc. 11th World Orchid Conference*, Miami. pp.290-293

Pritchard, H.W. and Prendergast, F.G. 1989. Facators influencing the germination and storage of orchid pollen. In: Pritchard, H.W. (ed.). *Modern Methods in Orchid Conservation : The Role of Physiology Ecology and Management.* Cambridge University Press, Cambridge, U.K. pp.1-16

Randhawa, G.S. and Nair, P.K.R. 1960. Studies on floral biology of plum grown under subtropical conditions.

* Rao, A.N. and Chin, L.W. 1973. Pollen viability and germination in some orchid hybrids. *Gardens Bulletin*. 26(2):245-257

Rao, V.N.M. and Khadar, J.B.M. 1962. Estimation of pollen production in fruit crops. *Madras agric. J.* 49(5):152-156

Ravidnran, P.N. 1977. *In vitro* germination and growth of cocoa pollen. *J. Pln. Crops*. 5(2):109-111

Rubluo, A., Chavez, V. and martinez, A. 1989. *In vitro* seed germination and re-introduction of *Bletia urbana* (Orchidaceae) in its natural habitat. *Lindleyana*. 4(2):68-73

* Sahid, C.A. 1980. Seed germination of a *Dendrobium* hybrid on several culture media. *Bulletin Penelitian Hortikultura* 6(4):577-590

* Schumucker, T. 1935. Uber den Einfluss Von Bor Saurc auf pflanzen insebenson-deve keimende pollen koner. *Plants*. 23:264-83

Seaton, P.T. 1985. Investigations to establish improved techniques for seed storage, germination and culture of seedlings of *Cattleya aurantiaca*. M.Phil. Thesis, The Polytechnic, Wolverhampton.

Seaton, P.T. 1994. Orchid seed and Pollen storage. *Am. Orchid Soc. Bull.* 63(8):918-922

Seaton, P.T. and Hailes, N.S.J. 1989. Effect of temperature and moisture content on the viability of *Cattleya aurantiaca* seed. In: Pritchard, H.W. (ed.). *Modern Methods in Orchid Conservation : The Role of Physiology Ecology and Management*. Cambridge University Press, Cambridge, U.K. pp.17-29

Sharma, D.K. and Singh, R.N. 1970. Studies on some pollination problems in mango. *Indian J. Hort.* 27:1-15

Singh, B.D. 1990. *Plant Breeding*. Kalyani Publishers, Ludhiana. p.46

Singh, F. 1992. *In vitro* propagation of orchids 'State of the art'. *J. Orchid Soc. India.* 6(1,2):11-14

Singh, F. 1993. *In vitro* Orchid seed Germination and cloning of orchids - A success story. In: Prakash, J. and Pierik, R.L.M. (Ed.). *Plant Biotechnology - Commercial Prospects and Problems*. Oxford & IBH Publishing Co., New Delhi. pp.85-110

Singh, F. and Prakash, D. 1984. *In vitro* propagation of *Thunia alba* (Wall.) Reichb. F. through flower stalk cuttings. *Scientia Horticulturae.* 24:385-390

Singh, M., Verma, B.P., Rawat, M.L. and Dhar, L. 1978. Studies on the morphology and viability of the pollen grains of loquat. *Prog. Hort.* 10(4):45-51

Singh, S.N. 1959. Germination of pollen grains of *Vitis vinifera. Curr. Sci.* 28:258

Singh, S.N. 1961a. Studies on morphology and viability of the pollen grains of mango. *Hort. adv.* 5:121-24

Singh, S.N. 1961b. Longivity of Papaya (*Carica papaya* L.) pollen. *Indian J. Hort.* 17:238-243

Slater, A.T. 1991. Interaction of the stigma with the pollinium in *Dendrobium speciosum. Australian J.* of *Bot.* 39(3):273-282

*Soedjono, S. 1988. Simple medium for regeneration of protocorm like bodies of *Dendrobium* Walter Ocimae. *Bulletin Penelitian Hortikultura* 16(1):73-78

Srivastava, D. 1982. Studies on the pollen biology of certain cultivated Malvaceae. *In* Nair, P.K.K. (Ed.) *Advances in Pollen spore Research.* Vol.9. Today and Tomorrows Printers and Publishers, New Delhi. pp.10-92

Stanley, R.G. and Linskens, H.F. 1974. *Pollen - Biology, Biochemistry and Management.* 1st Ed. Springer-Verlang Berlin Heidelberg, New York. pp.39-85

Strauss, M.S. and Arditti, J. 1980. Post pollination phenomena in Orchid flowers. X. Transport and fate of auxin. *Bot. gaz.* 143(3):286-293

Sudeep, R. 1994. Standardisation of medium supplements for shoot proliferation in *Dendrobium*. M.Sc.(Hort.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur.

*Thacenko, G.V. 1960. The influence of the quantity of pollen on fruit setting in vines (Russian). *Agrobiologija*, 3:459-61

Thomas, W. and Thomas, B. 1992. Convenient combination. *Am. Orchid. Soc. Bull.* 61:582-587

Thompson, A.H. and Batjer, L.P. 1950. The effect of boron in the germination and pollen tube growth for several deciduous fruit trees. *Proc. Amer. Soc. Hort. Sci.* 56:227-230

Tom and Sheeham, M. 1979. *Orchid genera Illustrated*. Van Nostrand Reinhold Company, New York. pp.12-16, 78-81

*Tsuchiya, I. 1954. Germination of orchid seeds from premature pods. *Na Pua Okika O Hawaii. Nei*. 4:11-16

Vacin, E. and Went, F. 1949. Some pH changes in nutrient solutions. *Bot. Gaz.* 110:605-613

*Vasil, J.K. 1958. Storage experiments with pollen of cultivated fruit trees and vegetables. *Sci. Cult.* 24(5):233-236

Vasil, J.K. 1960. Studies on pollen germination of certain *Cucurbitaceae*. *Amer. J. Bot.* 47:239-247

Vilasini, G., George, M.K. and Pillai, P.K. 1966. Studies on Pollen Morphology, Production and viability in different varieties of 'Shoe flower' (*Hibiscus rosasinensis* L.) *Agric. Res. J. Kerala* 4(2):24-27

*Visser, J.K. 1955. Germination and storage of pollen. *Medeol Land Hogersch Wagningin*. 55(1):1-68

Weinright, H. 1988. Overcoming problems in establishing micropropagules - guidelines for growers. *Prog. Hort.* 2(3):67-72

Withner, C.L. 1951. Effect of plant hormones and other compounds on growth of orchids. *Am. Orchid Soc. Bull.* 20:276-278

Wodehouse, R.P. 1935. *"Pollen Grains". Their structure, Identification and Significance in Science and Medicine.* Mc. Graw Hill Publications, New York.

*Woittiez, R.D. 1979. Sticking of pollen to stigma. *Acta Botanica Neerlandica* 28(2/3):237-238

Yam, T.W. and Weatherhead, M.A. 1988. Germination and seedling development of some Hongkong orchids. 1. *Lindleyana* 3(3):156-160

*Ye, X.L., Cheng, S.J., Wang, F.X. and Qian, N.F. 1988. Immature Seed Morphology and the *in vitro* development of *Dendrobium candidum. Acta Botanica Yannanica* 10(3):285-290

Yeung, E.C. 1988. The development and structure of the stigma of *Epidendrum ibaguense* (Orchidaceae). *Lindleyana.* 3(2):97-104

*Zimmer, K., Seemann, P. and Holters, J. 1981. Previcur-N application after tissue culture. *Devstscher Gartenbav* 36(29):1195-1197

Zirkle, C. 1937. Acetocarmine mounting media. *Science.* 85:528

Ziu, M. 1986. *In vitro* hardening and acclimatization of tissue culture plants. In: Winther, L.A. and Alderson, P.G.(ed). *Plant Tissue Culture and its Agricultural Applications.* Butterworths, London, pp.187-196

* Originals not seen

FLORAL BIOLOGY AND COMPATIBILITY STUDIES IN *Dendrobium*

By

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ABSTRACT OF A THESIS

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ABSTRACT

The investigation on 'Floral biology and compatibility studies in *Dendrobium*' was carried out in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, during the years 1993 to 1995.

Dendrobium hybrids selected for the study included New Pink, Hieng Beauty, Emma White, Sonia 28 Mutant B, Kasem White, (Kiomi Beauty x Banyat Pink), (Hawaiian Beauty x Kasem Pink), Sonia, Sonia # 28 and White Nern. The flowers presented an array of colours, ranging from creamy white to deep purple and with a beautiful blend of the two. Anthesis occurred between 8.30 am and 5.30 pm with peaks between 9.00 and 10.00 am and also 3.00 and 4.00 pm. Maximum stigma receptivity was observed between four to six days after anthesis.

Pollen grains were found agglutinated in masses called pollinia. The pollen output per pollinia ranged from 38,282 to 1,93,750 and the fertility percentage varied between 4.42 and 73.98, among the different hybrids. Best pollen germination was obtained in a medium comprising two per cent sucrose and one per cent agar. The medium supplemented with 75 ppm boric acid was the best for pollen tube elongation. Pollen viability was retained for the longest period when stored at 4°C.

High rate of self and cross incompatibility is encountered in *Dendrobium*. Hybrids New Pink, Emma White and (Kiomi Beauty x Banyat Pink) were self compatible. From hybridization studies it was evident that Hieng Beauty, Emma White and White nern were best suited as female parents while, New Pink and (Hawaiian Beauty x Kasem Pink) were better male parents. The hybrids Kasem

White and (Kiomi Beauty x Banyat Pink) were suitable as both male and female parents.

In the compatible crosses ovary swelled and developed into a pod. It matured in 85-110 days after pollination. Their germination frequency and the subsequent morphogenetic changes leading to seedling development was influenced by the hybrids involved, maturity of the pod and media used. Seedlings showed the best response in ½ MS medium supplemented with 2 ppm NAA, 2 ppm 2,4-D, 5 ppm BA and 1 g peptone.

Planting out of mature well developed seedlings (270 days after *in vitro* planting) in coconut husk pieces was found to be ideal for *ex vitro* establishment.

Appendices

APPENDIX-I

General analysis of variance for floral characters

Source of	df	Days for inflorescence Ist flower opening	Days for Ist flower opening to last flower opening	No. of flowers/ inflorescence	Length of inflorescence
Genotype	9	1.182**	1.630**	1.667**	1184.316**
Error	60	0.101	0.230	0.125	15.226

Source of	df	Internodal length		Size of flower	
		Basal internode	Apical internode	Length	Width
Genotype	9	1.286**	1.154**	7.093**	7.929
Error	40	0.129	0.149	0.102	0.115

** Significant at 1 per cent level

APPENDIX-II

General analysis of variance for pollen characters

Sources of variation	df	Pollen production	Pollen fertility (%)	df	Pollen size (μ)
Genotype	4	23.4**	0.525**	4	275.563*
Error	10	1.8	0.008	15	66.866

Pollen storage

Sources of variation	df	Days of collection	1 week	2 week	3 week	4 week	6 week
Treatments	4	0.002	0.056*	0.107**	0.169**	0.171**	0.138**
Error	10	0.006	0.016	0.002	0.001	0.003	0.002

Pollen tube length variation with respect to media

Source of variation	df	Pollen tube Length (μ)
Genotype	14	75.787**
Error	45	5.298

* Significant at 5 per cent level

** Significant at 1 per cent level