IMPROVEMENT OF *Dendrobium* THROUGH HYBRIDISATION AND *In vitro* MUTAGENESIS

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

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

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CERTIFICATE

Certified that this thesis, entitled "**Improvement of *Dendrobium* through hybridisation and *in vitro* mutagenesis**" is a record of research work done independently by **Smt.A.Sobhana,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the Advisory Committee of **Smt.A.Sobhana,** a candidate for the degree of **Doctor of Philosophy in Horticulture**, agree that the thesis entitled "**Improvement of *Dendrobium* through hybridisation and *in vitro* mutagenesis**" may be submitted by SmtA.Sobhana, in partial fulfilment of the requirement for the degree.

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EXTERNAL EXAMINER

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INTRODUCTION

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Orchids are the loveliest and spectacular among all the flowering plants in the world. These extraordinary plants belong to the family Orchidaceae, which represents one of the largest diverse and most advanced groups of flowering plants. It is also one of the highly evolved and successful families with complicated flower structures among the monocotyledons.

The exquisite beauty of flowers, variety of fragrance, brilliance in colours, remarkable range of sizes, manifold shapes, fantastic forms, long vase life and wide distribution on earth had aroused the highest admiration for these charming plants throughout the world.

Majority of the cultivated orchids is native to tropical countries and occurs in greatest diversity in the humid tropical forests. They are located in almost all the countries.

India harbours a varied flora of both tropical and temperate orchids having commercially potential genotypes. India's rich flora of orchids is represented by more than 150 genera and about 1300 species, which are the integral part of our nation's wonderful heritage. The major areas of distribution in our country are the eastern and western Himalayas and south Indian hills. However, the natural orchid wealth in the country is yet to be judiciously utilized and managed. India is far behind in the orchid trade when compared to the commercial farms of Thailand, Singapore, Malaysia, Japan, New Zealand and Australia. In these countries, growing of orchids has become a multi million dollar business. But the business potential in India still remains untapped.

Eventhough our country abounds in rich orchid habitat, very little work has been done on breeding of orchids. Orchid breeding is an endless proposition

and there is always room for the improvement of the existing cultivars and creation of new forms. The major breeding objectives are creation of new colour combinations, improvement of flower size, enhancement of number and length of inflorescence, compactness of flowers, extension of flowering season, creations of miniature forms, fragrant varieties as well as extending the blooming period and vase life.

Dendrobium is one of the best grown genera of orchids in the tropics and is widely used in the commercial cut flower production in Kerala. This is the second largest genus in the family Orchidaceae comprising of about 1340 species. This complex and extremely large genus has sympodial, epiphytic plants varying immensely in vegetative and floral characters.

Dendrobiums have long gracefully arching sprays of numerous, attractive, fully packed flowers with magnificent spectrum of colours. The cut flowers as well as potted plants fetch a remarkable appreciation in the trade because of the long lasting elegant floral display. Their unmatched ornamental value aroused interest and merited the attention of quite a large number of growers and thus the orchid cultivation has emerged as a very rewarding vocation in Kerala. Purple, white and a combination of these two colours are mostly preferred in dendrobiums.

All the hybrids grown in our state are exotic. In Kerala, the main source of *Dendrobium* orchids is South East Asian countries, especially Thailand and Singapore. Due to the competition in the trade, we seldom receive first quality plants from these countries. It has thus become inevitable to produce our own hybrids, which can compete with those produced elsewhere. Besides, hybrids produced from the plants that are found to perform well under our conditions, will have a better adaptability here.

Hybridisation is a potential method of orchid breeding. It is the most fascinating branch of Orchidology with uninhibited inter mingling of genomes not only at species level but also at generic level. This has contributed greatly to the impressive rates of evolution in the family, which turns out new species and varieties at a very rapid rate. Artificial pollination is also practiced to produce a large number of plants in a shorter time through embryo culture, as the conventional method of propagation yields less number of plants only. Self pollination has also resulted in abundant superior offsprings with desirable qualities for cut flower purposes.

Another method of crop improvement is mutation breeding through the use of ionising radiation and chemical mutagens. The response of mutagens under *in vitro* condition has been studied in many horticultural crops including ornamental crops with great success. It also facilitates handling a large number of irradiated materials within a limited space.

Before starting any hybridisation programme, the basic knowledge of the floral biology of any plant is of utmost importance. In order to select suitable parents for the improvement programme, compatibility between the parents is to be understood. The characters associated with the hybrid vigour and correlation between characters have to be known in advance before going for selection of the parents.

Embryo culture becomes a part of the hybridisation programme in orchids since the hybrid seedlings can be raised through aseptic culture only due to the lack of functional endosperm. Enhancement of the seedling development through nutritional manipulation under aseptic and field conditions becomes necessary to reduce the time lapse between sowing of seeds and flowering in the hybrids. Keeping these points in view, the present investigation was undertaken with the following objectives.

1) To evaluate the important *Dendrobium* varieties for their vegetative and floral characters including floral biology.
2) To assess the compatibility among the different *Dendrobium* varieties.
3) To improve selected varieties through hybridsation and *in vitro* mutagenesis for new colour combinations, flower size, flower number etc. and
4) To evaluate the hybrid seedlings in the field for superior characters.


REVIEW OF
LITERATURE

REVIEW OF LITERATURE

Orchids, renowned for their beautiful flowers, rank high in the floriculture industry all over the world. The superior design and very complicated floral machinery of orchids have placed them in the foremost rank in the plant kingdom. India is one of the major orchid habitats in the world.

Dendrobium is the popular and second largest genus having contributed to the trade of floriculture. It possesses marvellously showy flowers exhibiting a wide range of forms and colours and with a long keeping quality.

Dendrobiums are improved for various characters like spike length, number of flowers, extension of flowering season, expansion of range of flower colours, improving vase life, creating miniature forms, imparting fragrance etc.

The knowledge of floral biology, anthesis, pollen and method of pollination has become indispensible in the field of breeding of ornamental plants owing to its potentialities in the breeding for improving various non synchronous cultivars.

The floral morphology and pollination mechanisms have been evolved as a result of the continued evolutionary adjustment of flowers and their pollen vectors.

Natural pollination is very much limited in most of the orchids due to the peculiar floral structure. Assisted pollination can lead to efficient fertilization and further development of the pod. Knowledge of the stage of pollination, proper medium for germination of pollen grains, enhanced germination of seeds and fast seedling development etc. are highly essential for the successful breeding and evolving good hybrids in dendrobium. Hence an attempt has been made to review the literature on floral biology including pollen studies, pollination mechanisms,

pod culture, *in vitro* seedling development, genetic improvement through mutation and field establishment of the hybrids. A brief account of the same is presented below.

2.1 Genetic analysis and variability studies

Variability means the differences or variations present among single species or different species. It may be due to environment or due to genotype or both. In plant breeding programmes, an insight of the magnitude of variability present in a crop species is important as it provides the basis for effective selection.

Johnson *et al.* (1955) found it more useful to estimate heritability value together with genetic advance in predicting the expected progress to be achieved through selection.

Work towards these lines is very much limited in orchids. Genetic analysis of some characters of orchids grown in the plains of Bengal was done by Rehman *et al.* (1993). According to them high amount of genetic variance (r^2g) was recorded for length of inflorescence (506.07) and number of flowers per inflorescence (178.78). The heritability estimates were also very high for length of inflorescence (78.94%), number of flowers per inflorescence (95.00%) and flower size (85.15%). It is suggested that for selection of improved genotypes or lines, attention should be paid on all these three important characters.

2.2 Floral morphology

A wide range of colour variation exists in orchid flowers, the prominent being white and purple closely followed by yellow and green occurring in pure form or in every possible combinations.

The orchid flower is zygomorphic, consisting of three sepals and three petals and the column or gynostemium having the reproductive parts. The sepals are also known as petaloid sepals since they are almost invariably coloured and alike in appearance, except in some species. They are usually narrower than petals. The fanciful shape of these parts often makes orchid flower looking quite complex. Two of the petals are similar and the third one is highly modified and enlarged and is called the lip or labellum. The two identical wide petals spread outward and the lip ruffles downward. The petals may be similar to sepals in size but in a few cases, as in *Habenaria* and *Coelogyne species* they are filiform or may be fimbriate as in *Bulbophyllum fimbriatum*. The lip is the most prominent and distinctive part of the flower. The colour, shape and size of the lip vary in different genera. It is embellished with its own markings. Its structure also attracts insect visitors which help in pollination and fertilization of the flowers. It may be very simple, flattened, lobed, elongated to a slender spur, 'slipper' like appearance as in *Paphiopedilum spp.*, magnificient frill like appearance as in *Coelogyne* or tubular as in *Cattleya*, may be toothed, notched or sac like also.

The waxy structure called column or gynostemium, situated in the centre of the flower, is the unique structure and primary feature distinguishing orchids from all other kinds of plants. In the evolution of orchids male and female organs fused to form the column. It is delicately coloured, attractively shaped and often is decorated with wings or a cap or a fringed bonnet. It is the most diagnostic and delightful part which is cleverly designed both outwardly and from the point of view of the functions. The column bears at the tip its anthers inside which the pollen forms compact waxy masses termed pollinia. Out of the six stamens only one is fertile; in most cases, they are called monandrae. In *Paphiopedilum* two stamens are fertile, called diandrae. Only one fertile stamen is present in *Dendrobium, Cymbidium, Epidendrum, Vanda* etc. The pollen of orchids cannot be dispersed by wind and carried to stigma to effect fertilization.

Just below the anther the pistillate part is seen which consists of the receptive organ, stigma, a shiny depression filled with the extremely sticky fluid. The ovary is situated below the sepals, the portion which is generally recognised as stalk of the flower. Ovary is inferior and is tri carpellary consisting of numerous ovules. The partition wall between the stamen and stigma is called rostellum. The floral architecture of orchids is thus highly complex showing an extreme specialisation to effect fertilization (Mukharjee, 1990).

The flowers are produced singly or in spikes depending on genera and species. Sometimes spikes are branched and long.

The orchid flowers show incredible ability to mimic insects, birds etc. Some of them have the appearance of ladies' slipper (*Paphiopedilum*), others may assume various shapes of animals or bees; *Ophrys apifera* looks like a bee, *Coeloglossum viride* looks like a minute frog, *Peristeria elata* looks like a small dove and *Bulbophyllum purpureorhachis* simulates a lizard. They are bisexual, much more rarely unisexual as in *Catasetum* and *Cycnoches.*

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 in the uppermost position. The buds become resupinate just before or during opening, by twisting of the pedicel. The degree of twisting depends on the orientation of the inflorescence relative to the ground and the position of the pedicel. This twisting of the pedicellate ovary just before flowering, making lip the lower most floral segment, is also reported by Bose and Bhattacharjee (1980). A few exceptions are *Epidendrum*, some species of *Catasetum, Nephelaphyllum* and *Polystachya* where lip is the uppermost part in the blossom.

2.3 Floral biology

Variations in floral biology were observed in different genera and species of orchids.

2.3.1 Anthesis

Croat (1980) stated that modes of flowering behaviour probably have a direct influence on pollination biology and evolution. The details of anthesis vary from one crop species to another.

In vanilla, the flower opening commenced between 10.30 am and 1.00 pm and was completed by 6.00 pm (Nair and Mathew, 1986). They also reported that on an average, 49 days were taken from flower bud initiation to the anthesis of the first flower and 74 days for completing the anthesis in a cluster.

In a study on the reproductive biology of *Stelis argentata,* Christensen (1992) observed that new flowers opened primarily in the mornings, in the later afternoons and during rainy weather. Flowers lasted up to nine days but most pollinia were removed during the first two days of anthesis.

According to Varghese (1995), the *Dendrobium* flowers retained freshness for 45-50 days on the inflorescence. She also reported the floral resupination in *Dendrobium.* The flowers opened acropettally in an inflorescence with maturation initiating from the basal portion. In her study she could find the peak anthesis period in *Dendrobium* as between 9 am and 10 am and also between 3 pm and 4 pm.

2.3.2 Stigma receptivity

The wet stigmas of orchids differ markedly from the normal type of wet stigmas by containing detached secretary cells (eleutherocytes) in a mucilaginous matrix (Calder and Slater, 1985).

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

According to Devi and Deka (1992) stigma remained receptive throughout the day and for 3 days after anthesis in *Spathoglotis plicata*, 4 days in *Aerides odoratum*, 5 days in *Dendrobium amoenum* and 11 days in *Phaius tankervilleae*.

According to Varghese (1995), maximum stigma receptive period was between 4 and 6 days after anthesis, although stigma remained receptive from the first day of anthesis to the 9th day.

2.4 Pollen studies

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 anthesis, pollen production, pollen morphology, pollen dissemination, pollination, pollen germination and fertilization (Srivastava, 1982).

2.4.1 Pollen production

Lobanov (1950) reported that plants with large quantity of pollen resulted in greatest fertilization in the intra varietal and inter varietal crossings in fruit plants.

Oberle and Georgtzen (1952) demonstrated a method of determining the number of pollen grains per anther in grape vines with the help of a haemocytometer. A marked variation was observed in the number of pollen grains by different species and different varieties of the same species. The accuracy of

haemocytometer in estimating pollen production was further confirmed and modified by Rao and Khader (1962) in fruit crops.

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

Variation in atmospheric conditions also affected pollen production (Brooks and Puri, 1963, Sharma and Singh, 1970).

Significant variation was observed among the different hybrids of *Dendrobium* for pollen out put per pollinium, which ranged from 38282 to 193750 (Varghese, 1995).

2.4.2 Pollen morphology

According to Erdtman (1952) pollen morphology was a useful means of classifying plants into families, tribes, genera, species etc. He also stated that the pollen grains possessed a unique form and performed a special and vital function. The pollen morphology analysis has been used as an effective means to throw light on taxonomy, phytogeny and evolution of angiosperms (Nair, 1970).

In orchids, pollen grains are found as polyads. Individual pollen grains of the group are not regularly arranged and are so pressed together that the outline of the individual grain becomes angular (Moore and Webb, 1978). Pollen of the orchid flower is in an agglutinated mass called pollinium and is not powdery. Each orchid flower has got two to eight pollinia under the anther cap depending on the genus. In *Dendrobium*, pollinia are in two pairs each being tightly pressed and ovoid in shape (Sheehan and Sheehan 1979). *Cattleya* has four pollinia, *Laelia* and *Eria* each has eight pollinia.

According to Abraham and Vatsala (1981), in most of the members of Orchidaceae, pollen exists as tetrads. They are held together by means of elastic threads of tapetal origin. The tetrad nature of the pollen grains was also reported by Varghese (1995).

2.4.3 Pollen viability

Fertility of pollen should be tested for each variety/species since the appearance alone is not always a good index of viability (Stanley and Linskens, 1974). They also emphasised the importance of pollen viability in hybridisation and suggested various methods for testing the viability of pollen grains. To assess the viability of pollen grains, staining with different chemicals and dyes has been adopted. Zirkle (1937) described a method for mounting pollen grains in acetocarmine. The grains which stained well and looked plump and normal were taken as viable and the unstained shrivelled ones as non-viable.

According to Micic *et al.* (1987), the most suitable and effective test for determining pollen viability was staining with acetocarmine and it gave better results compared to germination test.

Pollen viability was reduced considerably one day after anthesis in vanilla (Nair and Mathew, 1986). Fruit set was noticed even when the flowers were forced to open just before opening and hand pollinated with the pollen of the same flower. The average percentage of fruit set were 70, 15 and 10 when 1, 2 and 3 days old pollen were used.

Das and Ghoshal (1988) reported a low percentage of pollen sterility in *Dendrobium chrysotoxum* and *D. transparens*. They also observed the tetrad nature of pollen.

2.4.4 Pollen germination

Germination tests were reported to be more accurate than stain tests which gave only a crude estimate of pollen viability.

Pollen fertility is usually ensured by *in vitro* germination of pollen grains. The pollen grains will be germinated in an artificial medium containing the required nutrients especially sugars. Addition of agar, gelatin, boric acid etc. can enhance pollen germination according to the kind of the plant. The stimulating effect of boron on pollen germination and tube growth was discussed by Schumucker (1935). According to him 1 to 10 ppm boric acid enhanced pollen germination and tube growth.

Munzen (1960) reported 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. According to Johri and Vasil (1961) the effect of boron was far better than the effect of any known hormones, vitamins or any other chemical substance. The 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 (Gausch and Dugger, 1953). Addition of agar and gelatin promoted better germination (Agarwal *et al.*, 1957).

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.

Stanly and Linskens (1974) suggested that sugar supplied moisture, carbohydrate and other nutrients for germination.

According to Varghese (1995), there existed a direct relationship between pollen size, pollen fertility and pollen production of *Dendrobium*

hybrids. She also reported that pollen failed to germinate in sucrose alone. Two per cent sucrose along with one per cent agar was found to be a suitable medium for germination. Pollen germination and tube growth were further increased by the addition of 75 mg l^{-1} boric acid along with 2 per cent sucrose and one per cent agar. Successful pollen germination was obtained 12-22 hours after incubation. Considerable difference existed between hybrids with respect to germination and it ranged from 4.42 to 73.98 per cent.

In a study by Chaichareon (1995), the growth of pollen tubes of diploid plants of *Dendrobium superbiens* was slow and many pollen grains did not germinate after 3 days whereas pollen tubes of allotetraploids were long and numerous on the third day. Tetraploids had higher fertility than diploids and many seeds they produced had perfect embryos.

Pollinia of *Spathoglottis plicata* germinated after 5-6 hours of incubation and continued upto 30-36 hours and that of *Cymbidium ensifolium* germinated after 14 hours of incubation and the germination rate progressively increased upto 12 hours, in Brewbaker medium with 10 per cent sucrose (Latha and Namboodiri, 1999).

2.5 Compatibility studies

Self incompatibility was defined by Brewbaker and Shapiro (1959) as the inability of a plant producing functional male and female gametes to set seed when self pollinated. Many of the cases of apparent self incompatibility and cross sterility commonly encounted among cultivated orchid hybrids can be attributed to one of the two causes, hybrid sterility or polyploidy (Lenz and Wimber, 1959).

According to the oppositional S allele hypothesis proposed by East and Mangelsdorf (1925) pollen grains that possess S alleles identical to one of those in

the pistil will not be functional on that particular pistil or stylar inhibition of pollen tube growth occur.

According to Duncan and Curtis (1943) the self incompatible orchids always have homomorphic, gametophytic pollyallelic incompatibility with stigmatic inhibition of pollen germination.

In *Dendrobium*, self incompatibility is generally expressed by abscission of the pollinated flower and ovary 3 to 21 days after pollination.

Boric acid stimulated pollen tube growth in both compatible and incompatible crosses and calcium enhanced the pollen growth in the compatible crosses (Kendall, 1968). There was some evidence that the incompatibility reactions were affected by the application of large amount of pollen to the stigma.

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).

Intersectional crosses were performed between species of sections Phalaenanthe (P), Ceratobium (C), Eleutheroglossum (E) and Latourea (L). Cross compatibility and meiotic pairing studies indicated that, Phalaenanthe and Ceratobium were closely related to each other while Latourea was distantly related to them. Cultivars suitable for cut flower production have been developed from amphidiploid hybrids derived from crosses between species of Phalaenanthe and Ceratobium (Kamemoto, 1987).

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

In a compatibility study involving certain species of *Dendrobium*, Johansen (1990) observed that they did not produce seed pods after self pollination; shrivelling and yellowing of ovary were noticed and there was no more development. Premature abscission of the whole flower at the base of the pedicel occurred ultimately. In the self compatible species, the ovary continued its growth. The variation in maturation time for the capsules in different species was very much pronounced, ranging from 43 days in *D. salaccense* to 441 days in *D. heterocarpum.*

Johansen (1990) also reported the different sizes for capsules of same parents in reciprocal crosses. Post pollination phenomona after interspecific pollinations were similar to those that occurred after self pollinations or intraspecific cross pollinations. After the application of NAA or IAA to the stigma of the partly self compatible Madame Pompadour, parthenocarpic fruit formation occurred (no pollen applied to stigma). In self incompatible plants auxin induced flower abscission only.

Johansen (1990) also indicated that auxin content in the pollinia initiated the incompatibility response and the production of ethylene. The compatibility substance was specifically recognised by the eleutherocytes. In the 29 interspecific and 47 intergeneric crossings performed to determine compatibility between species belonging to the same genus and to different genera for production of hybrids through embryo culture, ovaries swelled in many crosses but did not develop pods (Devi and Deka, 1994). 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 the 13 hybrid capsules formed, seeds of only three crosses germinated.

2.6 Pollination biology

Pollination is the simple process of transferring the pollen from one flower to the stigmatic surface of another flower. Since the pollinia are placed at a raised position on the column, insects attracted can easily locate them and carry the pollen (Northen, 1970).

Orchids are pollinated by bees, flies, moths, butterflies, beetles, ants, spider or even birds. In *Habenaria obtusata* the pollination is by mosquitoes.

The pollen fastening mechanism in orchid is so designed that it applies the pollen to the insect as it leaves the flower, causing it to carry the pollen off and deposit it on the next flower it visits, ensuring cross pollination (Northen, 1970). In cattleya, when the insect visits the flower for necter, by the movement of the insect, the glands which secrete a sticky fluid in the rostellum get ruptured and the insect gets smeared with the glue. The fuzzy tails (caudicle) in pollinia are caught in the glue. Thus the insect flies away with the pollen attached to it.

To achieve cross pollination, certain orchids have adopted many contrivances like mimicry. An example is *Ophyrus*. It resembles a female wasp and emits a similar odour to attract male wasps. In the attempt to mate with the plant, the male wasp picks up the pollinia and deposits it on another flower (Northen, 1970).

Orchids like *Peristeria elata* are equipped with a vibratile lip. They are so designed and delicately balanced that an insect of just the right weight is

catapulted into the anther with enough force to open the anther cap and brings out the pollen masses. The orchids have various kinds of sensing devices that cause pollen to be thrown on an insect in the exact spot and the same can be transferred on the stigma of another flower.

Foraging insects were guided into the flower of *Dendrobium speciosum* by the colour gradation (yellow) of the perianth (Slater and Calder, 1988) for causing pollination.

2.6.1 Artificial pollination and hybridisation

Most orchid flowers have remarkable long lasting quality unless they are fertilized. If the sex organ is disturbed in any way or fertilized, the flower withers within a short period.

In the production of orchid hybrids, the transfer of pollinia from one flower to the stigma of another flower is made. Pollinia can be obtained by carefully removing the anther cap with the help of a match stick, and pollinia will stick to the match stick and can be transferred to the stigmatic surface. This is a simple process, but it took many years by the commercial hybridizers to take up such interesting ventures even after flowering of the first hybrid. The first orchid hybrid was *Calantha dominii*, a cross between *Calanthe musuca* and *Calantha furcata*, done in 1856 (Bose and Bhattacharjee, 1980).

In hybridisation, selection of good and healthy plant and flower by visual observation also accounts to a great extent. Much thought is to be given on the genetic characters of the parent plants and their nature of inheritance. Very young plant or seedlings, blooming for the first time should not be selected as mother plant (Bose and Bhattacharjee, 1980). *Dendrobium nobile* and *D. phalaenopsis* exhibit their characters too much in the offsprings. *Cattleya*

labiata strongly impresses its general features if crossed with any other species of *Cattleya* or *Laelia*.

Many of the Indian species have shown dominance of their attractive characters in different crosses. These species have earned world wide recognition not only because of their attractiveness but also due to their ability to transmit their important characters to the hybrids (Bose and Bhattacharjee, 1980). Some of the examples are *Aerides multiflorum, Cymbidium devonianum, C. lowianum, C. traceanum, C. elegans, Dendrobium aggregatum, D. chrysotoxum, D. formosum, D. nobile, Paphiopedilum venustum* etc. Many intergeneric hybrids with fantastic characters were produced in orchids.

Inbred progenies obtained from the amphidiploid *Dendrobium* Jaquelyn Thomas Y 166-1 were produced through selfings, sibmatings and back crosses. An outcross was also included. Selection and inbreeding were effective in increasing flower size and improving colour purity. The characters like flower size, flower colour, total initiated flowers, vase life and bud drop were primarily influenced by parental genotypes since inbreeding decline was not apparent. Continued inbreeding of individuals selected for larger and wider flowers led to decreased number of inflorescence and shorter plants (Bobisud and Kamemoto, 1982).

In a trial on the reciprocal crosses between *Dendrobium nobile* and 8 other species and the crosses between *D. moniliforme* and 21 other species in the Eugenanthe section, it was found that *D. nobile* was reciprocally compatible only with *D. haniffii, D. linowianum* and *D. moniliforme*, each of which resulted in 100 per cent seed set when used as a pollinator. With *D. moniliforme*, however, all cross were fertile, the seed set varying between 17 and 100 per cent (Ando, 1983).

According to Nair and Mathew (1986), hand pollination could be undertaken with 97.65 to 100 per cent success any time between 6 am and 6 pm on the day of flower opening in vanilla.

Selfings and crossings were performed in different *Dendrobium* species viz., *D. chrysotoxum, D. crepidatum, D. pierardii, D. primulinum* and *D. transparens*. Self pollination was not successful in *D. chrysotoxum* and *D. pierardii*. However, sibmating between two plants of *D. chrysotoxum* resulted in fruit set. Reciprocal crosses between these two latter species were unsuccessful. Reciprocal crosses were achieved between *D. crepidatum* and *D. transparens* and between *D. crepidatum* and *D. pierardii*. Unidirectional crosses were successful in *D. primulinum* x *D. crepidatum* and *D. transparens* x *D. pierardii* (Das and Ghoshal, 1988).

Studies on *Dendrobium speciosum* showed that pollination was effected by the deposit of 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). Sticking of the pollen to the stigma is mainly determined by the wetness of the pollen or stigma.

According to Seaton (1994), cross pollination is to be preferred to self pollination since it is more likely to lead to the production of vigorous seedlings. Pollinia should not be left on the parent plant for too long because it may succumb to fungal infection and they should not be removed until the pollen has had sufficient time to mature.

In order to extend the flowering season, expand the range of flower colour and shapes and to increase floriferousness of phalaenopsis type dendrobium, hybridisation has been conducted between members of this group

and those in section spathulata, the so called antelope dendrobium (Davidson, 1994).

Amore and Kamemoto (1997) reported a regular peloria in *Dendrobium* hybrid D'Bush Pansy. Unlike the typical lip of *Dendrobium*, the lip of this hybrid is similar to the two lateral sepals, and the flat petals and sepals resemble those of pansy. This was crossed with several *Dendrobium* plants with normal lip and concluded that pansy lip is recessive to normal lip, controlled by a single recessive gene pair.

2.7 Post pollination phenomena

Pollination not only shortens the life of flower but also induces numerous and remarkable changes in its morphology and colouration. The changes after pollination in the orchid flower is regulated by the additional substance produced by the pollinated flowers or from the pollen (Strauss and Arditti, 1980).

In *Cymbidium goeringii* and *Paphiopedilum insigne* var. Sanderae, ovule formation occurred 43 to 45 days and 58 to 60 days, respectively, after pollination (Nagashima, 1982). Maturation of embryo took in 115 to 120 days and 195 to 200 days, respectively. The seeds germinated in 80 and 165 days. Highest germination was obtained in each of the species when seeds were harvested with the embryos almost mature.

In *Blettilla striata* and *Calanthe discolor*, ovaries reached their final size in 50 to 60 days and 30 to 50 days, respectively, after pollination. Embryos and seeds of both species developed in 80 to 110 days after fertilization (Nagashima, 1982).

According to Hegde (1984), pods of *Dendrobium species* matured in 9 to 17 months.

Ethylene produced in abundance after pollination and emasculation, also resulted the post pollination phenomena including its own production and the senescence of some floral segments (Chadwick *et al.*, 1986). According to Bose and Yadav (1989), the capsules becoming brownish or yellowish was the sign of maturity.

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

The post pollination phenomena within *Dendrobium speciosum* included a more intense perianth colour and the closure of the perianth (Slater, 1991). This was followed by the swelling of the column and ovary and after 4 days cell division in the ovary. Following pollination the pollinia broke apart as the tetrads dissociated from each other, the pollen grain progressively hydrated and germinated from the outside. The stigmatic mucilage appeared to be essential in the hydration and germination of the pollen. The detached cells of the stigma located near the entrance to the stylar canal were seen to loose the starch from the amyloplasts after the pollen tubes had passed.

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

According to Porat (1994) there was a rapid acceleration of the wilting process after pollination. This began only after 24 hours in *Phaleanopsis* hybrid

cv. Herbet Hager. Following pollination but not after emasculation there was an increase in ethylene production and sensitivity to ethylene. This was not dependent on endogenous ethylene production. Enhancement of senescence in *Doritaenopsis, Dendrobium* and *Cymbidium* as well as three *Phalaenopsis* cultivars was induced by successful pollination and not by emasculation. Wilting of flowers was accompanied by a loss of water from the cells of the upper layer of petals, leading to their upward folding.

2.8 Green pod culture

A major advancement in increasing the germination of orchid seeds and reducing flowering time was the development of green pod culture (Withner, 1943 and Tsuchiya, 1954). This 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.

Mitra (1986) revealed that seeds obtained from green pods after 8-12 weeks of anthesis germinate readily in a large number of species. The mature seeds, on the other hand, are hard to germinate due to some dormancy factors. A study of the degree of maturity of the orchid seeds revealed gradual changes in their enzyme compliments. It would be desirable to identify the critical stage when seeds pass on to the dormant stage.

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.

According to Linden (1980) the stage of seed maturation also influenced the rate of *in vitro* germination.

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 (Singh, 1993).

Immature seeds from unripe green capsules of *Dactylorhiza hatagirea*, collected 16 weeks after pollination were successfully germinated on modified Knudson-C medium (Vij *et al.*, 1995).

A decrease in the germination response of *Vanda* seeds with progressive age was reported by Sharma (1998).

2.9 Embryo culture studies

The orchid plant with its wide variations in growth, flowering, seed production and germination has got many adaptive characteristics. The most suitable example for the adaptive feature is the physiology of orchid seed germination. The first published description of an orchid seed is by Theophrastus (Salisbury, 1804). It was later found that the seeds germinated in the natural condition only when infected by a fungus, i.e., due to mycorrhizal association (Bernard, 1899). However, Knudson (1922) could germinate orchid seeds in a simple nutrient medium containing sugars (Arditti, 1979). A detailed review of work on orchid seed germination was compiled by Arditti (1967 and 1977). Orchid seed culture is also described by Bose and Bhattacharjee (1980) and Abraham and Vatsala (1981).

2.9.1 Nature of orchid seed

Orchid seeds are unique in many ways. They are extremely small, dust like, weighing 0.3 to 14 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). The seeds produced are in large numbers ranging from 1300 to 4 million (Arditti, 1967). Singh, (1993) described the seed as follows. Seed contains an undifferentiated embryo composed of 80-100 cells and without a functional endosperm. They are situated in the middle of the testa, being attached to it by a few strands. Testa cells are dead, vary in size and have longitudinal and transverse walls of different thickness, which gives them a net like appearance.

Two major groups of orchid seeds are usually distinguished. One group has relatively differentiated embryos, including rudimentary cotylendons as in *Bletilla hyacinthins* (Harley, 1951). However, majority of the species have relatively undifferentiated embryos and no endosperm (Maheshwari and Narayana Swami, 1952).

2.9.2 Seed germination

The seed germination in orchid is a complex process. A single capsule produces several millions of seeds. However, the percentage of germination and number of plants developed are very low due to lack of any functional endosperm.

In orchids, the germination and development of a seedling is not like in any other angiosperm. The rudimentary embryo enclosed in the seed coat develops like a dormant bud. In the process of development, the seed may or may not develop chlorophyll, but it swells in size and bursts out of the seed coat. A cone shaped spherical seedling is formed and this is called protocorm stage (Bernard, 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 Bills, 1965, Singh, 1993).

The process of germination proceeds symbiotically in nature, with the association of some root fungus and asymbiotically in aseptic conditions. The significance of fungus and its importance was well established by Bernard (1899).

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. mucoroides* and *Corticium catonii* (Singh, 1993).

2.9.3 Asymbiotic seed germination

Knudson (1922) 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).

2.9.4 Seed sterilization

The orchid seeds are usually cultured in completely aseptic condition. Hence the seeds are to be sterilized before inoculation into the medium. Orchid seeds can resist chemical treatments for sterilization (Redlinger, 1961 and Jordan, 1965).

Seeds of *Vanda* "Miss Jaoquim" treated with 5 per cent clorox for 10 minutes and washed with sterile water and inoculated on a culture medium produced seedlings in 10 to 12 weeks. Whereas seeds without seed treatments did

not germinate. Seeds directly transferred to the medium without exposure to outside, germinated well and produced strong seedlings within 8 to 10 weeks (Rao and Avadhani, 1963). *Bletilla* capsules sterilized with alcohol and inoculated directly into the medium produced 100 per cent germination and vigorous seedlings (Luks and Shevchenko, 1977).

According to Yanagawa *et al.* (1995) spraying the surface of a medium with 0.5 per cent sodium hypochlorite solution after sowing nonsterilized orchid seeds on the medium proved to be a feasible method of sowing orchid seeds under nonsterile conditions.

Pod sterilization after dipping in alcohol followed by flaming was reported in *Dendrobium ovatum* by Pyati and Murthy (1995) and in *Vanda* by Sharma (1998). According to Devi *et al.* (1998) pod sterilization in *Vanda coerulea* can be effected by alcohol treatment followed by flaming and 0.1 per cent mercuric chloride for 5 minutes.

2.9.5 Embryogenesis and germination

According to 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.

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 time in seeds collected when the embryos were at pre-tetrad or intermediary stages. Highest germination percentage 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. Protocorm arose from the embryos and could form calli or plantlets; the calli 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.*, 1988).

Stored lipids and proteins are utilized for protocorm formation and later, the accumulated starch is utilized for organogenesis in *Spathoglottis plicata* (Krishnan *et al.*, 1993).

According to Singh (1993), when the seeds are inoculated onto a nutrient medium under *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 differentiation 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 directions. 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 (Singh, 1993).

2.9.6 Culture media, components and media supplements

Eventhough sugar is one of the important components in the media, when chemically pure sugar was used there was no germination (Noggle and Wynd, 1943).

Yeast extract was reported to have inhibitory effect on germinating seeds and developing seedlings of *Dendrobium* and *Brassolaeleocattleya* (Kano, 1965). The inhibitory effect of coconut water in the initial stages of seed germination of *Dendrobium* seeds was reported by Kotomori and Murashige (1965). The favourable effect of peptone on the protocorm growth and proliferation of *Cattleya, Dendrobium* and *Vanda* was reported by Morel (1974). Different nitrogen sources have different effects on growth of seedlings. Organic nitrogen sources such as proteins, peptones, amino acids, urea and others are superior to inorganic ones. Several orchid species have been known to grow better on ammonia (Arditti, 1979).

The importance of thiamine in the culture of seeds of *Orchis latifolia* has been emphasized by Mead and Bulard (1979). According to Mathews and Rao (1980), peptone is not effective during the early stages of seed germination but promotes protocorm growth and proliferation of *Vanda*. The successful use of yeast extract for seed germination and protocorm proliferation in many orchid species was also reported by Mathews and Rao (1980).

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 extracts to Knudson C medium. Vacin and Went medium with 15 per cent coconut water plus 10 ppm NAA led to the rapid proliferation of protocorm like bodies and seedling formation and growth in *Dendrobium* cv. Jacqueline Thomas.

The nutritional requirements of the seedlings differ from those of germination and protocorm stages (Mitra, 1986). An indirect seedling development via callus formation is indicated in many species and is useful for

obtaining a larger number of hybrid plants from a single embryo, thereby facilitating hybrid analysis in replicants.

According to Mitra (1987) ammonium nitrate was found to be the best nitrogen source as it supported growth in a large number of orchid species. Further, ammonium salts promote growth during early germination and protocorm development. After leaves and roots are formed the protocorm prefers nitrate for their continued growth. The addition of vitamins or 2,4-D was found deleterious (Soediono, 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 lux). All seedlings developed to the adult stage, forming leaves, pseudobulbs and roots after 90 days of *in vitro* culture. Kumaria and Tandon (1991) suggested that *Dendrobium fimbriatum* var. *oculatum* seeds require a medium containing high concentrations of nutrient salts and vitamins for germination and development. Highest 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 roots 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^{-1} casein hydrolysate or 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 were maximum in peptone supplemented medium. In Knudson-C medium supplemented with either CW (20%), IAA (1 mg l^{-1}), NAA (1 mg l^{-1}) or 2,4-D (1 mg l^{-1}), an 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).

In vitro germination of seeds produced by selfing and crossing of different species was studied in modified Knudson-C and Burgeff N_3 media. Of the two media, the Burgeff N_3 original medium was of wider application and the seeds from green pods of *Dendrobium chrysotoxum, D. pierardii* x *D. crepidatum, Aeridis multiflorum* and *Cymbidium aloifolium* germinated successfully on it. Both media supplemented with NAA (1 mg l^{-1}) were suitable as media to which seedlings could be transferred after the initiation of the second leaf (Das and Ghoshal, 1989).

According to Devi *et al.* (1990), seed germination was 50-60 per cent higher in Vacin and Went medium for *Dendrobium farmeri* and *D. primulinum* and in Nitsh medium for *D. moschatum* and *D. fimbriatum*. Addition of 15 per cent coconut milk and 5 per cent banana extract + 5 per cent pineapple juice to Vacin and Went medium enhanced germination and resulted in accelerated seedling leaf and root growth in *D. farmeri* and *D. primulinum*. Similar result was obtained for *D. moschatum* and *D. fimbriatum* when 15 per cent coconut milk + 1 mg NAA l^{-1} were added to Nitsch medium. After a certain stage of

development orchid seedlings require no exogenous supply of sugars and it can be drastically reduced (Singh, 1993).

Optimum protocorm proliferation occurred in the light (11 hour per day) at 25°C, in half strength MS medium containing three per cent sucrose (Zhang *et al.*, 1992).

Sharon *et al.* (1992) raised protocorms of *Dendrobium* Snowfire from immature seeds using VW medium supplemented with 15 per cent coconut water.

The efficacy of a low cost medium prepared by using a commercial fertilizer formulation, table sugar and 15 per cent coconut water for raising seedlings of *Phaius tankervilleae, Dendrobium moschatum* and *D. fimbriatum* var. *oculatum* was examined in comparison with MS, Knudson C and Nitsch media (Malemnganba *et al.*, 1994). Nitsch medium and low cost medium favoured the early development of protocorms and shoots at a higher frequency than the other media. Use of low cost medium represented a saving of 70 per cent in costs.

When peptone 1000 mg l^{-1} was used along with ¼ MS + BA 20 mg l^{-1} + NAA 1 mg l^{-1}, maximum number of shoots (7.83), leaves (11) and roots (5.67) were obtained after 12 weeks of culture period in *Phalaenopsis* (Bhasker, 1996). The beneficial effect of coconut water on multiple shoot production, number of leaves and leaf length was also reported by Bhasker (1996) in *Phalaenopsis*.

2.9.7 Effect of growth regulators on seed germination

Orchid seed germination and seedling growth are to a great extent influenced by the growth regulators. Auxins, gibberellins and cytokinins play an important role in seed germination and growth. Experiments with IAA did not

give any satisfactory results for orchid seed germination. IAA impeded germination and caused elongation of the protocorms (Hadley and Harvis, 1968).

It was also reported that only traces of auxin were present in *Cypripedium* seeds and none at all in *Calanthe* and *Dendrobium* seeds (Poddubnaya and Arnoldi, 1960, Poddubnaya *et al.*, 1961). Root development of *Cypripedium* seedlings on Burgeff N_3F medium was stimulated by the addition of NAA at 1.5 mg l^{-1} (Boesman, 1962). Noticeable difference in growth and development of both control and treated plants were observed as the plants developed and grew older. This may be because no hormone was produced during the early stages of germination and growth but production was initiated and increased as the seedlings grew older and leaves and roots were produced (Arditti, 1965).

In media containing NAA, the roots were healthy and thick and within 15 to 18 days after transfer, shoot growth was less marked (Bose and Mukherjee, 1974).

Better protocorm proliferation was obtained in three interspecific *Vanda* hybrids, when NAA (1 mg l^{-1}) was used along with other additives (Mathews and Rao, 1980).

In a study on *Cymbidium longifolium* seed germination, it was shown that when supplemented with vitamins (2 mg l^{-1}, each, of thiamine HCl and Pyridoxine HCl, 0.3 ml l^{-1} of biotin and 0.03 mg l^{-1} of folic acid), hormones (0.2 mg l^{-1} each of indole acetic acid, 0.4 mg l^{-1} of kinetin) and amino acids (3.0 mg l^{-1}, each, of tryptophan and asparagine) the Vacin and Went medium supported 80 per cent germination in 30 days (Muralidhar and Mehta, 1986).

Based on earlier literature it was reported that IAA, IBA and NAA enhance seed germination and seedling growth in a large number of orchid species (Singh, 1993).

Several growth regulators were evaluated in *in vitro* cultures of *Dendrobium moniliforme* (Lim *et al.*, 1993) and seen that IBA and kinetin gave good shoot production. IBA at 0.1 mg l^{-1} was the best for producing many tall and rooted shoots.

According to Bhasker (1996) a combination of adenine 8 ppm + BA 16 ppm recorded maximum number of shoots (5) and leaves (7.67) in *Phalaenopsis*.

2.9.8 Effect of charcoal

Charcoal has been used as a purifying and decolourising compound for liquids since 18th century. The addition of activated charcoal to plant tissue culture medium may have either beneficial or harmful effects. It helps in the rapid and better development of shoots and roots of seedlings. The effects may be attributed to at least four factors, viz., establishing polarity by darkening of the medium, irreversible adsorption of inhibitory compounds, irreversible adsorption of plant growth hormones and other organic compounds and improving aeration of the culture medium (Fridborg and Eriksson, 1975, Fridborg *et al.*, 1978, Weatherhead *et al.*, 1978).

Prof.R. Ernst at the University of California was the first to use charcoal in seedling media. *Paphiopedilum* and *Phalaenopsis* seedlings grew well on media containing charcoal (Ernst, 1974).

Charcoal has the capacity to adsorb hormones and vitamins and thereby inhibit growth (Fridborg and Eriksson, 1975). Therefore it should be used

carefully in media which contain these components. However, if seedlings, plantlets, explants and tissues grow well on a medium, which contains both charcoal and the additives it adsorbs, there is no reason to omit the former. The elongation of the root tips of terrestrial orchid *Blettila striata* was better in graphite or charcoal containing culture media (Yam and Weatherhead, 1988).

2.10 Callusing

The incidence of calli in seed culture has been observed by many workers (Curtis and Nichols, 1948, Withner, 1959, Rao and Avadhani, 1964, Mitra *et al.*, 1976 and Vij *et al.*, 1981). The immature condition of seeds as well as the undifferentiated embryos may be the probable factors for the callusing of seeds. The callus mass formed from undifferentiated embryo is analogous to a highly proliferated protocorm. For many orchids the embryo possesses a natural capacity to cleave into additional embryos and it is perhaps augmented under artificial cultural condition (Mitra, 1986).

The addition of peptone to a medium containing IAA or NAA and or without kinetin or BAP favoured callusing of embryos or protocorms. Also media containing yeast extract, IAA and BAP or yeast extract and 2,4-D favoured callusing of germinating embryos or protocorms (Mitra, 1986).

2.11 PLB formations

Intuwong and Sagawa (1974) could produce yellow coloured PLBs by shoot tip culture in *Phalaenopis* in 2 per cent sucrose.

Soediono (1983) observed that proliferation of *Dendrobium* PLBs and plantlet formation were rapid when the medium contained 10 mg l^{-1} NAA.

Leaf elongation, differentiation and the formation of PLBs from shoot apices of *Cymbidium* were enhanced by BA 0.1 mg l^{-1} and inhibited by NAA 0.5 mg l-1 (Kim and Kako, 1984).

Phalaenopsis PLBs could be induced from *in vitro* leaf (Tanaka and Sakanishi, 1977, Latha and Seeni, 1991). The importance of coconut water for inducing PLB formation from shoot tip explants of *Phalaenopsis* without using plant growth regulators was reported by Ichihashi (1992).

In *Dendrobium*, wounded protocorms proliferated to form a mass of callus and then differentiated into somatic embryos, where as the intact protocorms developed directly into seedlings (Sharon *et al.*, 1992).

Bhasker (1996) could obtain 100 per cent success in PLB formation from *in vitro* leaf with BA 25 ppm + adenine 10 ppm + NAA 1 ppm and the time taken for PLB formation was also minimum. Further growth of PLB into plantlet was the best in ¼ MS medium containing BA 15 ppm + NAA 1 ppm, followed by adenine 8 ppm + BA 16 ppm.

2.12 Mutation studies

Genetic diversity which is the backbone of crop improvement, may be introduced deliberately by employing ionising radiations and chemical mutagens.

Tissue culture greatly facilitates the application of mutagens to tissues or cells and makes it easy to handle a large number of irradiated materials and also for the selection of the trait at tissue level.

Ionising radiations can interact with cells to produce a genetic effect. Dosages of gamma rays ranging from 2000 to 4000 R at a rate of 900 R/hour seemed to be optimum for the purpose of inducing mutations in *Cymbidium* protocorms (Harn, 1970). According to him, whole protocorm treatment was

preferred to protocorm pieces. Changywal (1970) studied the radio sensitivity of *Cymbidium* protocorms.

Investigations have contributed much to the induction of mutation *in vitro* (Nickell, 1973). Successful mutagenesis has also been reported in *Cymbidium* (Wimber and Vancott, 1967), *Dendrobium* (Chaichareon, 1973; Sanguthai *et al.*, 1973) and *Vanda* (Sanguthai and Sagawa, 1973) using colchicine and chemical mutagens.

External chemical or physical agents can cause mutations either directly or indirectly after enzymatic activation by interfering with enzymes necessary for chromosome segregation or duplication (Vajrabhaya, 1977). According to him chromosome or gene mutation may occur spontaneously *in vitro*. Any of the affected cells may divide and form chimeras which subsequently become complete plantlets with new characterstics and such occurrances have also been found in orchids.

Dendrobium mericlones 2 to 2.5 cm long can tolerate gamma radiation upto 5000 R without much apparent loss of viability. Some of the irradiated *Dendrobium* Pompadour produced flowers with modifications in shade or colour or both (Vajrabhaya, 1977).

Young offshoots of uniform growth of lady's slipper orchid (*Paphiopedilum villosum*) were treated with four concentrations (0.25 to 2.00%) of EMS for 48 hours. Three mutants with changed shape and colour patterns were obtained (Arora and Jana, 1980).

Effect of mutagens on *Spathoglottis plicata* was reported by Mazumder and Bhowmik (1997). They irradiated the protocorms with different doses of gamma rays ranging from 0.5 to 5.5 KR and also treated with 0.1 to 0.5 per cent EMS solution. The survival percentage, plant height, leaf length, root length,

number of roots and fresh weight deceased with increase in concentrations of mutagens. Based on chlorophyll mutations, 8 types of mutants were isolated.

2.13 Hardening and planting out

The media used for planting out should provide good support to the plants, supply water and ensure good drainage and also aeration around the roots.

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. Sutter *et al.* (1985) found that the survival of plantlets depended as much on the vigorous growth of the newly produced leaves as on the number of the leaves present at the time of planting out.

Ziu (1986) reported that the success in acclimatization of *in vitro* cultured plants is dependent not only on the post transfer growth conditions but also on the pretransfer culture conditions.

The survival rate of *Cymbidium kanran* plantlets transferred to pots in the green house was increased from 53 to over 80 per cent by soaking the moss peat compost for 30 minutes in a disinfectant solution of 0.1 per cent benlate (Kim *et al.*, 1988). Best growth of two year old seedlings was obtained in forest top soil followed by forest top soil + shredded pine bark (1:1).

Wainwright (1988) observed that the environment in a tissue culture container is that of very high humidity, low light levels and usually a constant temperature. 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*.

Seeni and Latha (1990) from TBGRI, reported the post transplanting growth of *Phalaenopsis* hybrid seedlings. They observed that axenic seedlings of the hybrid cultivar Fire Water Ponce recorded 100 per cent survival even without any acclimatization or pre-treatment of the seedlings. The medium which was found satisfactory was broken tiles, followed by charcoal, cassava pith, rubber seed husk and coconut husk. The foliar nutrient solution, which was found to be most effective in terms of rapid leaf and root growth was a combination of commercial diammonium phosphate and potassium nitrate (20:10:10 NPK).

Kumar (1992) observed that *in vitro* seedlings of *Dendrobium* recorded best survival per cent on pure charcoal, followed by cassava pith and rubber seed husk when kept at a temperature between 29° and 35°C with a RH between 70 and 90 per cent and illumination between 1000 and 1500 lux at pot level. Seedlings were watered daily and sprayed with a NPK nutrient solution on alternate days.

Lakshmidevi (1992) used brick and charcoal in equal poportions as the potting media for *Dendrobium fimbriatum* and *D. moschatum* and obtained 20 and 40 per cent survival of plantlets, respectively. Sharma and Tandon (1992) reported that a survival rate of 65 per cent was observed when the plantlets were transferred to a potting substrate comprising of charcoal fragments, brick bits and

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In another study, the seedlings ready for transplanting were washed carefully in moving water to remove all traces of agar and planted in community pots with 1:1 mixture of tree fern fibre and charcoal. These were found to establish well (Singh, 1993). Seedlings generally grow better in groups than singly, due to community effect.

Acclimatization was best done in peatmoss for improving stem diameter and plantlet height, although perlite was best for increasing root number (Lim *et al.*, 1993). Sudeep (1994), on the other hand, reported maximum survival rate of *Dendrobium* plantlets in a potting medium of coconut husk alone.

2.14 Nutrient regulation in young seedlings

The elements N, P and K in the ratio of 20:20:20 used every week followed by a 10:30:20 (NPK) mixture was found to be successful recommendation for various orchids, growing under South Indian conditions (Abraham and Vatsala, 1981).

Applications of dichlorophenoxy triethyl amine to seedlings of *Phalaenopsis* increased seedling survival, long term vegetative plant growth and greatly accelerated flowering when compared to controls (Keithly and Yokoyama

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The growth enhancing properties of dichlorophenoxy triethyl amine (DCPTA) was tested on transplanted seedlings of some orchid hybrids by Keithly *et al.* (1991).

The beneficial effect of 17:17:17 NPK complex, when sprayed at weekly intervals to *Cymbidium traceanum* was reported by Sobhana and Rajeevan (1995).

According to Bhasker (1996) the nutrient solution 30:10:10 (0.5%) and 17:17:17 (0.1%), sprayed at fortnightly intervals, recorded highest survival percentage after 12 weeks of planting out of *Phalaenopsis in vitro* plants. The growth characters, viz., plant height, leaf number, leaf length and width, root number and root length were found to be maximum for the plants sprayed with 17:17:17 (0.1%).

2.15 Effect of growth regulators on orchids

Naturally occurring hormones play an important role during the process of plant growth and development. The flowering intensity of Vanda Miss Joaquim was found to be inversely correlated with the auxin level in the shoot apex (Yadav and Bose, 1989).

Exogeneous application of growth regulators may induce or promote flowering, prevent or delay it. Plants of *Phalaenopsis schilleriana* sprayed with auxin solution were less abundant in flowering and it was suggested that there seemed to be an excess auxin which inhibited the production of inflorescence (Yadav and Bose, 1989). According to them gibberellins were also found to regulate flowering in orchids.

In certain species of *Cymbidium*, GA_3 application also induces flowering. The length of flower spike and size of individual flowers were also increased by GA_3 (Yadav and Basak, 1999). Cytokinin induces flowering in *Dendrobium*. Treatment of mature pseudobulbs with BA also stimulated flowering. BA at 4000 ppm prevents decrease in flower number. Antiauxins and growth retardants were found effective in stimulating flowering in Aranda (Yadav and Basak, 1999).

MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation entitled 'Improvement of *Dendrobium* through hybridization and *in vitro* mutagenesis' was carried out in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during this period 1996-1999. Ten important varieties of *Dendrobium* and six wild species maintained in the orchidarium of the All India Co-ordinated Research Project on Floriculture, Vellanikkara were used for the study. The varieties included Sonia 28, New Pink, Emma White, Pink Tips, Hieng Beauty, Promott-II, Candy Stripe, Banyat Pink, Sakura Pink and Sabine. The wild species used were *Dendrobium moschatum, D. fimbriatum, D. chrysanthum, D. densiflorum, D. crumenatum* and *D. pierardii* (Plates 1-15).

The study comprised of the following major experiments.

3.1 Morphological description of the selected *Dendrobium* varieties

3.2 Study on floral biology of the selected *Dendrobium* varieties

3.3 Compatibility among the *Dendrobium* varieties and wild species

3.4 Green pod culture and refinement of the embryo culture medium

3.5 *In vitro* mutagenesis

3.6 Hardening and field evaluation of the hybrid seedlings

3.1 Morphological description of the selected *Dendrobium* varieties

The *Dendrobium* varieties were selected based on the morphological and floral characters. Observations were recorded on the following characters.

Dendrobium Parents used in the crosses

Plate - 2 Candy Stripe

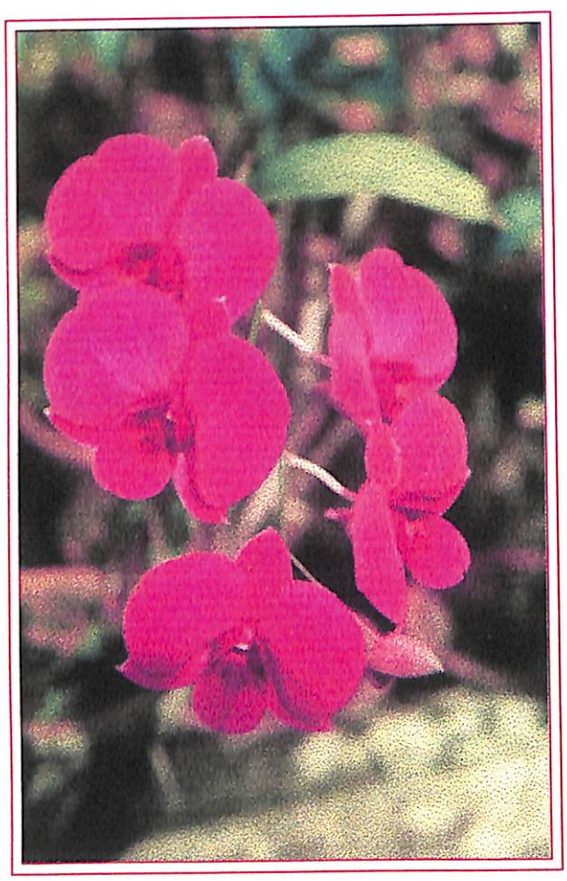


Plate - 1 Banyat Pink

Plate - 3 Emma White

Plate - 4 Hieng Beauty

Dendrobium Parents used in the crosses

Plate - 5 Pink Tips

Plate - 6 New Pink

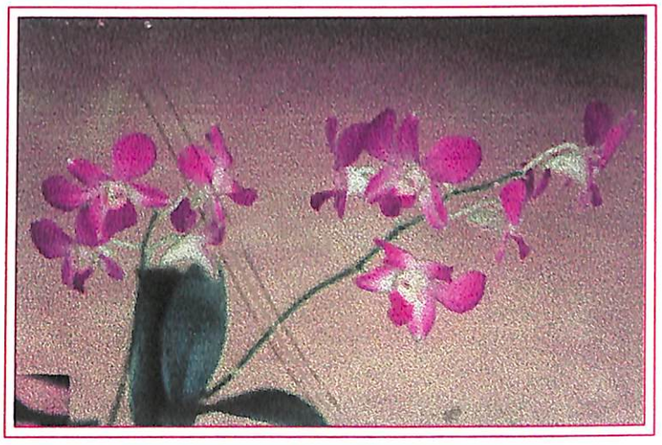


Plate - 7 Pramott - II

Plate - 8 Sabine

Dendrobium Parents used in the crosses

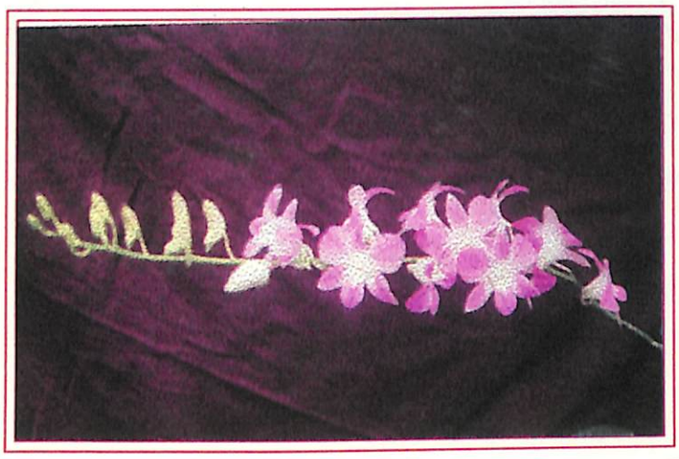


Plate - 9 Sakura Pink

Plate - 10 Sonia 28

Plate - 11 *Dendrobium chrysanthum*

Plate - 12 *Dendrobium crumenatum*

Dendrobium Parents used in the crosses

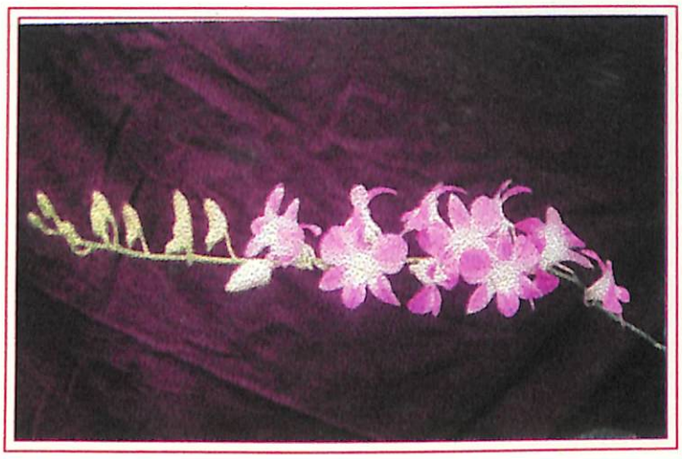


Plate - 9 Sakura Pink

Plate - 10 Sonia 28

Plate - 11 *Dendrobium chrysanthum*

Plate - 12 *Dendrobium crumenatum*

Dendrobium Parents used in the crosses

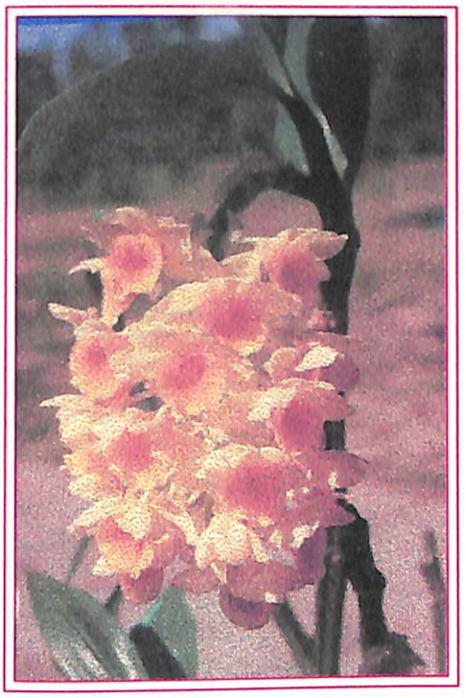


Plate - 13 *Dendrobium densiflorum*

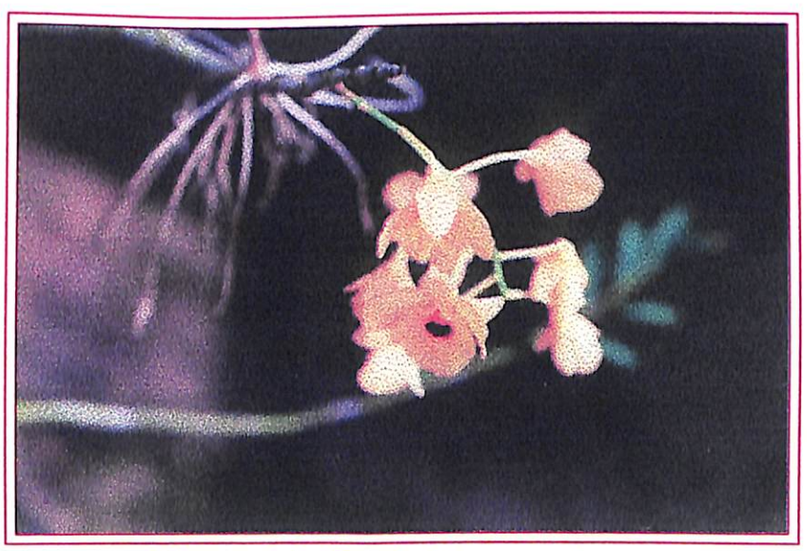


Plate - 14 *Dendrobium moschatum*

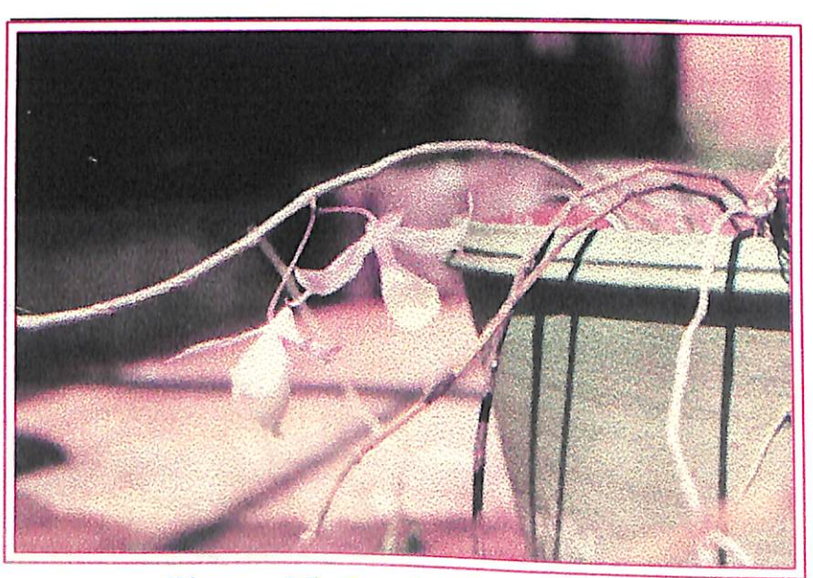


Plate - 15 *Dendrobium pierardii*

a) Morphological characters

i) Number of shoots
ii) Shoot height
iii) Number of leaves
iv) Leaf size

b) Floral characters

i) Number of flowers/inflorescence
ii) Days from spike emergence to first flower opening
iii) Days from first flower opening to last flower opening
iv) Days for wilting of first flower
v) Days for wilting of all flowers
vi) Flower size
vii) Spike length
viii) Internodal length
ix) Flower colour
x) Vase life
xi) Blooming period

3.2 Floral biology

3.2.1 Anthesis

The selected plants were marked at the time of spike emergence. The mature buds in the spike were tagged at the full bud stage. The time of opening of each bud was observed at hourly intervals and the time of full opening of the flowers was noted.

3.2.2 Stigma receptivity

A preliminary trial was conducted in New Pink to find out the correct stigma receptivity time. Five flowers of New Pink were hand pollinated with their

own pollen at different times of the day starting from 8 am to 5 pm, at 2 hourly intervals. The pollination leading to fruit development was considered as a reflection of viable stigma.

The study on stigma receptivity was conducted in all the ten varieties. The flowers were pollinated from the day of anthesis to ten consecutive days to find out the correct stigma receptivity period.

3.2.3 Pollen studies

All the *Dendrobium* varieties and species mentioned above were used for this study. Different aspects of pollen such as production, morphology, fertility and germination were studied.

3.2.3.1 Pollen production

Number of pollen grains per pollinium was estimated using a haemocytometer. Mature pollinia were collected from fully opened flowers (3-4 days old). In a glass vial 0.1 ml distilled water containing 0.05 per cent teepol was taken, the pollinium transferred to this vial and crushed gently to get an even dispersion of pollen grains. A drop of this suspension was drawn in a fine pipette and transferred to each of the two counting chambers of an improved Neubauer haemocytometer. Each chamber had an area of 0.0025 mm^2, divided into square millimeter areas. The counting chambers were 0.1 mm in depth so that the volume of solution that can be held in each chamber was 0.00025 ml.

The pollen grains in each of the counting chambers were counted under the low power magnification of the microscope. For each flower ten such estimates were made. Then the number of pollen grains per pollinium was calculated as follows.

a) Volume of each chamber = 0.00025 ml
b) Quantity of solution = 0.1 ml
c) Number of chambers occupied by the solution = 0.1/0.00025 = 400

If x is the number of pollen grains per chamber, total number of pollen grains per pollinium = 400 x.

3.2.3.2 Pollen morphology

Pollinia were collected from the fully opened flowers, 3-4 days after anthesis. Each pollinium was placed in a drop of water to enable proper dispersion of pollen grains to study the pollen morphology.

3.2.3.2.1 Pollen size and shape

Pollen grains were dispersed on a drop of aceto carmine:glycerine mixture (1:1) and mounted on a clean microscopic slide. This was covered with a zero cover glass and kept for 30 minutes for proper staining. Diameter of ten normal, plumpy, well shaped and well stained pollen grains from each variety was measured at random using a standard ocular micrometer under the low power of a microscope. The mean diameter was expressed in microns from which the size was worked out. The shape of the pollen grains was studied under the high power magnification.

3.2.3.3 Pollen fertility

The fertility of pollen grains was estimated by acetocarmine staining technique.

Pollen grains were dispersed in a drop of aceto carmine:glycerine mixture (1:1) on a clean microscopic slide and kept for 30 minutes for proper staining. This was examined under the low power of a microscope. Pollen fertility was estimated by counting fertile and sterile pollen grains separately. Pollen grains, which stained well, looked plumpy, well filled and well shaped were considered as fertile. Unstained, small, or shriveled or mis-shaped pollen grains were counted as sterile (Zirkle, 1937). The observations were made in five

different microscopic fields. This was repeated using three such slides of pollen in each variety and species. Pollen fertility was worked out as follows.

$$\text{Percentage of fertile pollen} = \frac{\text{Number of plumpy and well stained pollen grains}}{\text{Total number of pollen grains}} \times 100$$

3.2.3.4 Pollen germination

Good germination of pollen grains of *Dendrobium* was reported in a medium of 2 per cent sucrose + 1 per cent agar containing 75 ppm boric acid (Varghese, 1995). Based on this, the germination studies were conducted in the following media using the pollen grains of New Pink.

Sucrose – 1%, 2%

Agar – 0.5%, 1% and 2%

Boric acid – 75 mg l^{-1}

Pollinia were collected on the third day after anthesis and the fresh pollen grains were placed in cavity slides containing the media and allowed to rest as hanging drops. These were kept in a desiccator. A humid environment was provided for germination by pouring water in the desiccator. Germination counts were recorded after 24 hours in five different microscopic fields under the low power of the microscope and the germination worked out as follows

$$\text{Pollen germination percentage} = \frac{\text{Number of pollen grains germinated}}{\text{Total number of pollen grains}} \times 100$$

3.3 Compatibility among the *Dendrobium* varieties and wild species

The extent of fruit development (number) in each of the cross was recorded as a measure of compatibility.

All the *Dendrobium* varieties and wild species were used for this study. Self compatibility was assessed in the varieties by using the pollen grains of the same plant. Crosses were made in all possible combinations between the varieties and the species. Since regular flowering was not obtained in the wild species, certain crosses could not be done. The wild species were used as male parents only.

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

New Pink	x New Pink
New Pink	x Emma White
New Pink	x Sonia 28
New Pink	x Pink Tips
New Pink	x Candy Stripe
New Pink	x Sakura Pink
New Pink	x Hieng Beauty
New Pink	x Banyat Pink
New Pink	x Pramott-II
New Pink	x Sabine
New Pink	x *D. crumenatum*
New Pink	x *D. fimbriatum*
New Pink	x *D. moschatum*
New Pink	x *D. chrysanthum*
Emma White	x Emma White
Emma White	x Sonia 28
Emma White	x Pink Tips
Emma White	x Candy Stripe
Emma White	x Sakura Pink
Emma White	x Hieng Beauty

Emma White	x Banyat Pink
Emma White	x Pramott-II
Emma White	x Sabine
Emma White	x New Pink
Emma White	x *D. densiflorum*
Emma White	x *D. fimbriatum*
Emma White	x *D. moschatum*
Emma White	x *D. chrysanthum*
Emma White	x *D. pierardii*
Sonia 28	x Sonia 28
Sonia 28	x Pink Tips
Sonia 28	x Emma White
Sonia 28	x Candy Stripe
Sonia 28	x Sakura Pink
Sonia 28	x Hieng Beauty
Sonia 28	x Banyat Pink
Sonia 28	x Pramott-II
Sonia 28	x Sabine
Sonia 28	x New Pink
Sonia 28	x *D. crumenatum*
Sonia 28	x*D. densiflorum*
Sonia 28	x *D. fimbriatum*
Sonia 28	x *D. moschatum*
Sonia 28	x *D. pierardii*
Pink Tips	x Pink Tips
Pink Tips	x Emma White
Pink Tips	x Sonia 28
Pink Tips	x Candy Stripe
Pink Tips	x Sakura Pink

Pink Tips	x Hieng Beauty
Pink Tips	x Banyat Pink
Pink Tips	x Pramott-II
Pink Tips	x Sabine
Pink Tips	x New Pink
Pink Tips	x *D. crumenatum*
Pink Tips	x *D. densiflorum*
Pink Tips	x *D. fimbriatum*
Pink Tips	x *D. chrysanthum*
Candy Stripe	x Candy Stripe
Candy Stripe	x Emma White
Candy Stripe	x Sonia 28
Candy Stripe	x Pink Tips
Candy Stripe	x Sakura Pink
Candy Stripe	x Hieng Beauty
Candy Stripe	x Banyat Pink
Candy Stripe	x Pramott-II
Candy Stripe	x Sabine
Candy Stripe	x New Pink
Candy Stripe	x *D. densiflorum*
Candy Stripe	x *D. fimbriatum*
Candy Stripe	x *D. moschatum*
Candy Stripe	x *D. chrysanthum*
Sakura Pink	x Sakura Pink
Sakura Pink	x Emma White
Sakura Pink	x Sonia 28
Sakura Pink	x Pink Tips
Sakura Pink	x Candy Stripe
Sakura Pink	x Hieng Beauty

Sakura Pink	x Banyat Pink
Sakura Pink	x Promott-II
Sakura Pink	x Sabine
Sakura Pink	x New Pink
Sakura Pink	x *D. crumenatum*
Sakura Pink	x *D. fimbriatum*
Sakura Pink	x *D. moschatum*
Sakura Pink	x *D. chrysanthum*
Sabine	x Sabine
Sabine	x Emma White
Sabine	x Sonia 28
Sabine	x Pink Tips
Sabine	x Candy Stripe
Sabine	x Sakura Pin
Sabine	x Hieng Beauty
Sabine	x Banyat Pink
Sabine	x Pramott-II
Sabine	x New Pink
Sabine	x *D. crumenatum*
Sabine	x *D. densiflorum*
Sabine	x *D. fimbriatum*
Sabine	x *D. pierardii*
Banyat Pink	x Banyat Pink
Banyat Pink	x Emma White
Banyat Pink	x Sonia 28
Banyat Pink	x Pink Tips
Banyat Pink	x Candy Stripe
Banyat Pink	x Sakura Pink
Banyat Pink	x Hieng Beauty

Banyat Pink	x Pramott-II
Banyat Pink	x Sabine
Banyat Pink	x New Pink
Banyat Pink	x *D. densiflorum*
Banyat Pink	x *D. moschatum*
Banyat Pink	x *D. chrysanthum*
Hieng Beauty	x Hieng Beauty
Hieng Beauty	x Emma White
Hieng Beauty	x Sonia 28
Hieng Beauty	x Pink Tips
Hieng Beauty	x Candy Strips
Hieng Beauty	x Sakura Pink
Hieng Beauty	x Banyat Pink
Hieng Beauty	x Pramott-II
Hieng Beauty	x Sabine
Hieng Beauty	x New Pink
Hieng Beauty	x *D. crumenatum*
Hieng Beauty	x D. densiflorum
Hieng Beauty	x *D. fimbriatum*
Hieng Beauty	x *D. pierardii*
Pramott-II	x Pramott-II
Pramott-II	x Emma White
Pramott-II	x Sonia 28
Pramott-II	x Pink Tips
Pramott-II	x Candy Stripe
Pramott-II	x Sakura Pink
Pramott-II	x Hieng Beauty
Pramott-II	x Banyat Pink
Pramott-II	x Sabine

Pramott-II	x New Pink
Pramott-II	x *D. crumenatum*
Pramott-II	x *D. densiflorum*
Pramott-II	x *D. chrysanthum*
Pramott-II	x *D. pierardii*

The parent plants were marked at the time of flowering. Hand pollination was carried out using a pointed needle at the correct stigma receptivity period of each hybrid as worked out from the preliminary studies. The pollinia from the male parent were collected on a clean butter paper and then transferred carefully to the stigmatic surface of the selected female parent.

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

a) Percentage of pod set
b) Number of days taken for maturity of pod for culturing.
c) Size of pod

3.4 Green pod culture and refinement of embryo culture medium

The pods were harvested at the green pod stage starting from 60 days to 150 days age, to standardise the correct maturity stage for culturing. This was done in New Pink selfed pod. Culturing was done under aseptic conditions.

3.4.1 Preparation of the pod

Pods were rinsed with tap water, teepol and distilled water. Then they were treated with a fungicide, emisan 0.1 per cent, for 20 minutes. Surface sterilization was carried out under perfect aseptic conditions.

Different surface sterilization treatments adopted were the following.

Surface sterilization treatments

Treatment No.	Concentration of Mercuric chloride (%)	Duration of treatment (seconds)
1	0.05	5
2	0.05	10
3	0.10	5
4	0.10	10
5	0.15	5
6	0.15	10
7	0.20	5
8	0.20	10
9	0.10 + (alcohol dip and flaming)	1
10	0.10 + (alcohol dip and flaming)	5
11	Alcohol dip and flaming alone	

The pods were then washed with distilled water, wiped dry and moved to the laminar air flow cabinet. The cabinet was irradiated with UV rays for 20 minutes. The working table was also thoroughly wiped with absolute ethyl alcohol. Sterilized forceps, petridishes and surgical blades were used.

3.4.2 Inoculation

After surface sterilization the two ends of the pods were cut using a sharp sterile blade. The pods were longitudinally split open and the seeds were slowly separated. Then the cotton plug of the culture vessel was removed, the vessel neck flamed and the seeds were dusted into the medium using a sterile foreceps.

The neck of the culture vessel was again flamed and the plug was replaced. The culture vessels were then kept in the culture room.

The following observations were recorded during the seed germination and seedling development.

a) Germination percentage - expressed as very low-VL (below 25%), low-L (25-50%), high-H (50-75%) and very high-VH (above 75%)
b) Number of days for greening
c) Number of days for protocorm formation
d) Number of days for differentiation of leaves
e) Number of days for shoot and root formation
f) Number of days taken for planting out

3.4.3 Culture media

The basal media used in the present study were full strength MS (Murashige and Skoog, 1962), ½ strength MS (50% concentration of inorganic salts), ¼ strength MS (25% concentration of inorganic salts), Vacin and Went (Vacin and Went, 1949) and Knudson-C (Knudson, 1922).

The compositions of the different media are given in Appendices-I, II and III.

3.4.3.1 Media preparation

MS medium was prepared according to the standard procedure given by Gamborg and Shyluck (1981). Stock solutions were prepared by dissolving required quantities of major and minor nutrients in distilled water and were stored under refrigerated conditions. The chemicals used for the preparation of the media were of analytical grade from British Drug House (BDH), Sisco Research Laboratory (SRL), Merck or Sigma.

Specific quantities of stock solutions of chemicals were pipetted out into a 1000 ml standard flask. To this, the required quantity of sucrose, inositol

and phytohormones were added and the volume was made up with double glass distilled water. Then the pH of the solution was checked and adjusted to 5.8 using one per cent NaOH/HCl. To get the solid medium, agar was added and final volume of the medium was made upto 1000 ml. Agar was then dissolved in the medium by heating. Then the medium was poured hot into clean and dry culture vessels. They were plugged with non-absorbent cotton and sterilized at a pressure of 1.1 kg/cm^2 at 121°C for 20-30 minutes.

For preparing Vacin and Went as well as Knudson-C media, the prescribed chemicals for one litre were weighed and dissolved separately in distilled water. Then they were mixed, sucrose and phytohormones were added and the volume made upto 1000 ml. After this the pH was adjusted, agar was added and melted. The media were then poured into culture vessels, and sterilized.

After sterilization the culture vessels were transferred to an air conditioned culture room.

3.4.3.2 Refinement of the medium

The germinating embryos at the protocorm stage were sub-cultured to various media and their effect on the growth of the seedlings was studied.

3.4.3.3 Refinement with growth hormones

Different combinations of auxins (IBA and NAA) and cytokinins (Kinetin and BA) were used for the trial each at 2, 4, 6 and 8 ppm to study their effect.

3.4.3.4 Refinement with media supplements

Certain media supplements like coconut water, banana pulp (Nendran) and peptone were also added to the media used for subculturing and the growth of the seedlings was studied. The concentrations used are listed below:

Name of the media supplement	Concentrations
Peptone	500 and 1000 mg l^{-1}
Coconut water	50, 100, 150 and 200 ml l^{-1}
Banana pulp	20, 40, 60 and 80 g l^{-1}
Adenine	2, 4, 6 and 8 mg l^{-1}

The following observations were recorded.

(a) Number of days for leaf production
(b) Number of days for shoot and root formation
(c) Shoot height
(d) Number of leaves
(e) Leaf length
(f) Number of roots
(g) Root length.

3.4.3.5 Effect of charcoal and sucrose

The influence of charcoal on germination and further growth of seedlings was studied employing different concentrations of charcoal, viz., 0.05, 0.10, 0.15 and 0.20 per cent.

Sucrose concentration was also found to have influence on seed germination and seedling growth. The growth was studied in ½ MS medium provided with reduced levels of sucrose, viz., 3.0, 2.0, 1.0 and 0.5 per cent.

Following were the observations recorded for the above trials.

a) Germination percentage
b) Number of days for greening, protocorm development and shoot and root formation

c) Shoot height

d) Number of leaves

e) Largest leaf length

f) Number of roots

g) Mean root length

3.5 *In vitro* mutagenesis

Gamma ray was used to irradiate the *in vitro* cultures. Different parts subjected to *in vitro* mutagenesis were protocorms, callus and PLBs. Callus was inducted from the protocorms and PLBs were produced from the *in vitro* leaf of the hybrids. The materials were exposed to various doses of gamma irradiation, viz., 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy and 60 Gy. Immediately after irradiation the materials were transferred to a fresh medium.

Following observations were recorded on the irradiated cultures.

a) Survival percentage of irradiated material

b) Days taken for leaf production

c) Days taken for shoot and root production

d) Number of shoots per seedling

e) Mean number of leaves per shoot

f) Days for planting out

Comparative evaluations were made during the development of irradiated and nonirradiated cultures.

3.6 Hardening and field evaluation of the hybrid seedlings

When the seedlings were fully grown and ready for transplanting to the field, they were removed from the culture vessels. For this the cotton plugs of the

culture vessels were removed and distilled water was poured into the vessels. They were shaken and kept aside for 15 minutes. This enabled easy pulling of the seedlings from the agar media without any breakage to the roots. The seedlings were taken out of the culture vessels carefully using a clean foreceps, without causing damage to the roots. The agar adhering to the roots were completely removed by thorough washing under the running tap water. Observations were then recorded on the following morphological characters.

a) Number of shoots
b) Shoot height
c) Number of leaves
d) Largest leaf length
e) Largest leaf width
f) Number of roots
g) Root length

After making observations, the seedlings were kept immersed in a fungicide solution (Bavistin 0.05% + Indofil 0.05%) for 10 minutes. Then they were spread on a blotting paper to remove excess moisture. Small community pots containing 1:1 mixture of charcoal and brick pieces were treated with a fungicide solution. The seedlings were then transplanted to these pots and kept under shade. Required humidity was maintained around the plants. Field establishment of each hybrid was recorded. The growth performance of the seedling plants was observed for a period of one year after planting and observations on the following characters were recorded.

a) Seedling height
b) Number of shoots
c) Number of leaves

d) Length and width of the largest leaf

e) Colour variations, if any, in the leaves and shoots

3.6.1 Effect of nutrient solutions

A study was carried out in the established healthy seedlings to find out the best combination of nutrients for the growth of seedlings. The nutrient solutions tried are given below.

Various nutrient formulations and their time of application

Nutrient solutions	Concentrations	Interval of application
MS stock solution	1/10 strength	alternate day
MS stock solution	1/10 strength	twice/week
MS stock solution	1/10 strength	once/week
30:10:10 Green Care	0.1%	alternate day
30:10:10 Green Care	0.1%	twice/week
30:10:10 Green Care	0.1%	once/week
30:10:10 Green Care	0.2%	alternate day
30:10:10 Green Care	0.2%	twice/week
30:10:10 Green Care	0.2%	once/week
17:17:17 Complex	0.1%	alternate day
17:17:17 Complex	0.1%	twice/week
17:17:17 Complex	0.1%	once/week
30:10:10 Green care (0.1%) +17:17:17 Complex (0.1%)		alternate day

Number of plants per treatment = 6

Number of plants kept for taking observations = 6

Seedlings of the crosses New Pink x Emma White and Emma White x New Pink were used for the trial.

Observations were recorded at monthly intervals, for a period of ten months, on the following morphological characters.

a) Number of shoots

b) Number of leaves

3.6.2 Effect of growth regulators

A trial was carried out in young seedlings to find out the effect of growth hormones like benzyl adenine and gibberellic acid on the growth. Details of the treatments are given below.

Concentration of growth hormones and the interval of application

Growth hormone	Concentration (mg l^{-1})	Interval of application
BA	50	Monthly
BA	50	Fortnightly
BA	25	Monthly
BA	25	Fortnightly
GA_3	10	Monthly
GA_3	10	Fortnightly
GA_3	5	Monthly
GA_3	5	Fortnightly

Number of plants/treatment = 4

Monthly observations were made on the following characters.

a) Number of shoots

b) Shoot height

c) Number of leaves

3.6.3 Flowering of hybrids

The hybrids were maintained in the field by giving regular dose of fertilizers and plant protection chemicals. The fertilizer complex Green Care 30:10:10 @ 0.2 per cent was given twice a week to supply the required nutrients.

When the hybrids started flowering, the following characters were recorded.

a) Days from planting to flowering

b) Days from spike emergence of first flower opening

c) Days for opening of all flowers

d) Number of flowers per spike

e) Flower size

f) Flower colour

g) Spike length

h) Days for wilting of the flowers

Statistical analysis

The data recorded from various experiments were statistically analysed in Complete Randomised Design suggested by Panse and Sukatme (1985).

The morphological and floral characters were also analysed and data processed for the analysis of variance, genotypic and phenotypic coefficients of variation and heritability. The analysis techniques suggested by Fisher (1954) was employed for the estimation of the various genetic parameters.

$$\text{Genotypic coefficient of variation (GCV\%)} = \frac{\sigma g \times 100}{X}$$

$$\text{Phenotypic coefficient of variation (PCV\%)} = \frac{\sigma p \times 100}{X}$$

where

$$X = \frac{\text{Grand total}}{\text{Number of replications x Number of treatments}}$$

σg = Genotypic standard deviation

σp = Phenotypic standard deviation

$$\text{Heritability } (h^2) = \frac{\sigma g^2}{\sigma p^2}$$

σg^2 = Genotypic variance

σp^2 = Phenotypic variance

The genotypic and phenotypic correlation coefficients were worked out to study the extent of association between the characters adopting the formula suggested by Johnson *et al.* (1955).


RESULTS

RESULTS

The results of the present investigations entitled "Improvement of *Dendrobium* through hybridisation and *in vitro* mutagenesis" are presented under the following heads

1. Vegetative and floral characters of the *Dendrobium* varieties
2. Variation and correlation studies
3. Genotypic path coefficient analysis
4. Floral morphology
5. Floral biology
6. Pollen studies
7. Compatibility studies in different *Dendrobium* varieties and wild species
8. Green pod culture and refinement of culture media
9. *In vitro* mutagenesis
10. Planting out and hardening
11. Field evaluation of hybrid seedlings

4.1 Vegetative and floral characters

Morphological description of the ten *Dendrobium* varieties used in the study is presented in Table 1.

4.1.1 Vegetative parameters

Among the hybrids, New Pink had the maximum number of shoots (9.00) which was significantly different from all others except Hieng Beauty. Emma White, Sakura Pink and Pramott-II had the least number of shoots (5.67, each). The plant height ranged from 22.17 cm (Sakura Pink) to 30.17 cm (Sonia 28) and significant difference was noticed among the different hybrids. Sonia 28, New Pink and Hieng Beauty had almost similar heights (27.33-30.17 cm) and all other hybrids had shoots of almost equal height. Regarding the

Table 1. Morphological and floral description of *Dendrobium* varieties

Sl. No.	Variety	Number of shoots	Height of shoots	Total number of leaves	Average length of leaf (cm)	Average breadth of leaf (cm)	Days from spike emergence to first flower opening	Number of flowers/ spike	Size of flowers	
									Length (cm)	Breadth (cm)
1	Hieng Beauty	7.67	27.33	32.33	15.17	4.03	34.00	7.00	6.67	6.53
2	Emma White	5.67	23.17	32.33	15.17	4.77	37.67	7.00	5.73	5.73
3	Pink Tips	7.00	24.17	27.33	14.17	3.77	38.33	8.33	6.47	6.47
4	Sonia-28	7.33	30.17	32.67	13.50	3.30	35.00	9.67	7.70	7.43
5	Candy Stripe	7.33	28.50	33.33	14.87	4.50	40.00	11.67	7.00	6.83
6	New Pink	9.00	29.07	29.00	15.13	4.13	40.67	7.33	8.27	7.90
7	Pramott-II	5.67	23.33	23.33	14.93	3.97	34.00	6.33	6.07	5.63
8	Sabine	7.00	22.00	22.33	12.90	4.00	40.00	5.67	8.10	7.20
9	Banyat Pink	7.00	22.50	25.00	14.40	3.27	33.33	5.67	7.97	7.53
10	Sakura Pink	5.67	22.17	21.33	13.57	3.07	37.33	7.33	5.60	5.60
	CD(0.05)	1.12	2.45	4.39	0.59	0.20	1.96	1.55	0.31	0.25

Contd.

Table 1. Morphological and floral description of *Dendrobium* varieties

Sl. No.	Variety	Number of shoots	Height of shoots	Total number of leaves	Average length of leaf (cm)	Average breadth of leaf (cm)	Days from spike emergence to first flower opening	Number of flowers/ spike	Size of flowers	
									Length (cm)	Breadth (cm)
1	Hieng Beauty	7.67	27.33	32.33	15.17	4.03	34.00	7.00	6.67	6.53
2	Emma White	5.67	23.17	32.33	15.17	4.77	37.67	7.00	5.73	5.73
3	Pink Tips	7.00	24.17	27.33	14.17	3.77	38.33	8.33	6.47	6.47
4	Sonia-28	7.33	30.17	32.67	13.50	3.30	35.00	9.67	7.70	7.43
5	Candy Stripe	7.33	28.50	33.33	14.87	4.50	40.00	11.67	7.00	6.83
6	New Pink	9.00	29.07	29.00	15.13	4.13	40.67	7.33	8.27	7.90
7	Pramott-II	5.67	23.33	23.33	14.93	3.97	34.00	6.33	6.07	5.63
8	Sabine	7.00	22.00	22.33	12.90	4.00	40.00	5.67	8.10	7.20
9	Banyat Pink	7.00	22.50	25.00	14.40	3.27	33.33	5.67	7.97	7.53
10	Sakura Pink	5.67	22.17	21.33	13.57	3.07	37.33	7.33	5.60	5.60
	CD(0.05)	1.12	2.45	4.39	0.59	0.20	1.96	1.55	0.31	0.25

Contd.

Table 1. Continued

Sl. No.	Variety	Length of inflorescence (cm)	Days for opening of all florets	Days for wilting of all florets	Internodal length		Vase life (days)	Period of blooming	Colour of flowers
					First (cm)	Last (cm)			
1	Hieng Beauty	31.50	14.67	43.33	3.47	2.10	16.33	Throughout the year	White with deep purple shade towards innerside of petals, sepals and lip
2	Emma White	29.33	14.33	49.67	3.20	2.07	16.33	Throughout the year	White sepals, petals and lip
3	Pink Tips	28.50	18.33	56.33	2.97	1.87	15.33	Mostly April to November	Very light pink petals, sepals and lip. Petals larger than sepal.
4	Sonia-28	35.60	18.00	57.00	3.07	2.27	18.00	Throughout the year	Deep purple petals and lip with white colouration towards the centre. Sepals light purple than petals and lip with more white colouration towards centre - petals a little larger than sepals.
5	Candy Stripe	34.50	19.33	43.33	3.93	2.20	13.00	Throughout the year	Deep purple lines on light purple petals, sepals and lip is deep purple.
6	New Pink	31.83	12.33	55.00	3.03	1.90	18.33	Throughout the year	Deep purple petals and lip, light purple sepals. White towards the centre. Petals larger than sepals
7	Pramott-II	29.17	13.67	44.67	2.90	1.70	13.33	More during June to December	Deep purple petals and light purple sepals, lip is deep purple. Petals and sepals of almost equal size.
8	Sabine	27.27	11.67	42.33	3.13	2.50	15.00	Mostly during April to October	Intensively deep purple and velvetty petals, sepals and lip. Phalaenopsis type broad petals and sepals.
9	Banyat Pink	22.83	10.67	39.33	2.60	2.37	17.00	Mostly during June to December	Petals and lip deep purple, broad petals, sepals are lighter than petals.
10	Sakura Pink	24.50	13.33	42.67	2.60	1.60	15.67	Mostly during May to October	Light purple petals sepals and lip. Petals and sepals separate and of almost equal size.
	CD (0.05)	3.16	2.28	3.36	0.23	0.34	1.35		

number of leaves, Sakura Pink had the least number (21.33) and Candy Stripe, the highest (33.33).

There was significant variation among the hybrids in leaf size also. Hieng Beauty, Emma White and New Pink had the maximum leaf length (15.17 cm, each) and Emma White and Candy Stripe, the maximum leaf breadth (4.77 cm and 4.50 cm, respectively). Minimum length of leaf was recorded in Sabine (12.90 cm) and minimum breadth in Sakura Pink (3.07 cm).

4.1.2 Floral characters

Candy Stripe, New Pink and Sabine took maximum number of days (approximately 40 days) from spike emergence to first flower opening and the least by Sakura Pink (31.33 days). Studies on the other floral characters revealed that Candy Stripe had the maximum number of flowers (11.67) which was significantly different from all others. The least number was borne by Sabine and Banyat Pink (5.67). New Pink had the maximum sized flowers (8.27 x 7.90 cm) closely followed by Sabine (8.10 x 7.20 cm) and Banyat Pink (7.97 x 7.53 cm). Smallest flowers among the varieties were those of Sakura Pink and Emma White (5.60 cm and 5.73 cm, respectively) which were significantly different from others.

Sonia 28 and Candy Stripe had longest spikes (35.6 and 34.5 cm, respectively) followed by New Pink and Hieng Beauty. The smallest spike was that of Banyat Pink (22.83 cm).

Maximum time for opening of all florets was taken by Candy Stripe (19.33 days), followed by Pink Tips and Sonia 28 (18.33 and 18.00 days, respectively). Banyat Pink took minimum time (10.67 days) for opening of all florets.

The flowers lasted for maximum number of days in Sonia 28 and Pink Tips (57.00 and 56.33 days, respectively). Banyat Pink took only 39.33 days for

the wilting of all florets. The internodal length between the first two flowers ranged from 2.60 cm (Sakura Pink) to 3.93 cm (Candy Stripe) and the internodal length between the last two flowers ranged from 1.60 cm (Banyat Pink) to 2.50 cm (Sabine).

A study on the vase life of the spike till the wilting of the first (basal) floret, revealed that the vase life ranged from 13 days in Candy Stripe, to a maximum of 18.33 days in New Pink. Significant differences were noticed among the varieties in this character also.

Year round flowering occurred in the varieties Hieng Beauty, Emma White, Sonia 28, Candy Stripe and New Pink. Pink Tips and Sabine had maximum flowering during April to November. In Banyat Pink and Pramott-II flowering was observed mostly during June to December. Sakura Pink flowered mostly during May to October.

4.2 Variation and correlation studies

4.2.1 Coefficients of variation

The genotypic and phenotypic coefficients of variation are presented in Table 2.

In general PCV was slightly higher than GCV for all the characters. Both PCV and GCV were highest for number of flowers per spike (PCV = 26.39 and GCV = 23.55), followed by days for opening of all florets (PCV = 21.64, GCV = 19.64) and number of shoots (PCV = 20.79, GCV = 15.38). The lowest coefficients of variations were observed for length of leaf (PCV = 6.01, GCV = 5.52).

4.2.2 Heritability

The data pertaining to the heritability of 15 characters are presented in Table 2.

Table 2. Range, Mean, PCV, GCV and heritability for 15 characters in *Dendrobium*

Sl. No.	Characters	Range	Mean	PCV (%)	GCV (%)	Heritability (%)
1	No. of shoots	5.67-9.00	7.13	20.79	15.38	54.7
2	Plant height (cm)	22.00-30.17	25.24	13.38	12.13	82.2
3	No. of leaves	21.33-33.33	27.90	18.34	15.89	75.0
4	Length of leaf (cm)	12.90-15.17	14.30	6.01	5.52	84.2
5	Breadth of leaf (cm)	3.07-4.77	3.88	14.24	13.92	95.6
6	Number of days taken from spike emergence to flowering	31.33-40.67	36.43	9.41	8.88	88.9
7	No. of flowers per spike	5.67-11.67	29.50	26.39	23.55	79.7
8	Length of flower (cm)	5.60-8.27	6.96	14.57	14.34	96.9
9	Breadth of flower (cm)	5.60-7.90	6.69	12.64	12.45	97.0
10	Length of inflorescence (cm)	22.83-35.60	29.50	14.63	13.23	81.8
11	Days for opening of all florets	10.67-19.33	14.63	21.64	19.64	82.4
12	Days for wilting of all florets	39.33-57.00	47.37	14.28	13.67	91.6
13	Internodal length - first flower (cm)	2.60-3.93	3.13	11.90	11.13	87.5
14	Internodal length - last flower (cm)	1.60-2.50	2.06	16.13	12.95	64.5
15	Vase life up to wilting of first flower (days)	13.00-18.00	15.83	11.87	10.78	82.5

The heritability was of moderate to high magnitude. The characters which showed maximum heritability were breadth of flower (97.0), length of flower (96.9) and breadth of leaf (95.6). Heritability was the lowest for number of shoots (54.7).

4.2.3 Correlation studies

The genotypic and phenotypic correlations of various morphological and floral characters with flowers per spike were worked out (Tables 3 & 4). The characters having significant correlation with flowers per spike were mean height of shoots, total number of leaves, length of inflorescence, days for opening of all florets and inter nodal length between first two flowers. All the characters showed positive correlation. Days for opening of all florets registered the highest positive correlation (r_g = 0.92, r_p = 0.84). This was followed by length of inflorescence (r_g = 0.74, r_p = 70). Lowest positive correlation was observed for mean width of leaves (r_g = 0.19, r_p = 0.16). The only character showing negative correlation was vase life up to the wilting of first flower (r_g = -0.25, r_p = -0.14).

4.2.4 Inter-correlation among different characters

In general, genotypic correlation coefficients were higher than phenotypic correlation coefficients. Highest positive correlation among all the characters was observed between length of flowers and breadth of flowers (r_g = 0.97), r_p = 0.95). Number of shoots had highest positive correlation with breadth of flowers (r_g = 0.75, r_p = 0.58). Mean height of shoots was highly correlated with length of inflorescence (r_g = 0.96, r_p = 0.76). Total number of leaves showed highest positive correlation with length of inflorescence (r_g = 0.83, r_p = 0.71), followed by number of flowers per spike and days for opening of all flowers. Days from spike emergence to first flower opening registered significant positive correlation with average breadth of leaves and number of shoots. Length of flower showed highest positive correlation with breadth of flower (r_g = 0.97,

Table 3. Genotypic correlation coefficients (r_g) among different characters in *Dendrobium*

Cha-racter	No. of shoots	Mean weight of shoots	Total no. of leaves	Mean length of leaves	Mean breadth of leaves	Days from spike emerg-ence to first flower opening	No. of flower per spike	Length of flowers	Breadth of flowers	Length of inflores cence	Days for open-ing of all florets	Days for wilting of all florets	Internod al length - first flower	Internod al length - last flower	Vase life upto wilting of first flower
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	-														
2	0.587*	-													
3	0.376	0.834**	-												
4	0.088	0.304	0.563*	-											
5	0.051	0.159	0.546*	0.593*	-										
6	0.557*	0.303	0.359	0.085	0.683**	-									
7	0.276	0.726**	0.684**	0.086	0.185	0.298	-								
8	0.620*	0.389	0.045	-0.247	-0.119	0.446	-								
9	0.746**	0.529*	0.209	-0.155	-0.129	0.442	-0.062	0.969**	-						
10	0.361	0.961**	0.833**	0.258	0.436	0.443	0.743**	0.149	0.232	-					
11	0.264	0.566*	0.656**	0.048	0.186	0.207	0.924**	-0.260	-0.124	0.716**	-				
12	0.589*	0.536*	0.429	0.032	0.065	0.376	0.351	0.093	0.217	0.550*	0.466	-			
13	0.183	0.537*	0.770**	0.594*	0.614**	0.363	0.611**	0.091	0.159	0.594*	0.509*	-0.172	-		
14	0.236	0.114	0.249	-0.326	0.135	0.378	-0.017	0.728*	0.666**	0.132	-0.092	0.219	0.367	-	
15	0.421	0.345	0.245	-0.051	-0.330	-0.082	-0.246	0.447	0.554*		-0.312	0.476	-0.339	0.159	-

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

Table 4. Phenotypic correlation coefficients (r_p) among different characters in *Dendrobium*

Character	No. of shoots	Mean weight of shoots	Total no. of leaves	Mean length of leaves	Mean breadth of leaves	Days from spike emergence to first flower opening	No. of flower per spike	Length of flowers	Breadth of flowers	Length of inflore scence	Days for opening of all florets	Days for wilting of all florets	Internodal length - first flower	Internodal length - last flower	Vase life upto wilting of first flower
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	-														
2	0.475	-													
3	0.231	0.634*	-												
4	0.080	0.241	0.410	-											
5	0.003	0.138	0.441	0.584	-										
6	0.515	0.296	0.258	0.112	0.654*	-									
7	0.241	0.615*	0.559	0.089	0.159	0.277	-								
8	0.496	0.342	0.081	-0.233	-0.128	0.411	-0.015	-							
9	0.582*	0.461	0.223	-0.147	-0.138	0.405	0.106	0.949**	-						
10	0.297	0.763*	0.709*	0.243	0.385	0.393	0.703	0.170	0.241	-					
11	0.278	0.504	0.545	0.093	0.193	0.242	0.838*	-0.213	-0.097	0.651*	-				
12	0.462	0.507	0.386	0.038	0.071	0.345	0.358	0.110	0.223	0.510	0.493	-			
13	0.160	0.469	0.679*	0.474	0.550	0.299	0.537	0.099	0.164	0.527	0.439	-0.135	-		
14	0.069	0.123	0.284	-0.245	0.092	0.276	0.028	0.612*	0.577*	0.094	-0.046	-0.139	0.360	-	
15	0.266	0.288	0.172	-0.017	-0.279	-0.057	-0.139	0.412	0.512	0.005	0.227	0.440	-0.299	0.163	-

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

r_p = 0.95), followed by internodal length between last two flowers (r_g = 0.73, r_p = 0.61). Breadth of flowers showed high significant correlation with number of shoots and the internodal length between last two flowers, besides the length of flowers.

Length of inflorescence was positively correlated with days for opening of all florets and total number of leaves, besides mean height of shoots. The internodal length of first flower recorded highest correlation with total number of leaves followed by mean breadth of leaves and number of flower per spike. Vase life up to wilting of first flower registered negative correlation with some characters and the internodal length of first flower had highest magnitude among them. Vase life had (r_g = 0.33, r_p = 0.29) highest positive correlation with breadth of flowers.

4.3 Genotypic path coefficient analysis

Fifteen characters, which showed significant correlation with yield (number of flowers), were selected for path coefficient analysis.

The direct and indirect effects of the component characters on yield characters i.e., the number of florets per spike are furnished in Table 5.

Days from spike emergence to first flower opening had the highest positive direct effect (1.596) followed by breadth of flower (0.815). Other characters, which had positive direct effect were days for opening of all florets (0.450), length of inflorescence (0.291), total number of leaves (0.070) and average length of leaves (0.010). But length of flower, average breadth of leaves, number of shoots and height of shoots exhibited a negative direct effect (-1.151, -1.419, -0.659, -0.004, respectively).

Number of shoots had high indirect positive effect through days from spike emergence to flower opening (0.893) followed by breadth of flower (0.616). But it had high negative indirect effect through length of flower (-0.722).

Table 5. Genotypic path coefficient analysis of florets/spike

Character	No. of shoots	Mean height of shoot	Total leaf number	Mean leaf length	Mean leaf breadth	Days from spike emerg-ence to first flower opening	Length of flower	Breadth of flower	Length of inflores-cence	Days for opening of all flowers	Days for wilting of all flowers	Inter-nodal length (first)	Inter-nodal length (last)	Vase life
No. of shoots	**-0.659**	-0.002	0.027	0.001	-0.075	0.893	-0.722	0.616	0.106	0.119	-0.221	0.138	-0.099	0.157
Mean height of shoot	-0.391	**-0.004**	0.059	0.003	-0.228	0.485	-0.451	0.434	0.283	0.236	-0.199	0.404	-0.046	0.127
Total leaf number	-0.254	-0.003	**0.070**	0.006	-0.783	0.580	-0.050	0.170	0.245	0.298	-0.160	0.579	-0.099	0.091
Mean leaf length	-0.058	-0.001	0.040	**0.010**	-0.843	0.133	0.285	-0.127	0.076	0.021	-0.012	0.448	0.133	-0.019
Mean leaf breadth	-0.035	-0.001	0.039	0.006	**-1.419**	1.093	0.137	-0.105	0.128	0.084	-0.024	0.461	-0.055	-0.121
Days from spike emergence to first flower opening	-0.369	-0.001	0.026	0.001	-0.972	**1.596**	-0.516	0.362	0.130	0.093	-0.140	0.274	0.154	-0.030
Length of flower	-0.414	-0.001	0.003	-0.003	0.169	0.715	**-1.151**	0.790	0.043	-0.118	-0.034	0.068	-0.295	0.163
Breadth of flower	-0.498	-0.002	0.015	-0.002	0.183	0.708	-1.116	**0.815**	0.068	-0.057	-0.081	0.119	-0.270	0.202
Length of inflorescence	-0.240	-0.004	0.059	0.003	-0.623	0.710	-0.171	0.190	**0.291**	0.324	-0.204	0.446	-0.054	0.018
Days for opening of all flowers	-0.174	-0.002	0.047	0.000	-0.264	0.329	0.302	-0.102	0.210	**0.450**	-0.172	0.383	0.038	-0.115
Days for wilting of all flowers	-0.393	-0.002	0.030	0.000	-0.092	0.602	-0.106	0.177	0.161	0.210	**-0.370**	-0.130	0.090	0.174
Internodal length (first)	-0.122	-0.002	0.055	0.006	-0.876	0.585	-0.104	0.130	0.174	0.231	0.064	**0.747**	-0.148	-0.124
Internodal length (last)	-0.163	0.000	0.017	-0.003	-0.195	0.613	-0.845	0.547	0.039	-0.042	0.083	0.275	**-0.402**	0.058
Vase life	-0.284	-0.001	0.018	0.001	0.472	-0.133	-0.516	0.453	0.015	-0.142	-0.188	-0.255	-0.064	**0.364**

Digonal values (in bold) indicate direct effect

Residual = -0.1002

The indirect effect of all characters through number of shoots was negative.

The days taken from spike emergence to first flower opening had highest indirect positive effect through breadth of flower (0.362) followed by length of first internode (0.274), length of inflorescence (0.130) and very little positive effect through days for opening of all florets (0.093). It had a high negative indirect effect through breadth of leaf (-0.972).

Eventhough average length of leaf had very little direct effect (0.010), it had high indirect effect through breadth of leaf (-0.843) followed by breadth of flower (-0.127), vase life (-0.019) and days for wilting of all flowers (-0.012). It had indirect positive effect with all other characters.

Length of flower had highest positive indirect effect through breadth of flower (0.790) and days from spike emergence to first flower opening (0.715). Breadth of flowers had highest indirect negative effect through length of flower (-01.116) and highest positive indirect effect through days from spike emergence to first flower opening (0.708).

Though the direct effect of length of inflorescence was only 0.291, it had a high indirect positive effect through days from spike emergence to first flower opening (0.710).

Internodal length between first two flowers had indirect positive effect through the days for spike emergence to first flower opening (0.585) and indirect negative effect through average breadth of leaves (-0.876).

The internodal length between last two flowers had a negative indirect effect through length of flower (-0.845) and a positive indirect effect through the days from spike emergence to first flower opening (0.613) followed by breadth of flower (0.547).

Vase life had positive indirect effect through the days from spike emergence to first flower opening (0.472) and breadth of flower (0.453). But it had negative indirect effect through length of flower (-0.516).

4.4 Floral morphology

Dendrobiums are sympodial epiphytes with flowers borne on racemose inflorescence. The individual florets are attached directly to the axis.

The 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, some times referred as petaloid sepals. Of the inner petals, lateral two are similar and to a little broader than the sepals. The third petal is highly modified and enlarged and is called the 'lip' or 'labellum'. The two identical petals spread outward and the lip ruffles downward. It is three lobed and is embellished with its own markings of colour and is fantastically decorated with fringed margins and feathery out growths.

The gynostemium or column 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 pollinium. The pollinium lies in a cavity covered by a fringed cap. There are two pairs of pollinia in dendrobium. Just below the anther, is the stigma which is separated by a partition structure called rostellum. The stigma is a shiny depression filled with extremely sticky fluid. A typical dendrobium flower is shown in Fig.1.

Studies on the floral characters of the *Dendrobium* varieties revealed that the flowers opened acropetally after the emergence of the inflorescence. It took approximately one month from the inflorescence emergence to first flower opening which varied according to the varieties.

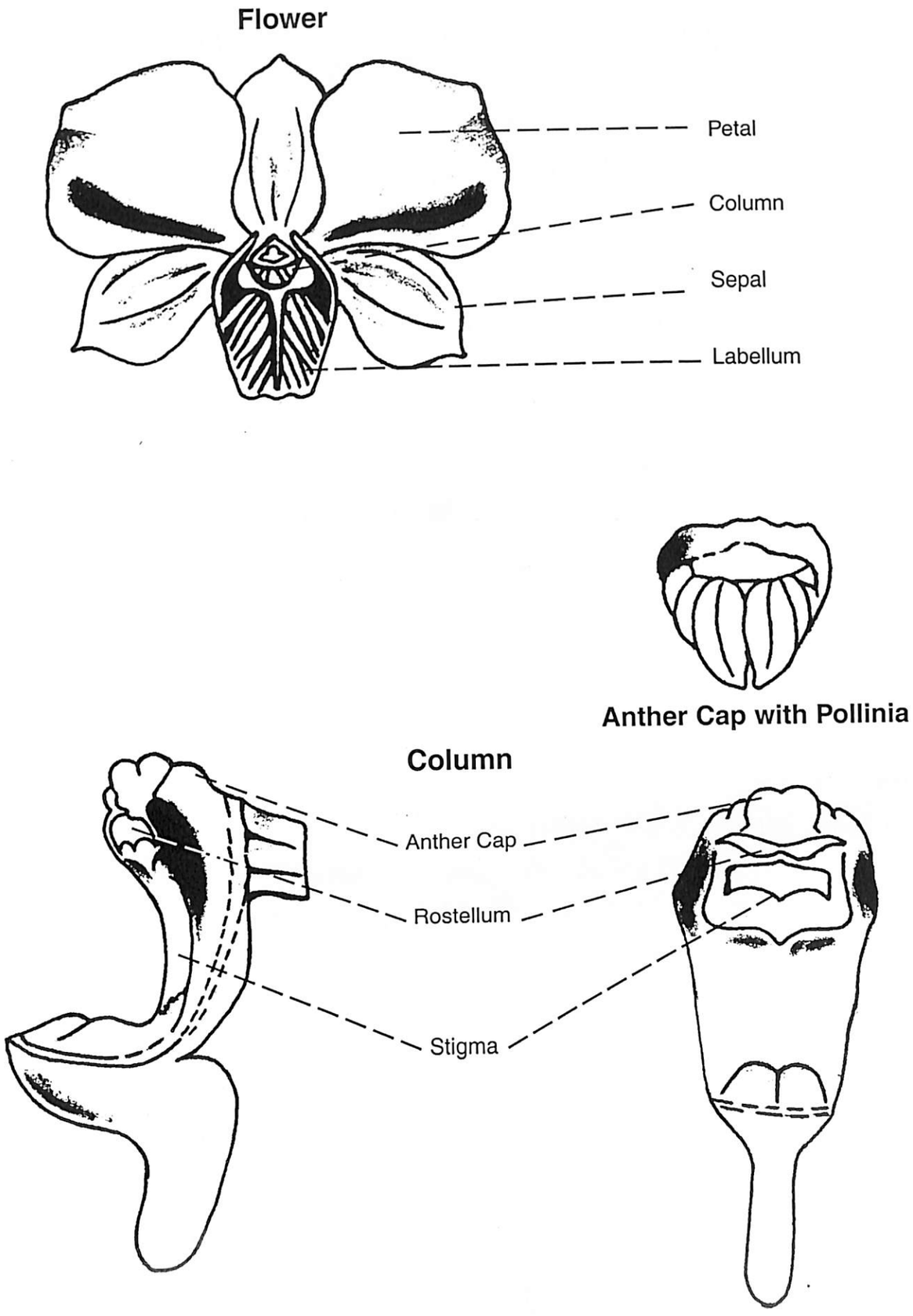


Fig. 1 Floral parts of a *Dendrobium* flower

4.5 Floral biology

Anthesis and stigma receptivity time noticed in the varieties are presented below.

4.5.1 Anthesis

Data on anthesis time are presented in Table 6.

In all the *Dendrobium* varieties studied, flower opening occurred during the day time. The time of opening started from 7.30 am and lasted up to 2.30 pm which varied in different varieties. It took almost a day for the complete opening of a flower, depending on the variety. A varietal difference could be observed in the opening time of the flowers (Table 6) and all the flowers in an inflorescence did not open at the same time of the day. The time interval between the opening of the florets of a spike was also not uniform.

In Candy Stripe, Pink Tips and Emma White, the flower opening started from 7.30 am to 11.30 am where as the flowers of Sabine, Sonia-28, Pramott-II, New Pink and Hieng Beauty started opening around 8.00 to 8.30 am and lasted up to 11.30 am. In Sakura Pink, the opening started at 9.00 am and lasted up to 12.30 pm and in Banyat Pink the opening started during 11.00 am to 2.30 pm. The opening of successive flowers was found from base to apex. In Hieng Beauty, Emma White, Candy Stripe and Sakura Pink, the flowers of the inflorescence opened almost uniformly at an interval of 24 hours while those of New Pink, Sonia-28, Pramott-II, Pink Tips, Sabine and Banyat Pink, took almost two days to complete the anthesis of each flower bud. The longevity of each flower on the plant was for 35 to 45 days.

4.5.2 Stigma receptivity

Stigma receptivity after anthesis, based on successful pod set in each of the variety studied, is presented in Table 6.

Table 6. Anthesis time and stigma receptivity in *Dendrobium* varieties

Variety	Anthesis time	Maximum stigma receptivity
Candy stripe	7.30 am - 11.30 am	2^{nd} - 5^{th} day
Emma White	7.30 am - 11.30 am	2^{nd} - 5^{th} day
Hieng Beauty	8.30 am - 11.30 am	2^{nd} - 6^{th} day
New Pink	8.30 am - 11.30 am	3^{rd} - 10^{th} day
Pramott-II	8.30 am - 11.00 am	2^{nd} - 6^{th} day
Pink Tips	7.30 am - 11.00 am	2^{nd} - 8^{th} day
Sabine	8.00 am - 11.00 am	2^{nd} - 5^{th} day
Sakura Pink	9.00 am - 12.30 pm	2^{nd} - 6^{th} day
Sonia 28	8.00 am - 11.00 am	2^{nd} - 6^{th} day
Banyat Pink	11.00 am - 2.30 pm	1^{st} - 5^{th} day

Table 7. Fertility pattern of New Pink flowers, hand pollinated at different periods after flower opening

Time of pollination	Total number of flowers pollinated	Pod set	
		Number of flowers setting pods	Percentage of pod set
8 am	5	4	80
9 am	5	5	100
11 am	5	5	100
1 pm	5	4	80
3 pm	5	5	100
5 pm	5	4	80

Irrespective of the time of the day at which pollination was carried out, successful pod set could be obtained from 8 am to 5 pm (Table 7).

Maximum stigma receptivity period as observed by pod set was for New Pink (3rd to 10th day after anthesis) followed by Pink Tips (2nd to 8th day after anthesis). Almost all the hybrids were found to have receptive stigma from the second day of anthesis. In Banyat Pink alone the stigma became receptive on the first day of anthesis and remained receptive till fifth day.

Maximum stigma receptivity was observed in all the hybrids during second to fifth day after anthesis.

4.6 Pollen studies

Pollen characters like pollen production, pollen size and pollen viability differed considerably in the varieties as well as wild species under study. Data in this regard are presented in Table 8.

4.6.1 Pollen production

Pollen production per anther showed significant variations among different *Dendrobium* varieties and wild species. It was the lowest in *Dendrobium pierardii* (2720) and maximum pollen production was in Emma White ((13120). New Pink also had a substantial pollen out put (11040).

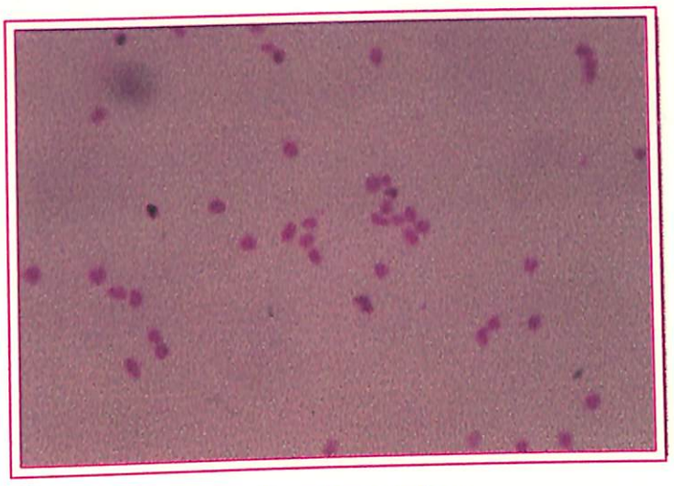
4.6.2 Pollen morphology

Pollen grains in *Dendrobium* were found in agglutinated masses called pollinia. The flower consisted of two pollinia with two lobes each. The pollinium was oval in shape. The pollinia appeared to be yellow coloured in all the varieties except in Hieng Beauty in which it was light yellow in colour. The pollen grains were found as spherical to rectangular in shape and were found to exist as tetrads (Plate 16). The pollen grains of the various hybrids and wild species were almost similar in shape.

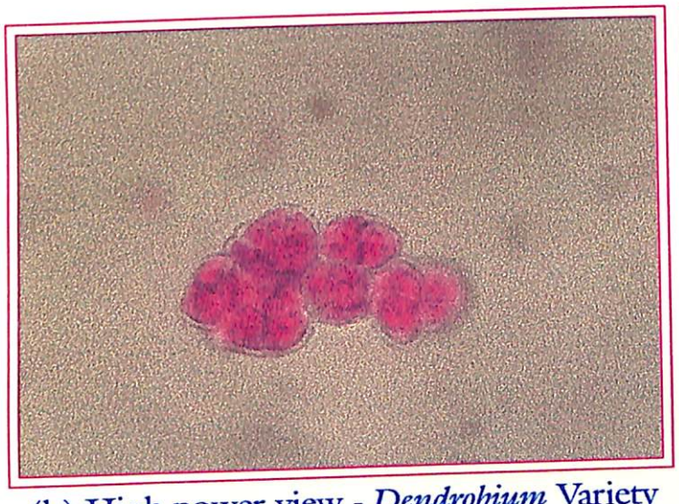
Table 8. Pollen characters of *Dendrobium* varieties and wild species

Variety/Species	Pollen fertility (%)	Pollen germination (%)	Pollen diameter (μ)	Pollen production per pollinium (%)
Candy Stripe	74.73	64.18	23.34	8800.00
Emma White	86.09	80.63	34.82	13120.00
Hieng Beauty	71.08	59.43	31.90	7760.00
New Pink	91.93	75.00	44.35	11040.00
Pink Tips	82.85	66.25	32.68	7200.00
Pramott-II	62.61	48.35	29.63	4880.00
Sabine	73.85	57.06	47.46	4160.00
Sakura Pink	73.19	60.01	34.62	5960.00
Sonia 28	78.98	69.76	42.01	9600.00
Banyat Pink	68.02	56.48	34.23	7920.00
D. crumenatum	68.33	20.92	28.66	3040.00
D. densifolium	65.61	34.73	23.34	4000.00
D. fimbriatum	54.34	32.55	19.84	4080.00
D. moschatum	64.83	20.94	18.68	3800.00
D. chrysanthum	46.77	20.71	20.00	4560.00
D. pierardii	68.27	14.22	24.51	2720.00
CD(0.05)	18.00	14.87	8.65	1553.48
SEm±	6.21	5.14	3.06	549.32

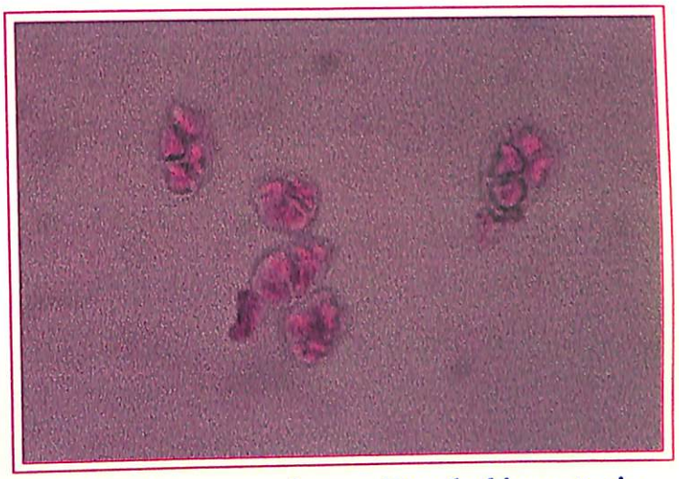
Plate - 16 Pollen grains of *Dendrobium*



(a) *Low power view*



(b) High power view - *Dendrobium* Variety



(c) High power view - *Dendrobium species*

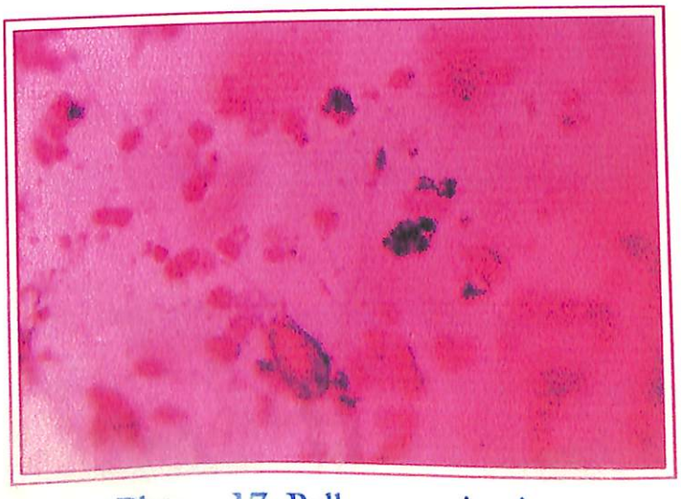


Plate - 17 Pollen germination

4.6.3 Pollen size

Significant difference could be noticed with regard to pollen size in different varieties and wild species (Table 8). It ranged from 18.68 µ in *D. moschatum*, to 47.46 µ in Sabine. Comparatively larger size was observed in New Pink (44.35 µ) and Sonia 28 (42.01 µ). It was observed that wild *Dendrobium* species had comparatively small sized pollens.

4.6.4 Pollen fertility

Pollen fertility was found to be maximum (91.93%) in New Pink followed by Emma White (86.09%) and Pink Tips (82.85%). Significantly higher percentage of fertile pollens were also observed in Sonia 28 (78.98%), Candy Stripe (74.73%), Sabine (73.85%) and Sakura Pink (73.19%). Fertility was the lowest in *D. chrysanthum* (46.77%).

4.6.5 Standardisation of media for pollen germination

The germination percentage of pollen grains varied significantly with different levels of sucrose and agar (Table 9). Maximum germination was obtained with 2 per cent sucrose + 1 per cent agar (80.33%), which was on par with the treatment 2 per cent sucrose + 2 per cent agar (78.00%) (Plate 17). These two were significantly superior to all other treatments. Addition of boric acid 75 mg l^{-1} could not increase the germination percentage of pollen grains except in 1 per cent sucrose + 1 per cent agar, which enhanced the germination from 26.67 per cent to 41.67 per cent. Lowest germination percentage was with 1 per cent sucrose + 1 per cent agar (26.67%).

4.6.6 Percentage germination of pollen grains in *Dendrobium* varieties and wild species

The previous trial indicated the superiority of sucrose 2 per cent + agar 1 per cent as well as sucrose 2 per cent + agar 2 per cent for pollen germination.

Table 9. Effect of different media on pollen germination of Emma White

Treatments			Germination percentage
Sucrose (%)	Agar (%)	Boric acid (mg l^{-1})	
1.0	0.5	-	61.54
1.0	1.0	-	26.67
2.0	1.0	-	80.33
2.0	2.0	-	78.00
1.0	0.5	75	45.96
1.0	1.0	75	41.67
2.0	1.0	75	63.64
2.0	2.00	75	33.33
CD (0.05)			8.09
SEm±			2.97

Using the former medium germination tests were conducted in the various *Dendrobium* varieties and wild species and the data are presented in Table 8.

The varieties and wild species showed significant differences in the germination percentage.

The results indicated that Emma White had the maximum germination percentage (80.63%) and the minimum was that of *D. pierardii* (14.22%). In general, lower percentage of pollen germination was noticed for wild species.

4.7 Compatibility studies

4.7.1 Self compatibility in different *Dendrobium* varieties

Self pollination was attempted in the ten *Dendrobium* varieties. The details on pod set are presented in Table 10. Five flowers were used for each selfing and the pod set was noted (Plates 18 & 19). Out of the ten varieties, only in Hieng Beauty, selfing was not found successful. Bulging of the ovary was noticed as in the case of set pods, but after one month, yellowing was seen leading to the dropping of the full flower.

In self compatibility studies, maximum pod set (100%) was observed in Pink Tips, Emma White and New Pink confirming to successful self pollination. Sakura Pink and Sonia 28 had 80 per cent pod set whereas in Candy Stripe, Pramott-II and Banyat Pink, the self compatibility was to the extent of 60 per cent. Sabine had the least pod set (40%) on selfing.

Slight variation was observed in the time taken for the maturity of the pods for culturing and it varied from 95 days in Candy Stripe to 120 days in Pink Tips.

Table 10. Self compatibility in *Dendrobium* varieties

Name of varieties	No. of flowers pollinated	No. of flowers setting pods	Pod set percentage	Days for maturity for culturing	Remarks
Sonia 28	5	4	80	105	
Hieng Beauty	5	-	-	-	Three flowers had bulging of ovary but after one month yellowing was noticed and pods dropped
Candy Stripe	5	3	60	95	
Pink Tips	5	5	100	120	
Emma White	5	5	100	105	
Pramott-II	5	3	60	105	
Sakura Pink	5	4	80	110	
Banyat Pink	5	3	60	110	
Sabine	5	2	40	120	
New Pink	5	5	100	105	

4.7.2 Cross compatibility studies

The compatibility chart of the varieties and species is presented in Table 11. All the ten varieties and six wild species were used in all possible combinations.

The response of different varieties to crossing was different. Emma White had the maximum cross compatibility (Table 11) and it was compatible with all the nine varieties and four species. Its incompatibility was noticed with *D. densiflorum.* Maximum incompatible crosses were observed on Sabine.

The response of wild species as male parents also varied in different varieties.

Emma White, New Pink and Pink Tips were good male parents. These three varieties gave 100 per cent pod set when crossed to the all other varieties.

Out of the six *Dendrobium* species, *D. fimbriatum* gave the maximum cross compatibility when used as male parent. Out of the eight crosses made with *D. fimbriatum*, seven were successful. In the case of *D. moschatum* also, out of the six crosses, five were found successful on other varieties.

Pod size was measured in all the hybrid pods and variations were observed in different crossings/selfings. In general, slightly smaller pods were observed in all the crosses involving the wild species.

In certain crosses, ovary swelling was observed but it was fallen after 30 days before reaching maturity. Crosses of Pramott-II x Sonia-28, as well as Hieng Beauty x Sabine, fall in this category.

4.7.3 Post pollination phenomena

Observations relating to post pollination phenomena indicated a series of changes (Table 12-21). In the unsuccessful crosses, either the flower remained

Table 11. Self and cross compatibility in ten *Dendrobium* hybrids and six wild species

	Emma White	Candy Stripe	Hieng Beauty	New Pink	Pramott-II	Pink Tips	Sabine	Sakura Pink	Sonia 28	Banyat Pink	*D. cru*	*D. d*	*D. f*	*D. m*	*D.chr*	*D. p*
Emma White	S	C	C	C	C	C	C	C	C	C	N	Cx	C	C	C	C
Candy Stripe	C	S	C	C	C	C	Cx	C	C	C	N	C	Cx	C	C	N
Hieng Beauty	C	C	Sx	C	Cx	C	C	Cx	C	C	Cx	C	C	N	N	C
New Pink	C	C	C	S	Cx	C	Cx	C	C	C	C	N	C	C	C	N
Promott-11	C	C	C	C	S	C	C	Cx	Cx	C	Cx	C	N	N	Cx	C
Pink Tips	C	C	C	C	Cx	S	Cx	C	C	Cx	C	Cx	C	N	Cx	N
Sabine	C	Cx	Cx	C	Cx	C	S	C	C	Cx	Cx	C	C	N	N	C
Sakura Pink	C	C	C	C	C	C	Cx	S	C	Cx	C	N	C	C	Cx	N
Sonia 28	C	C	C	C	C	C	C	Cx	S	Cx	C	Cx	C	C	N	C
Banyat Pink	C	Cx	C	C	C	C	C	Cx	C	S	N	C	N	Cx	C	N

C - Cross compatible
S - Self compatible
Sx - Self incompatible
Cx - Cross incompatible
N - Not done

D. cru - *D. crumenatum*
D. d - *D. densiflorum*
D. f - *D. fimbriatum*
D. m - *D. moschatum*
D. chr - *D. chrysanthum*
D. p - *D. pierardii*

intact, retaining its freshness or the flowers faded and abscised 2-3 days after pollination.

In the case of successful crosses, ovary remained as the part of the spike and gradually enlarged to form the pod, whereas the petals and sepals withered.

In some cases the ovaries swelled after pollination but the bulged ovaries were found to fall after about a month without developing into pods. This happened in selfed Hieng Beauty, Banyat Pink x Pramott-II and Pramott-II x Sonia 28.

The time taken for culturing of the pods varied from 90 days in Candy Stripe x Emma White and Emma White x Sabine to 135 days in some crosses involving wild species. The pods of the crosses with wild species took more time for culturing, ranging from 120 days to 135 days.

The compatibility observed in different varieties are presented below:

4.7.4 Candy Stripe

Candy Stripe was found to be compatible with all the eight varieties, except Sabine (Table 12). When *D. fimbriatum* was used as male parent no pod set could be obtained and the swollen ovary was found to fall after 20 days of pollination.

The pod size ranged from 4.5 cm (Candy Stripe selfed) to 5.7 cm (Candy Stripe x Pramott-II) and the time taken for pod culture ranged from 90 days (Candy Stripe x Emma White) to 135 days (Candy Stripe x *D. densiflorum*).

4.7.5 Emma White

Maximum compatibility was observed in Emma White in which all the crosses were successful except with *D. densiflorum* (Table 13). Pod size ranged from 4.8 cm (Emma White x *D. moschatum*) to 5.8 cm (Emma White x New Pink).

Table 12. Details of crosses made on Candy Stripe

Sl. No.	Crosses	Post pollination changes	Pod size (cm)	Time taken for culturing (days)	Remarks
1	Candy Stripe x self	Set	4.5	95	
2	Candy Stripe x Emma White	Set	5.0	90	
3	Candy Stripe x Hieng Beauty	Set	5.8	105	
4	Candy Stripe x New Pink	Set	4.6	95	
5	Candy Stripe x Pink Tips	Set	5.2	105	
6	Candy Stripe x Pramott-II	Set	5.7	105	No germination obtained
7	Candy Stripe x Sabine	No set	-	-	Flower abscised
8	Candy Stripe x Sakura Pink	Set	5.8	105	
9	Candy Stripe x Sonia 28	Set			
10	Candy Stripe x Banyat Pink	Set	5.1	120	
11	Candy Stripe x *D. crumenatum*	Not done			
12	Candy Stripe x *D. densiflorum*	Set	5.0	135	
13	Candy Stripe x *D. fimbriatum*	No set			Ovary swollen but fallen after 20 days
14	Candy Stripe x *D. moschatum*	Set	5.2	130	
15	Candy Stripe x *D. chrysanthum*	Set	5.0	125	
16	Candy Stripe x *D. pierardii*	Not done			

Table 13. Details of crosses made on Emma White

Sl. No.	Crosses	Post pollination changes	Pod size (cm)	Time taken for culturing (days)	Remarks
1	Emma White x self	Set	5.1	105	
2	Emma White x Candy Stripe	Set	5.1	125	
3	Emma White x Hieng Beauty	Set	5.3	105	
4	Emma White x New Pink	Set	5.8	110	
5	Emma White x Pramott-II	Set	5.3	110	Yellowing of pod noticed
6	Emma White x Pink Tips	Set	5.2	115	
7	Emma White x Sabine	Set	6.0	90	
8	Emma White x Sakura Pink	Set	5.5	105	
9	Emma White x Sonia 28	Set	5.2	120	
10	Emma White x Banyat Pink	Set	4.8	105	
11	Emma White x *D. crumenatum*	Not done			
12	Emma White x *D. densiflorum*	No set			Flower absicised
13	Emma White x *D. fimbriatum*	Set	5.0	125	
14	Emma White x *D. moschatum*	Set	4.8	120	Pod broken slightly at the tip
15	Emma White x *D. chrysanthum*	Set	4.9		Yellowing and rotting seen after 90 days
16	Emma White x *D. pierardii*	Set	4.8	120	

It took a minimum of 90 days (Emma White x Sabine) to a maximum of 125 days (Emma White x Candy Stripe, Emma White x *D. fimbriatum*) for pod culture.

4.7.6 Hieng Beauty

Pod set was not obtained with Pramott-II, Sakura Pink and *D. crumenatum*. Pod size ranged from 4.4 cm (Hieng Beauty x *D. fimbriatum*) to 5.9 (Hieng Beauty x Banyat Pink) and the time taken for culturing ranged from 95 days to 125 days (Table 14).

4.7.7 New Pink

New Pink did not set pods with Pramott-II and Sabine. With all others tried, pod set was obtained (Table 15). It was also observed that New Pink set pods with all the four wild species tried. Pod size ranged from 4.9 cm in New Pink x *D. chrysanthum* to 6.5 cm in New Pink x Sonia-28. The time for pod culture ranged from 105 to 130 days.

4.7.8 Pink Tips

Pod set could not be obtained with Pramott-II, Sabine, Banyat Pink, *D. densiflorum* and *D. Chrysanthum* but it was obtained with the other six varieties and two species (Table 16). Pod size ranged from 5 to 6.5 cm and it took a minimum of 105 days to a maximum of 135 days for culturing of pods.

4.7.9 Pramott-II

Pod set was observed with all varieties except with Sakura Pink, Sonia 28, *D. crumenatum* and *D. chrysanthum* (Table 17). Pod size ranged from 5 cm in Pramott-II x *D. pierardii* to 6.1 cm in Pramott-II x Candy Stripe as well as Pramott-II x Banyat Pink.

Table 14. Details of crosses made on Hieng Beauty

Sl. No.	Crosses	Post pollination changes	Pod size (cm)	Time taken for culturing (days)	Remarks
1	Hieng Beauty x self	Not set (bulging of ovary seen but yellowing noticed and fallen after 1 month)			
2	Hieng Beauty x Candy Stripe	Set	5.3	110	
3	Hieng Beauty x Emma White	Set	5.1	95	
4	Hieng Beauty x New Pink	Set	5.4	95	
5	Hieng Beauty x Pramott-II	No set	-	-	No change to flowers
6	Hieng Beauty x Pink Tips	Set	5.2	105	
7	Hieng Beauty x Sabine	Set	(Swelling of ovary seen but yellowing noticed and fallen after 34 days)		
8	Hieng Beauty x Sakura Pink	No set			
9	Hieng Beauty x Sonia 28	Set	5.4	95	
10	Hieng Beauty x Banyat Pink	Set	5.9	105	
11	Hieng Beauty x *D. crumenatum*	No set			Flower abscised
12	Hieng Beauty x *D. densiflorum*	Set	4.5	(Yellowing of pod noticed after 100 days hence culturing not successful)	
13	Hieng Beauty x *D. fimbriatum*	Set	4.4	125	
14	Hieng Beauty x *D. moschatum*	Not done			
15	Hieng Beauty x *D. chrysanthum*	Not done			
16	Hieng Beauty x *D. pierardii*	Set	4.80	120	

Table 15. Details of crosses made on New Pink

Sl. No.	Crosses	Post pollination changes	Pod size (cm)	Time taken for culturing (days)	Remarks
1	New Pink x self	Set	5.9	105	Large sized pods noticed in general
2	New Pink x Candy Stripe	Set	6.4	110	
3	New Pink x Emma White	Set	6.5	105	
4	New Pink x Hieng Beauty	Set	5.2	110	
5	New Pink x Pramott-II	No set	-	-	
6	New Pink x Pink Tips	Set	5.8	105	
7	New Pink x Sabine	No set	-	-	No change to the flower
8	New Pink x Sakura Pink	Set	5.5	115	
9	New Pink x Sonia 28	Set	6.5	105	Pod broken during culturing
10	New Pink x Banyat Pink	Set	6.3	120	
11	New Pink x *D. crumenatum*	Set	5.1	130	
12	New Pink x *D. densiflorum*	Not done			
13	New Pink x *D. fimbriatum*	Set	5.0	125	
14	New Pink x *D. moschatum*	Set	4.9	125	
15	New Pink x *D. chrysanthum*	Set	4.5	130	
16	New Pink x *D. pierardii*	Not done			

Table 16. Details of crosses made on Pink Tips

Sl. No.	Crosses	Post pollination changes	Pod size (cm)	Time taken for culturing (days)	Remarks
1	Pink Tips x self	Set	5.8	120	
2	Pink Tips x Candy Stripe	Set	6.1	105	
3	Pink Tips x Emma White	Set	5.5	105	
4	Pink Tips x Hieng Beauty	Set	5.7	115	
5	Pink Tips x New Pink	Set	6.0	110	
6	Pink Tips x Pramott-II	No set	-	-	Flower abscised
7	Pink Tips x Sabine	No set	-	-	Flower abscised
8	Pink Tips x Sakura Pink	Set	6.5	110	
9	Pink Tips x Sonia 28	Set	5.5	115	
10	Pink Tips x Banyat Pink	No set	-	-	No change to the flower
11	Pink Tips x *D. crumenatum*	Set	5.0	135	
12	Pink Tips x *D. densiflorum*	No set	-	-	No change to the flower
13	Pink Tips x *D. fimbriatum*	Set	5.0	135	Pod broken at the tip
14	Pink Tips x *D. moschatum*	Not done			
15	Pink Tips x *D. chrysanthum*	No set	-	-	Flower abscised
16	Pink Tips x *D. pierardii*	Not done			

Table 17. Details of crosses made on Pramott-II

Sl. No.	Crosses	Post pollination changes	Pod size (cm)	Time taken for culturing (days)	Remarks
1	Pramott-II x self	Set	5.4	105	
2	Pramott-II x Candy Stripe	Set	6.1	110	
3	Pramott-II x Emma White	Set	5.8	105	
4	Pramott-II x Hieng Beauty	Set	5.9	105	
5	Pramott-II x New Pink	Set	5.8	100	
6	Pramott-II x Pink Tips	Set	5.5	110	
7	Pramott-II x Sabine	Set	5.8	115	
8	Pramott-II x Sakura Pink	No set	-	-	Flower abscised
9	Pramott-II x Sonia 28	No set	-	-	Ovary swelling noticed but fallen after 30 days
10	Pramott-II x Banyat Pink	Set	6.1	110	
11	Pramott-II x *D. crumenatum*	No set	-	-	
12	Pramott-II x *D. densiflorum*	Set	5.2	120	
13	Pramott-II x *D. fimbriatum*	Not done			
14	Pramott-II x *D. moschatum*	Not done			
15	Pramott-II x *D. chrysanthum*	No set	-	-	Flower abscised
16	Pramott-II x *D. pierardii*	Set	5	125	

4.7.10 Sabine

Pod set was observed to be comparatively less. Set was obtained with Emma White, New Pink, Pink Tips, Sakura Pink, Sonia-28, *D. densiflorum*, *D. fimbriatum* and *D. pierardii* (Table 18). The size of pod ranged from 4.8 cm in Sabine x *D. fimbriatum* to 6.3 cm in Sabine x New Pink. It took 105 to 135 days for pod culturing.

4.7.11 Sakura Pink

Pod set could not be obtained with Sabine, Banyat Pink and *D. chrysanthum* (Table 19). Pod size ranged from 5.1 to 6.0 cm and it took 100 days (Sakura Pink x New Pink) to 135 days (Sakura Pink x *D. fimbriatum* and Sakura Pink x *D. moschatum*) for culturing.

4.7.12 Sonia 28

Pod set was obtained with all other varieties except Sakura Pink, Banyat Pink and *D. densiflorum* (Table 20). Pod size ranged from 4.2 to 6.0 cm and the time taken for culturing ranged from 95 to 135 days.

4.7.13 Banyat Pink

Banyat Pink could not set pods with Candy Stripe, Sakura Pink and *D. moschatum* (Table 21). The size of pods ranged from 5.0 cm in Banyat Pink x Pramott-II to 6.5 cm in Banyat Pink x New Pink. Time taken for culturing ranged from 115 to 130 days.

4.8 Green pod culture

The pods were harvested at the green pod stage when the embryo got matured before the breakage of the pod.

Table 18. Details of crosses made on Sabine

Sl. No.	Crosses	Post pollination changes	Pod size (cm)	Time taken for culturing (days)	Remarks
1	Sabine x self	Set	6.4	120	
2	Sabine x Candy Stripe	No set	-	-	Flower abscised
3	Sabine x Emma White	Set	5.6	110	
4	Sabine x Hieng Beauty	No set	-	-	Ovary bulged but fallen after 30 days
5	Sabine x New Pink	Set	6.3	115	
6	Sabine x Pink Tips	Set	5.8	105	
7	Sabine x Pramott-II	No set	-	-	flower abscised
8	Sabine x Sakura Pink	Set	5.6	110	Pod yellowing seen and broken during culturing
9	Sabine x Sonia 28	Set	6.0	105	
10	Sabine x Banyat Pink	No set	-	-	Flower abscised
11	Sabine x *D. crumenatum*	No set	-	-	Flower abscised
12	Sabine x *D. densiflorum*	Set	4.9	135	
13	Sabine x *D. fimbriatum*	Set	4.8	130	
14	Sabine x *D. moschatum*	Not done			
15	Sabine x *D. chrysanthum*	Not done			
16	Sabine x *D. pierardii*	Set	5.1	135	

Table 19. Details of crosses made on Sakura Pink

Sl. No.	Crosses	Post pollination changes	Pod size (cm)	Time taken for culturing (days)	Remarks
1	Sakura Pink x self	Set	5.5	110	
2	Sakura Pink x Candy Stripe	Set	5.3	105	
3	Sakura Pink x Emma White	Set	5.5	105	
4	Sakura Pink x Hieng Beauty	Set	5.9	115	
5	Sakura Pink x New Pink	Set	5.3	100	
6	Sakura Pink x Pink Tips	Set	5.8	105	
7	Sakura Pink x Pramott-II	Set	5.2	110	
8	Sakura Pink x Sabine	No set	-	-	Flower abscised
9	Sakura Pink x Sonia 28	Set	6.0	110	
10	Sakura Pink x Banyat Pink	No set	-	-	No change to the flower
11	Sakura Pink x *D. crumenatum*	Set	5.1	125	
12	Sakura Pink x *D. densiflorum*	Not done			
13	Sakura Pink x *D. fimbriatum*	Set	5.1	135	
14	Sakura Pink x *D. moschatum*	Set	5.2	135	
15	Sakura Pink x *D. chrysanthum*	No set	-	-	Flower abscised
16	Sakura Pink x *D. pierardii*	Not done			

Table 20. Details of crosses made on Sonia 28

Sl. No.	Crosses	Post pollination changes	Pod size (cm)	Time taken for culturing (days)	Remarks
1	Sonia 28 x self	Set	5.8	105	
2	Sonia 28 x Candy Stripe	Set	5.3	110	
3	Sonia 28 x Emma White	Set	5.4	100	
4	Sonia 28 x Hieng Beauty	Set	5.6	105	
5	Sonia 28 x New Pink	Set	6.1	95	
6	Sonia 28 x Pramott-II	Set	5.8	100	
7	Sonia 28 x Pink Tips	Set	4.9	105	
8	Sonia 28 x Sabine	Set1	6.0	115	
9	Sonia 28 x Sakura Pink	No set	-	-	Ovary bulged but fallen after 25 days
10	Sonia 28 x Banyat Pink	No set	-	-	Flower abscise
11	Sonia 28 x *D. crumenatum*	Set	4.2	135	
12	Sonia 28 x *D. densiflorum*	No set	-	-	Pedicel enlarged but dropped after 16 days
13	Sonia 28 x *D. fimbriatum*	Set	4.8	125	
14	Sonia 28 x *D. moschatum*	Set	4.8	135	
15	Sonia 28 x *D. chrysanthum*	Could not be done			
16	Sonia 28 x *D. pierardii*	Set	4.2	-	Rotting observed after 100 days and hence no germination

Table 21. Details of crosses made on Banyat Pink

Sl. No.	Crosses	Post pollination changes	Pod size (cm)	Time taken for culturing (days)	Remarks
1	Banyat Pink x self	Set	6.1	110	
2	Banyat Pink x Candy Stripe	No set	-	-	Pedicel slightly bulged initially but fell
3	Banyat Pink x Emma White	Set	6.4	115	
4	Banyat Pink x Hieng Beauty	Set	5.6	115	
5	Banyat Pink x New Pink	Set	6.5	120	
6	Banyat Pink x Pramott-II	Set	5.0	-	Yellowing of pod noticed after 75 days
7	Banyat Pink x Pink Tips	Set	5.5	115	
8	Banyat Pink x Sabine	Set	5.4	115	
9	Banyat Pink x Sakura Pink	No set	-	-	No change to flower
10	Banyat Pink x Sonia 28	Set	5.9	115	
11	Banyat Pink x *D. crumenatum*	Not done			
12	Banyat Pink x *D. densiflorum*	Set	5.2	125	
13	Banyat Pink x *D. fimbriatum*	Not done			
14	Banyat Pink x *D. moschatum*	No set	-	-	Flower abscised
15	Banyat Pink x *D. chrysanthum*	Set	5.1	130	
16	Emma White x *D. pierardii*	Not done			

Plate - 18 Pod setting in *Dendrobium*

Plate - 19 *Dendrobium* pods

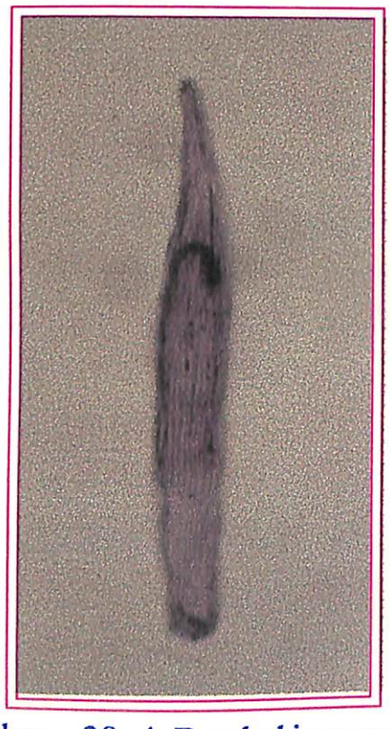


Plate - 20 A *Dendrobium* seed

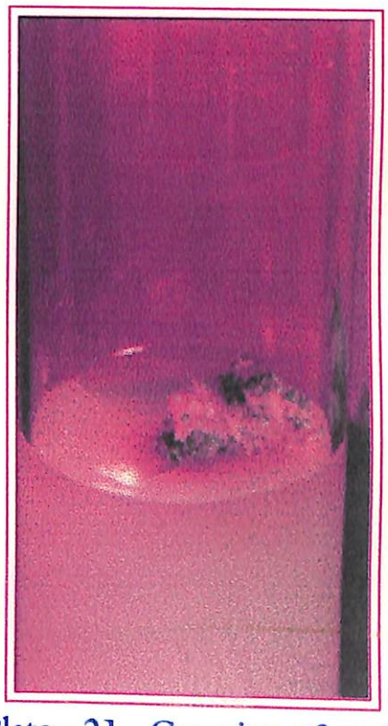


Plate - 21 Greening of seeds

4.8.1 Standardisation of surface sterilization of the pod

The results of the trial on different methods of surface sterilization of pods are presented in Table 22.

Significant differences among the surface sterilization treatments were observed for the control of rate of contamination and survival of cultured pods.

Among the different treatments, none recorded total survival. Maximum survival percentage with effective control on contamination was obtained with the combination in which the pod was kept in mercuric chloride (0.1%) for one minute and then flamed after a dip in 70 per cent alcohol. A survival percentage of 85 was recorded for this treatment. The survival percentage with the surface sterilization of dipping in 70 per cent alcohol alone and flaming is 80. Whereas mercuric chloride treatment (0.1%) for 5 minutes followed by alcohol dip before flaming resulted in 65 per cent survival. Eventhough contamination was less, more cultures were found to be dead in this, compared to the former two treatments.

Maximum contamination was found to be with the surface sterilization using 0.05 per cent mercuric chloride treatment for 5 minutes (80%) followed by 0.05 per cent for 10 minutes and 0.1 per cent for 5 minutes (60%) each.

Survival rate was minimum (10%) for treatments involving mercuric chloride 0.05 per cent for 5 minutes and 0.2 per cent for 10 minutes, without alcohol dip.

4.8.2 Age of the pod

Data are presented in Table 23. When 60 days old pod was used for culturing, the embryo did not turn green even after three months, where as high percentage of germination was obtained by culturing 70 days old pod.

Table 22. Effect of surface sterilization on the survival of New Pink x Emma White seeds

No. of tubes - 20/treatment
Medium - ½ MS + BA - 1 mg l^{-1}
+ IBA - 1 mg l^{-1}

Treatment	Time (seconds)	Percentage of		
		Contamination	Dead/ bleached	Survival
Mercuric chloride (%)				
0.05	5	80	5	10
0.05	10	60	5	35
0.1	5	60	10	30
0.1	10	25	35	40
0.15	5	25	50	25
0.15	10	10	70	20
0.2	5	15	65	20
0.2	10	5	85	10
0.1 (5 minutes) + alcohol dip and flaming		5	30	65
0.1 (1 minute) + alcohol dip and flaming		10	5	85
Alcohol dip and flaming alone		15	5	80

Table 23. Effect of age of pod on germination of New Pink selfed seed
Medium - ½ MS + Kin - 1 mg l^{-1} + IBA - 1 mg l^{-1}

Age of pod (days)	Germination	Number of days to greening	Remarks
60	-	-	Seeds not separated and sticking to the pod
70	H	33.67	Seeds not separated and sticking to the pod
80	VH	27.00	Well separated seeds inside the pod
90	VL	26.33	Well separated seeds inside the pod
100	VH	24.00	Well separated seeds
110	VH	22.67	Well separated seeds
120	H	29.00	Slight yellowing of pod noticed
130	VL	32.00	Yellowing of pod noticed
140	VL	37.00	Pod broken at the time of culturing
150	VL	33.00	Pod broken in the field
CD (0.05)		3.65	
SEm±		1.23	

Very high germination was obtained with 90, 100, 110 and 120 days old pods. The pods of 130 and 140 days age also gave comparatively high germination. Lowest germination was obtained with 150 days old pods.

Regarding the time taken for greening *in vitro*, it was minimum with the pods of age 110 days (22.67 days), which was significantly superior to all others except, 100 days old pod, where it was 24 days (Fig. 2).

Significant differences were observed in the time taken for greening of seeds of pods belonging to different age groups.

Maximum time for greening was taken by the pods of age 140 days (37 days) which was on par with that of 70 days old pods (33.67 days). Cracking of the pods were noticed at 140 and 150 days.

4.8.3 Embryo culture of the hybrids

Green pod culture was resorted to, by taking the 90 to 100 days old pods, the age of which differed according to the crosses. A *Dendrobium* seed is shown in Plate 20.

4.8.4 Stages of germination

After about 15-20 days of inoculation, greening of the seeds (Plate 21) was noticed. The germinated seeds got bulged and swollen structures (protocorms) were formed in 45-60 days. The protocorms increased in size and from these, leaves were produced in about 75-90 days. Shoot and root formation occurred in 100-120 days (Plates 23 & 24).

4.8.5 Culture details of the hybrids

The germination frequency and the subsequent morphogenetic changes leading to the seedling development varied with the hybrids and the culture details are presented below.

When the different hybrids were cultured, some of them did not germinate. Among those germinated, some did not reach the seedling stage.

Minimum germination percentage was observed with New Pink x *D.crumenatum*, Sonia 28 x *D.crumenatum* and Banyat Pink x *D. chrysanthum*. Very high germination was noticed New Pink x Emma White, Emma White x New Pink, New Pink x Pink Tips, Pink Tips x Sonia 28, Pink Tips x Emma White and Banyat Pink x New Pink (Tables 24-33).

Pink Tips selfed as well as Sonia 28 selfed gave highest germination. The days taken for each stage in culture varied significantly among the hybrids. Emma White selfed took the least number of days (253 days) for planting out and Sonia 28 x *D. crumenatum* took maximum days (385 days) for planting to the field.

4.8.5.1 Candy stripe

The data on the culturing of the different hybrids are presented in Table 24.

Selfed pods of Candy Stripe gave only very low germination. Out of the different crosses made on Candy Stripe, that with Pink Tips, Candy Stripe x New Pink and Candy Stripe x Banyat Pink recorded very high germination. Candy Stripe x Emma White, Candy Stripe x Pramott-II and Candy Stripe x *D. chrysanthum* did not germinate in culture. Germination percentage was the minimum in Candy Stripe x *D. densiflorum*. The germination of Candy Stripe x Hieng Beauty and Candy Stripe x *D. moschatum* were low, compared to other crosses. It took a minimum of 20 days for the greening of Candy Stripe x Sonia 28 seeds, and a maximum of 37.33 days for the germination of Candy Stripe x Banyat Pink seeds. Significant variation was observed among the hybrids for the time taken for greening also. Maximum time for protocorm development was taken

Table 24. Green pod culture details of the pods of crosses made with Candy Stripe

Crosses	Germination	Number of days taken for					Remarks
		Greening	Protocorm development	First leaf production	Shoot and root formation	Planting out	
Selfed	VL	31.33	64.33	91.33	127.69	293.33	Seeds were white, powdery and a few in number
Candy Stripe x New Pink	L	32.33	52.67	75.61	104.33	277.50	
Candy Stripe x Pink Tips	VH	21.67	53.33	83.67	113.33	325.00	
Candy Stripe x Hieng Beauty	VL	27.33	49.00	82.33	108.00	308.33	
Candy Stripe x Sonia-28	H	20.00	38.33	73.33	109.00	300.00	
Candy Stripe x Sakura Pink	L	26.00	45.67	79.00	113.33	305.00	
Candy Stripe x Banyat Pink	VH	37.33	60.33	After protocorm stage, no more development noticed			
Candy Stripe x Emma White	No germination						A slight yellowish greening seen after 13 days in some of the cultures. After that no more changes observed.
Candy Stripe x Pramott-II	No germination						
Candy Stripe x *D. densiflorum*	VL	32.67	72.33	100.00	140.00	375.00	
Candy Stripe x *D. moschatum*	VL	34.00	62.33	89.00	No more development		
Candy Stripe x *D. chrysanthum*	No germination						
CD (0.05)		4.61	3.95	6.33	10.22	12.85	
SEm±		1.55	1.33	2.11	3.37	4.08	

by the seeds of the cross Candy Stripe x *D. densiflorum*. But Candy Stripe x Sonia 28 took the minimum time for protocorm formation (38.33 days).

Among the various crosses made on Candy Stripe, that with *D. densiflorum* took maximum number of days for first leaf production (100 days), shoot and root formation (140 days) as well as for planting out (375 days).

In the case of Candy Stripe x Banyat Pink, eventhough good germination was obtained and protocorms were formed, no more development occurred after the protocorm stage.

Significant differences were observed among the different hybrids with regard to first leaf production, shoot and root formation and the time taken for seedling development. Minimum time for planting out was taken by Candy Stripe x New Pink (277.5 days).

A slight yellowish greening was observed after 13 days in 20 per cent cultures of Candy Stripe x Pramott-II but no more change was seen.

4.8.5.2 Emma White

Cultural details are presented in Table 25.

Emma White selfed seeds showed a high frequency of seed germination. The time taken to reach each stage during the development of seedlings in the culture, was also comparatively less.

It took only 253 days for planting out of the selfed seedlings, which was more in the case of all hybrids of Emma White.

Among the hybrids, maximum germination was with Emma White x New Pink and Emma White x Hieng Beauty. In all the eight hybrids, germination rate was low, the lowest being in Emma White x Sabine. Emma White x

Table 25. Green pod culture details of the pods of crosses made with Emma White

Crosses	Germination	Number of days taken for					Remarks
		Greening	Protocorm development	First leaf production	Shoot and root formation	Planting out	
Emma White x selfed	VH	20.33	41.67	70.67	109.67	253.00	Yellow coloured seeds with more number per pod
Emma White x Banyat Pink	VH	21.00	42.67	73.00	104.33	285.00	
Emma White x Pink Tips	VH	21.67	52.00	81.67	108.67	266.67	
Emma White x Sabine	VL	24.67	43.00	88.33	124.33	295.00	
Emma White x New Pink	VH	22.67	41.67	72.67	110.67	308.00	
Emma White x Candy Stripe	L	35.67	57.67	83.00	114.00	307.00	
Emma White x Sonia-28	L	28.33	57.33	86.00	109.67	288.33	
Emma White x Hieng Beauty	VH	23.33	50.00	85.00	121.67	308.00	
Emma White x Pramott-II	L	25.33	48.33	83.33	121.63	298.00	
Emma White x Sakura Pink	H	22.33	45.00	88.33	128.33	320.00	
Emma White x *D. pierardii*	L	35.00	66.67	90.67	135.00	315.00	
Emma White x *D. fimbriatum*	L	32.33	64.00	105.00	140.00	340.00	Two seedlings were obtained but no field establishment
Emma White x *D. moschatum*	L	50.67	76.67	No further development			
Emma White x *D. chrysanthum*	After 30 days yellowing only - no more change						
CD (0.05)		4.94	5.26	6.36	6.83	9.87	
SEm±		1.70	1.81	2.17	2.33	3.37	

D. chrysanthum did not germinate but yellowing of the seeds was noticed after 30 days with no further development.

Maximum time (50.67 days) for protocorm formation was observed with *D. moschatum* which took a maximum of 76.67 days for first leaf production. But further development of the protocorm into seedlings was not observed in this.

The time taken for greening ranged from 20.33 days in Emma White selfed to 50.67 days in Emma White x *D. moschatum*.

Protocorms were formed in a minimum of 41.67 days in Emma White selfed and it took maximum days (76.67) in the cross with *D. moschatum*.

Significant variation could be observed in the time taken for first leaf production (70.67 to 105.00 days) and root and shoot formation (104.33 days to 140.00 days).

For complete development of the seedlings it took 253.00 days (Emma White selfed) to 340.00 days (Emma White x *D. fimbriatum*).

4.8.5.3 Hieng Beauty

Data on the culture of the seeds obtained by the crossing on Hieng Beauty are presented in Table 26.

Out of the eight successful crosses, Hieng Beauty x New Pink and Hieng Beauty x Pink Tips had the highest germination.

Lowest germination percentage was obtained in Hieng Beauty x *D. fimbriatum*.

Significant variations were observed among the different crosses to reach different stage in the culture. Protocorm stage was attained in 12.33 days by Hieng Beauty x Emma White and 34.67 days by Hieng Beauty x *D. densiflorum*.

Table 26. Green pod culture details of the pods of crosses made with Hieng Beauty

Crosses	Germination	Number of days taken for					Remarks
		Greening	Protocorm development	First leaf production	Shoot and root formation	Planting out	
Hieng Beauty x Sonia-28	L	34.00	51.00	75.00	102.00	297.00	Creamy white coloured seeds more number of seeds per pod
Hieng Beauty x Banyat Pink	VH	30.00	45.67	68.33	100.00	298.00	
Hieng Beauty x Candy Stripe	VH	30.67	52.33	72.33	101.00	297.00	
Hieng Beauty x Pink Tips	VH	23.33	47.67	73.33	100.67	283.33	
Hieng Beauty x New Pink	VH	32.67	50.00	65.00	98.67	288.00	
Hieng Beauty x Emma White	H	12.33	31.00	60./67	91.33	290.00	
Hieng Beauty x *D. densiflorum*	L	34.67	61.00	85.00	116.67	296.67	Five seedlings only obtained
Hieng Beauty x *D. fimbriatum*	VL	29.00	No more development				
Hieng Beauty x *D. pierardii*	Yellowing seen after 25 days but no more development						
CD (0.05)		5.74	4.98	5.10	5.34		
SEm±		1.93	1.66	1.68	1.76	NS	

Minimum time for first leaf production as well as shoot and root formation was observed in Hieng Beauty x Emma White (31 days and 60.67 days respectively). Maximum time for protocorm formation, first leaf production, as well as shoot and root formation were attained by Hieng Beauty x *D. densiflorum* (61, 85, 116.67 days respectively).

The time taken for planting out of seedlings did not differ significantly among the hybrids.

4.8.5.4 New Pink

Significant differences were noticed among the hybrids with respect to the time taken for attaining each stage in the culture (Table 27).

Minimum germination was observed in New Pink x *D. chrysanthum.* Very high germination was noted in New Pink x Emma White, New Pink x Pink Tips, New Pink x Hieng Beauty and New Pink x Sonia-28. The time taken for greening and protocorm formation did not differ significantly among the different hybrids. The least time for greening (22.33 days) was taken by New Pink x Hieng Beauty and for protocorm formation by New Pink x *D. chrysanthum* (41.67 days).

New Pink x Hieng Beauty had taken the minimum time for first leaf formation (57.67 days) and New Pink selfed seeds took the minimum of 96 days for shoot and root formation.

Seedlings of New Pink x Hieng Beauty were planted at 257 days age which was the minimum among all the crosses.

Seeds of New Pink x *D. fimbriatum* and *D. crumenatum* did not develop further after the greening stage.

Table 27. Green pod culture deails of the pods of crosses made with New Pink

Crosses	Germin-ation	Number of days taken for					Remarks
		Greening	Protocorm development	First leaf production	Shoot and root formation	Planting out	
New Pink selfed	VH	26.33	45.00	60.33	96.00	290.00	Yellow coloured seeds, more number in all the crosses
New Pink x Hieng Beauty	VH	22.33	44.33	57.67	101.00	257.00	
New Pink x Sonia-28	VH	25.67	46.00	63.00	99.67	298.00	
New Pink x Sakura Pink	H	27.33	52.67	74.33	101.67	298.00	
New Pink x Banyat Pink	L	25.67	48.00	68.33	100.00	306.67	
New Pink x Pink Tips	VH	23.67	48.67	59.67	97.33	287.00	
New Pink x Emma White	VH	26.33	47.33	65.00	99.33	305.00	
New Pink x Candy Stripe	VH	24.33	52.00	61.33	97.33	288.00	
New Pink x *D. chrysanthum*	VL	28.00	41.67	66.33	98.67	278.00	
New Pink x *D. moschatum*	VL	30.33	48.67	73.00	111.00	331.67	
New Pink x *D. fimbriatum*	L	30.33	No more development after greening				
New Pink x *D. crumenatum*	VL	30.00					Pod was broken while culturing hence contamination was more and none could survive
CD (0.05)		NS	NS	4.90	4.53	28.08	
SEm±				2.35	2.17	9.52	

4.8.5.5 Pink Tips

Data on the culture details showing the significant variations are furnished in Table 28.

Selfed seeds of Pink Tips, Pink Tips x Emma White, Pink Tips x Sonia 28 and Pink Tips x New Pink gave high rate of germination. Pink Tips x *D. fimbriatum* gave the lowest germination. A minimum of 23.33 days was taken by Pink Tips x New Pink for germination, which was maximum with Pink Tips x *D. moschatum* seeds (32.00 days). The time taken for protocorm formation varied from 48 days (New Pink selfed) to 61 days (Pink Tips x *D. moschatum*).

The days for first leaf production varied from 82.33 in Pink Tips x Sonia 28 to 96.00 in Pink Tips x *D. moschatum.*

Significant variation existed with respect to the time taken for shoot and root production, which ranged from 111.67 days in Pink Tips x Sonia 28 to 130.00 days in Pink Tips x *D. moschatum* and Pink Tips x *D. crumenatum.*

The total time taken for planting out ranged from 288.00 days (Pink Tips selfed) to 358.33 days in Pink Tips x *D. fimbriatum.*

4.8.5.6 Pramott-II

The data showing the culture details are presented in Table 29.

Significant differences were observed among different hybrids with respect to the duration for attaining different stages in culture.

Highest germination rate was noticed in Pramott-II x Emma White and Pramott-II x New Pink and the lowest in Pramott-II x *D. pierardii* as well as Pramott-II x *D. densiflorum.* Pramott-II x Pink Tips seeds did not germinate in culture. It took 21.33 days for greening in Pramott-II x Emma White and 40.67 days in Pramott-II x *D. pierardii.*

Table 28. Green pod culture details of the pods of crosses made with Pink Tips

Crosses	Germination	Number of days taken for					Remarks
		Greening	Protocorm development	First leaf production	Shoot and root formation	Planting out	
Pink Tips Selfed	VH	24.33	48.33	79.00	114.00	288.00	A few seed with a creamy white colour
Pink Tips x Hieng Beauty	H	25.00	52.00	91.00	119.00	298.33	
Pink Tips x Emma White	VH	22.67	52.67	90.00	114.33	286.67	
Pink Tips x Sakura Pink	L	25.33	47.00	84.00	117.63	327.00	
Pink Tips x Candy Stripe	H	26.67	50.67	89.00	120.00	300.00	
Pink Tips x New Pink	VH	23.33	54.33	89.33	107.67	310.00	
Pink Tips x Sonia-28	VH	23.67	57.67	82.33	111.63	305.00	
Pink Tips x *D. fimbriatum*	VL	29.33	58.33	90.00	125.00	358.33	
Pink Tips x *D. crumenatum*	L	30.67	60.00	90.67	130.00	360.00	Field establishment very poor
CD (0.05)		4.00	5.90	6.53	6.51	12.45	
SEm±		1.37	2.02	2.23	2.22	4.22	

Table 29. Green pod culture details of the pods of crosses made with Pramott-II

Crosses	Germination	Number of days taken for					Remarks
		Greening	Protocorm development	First leaf production	Shoot and root formation	Planting out	
Pramott-II selfed	H	23.00	48.33	84.33	108.33	275.00	Seeds very less and creamy white coloured
Pramott-II x Emma White	VH	21.33	52.00	82.33	115.00	285.00	
Pramott-II x Banyat Pink	L	24.00	46.33	81.00	120.00	282.00	
Pramott-II x New Pink	VH	31.33	57.73	90.63	123.33	285.00	
Pramott-II x Candy Stripe	L	27.67	51.67	84.33	124.00	291.00	
Pramott-II x Sabine	H	28.33	41.67	73.33	106.67	270.33	
Pramott-II x Hieng Beauty	L	24.67	49.33	82.67	115.00	280.67	
Pramott-II x *D. densiflorum*	VL	39.33	71.67	95.00	135.00	325.00	
Pramott-II x *D. pierardii*	VL	40.67	68.33	93.00	128.00	315.67	
Pramott-II x Pink Tips	No germination						
CD (0.05)		4.58	8.23	8.20	11.23	12.29	
SEm±		1.54	2.77	2.76	3.78	4.1	

The number of days taken for protocorm formation in different hybrids ranged from 41.67 (Pramott-II x Sabine) to 71.67 (Pramott-II x *D. densiflorumi*). *D. densiflorum* recorded the maximum time for first leaf production (95 days) shoot and root formation (135 days) and for planting out (325 days).

4.8.5.7 Sabine

Data on the embryo culture details are presented in Table 30.

Out of the seven crosses, Sabine x Emma White did not germinate. Maximum germination was found in Sabine x Pink Tips. The lowest germination percentage was found in Sabine x Sakura Pink.

Greening of the seeds took place in 20 (Sabine x Pink Tips) to 36 days (Sabine x *D. densiflorum*) and the protocorm formation within 41 days (Sabine x Pink Tips) to a maximum of 74 days (Sabine x *D. pierardii*).

Sabine x *D. densiflorum* and Sabine x *D. pierardii* did not grow further after the protocorm stage.

Sabine selfed seeds and Sabine x Sakura Pink took the minimum time (81.00 days) for the first leaf production as well as for shoot and root formation (113.00 days). The minimum period for planting out of seedlings was 278.33 days (Sabine x Pink Tips) and the maximum was 353.00 days (Sabine x *D. fimbriatum*).

Significant variation occurred in all the characters studied.

4.8.5.8 Sakura Pink

Data on the embryo culture are presented in Table 31.

Two of the hybrids, Sakura Pink x *D. fimbriatum* and Sakura Pink x *D. chrysanthum* did not germinate.

Table 30. Green pod culture details of the pods of crosses made with Sabine

Crosses	Germination	Number of days taken for					Remarks
		Greening	Protocorm development	First leaf production	Shoot and root formation	Planting out	
Sabine Selfed	L	30.00	50.00	81.00	113.00	313.00	More number of seeds which were yellow coloured
Sabine x New Pink	L	25.67	52.67	89.00	115.00	316.67	
Sabine x Pink Tips	VH	20.00	41.00	82.67	116.33	278.33	
Sabine x Sonia-28	H	26.00	54.33	93.33	123.67	311.67	
Sabine x Sakura Pink	VL	24.00	50.67	81.00	113.00	296.67	
Sabine x *D. fimbriatum*	VL	34.67	67.67	93.33	143.33	353.00	
Sabine x *D. densiflorum*	L	36.00	72.33	No further growth			
Sabine x *D. pierardii*	VL	35.00	74.00	No further growth			
Sabine x Emma White	No germination						
CD (0.05)		3.54	7.70	10.20	9.22	16.24	
SEM±		1.18	2.54	3.31	2.99	5.27	

Table 31. Green pod culture details of the pods of crosses made with Sakura Pink

Crosses	Germination	Number of days taken for					Remarks
		Greening	Protocorm development	First leaf production	Shoot and root formation	Planting out	
Sakura Pink selfed	H	28.67	52.67	85.67	108.33	293.33	Light yellow coloured and moderately large number of seeds
Sakura Pink x Hieng Beauty	H	25.33	42.33	72.67	105.67	309.00	
Sakura Pink x New Pink	H	27.67	52.33	79.67	111.67	300.00	
Sakura Pink x Candy Stripe	L	27.33	54.00	83.33	110.00	303.00	
Sakura Pink x Emma White	H	26.00	50.00	80.33	117.33	296.67	
Sakura Pink x Sonia-28	H	22.00	49.33	79.67	118.33	317.00	
Sakura Pink x Pramott-II	H	22.33	51.67	90.67	108.33	288.00	
Sakura Pink x Pink Tips	H	25.00	51.33	75.00	107.67	286.67	
Sakura Pink x *D. moschatum*	L	32.33	57.67	No further development			
Sakura Pink x *D. crumenatum*	L	22.33	45	From the protocorm stage full contaminated			
Sakura Pink x *D. fimbriatum*	No germination						
CD (0.05)		3.74	5.35	NS	8.04	12.35	
SEm±		1.26	1.80		2.68	4.12	

Among the ten hybrids, lowest germination was observed in Sakura Pink x *D. moschatum* and Sakura Pink x *D. crumenatum*. The highest germination was noted in Sakura Pink x Emma White and Sakura Pink x Pink Tips.

Greening took place in 22 days in Sakura Pink x Sonia-28 and in 32.33 days in Sakura Pink x *D. moschatum*. The time taken for protocorm formation ranged from 42.33 days (Sakura Pink x Hieng beauty) to 57.67 days (Sakura Pink x *D. moschatum*). But the Sakura Pink x *D. moschatum* seeds did not show further growth after the protocorm stage. The time taken for first leaf production as well as for shoot and root formation was the least in Sakura Pink x Hieng Beauty (72.67 and 105.67 days, respectively).

Maximum time for planting out of the seedlings was taken by Sakura Pink x Pink Tips (286.67 days).

4.8.5.9 Sonia 28

Green pods from eleven crosses successfully carried out, were cultured and data are presented in Table 32.

Out of the different crosses, only in one cross, Sonia 28 x *D. pierardii*, germination was not obtained.

Germination percentage was the highest in Sonia 28 selfed seeds and Sonia 28 x New Pink. The lowest germination was in Sonia 28 x *D. crumenatum*.

Time taken for greening in different hybrids ranged from 20.00 days (Sonia 28 x Hieng Beauty) to 49.33 days (Sonia 28 x *D. crumenatum*).

Minimum time was taken by Sonia 28 x Hieng Beauty with respect to protocorm formation (42 days), first leaf production (79 days) shoot and root formation (117 days) as well as for planting out (288 days). Maximum number of days for planting out was observed in Sonia-28 x *D. moschatum* (385 days).

Table 32. Green pod culture details of the pods of crosses made with Sonia 28

Crosses	Germination	Number of days taken for					Remarks
		Greening	Protocorm development	First leaf production	Shoot and root formation	Planting out	
Sonia 28 selfed	VH	26.00	55.67	93.33	121.67	296.67	Yellow coloured seeds, many seeds noticed
Sonia 28 x Hieng Beauty	H	20.00	42.00	79.00	117.33	288.00	
Sonia 28 x Candy Stripe	H	28.33	56.67	91.67	125.00	311.67	
Sonia 28 x Pink Tips	L	23.67	44.00	86.00	118.33	323.33	
Sonia 28 x New Pink	VH	26.67	56.67	98.33	131.67	298.00	
Sonia 28 x Pramott-II	H	27.00	58.33	95.67	125.67	306.67	
Sonia 28 x Sabine	L	31.67	57.67	96.67	133.33	320.00	
Sonia 28 x Emma White	L	23.67	43.00	80.33	125.00	288.67	
Sonia 28 x *D. moschatum*	L	31.67	66.67	100.00	136.67	385.00	
Sonia 28 x *D. crumenatum*	VL	49.33	62.67	87.33	121.67	338.00	
Sonia 28 x *D. pierardii*	No germination						
Sonia 28 x *D. fimbriatum*	No germination						
CD (0.05)		4.24	7.94	7.99	6.77	11.98	
SEm±		1.51	2.70	2.66	2.31	4.00	

4.8.5.10 Banyat Pink

The embryo culture was carried out in eight crosses and in the selfed pods of Banyat Pink (Table 33).

Significant differences could be observed among the hybrids with respect to the different stages in culture.

Maximum germination was found in Banyat Pink x New Pink, Banyat Pink x Emma White and Banyat Pink x Hieng Beauty. The lowest germination rate was recorded in Banyat Pink x *D. chrysanthum* and Banyat Pink x Pramott-II. Germination was not obtained in Banyat Pink x *D. densiflorum*.

Time taken for greening ranged from 20.33 days (Banyat Pink x Pink Tips) to 32.33 days (Banyat Pink x Pramott-II).

Minimum time for greening protocorm formation (43.33 days), first leaf production (70.67 days). Shoot and root formation (108.33 days) as well as for planting out (282.00 days) were recorded in Banyat Pink x Pink Tips. Maximum time for planting out was taken by Banyat Pink x Sabine (315.00 days). It also showed maximum time for protocorm formation (69.67 days), first leaf production (101.00 days) as well shoot and root formation (125.33 days).

4.8.6 Refinement of culture media

4.8.6.1 Effect of media on seed germination

Different basal media were evaluated to find out their effect on seed germination and the results are presented in Table 34.

High rate of germination was observed in all the five media. Regarding the time taken for greening, significant difference was not found in different media, except MS at its full strength (28.00 days). VW and KC each took 22.67 days and MS (1/4 strength) took 23.00 days, followed by MS half strength (25.33 days).

Table 33. Green pod culture details of the pods of crosses made with Banyat Pink

Crosses	Germination	Number of days taken for					Remarks
		Greening	Protocorm development	First leaf production	Shoot and root formation	Planting out	
Banyat Pink selfed	H	25.00	48.67	78.00	123.33	308.00	More number of seeds with slight yellow colour
Banyat Pink x Sonia-28	VH	28.00	53.00	81.00	115.00	302.00	
Banyat Pink x Sabine	VL	29.33	69.67	101.00	125.33	315.00	
Banyat Pink x Emma White	VH	27.33	45.00	73.33	119.33	300.00	
Banyat Pink x Hieng Beauty	VH	23.67	55.33	73.33	112.67	295.00	
Banyat Pink x New Pink	VH	24.00	45.67	74.33	120.00	310.67	
Banyat Pink x Pink Tips	H	20.33	42.33	70.67	108.33	282.00	
Banyat Pink x Pramott-II	VL	32.33	56.67	86.00	Very few protocorms were developed and no seedlings obtained		
Banyat Pink x *D. chrysanthum*	VL	30.00	No more development observed				
Banyat Pink x *D. densiflorum*	No germination						
CD (0.05)		3.67	11.28	7.16	6.94	10.91	
SEm±		1.21	3.72	2.36	2.25	3.54	

Table 34. Effect of media on germination of New Pink x Emma White seeds

Each medium with Kin 1 mg l^{-1} and IBA - 1 mg l^{-1}

Media	Germination percentage	Number of days for greening	Number of days for protocorm development	Remarks
¼ strength MS	93.33	23.00	43.67	
½ strength MS	96.67	25.33	44.33	
Full strength MS	85.00	28.00	48.67	
VW	100.00	22.67	43.33	Dark green protocorm
KC	96.67	22.67	44.67	Dark green protocorm
CD (0.05)	9.54	2.56	1.87	
SEm±	4.28	1.15	0.84	

Table 35. Effect of media on seedling growth of New Pink x Emma White

Media - With kinetin 4 mg l^{-1} + IBA 4 mg l^{-1}

Culture period 5 months

Media	Seedling characters				
	Height (cm)	Number of leaves	Length of longest leaf (cm)	Number of roots	Mean length of roots (cm)
¼ Strength MS	3.33	5.00	3.67	4.67	3.80
½ Strength MS	2.80	4.00	3.33	4.33	3.07
Full strength MS	2.37	3.33	3.03	4.33	2.87
Vacin and Went	3.17	4.67	3.53	5.00	3.97
Knudson C	3.27	5.00	3.53	4.67	4.00
CD(0.05)	0.35	NS	0.32	NS	0.29
SEm±	0.11		0.10		0.09

Fig. 2. Effect of pod age on germination of New Pink selfed seeds

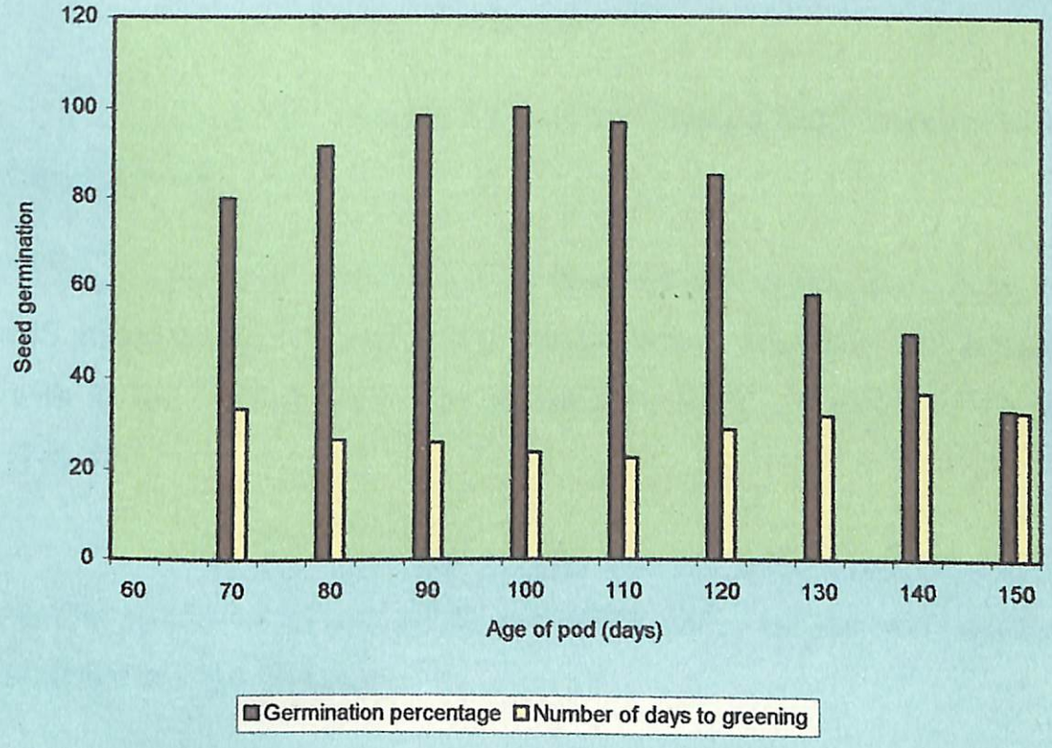
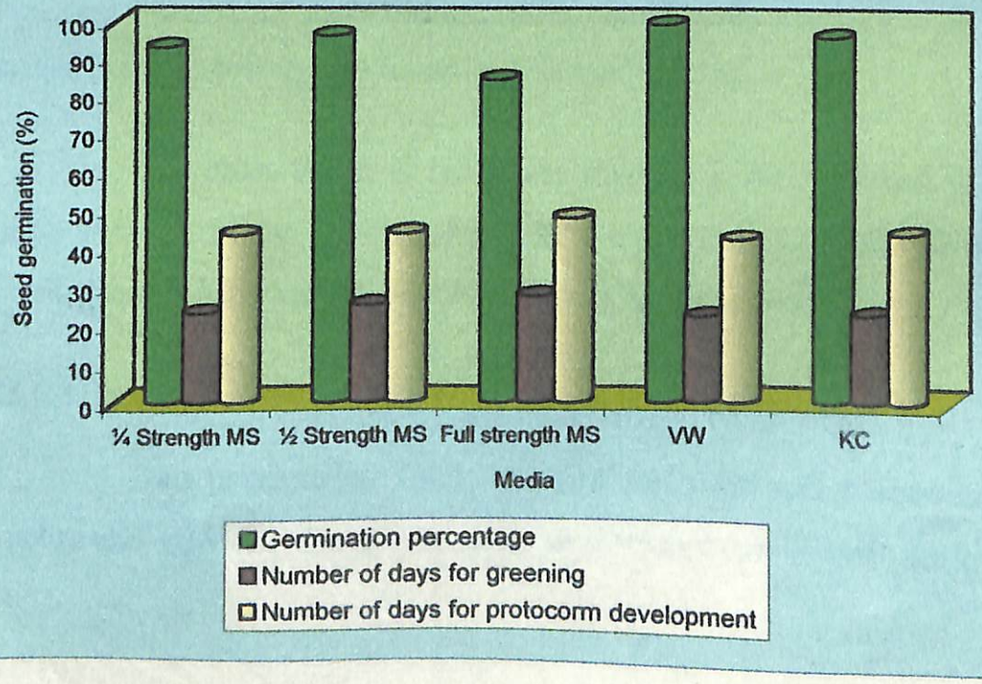


Fig. 3. Effect of media on germination of New Pink x Emma White seeds



A similar trend was noticed with regard to the time taken for protocorm development also. MS (full strength) medium took the maximum time of 48.67 days which was significantly higher than others (Fig. 3).

4.8.6.2 Effect of media on seedling growth

MS at ¼th strength, KC and VW showed better response in respect of seedling growth (Table 35).

Significant difference was observed among the above three media and MS at half strength as well as full strength levels. Maximum height was obtained with ¼ MS medium (3.33 cm) followed by KC (3.27 cm) and VW (3.17 cm) (Fig. 4).

Eventhough the leaf number did not differ significantly, maximum number was obtained with ¼ MS and KC (5, each) and minimum number for MS at its full strength (3.33).

Leaf length was highest in ¼ MS medium (3.67 cm), closely followed by VW and KC media (3.53 cm each), which were significantly higher than that in full strength MS (3.03 cm).

Number of roots did not differ significantly among the different media but the highest number was found in VW medium (5).

Maximum length of roots was obtained in KC, VW and ¼ strength MS media (4.0 cm, 3.9 cm and 3.8 cm, respectively), which were significantly superior to full strength MS medium which gave only 2.87 cm length.

4.8.6.3 Effect of kinetin and IBA on seed germination

Data presented in Table 36 reveal that there was a pronounced effect of kinetin and IBA on the germination of *Dendrobium* hybrid seeds. Very high

Table 36. Effect of Kinetin and IBA on the germination of New Pink selfed seeds

Medium - Vacin & Went

Kinetin ($mg\ l^{-1}$)	IBA ($mg\ l^{-1}$)	Germination percentage	Days for greening
0	0	75.00	32.67
1	0	83.33	30.67
2	0	83.33	30.67
1	1	100.00	24.33
2	1	100.00	25.00
1	2	93.33	26.00
2	2	100.00	26.67
CD (0.05)		14.04	3.94
SEm±		4.63	1.30

germination was obtained with kinetin 1 mg l^{-1} + IBA 1 mg l^{-1}, kinetin 2 mg l^{-1} + IBA 1 mg l^{-1} as well as kinetin 2 mg l^{-1} + IBA 2 mg l^{-1}. The germination percentage was low in the media without any growth regulators.

Regarding the time taken for greening, minimum days were taken by the media with kinetin 1 mg l^{-1} + IBA 1 mg l^{-1} (24.33 days) and kinetin 2 mg l^{-1} + IBA 1 mg l^{-1} (25 days).

Significant difference was noticed among the media with both the growth hormones and the media with only kinetin. Maximum number of days was taken for greening in the medium without any growth regulators.

4.8.6.4 Effect of kinetin, BAP and IBA on leaf as well as shoot and root production

Data pertaining to the results of the trial on the effect of kinetin, BAP and IBA on the time taken for leaf production as well as shoot and root formation are presented in Table 37.

Among the 20 treatments involving kinetin, BAP and IBA, days taken for first leaf production was minimum (25.33 days) for the treatment involving kinetin 8 mg l^{-1} and IBA 4 mg l^{-1} followed by kinetin 8 mg l^{-1} + IBA 2 mg l^{-1}.

Significant difference could not be obtained between treatments involving kinetin 4 mg l^{-1}, 6 mg l^{-1} along with IBA 2 mg l^{-1}, 4 mg l^{-1} and 6 mg l^{-1}. This was also on par with media containing BAP 4 mg l^{-1} + IBA 4 mg l^{-1}, BAP 6 mg l^{-1} + IBA 4 mg l^{-1} and BAP 8 mg l^{-1} + IBA 4 mg l^{-1}.

Kinetin and BAP, each at 8 mg l^{-1} along with IBA at 6 mg l^{-1} as well as 8 mg l^{-1} had almost the same effect.

Maximum time was taken in the medium containing BAP 2 mg l^{-1} and IBA 2 mg l^{-1} (33.00 days).

Table 37. Effect of Kinetin, BAP and IBA on leaf production as well as shoot and root formation (New Pink x Emma White)

Medium - VW
Stage - Protocorm

Kinetin (mg l^{-1})	IBA (mg l^{-1})	Days to first leaf production	Days to shoot and root formation	Remarks
2	2	29.67	51.33	
4	2	27.00	50.33	
4	4	26.00	46.67	Proliferation of protocorm
6	2	26.00	49.67	
6	4	28.00	47.67	
6	6	29.00	44.33	
8	2	26.33	44.00	
8	4	25.33	43.00	Proliferation of protocorm
8	6	29.67	44.33	
8	8	29.00	44.00	Proliferation of protocorm
BAP (mg l^{-1})				
2	2	33.00	53.00	
4	2	30.67	52.00	
4	4	27.00	47.00	
6	2	27.67	52.67	
6	4	27.33	47.66	Proliferation of protocorm
6	6	29.67	44.33	Proliferation of protocorm
8	2	30.67	45.00	
8	4	28.33	45.67	Proliferation of protocorm
8	6	29.67	46.33	Proliferation of protocorm
8	8	29.67	46.00	Proliferation of protocorm
CD(0.05)		3.17	3.46	
SEm±		1.11	1.21	

A similar trend was noticed with respect to the time taken for shoot and root formation also which was minimum with kinetin 8 mg l^{-1} + IBA 4 mg l^{-1} (43.00 days). Here also the longest period was taken for the treatment involving BAP 2 mg l^{-1} and IBA 2 mg l^{-1} (53.33 days).

There was no significant difference among the treatments involving 8 mg l^{-1}, kinetin and 8 mg l^{-1} BAP, 6 mg l^{-1}, kinetin + 6 mg l^{-1} IBA, 6 mg l^{-1} BAP + 6 mg l^{-1} IBA.

4.8.6.4 Effect of kinetin, BAP and NAA on seedling growth

Data pertaining to the effect of different concentrations of kinetin, BAP and NAA on the growth of seedlings are presented in Table 38 (Plate 22a).

4.8.6.4.1 Seedling height

It was seen that a seedling height of 2.73 cm was obtained with kinetin 8 mg l^{-1} + NAA 4 mg l^{-1}, which was followed by kinetin and NAA, each at 4 mg l^{-1} (2.70 cm), kinetin 8 mg l^{-1} + NAA 6 mg l^{-1} (2.70 cm), BAP 8 mg l^{-1} + NAA 6 mg l^{-1} (2.53 cm).

The minimum height of 1.83 cm was observed with media containing kinetin 2 mg l^{-1} + NAA 2 mg l^{-1} as well as BAP 2 mg l^{-1} + NAA 2 mg l^{-1}. Not much different was the treatment with kinetin 4 mg l^{-1} + NAA 2 mg l^{-1} (1.87 cm) and BAP 4 mg l^{-1} + NAA 2 mg l^{-1} (1.90 cm).

4.8.6.4.2 Number of leaves

From the table it is clear that the number of leaves was also affected by the growth regulators. It ranged from 3.00 in the media with kinetin 2 mg l^{-1} + NAA 2 mg l^{-1} to 5.8 in kinetin 4 mg l^{-1} + NAA 4 mg l^{-1} containing medium. Significant differences were found among different treatments.

Table 38. Effect of Kinetin, BAP and NAA on seedling growth and development (New Pink x Emma White)

Medium - VW
Culture period - 4 months

Kinetin (mg l^{-1})	NAA (mg l^{-1})	Seedling characters				
		Height (cm)	Number of leaves	Mean leaf length (cm)	Number of roots	Mean length of roots (cm)
2	2	1.83	3.00	1.96	2.47	1.27
4	2	1.87	4.40	2.23	2.77	1.73
4	4	2.70	5.80	3.33	4.60	3.00
6	2	1.93	3.40	2.36	2.83	2.10
6	4	2.27	3.73	2.73	4.40	2.60
6	6	2.07	3.40	2.00	2.87	2.87
8	2	2.17	4.00	2.13	2.53	1.67
8	4	2.73	4.60	2.13	3.47	2.53
8	6	2.70	5.00	2.83	3.53	2.97
8	8	2.30	4.37	2.97	4.73	3.03
BAP (mg l^{-1})						
2	2	1.83	3.13	1.87	3.07	1.53
4	2	1.90	3.93	2.07	4.30	1.23
4	4	2.30	4.70	2.80	4.40	2.33
6	2	2.00	4.07	2.37	2.80	1.63
6	4	2.20	4.40	3.03	3.73	2.83
6	6	2.00	3.13	2.27	3.20	2.90
8	2	2.30	3.90	2.23	2.73	1.30
8	4	2.50	4.47	1.90	3.13	2.47
8	6	2.53	4.47	2.80	3.53	2.80
8	8	2.23	4.40	2.73	4.53	2.90
CD(0.05)		0.51	1.17	0.49	1.00	0.54
SEm±		0.18	0.41	0.17	0.35	0.19

In the treatments involving BAP, maximum number of leaves was obtained with 4 mg l^{-1} BAP + 4 mg l^{-1} NAA (4.70) followed by 8 mg l^{-1} BAP + 4 mg l^{-1} and 6 mg l^{-1} NAA (4.47). Here also the minimum number (3.13) was with BAP 2 mg l^{-1} + NAA 2 mg l^{-1}.

4.8.6.4.3 Leaf length

A maximum leaf length of 3.33 cm was recorded for the treatment involving kinetin 4 mg l^{-1} + NAA 4 mg l^{-1} as well as kinetin 6 mg l^{-1} + NAA 2 mg l^{-1}. This was followed by BAP 6 mg l^{-1} + NAA 4 mg l^{-1} (3.30 cm). These three treatments were significantly superior to all others in increasing the length of leaves.

The least length of 1.87 cm was observed for the treatment with BAP 2 mg l^{-1} + NAA 2 mg l^{-1} and a length of 1.90 cm in kinetin 2 mg l^{-1} + NAA 2 mg l^{-1}.

4.8.6.4.4 Number of roots

Number of roots was maximum for the treatment with kinetin 8 mg l^{-1} + NAA 8 mg l^{-1} (4.73), closely followed by kinetin 4 mg l^{-1} + NAA 4 mg l^{-1} (4.60) and BAP 8 mg l^{-1} + NAA 8 mg l^{-1} (4.53).

Media with kinetin 6 mg l^{-1} + NAA 4 mg l^{-1} and BAP 4 mg l^{-1} + NAA 4 mg l^{-1} also had higher root number (4.40 each), which were on par with the above three treatments.

The lowest number was recorded in the medium containing kinetin 2 mg l^{-1} + NAA 2 mg l^{-1} (2.47).

4.8.6.4.5 Root length

Significant differences were observed in the treatments with respect to the length of roots. Significantly longer roots were found in the treatments, kinetin

Plate - 22 Seedling growth in different media

(a) With kinetin and NAA each at 4mg l^{-1} (left) and with kinetin and NAA each at 2 mg l^{-1} (right)

(b) Without peptone (left) and with peptone 1000 mg l^{-1} (right)

(c) With adenine 6 mg l^{-1} (left) and without adenine (right)

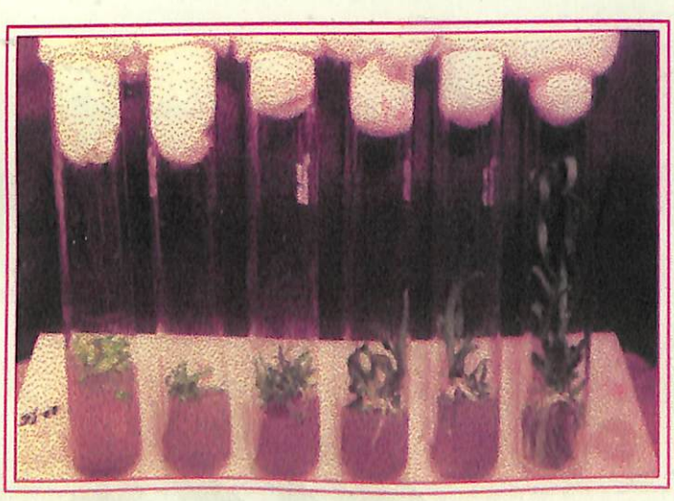


Plate - 23 Stages of seedling growth

Plate - 24 A fully grown seedling

8 mg l^{-1} + NAA 8 mg l^{-1} (3.03 cm), kinetin 4 mg l^{-1} + NAA 4 mg l^{-1} (3.00 cm), kinetin 8 mg l^{-1} + NAA 6 mg l^{-1} (2.97 cm), BAP 6 mg l^{-1} + NAA 6 mg l^{-1} (2.90 cm) as well as BAP 8 mg l^{-1} + NAA 8 mg l^{-1} (2.90 cm).

The least length was obtained in the medium with BAP 4 mg l^{-1} + NAA 2 mg l^{-1} (1.23 cm) followed by kinetin 2 mg l^{-1} + NAA 2 mg l^{-1} (1.27 cm).

4.8.6.5 Effect of kinetin, BAP and IBA on seedling growth

The data pertaining to the trial to find out the effect of kinetin, BAP and IBA on the growth of seedling are presented in Table 39.

4.8.6.5.1 Seedling height

Out of the 20 treatments tried, kinetin 8 mg l^{-1} + IBA 8 mg l^{-1} gave the maximum height (2.33 cm) closely followed by kinetin 4 mg l^{-1} + IBA 4 mg l^{-1} (2.23 cm) and kinetin 8 mg l^{-1} + IBA 6 mg l^{-1}. Significant differences were observed among the different treatments with regard to height of seedlings.

Minimum height of 1.17 cm was recorded for the treatment kinetin 2 mg l^{-1} + IBA 2 mg l^{-1} followed by BAP 2 mg l^{-1} + IBA 2 mg l^{-1} (1.23 cm). The increased levels of both kinetin and BAP resulted in the increments in height.

4.8.6.5.2 Number of leaves

Highest number of leaves was observed in the medium with kinetin 6 mg l^{-1} + NAA 2 mg l^{-1} as well as kinetin 8 mg l^{-1} + NAA 6 mg l^{-1} (4.73 each) followed by BAP 8 mg l^{-1} + IBA 6 mg l^{-1} (4.60). Kinetin 6 mg l^{-1} + NAA 4 mg l^{-1} (4.47) and kinetin 4 mg l^{-1} + NAA 4 mg l^{-1} (4.33) also gave good results with regard to leaf number.

The least number was observed with the treatment kinetin 8 mg l^{-1} + IBA 2 mg l^{-1} as well as BAP 2 mg l^{-1} + IBA 2 mg l^{-1}, followed by kinetin 2 mg l^{-1} + IBA 2 mg l^{-1} (2.47).

Table 39. Effect of Kinetin, BAP and IBA on seedling growth and development (New Pink x Emma White)

Medium - VW
Culture period - 4 months

Kinetin (mg l^{-1})	IBA (mg l^{-1})	Seedling characters				
		Height (cm)	Number of leaves	Mean leaf length (cm)	Number of roots	Mean length of roots (cm)
2	2	1.17	2.47	2.07	2.73	1.23
4	2	1.57	3.07	2.17	2.80	1.53
4	4	2.27	4.33	2.53	4.27	2.97
6	2	2.03	4.73	2.40	3.40	2.00
6	4	2.13	4.47	2.20	4.67	2.97
6	6	1.27	3.47	2.07	4.67	2.40
8	2	1.90	2.27	2.20	3.53	1.67
8	4	2.10	3.40	2.47	4.93	2.67
8	6	2.27	4.73	2.30	5.44	2.47
8	8	2.33	3.93	1.97	4.93	2.87
BAP (mg l^{-1})						
2	2	1.23	2.27	2.03	2.67	1.20
4	2	1.97	2.73	2.27	3.07	1.50
4	4	2.00	3.67	2.23	3.73	2.70
6	2	1.80	4.07	2.23	3.20	1.40
6	4	1.77	3.40	2.10	4.87	2.97
6	6	1.67	3.00	1.77	4.20	2.10
8	2	1.90	3.40	2.03	3.20	1.60
8	4	2.03	2.53	2.30	4.40	2.77
8	6	2.17	4.60	2.17	4.93	2.33
8	8	2.17	3.60	1.97	4.60	2.83
CD(0.05)		0.51	1.14	0.40	1.34	0.54
SEm±		0.18	0.40	0.14	0.47	0.19

4.8.6.5.3 Leaf length

Maximum leaf length was obtained in the medium with kinetin 4 mg l^{-1} + IBA 4 mg l^{-1} (2.53 cm) followed by kinetin 8 mg l^{-1} + IBA 4 mg l^{-1} (2.47 cm) and kinetin 6 mg l^{-1} + IBA 2 mg l^{-1} (2.40 cm).

Significant effects were noticed by the different combinations of kinetin, BAP and IBA on the length of leaves. The least length was obtained in the medium containing BAP 6 mg l^{-1} and IBA 6 mg l^{-1} (1.77 cm). BAP or kinetin along with IBA, each at 8 mg l^{-1} produced leaf length of 1.97.

4.8.6.5.4 Number of roots

Maximum number of 5.44 was obtained in the medium with 8 mg l^{-1} kinetin + 6 mg l^{-1} IBA. This was on par with kinetin 8 mg l^{-1} + IBA 4 mg l^{-1}, kinetin 8 mg l^{-1} + IBA 8 mg l^{-1} and BAP 8 mg l^{-1} + IBA 6 mg l^{-1} (4.93 in all). These were significantly superior to all other treatments.

A minimum of 2.67 roots was observed with the treatment involving BAP 2 mg l^{-1} + IBA 2 mg l^{-1} and 2.73 with that having kinetin 2 mg l^{-1} + IBA 2 mg l^{-1}.

4.8.6.5.5 Root length

Longest roots were observed in the treatments kinetin 4 mg l^{-1} + IBA 4 mg l^{-1}, kinetin 6 mg l^{-1} + IBA 4 mg l^{-1} as well as BAP 6 mg l^{-1} + IBA 4 mg l^{-1} (2.97 cm in each). Kinetin and BAP at 8 mg l^{-1} along with IBA 8 mg l^{-1} also gave good results (2.87 cm and 2.83 cm long roots). The minimum length of roots was recorded for BAP 2 mg l^{-1} + IBA 2 mg l^{-1} (1.20 cm) and kinetin 2 mg l^{-1} + IBA 2 mg l^{-1} (1.23 cm). Significant differences were observed among different treatments.

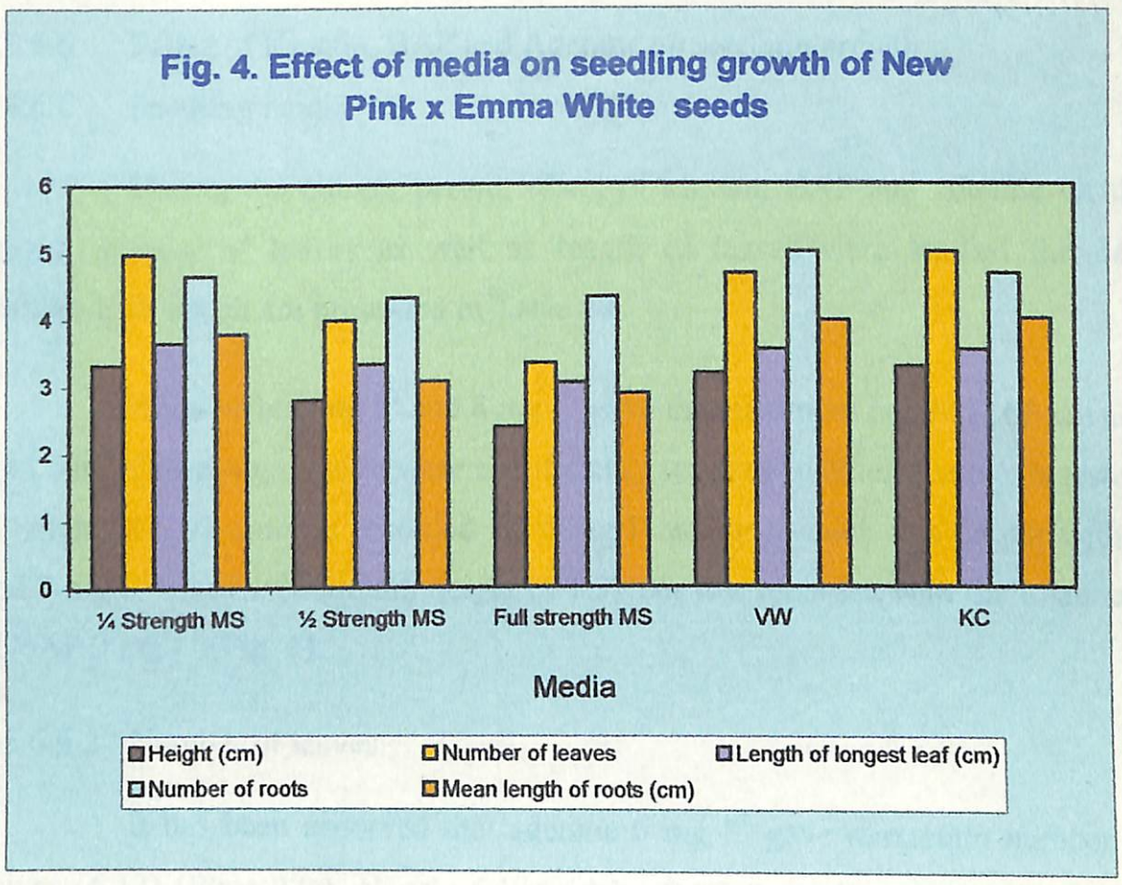


Fig. 4. Effect of media on seedling growth of New Pink x Emma White seeds
6
5
4
3
2
1
0
¼ Strength MS
½ Strength MS
Full strength MS
VW
KC
Media
Height (cm)
Number of leaves
Length of longest leaf (cm)
Number of roots
Mean length of roots (cm)

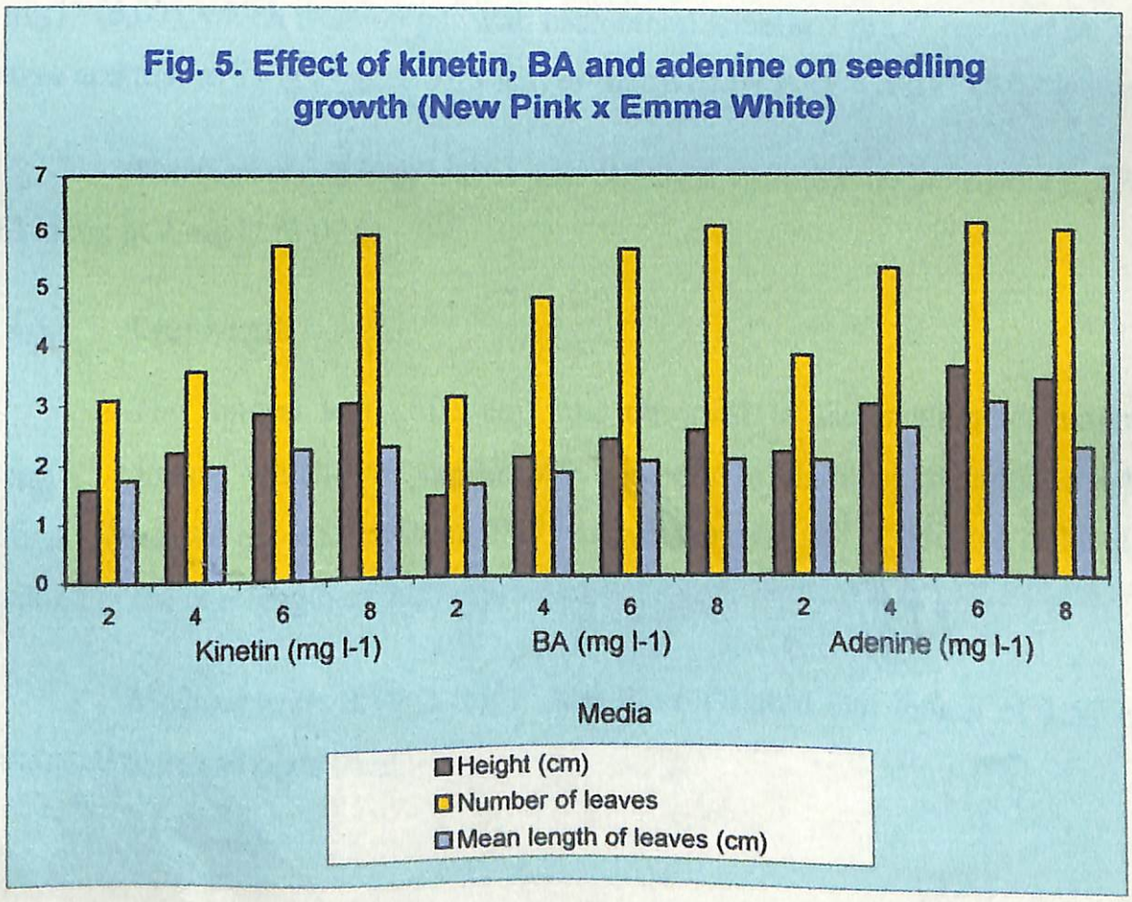


Fig. 5. Effect of kinetin, BA and adenine on seedling growth (New Pink x Emma White)
7
6
5
4
3
2
1
0
2
4
6
8
2
4
6
8
2
4
6
8
Kinetin (mg l-1)
BA (mg l-1)
Adenine (mg l-1)
Media
Height (cm)
Number of leaves
Mean length of leaves (cm)

4.8.6.6 Effect of Kinetin, BAP and Adenine on seedling growth

4.8.6.6.1 Seedling height

During the culture period, effect of kinetin, BAP and adenine on the height, number of leaves as well as length of leaves were studied the data pertaining to which are presented in Table 40.

Adenine at 6 mg l^{-1} and 8 mg l^{-1} gave the maximum height (3.63 cm and 3.43 cm) of seedlings, which were significantly superior to other treatments tested. A height of 2.97 cm was recorded for 4 mg l^{-1} adenine which was on par with 6 and 8 mg l^{-1} kinetin. Minimum height of 1.37 cm was recorded with the treatment of BAP 2 mg l^{-1} (Fig. 5).

4.8.6.6.2 Number of leaves

It has been observed that adenine 6 mg l^{-1} gave maximum number of leaves (6.13) (Plate 22c), closely followed by BAP 8 mg l^{-1} (6.07) and adenine 8 mg l^{-1} (6.00), which were on par with each other. Kinetin 8 mg l^{-1} resulted in 5.93 leaves and that at 6 mg l^{-1} gave 5.73 leaves followed by BAP 6 mg l^{-1} (5.67 leaves).

The least number of leaves was obtained with the medium having BAP or kinetin at 2 mg l^{-1} (3.07).

4.8.6.6.3 Leaf length

The longest leaf (3.03 cm) was observed in the medium containing 6 mg l^{-1} adenine, which was significantly superior to all other treatments. BAP 4 mg l^{-1} increased the leaf length to 2.57 cm, whereas 8 mg l^{-1} of adenine or kinetin resulted in the leaf length of 2.23 cm.

Medium with BAP 2 mg l^{-1} had the minimum leaf length of 1.57 cm among all the treatments tried.

Table 40. Effect of Kinetin, BAP and Adenine on seedling growth (New Pink x Emma White)

Medium - VW
Culture period - 4 months

Kinetin ($mg\ l^{-1}$)	Height (cm)	Number of leaves	Mean length of leaves (cm)
2	1.57	3.07	1.73
4	2.17	3.53	1.93
6	2.80	5.73	2.20
8	2.97	5.93	2.23
BAP ($mg\ l^{-1}$)			
2	1.37	3.07	1.57
4	2.03	4.80	1.77
6	2.33	5.67	1.97
8	2.50	6.07	2.00
Adenine ($mg\ l^{-1}$)			
2	2.13	3.80	2.00
4	2.97	5.33	2.57
6	3.63	6.13	3.03
8	3.43	6.00	2.23
CD (0.05)	0.23	0.89	0.23
SEm±		0.43	0.11

4.8.6.7 Effect of sucrose on seed germination

Difference in the sucrose levels in the medium was found to affect the germination (Table 41).

High germination was obtained irrespective of sucrose levels. But the medium with 3 per cent sucrose, took the maximum time for shoot and root development (101.33 days) which was significantly higher than the time taken in other media with less sucrose levels.

Germination percentage decreased with the lowering of the sucrose levels. The time taken for greening and protocorm development were minimum (26.67 and 45.33 days, respectively) with 3 per cent level, which were significantly different from those at 0.5 per cent level. Minimum time for shoot and root formation was taken by the cultures in the medium with 0.5 per cent sucrose, (88.67 days) which was significantly different from all others.

4.8.6.8 Effect of sucrose on seedling growth

Table 42 shows the effect of different levels of sucrose on seedling growth.

Significant difference could not be observed in height and number of leaves among the different treatments varying in sucrose concentration. Maximum height (3.00 cm) and number of leaves (5.67) were obtained with the sucrose concentration of 0.5 per cent and the minimum with 3 per cent.

The longest leaves were produced at 0.5 per cent sucrose level (3.6 cm) which was significantly superior to that with 3 per cent sucrose (3.2 cm). Regarding the number of roots and mean length of roots also 0.5 per cent sucrose gave the maximum values (6.40 and 4.03 cm, respectively), which were significantly superior to the treatments involving 3 per cent and 2 per cent sucrose.

Table 41. Effect of sucrose on the germination of New Pink x Emma White crossed seeds

Medium - VW + kinetin 4 mg l^{-1} + IBA 4 mg l^{-1}

Sucrose concentration (%)	Germination percentage	Days for greening	Days for protocorm development	Days for shoot and root formation
3.00	100.00	26.67	45.33	101.33
2.0	86.67	30.00	51.67	96.00
1.0	83.33	29.33	52.33	94.00
0.5	71.67	33.00	53.33	88.67
CD(0.05)	15.62	3.23	2.71	2.61
SEm±	4.79	0.99	0.83	0.80

Table 42. Effect of sucrose on seedling growth of New Pink x Emma White seeds

Medium - ½ MS + kinetin 4 mg l^{-1} + IBA 4 mg l^{-1}

Culture period - 4 months

Sucrose concentration (%)	Seedling characters				
	Height (cm)	Number of leaves	Length of longest leaf (cm)	Number of roots	Mean length of roots (cm)
3.0	2.63	4.67	3.20	4.60	3.17
2.0	2.77	5.00	3.57	4.73	3.40
1.0	2.93	5.00	3.53	5.67	4.03
0.5	3.00	5.67	3.60	6.40	4.03
CD(0.05)	NS	NS	0.16	1.01	0.52
SEm±			0.05	0.31	0.16

Significant difference was not noticed between 1.0 per cent and 0.5 per cent sucrose in any of the characters studied.

Medium with 3 per cent sucrose gave the least value in respect of height (2.63 cm), number of leaves (4.67), length of leaf (3.20 cm), number of roots (4.60) and length of roots (3.17 cm).

4.8.6.9 Effect of charcoal on germination and further growth

Data pertaining to the effect of charcoal on seed germination and further development are presented in Table 43.

All the treatments gave good germination. Time taken for greening also was not much affected by the concentration of charcoal in the culture medium, eventhough 0.5 per cent charcoal took the minimum number of days (26.33) for greening.

Significant difference could be obtained among the treatments with respect to the time taken for protocorm development. A culture medium containing 0.5 per cent charcoal took 43.33 days for protocorm development which was on par with 1.0 per cent (46.00 days) and 1.5 per cent (48.67 days) charcoal.

There was no significant difference between the treatments with 2 per cent charcoal and without charcoal in respect of time taken for protocorm development (52.00 and 55.00 days, respectively).

It was found that shoot and root formation occurred in 71.67 days in the treatment with 0.5 per cent charcoal, which was significantly superior to all other treatments. A little higher concentration of charcoal (1.0 per cent), took 74.67 days for shoot and root formation, which was significantly different for all other treatments. Time taken for shoot and root formation increased with increasing levels of charcoal, but the maximum time was taken in the medium without charcoal (86.33 days).

Table 43. Effect of charcoal on the germination of New Pink x Emma White seeds
Medium - VW + kinetin 4 mg l^{-1} + IBA 4 mg l^{-1}

Charcoal (g l^{-1})	Germination percentage	Days for greening	Days for protocorm development	Days for shoot and root formation	Remarks
0	96.67	29.33	55.00	86.33	After germination slow growth compared to charcoal media
0.5	96.67	26.33	43.33	71.67	Sturdy growth of seedlings
1.0	98.33	28.67	46.00	74.33	Healthy and plumpy protocorms, sturdy growth
1.5	93.33	29.67	48.67	78.00	Sturdy and healthy growth
2.00	96.67	29.00	52.00	79.67	Slow and stunted growth
CD(0.05)	NS	NS	5.48	2.87	
SEm±			1.74	0.91	

Table 44. Effect of charcoal on seedling growth (New Pink x Ema White)
Medium - VW + Kinetin 4 mg l^{-1} + IBA 4 mg l^{-1}
Culture period - 4 months

Charcoal (g l^{-1})	Seedling characters				
	Height (cm)	Number of leaves	Mean leaf length (cm)	Number of roots	Mean length of roots (cm)
0	2.00	3.33	2.30	4.00	2.77
0.5	2.50	4.67	2.73	5.67	3.27
1.0	2.53	5.00	2.77	5.73	3.73
1.5	2.57	4.33	2.60	5.73	3.67
2.0	2.40	3.67	2.43	5.47	3.33
CD(0.05	0.32	NS	0.22	0.76	NS
SEm±	0.10		0.07	0.24	

4.8.6.10 Effect of charcoal on seedling growth

Data pertaining to the trial with different levels of charcoal on seedling growth are presented in Table 44.

Height of the shoots was maximum with 1.5 per cent charcoal but significant difference was not observed among the charcoal levels of 0.5, 1.0, 1.5 and 2.0 per cent with respect to height. The minimum height of 2.00 cm was found in medium without charcoal, which was significantly different from others.

Regarding the number of leaves, there was no significant difference among the different treatments, eventhough the maximum number was obtained with 1.0 per cent charcoal (5.00) followed by 0.5 per cent charcoal (4.67).

Longest leaves were produced with 1.0 per cent charcoal (2.77 cm) followed by 0.5 per cent charcoal (2.73 cm), which were significantly superior to the medium with 2 per cent charcoal and without charcoal. Here also minimum length was noticed for medium without charcoal (2.3 cm).

Significant difference could not be observed among the different charcoal levels with respect to the number of roots eventhough the maximum number was with 1.0 and 1.5 per cent of charcoal (5.73). Medium without charcoal gave the least number (4.00).

Length of roots did not differ significantly among the treatments with and without charcoal. Maximum length observed was with 1.0 per cent charcoal (3.73) followed by 1.5 per cent charcoal (3.67 cm).

4.8.6.11 Effect of media supplements on leaf, shoot and root formation

Data pertaining to the effect of media supplements on first leaf production as well as shoot and root formation are presented in Table 45.

Table 45. Effect of media supplements on first leaf production and shoot and root formation (New Pink x Emma White)

Medium - VW + Kinetin 4 mg l^{-1} + IBA 4 mg l^{-1}
Culture period - 4 months

Media supplements (per litre)	Days to first leaf production	Days to shoot and root formation	Remarks
Nil	15.33	46.33	
Peptone 500 mg	14.67	45.67	
Peptone 1000 mg	15.00	43.67	Proliferation of numerous side shoots and sturdy growth
Coconut water 50 ml	16.33	47.00	
Coconut water 100 ml	14.00	44.67	
Coconut water 150 ml	13.67	43.00	More proliferation of side shoots when compared to others
Coconut water 200 ml	14.67	44.00	
Banana pulp 20 g	16.00	43.67	
Banana pulp 40 g	15.33	45.67	Sturdy and thick growth
Banana pulp 60 g	15.67	45.00	Sturdy and thick growth
Banana pulp 80 g	15.67	45.67	Growth become slow later
CD(0.05) SEm±	NS	NS	

Table 46. Effect of media supplements on seedling growth (New Pink x Emma White)

Medium - VW + Kinetin 4 mg l^{-1} + IBA 4 mg l^{-1}
Culture period - 4 months

Media supplements (per litre)	Seedling characters				
	Height (cm)	Number of leaves	Mean leaf length (cm)	Number of roots	Mean length of roots (cm)
Nil	2.17	4.07	2.20	4.40	2.37
Peptone 500 mg	2.43	4.87	2.63	5.33	2.93
Peptone 1000 mg	3.03	5.73	3.19	7.07	3.27
Coconut water 50 ml	2.27	4.73	2.53	4.40	2.13
Coconut water 100 ml	2.70	5.40	2.87	4.73	2.63
Coconut water 150 ml	3.20	5.93	3.30	7.00	3.47
Coconut water 200 ml	2.70	4.67	2.90	5.20	2.23
Banana pulp 20 g	2.20	4.53	2.47	4.47	2.13
Banana pulp 40 g	2.53	5.07	2.73	4.93	2.47
Banana pulp 60 g	2.37	4.33	2.47	5.00	2.50
Banana pulp 80 g	2.40	5.00	2.43	5.07	2.47
CD(0.05) SEm±	NS	NS	0.35 0.12	1.20 0.41	0.50 0.17

Significant difference could not be observed among the different treatments with respect of the time taken for first leaf production as well as shoot and root formation.

Minimum time of 13.67 days was taken for first leaf production in coconut water 150 ml l^{-1} followed by coconut water 100 ml l^{-1} (14.00 days).

Maximum time (16.00 days) was taken for the first leaf production in banana pulp 20 mg l^{-1}.

Forty three days were taken for the shoot and root formation in coconut water 150 ml l^{-1}, followed by peptone 1000 mg l^{-1} (43.67 days). Maximum time was taken by coconut water 50 ml l^{-1} (47.00 days) for shoot and root formation.

4.8.6.12 Effect of media supplements on seedling development

Effect of various media supplements, namely peptone, coconut water and banana pulp on the growth of the seedlings was studied and data presented in Table 46.

4.8.6.12.1 Seedling height

Eventhough a maximum height of 3.20 cm was attained in the treatment involving coconut water 150 ml l^{-1}, there was no significant difference among the different treatments tried. Peptone at 1000 mg l^{-1} could increase the height to 3.03 cm (Plate 22b) and coconut water at 100 ml l^{-1} as well as at 200 ml l^{-1}, increased the height to 2.7 cm, each. The least height (2.17 cm) was observed in the medium without any additives.

4.8.6.12.2 Number of Leaves

Out of the different additives used, coconut water at 150 ml gave maximum number of leaves (5.93) followed by peptone 1000 mg l^{-1} (5.73). The

least number (4.07) was obtained in medium without any additives eventhough there was no significant difference among the various treatments.

4.8.6.12.3 Leaf length

Coconut water at 150 ml l^{-1} and peptone 1000 mg l^{-1} produced the longest leaves (3.30 cm and 3.19 cm, respectively) which were significantly superior to all other treatments. Significant difference could be noticed among the different treatments and the least length was (2.20 cm) obtained in medium without additives.

4.8.6.12. 4 Number of roots

Maximum number of roots was observed in peptone 1000 mg l^{-1} (7.07), followed by coconut water 150 ml l^{-1} (7.00), which were significantly superior to all other treatments.

Banana pulp at different levels did not vary significantly in their effect on number of roots.

The least number of roots (4.40) was observed with coconut water 50 ml as well as in medium without the additives.

4.8.6.12.5 Root length

Coconut water at 150 ml l^{-1} could give a maximum length of roots (3.47 cm), which was significantly superior to all other treatments, except peptone 1000 mg l^{-1} (3.27 cm). Significant difference existed between the various treatments. Shortest roots were produced with banana pulp 20 mg l^{-1} and coconut water 50 ml l^{-1} (2.13 cm, each).

4.8.7 Callusing

Data pertaining to callus formation (Table 47) revealed that NAA 4 mg l^{-1} + 2,4-D 2 mg l^{-1} as well as the same with the addition of kinetin 2 mg l^{-1}

Table 47. Callusing from *in vitro* protocorms (Emma White selfed)

Medium - ½ MS

Growth hormone concentration (mg l^{-1})			Percent of cultures showing callusing	Remarks
NAA	2,4-D	Kinetin		
2	2	-	20	
2	2	2	25	
4	2	-	45	More callus proliferation
4	2	2	45	More callus proliferation
6	2	-	40	
6	2	2	40	More callus proliferation

Table 48. Plantlet development from *in vitro* callus

Medium - ¼ MS + 1.5% sucrose

Growth hormone concentration		Peptone (mg)	Percentage of cultures developing plantlets
Kinetin (mg l^{-1})	IBA (mg l^{-1})		
1	1	500	10.00
2	1	500	15.00
2	2	500	30.00
4	2	500	50.00
4	4	500	60.00
6	4	500	40.00
6	6	500	35.00

recorded the highest values (45%). This was closely followed by NAA at 6 mg l^{-1} + 2,4-D at 2 mg l^{-1} alone or along with kinetin 2 mg l^{-1} (40% callusing). Least callusing was observed in NAA 2 mg l^{-1} + 2,4-D 2 mg l^{-1} (20%). Maximum number of plantlets was recorded from callus in the medium containing kinetin 4 mg l^{-1} + IBA 4 mg l^{-1} + peptone 500 mg l^{-1} (60%) (Table 48).

4.8.8 PLB formation

Data on the PLB production are presented in Table 49. It was found that BAP 25 mg l^{-1} + NAA 2 mg l^{-1} gave the maximum percentage of cultures with PLB formation (60%) followed by BAP 25 mg l^{-1} + NAA 1 mg l^{-1} (50%). PLB formation was only 5 per cent in BAP 10 mg l^{-1} + NAA 1 mg l^{-1} and PLBs were not obtained in medium with BAP 5 mg l^{-1} + NAA 2 mg l^{-1} and in BAP 5 mg l^{-1} + NAA 1 mg l^{-1}.

A maximum of three plantlets were only obtained from PLBs in a medium containing BAP 10 mg l^{-1} + NAA 1 mg l^{-1} (Table 50).

4.9 *In vitro* mutagenesis

Protocorms, callus induced from protocorms and PLBs from *in vitro* leaf were subjected to irradiation by gamma rays under *in vitro* conditions. Doses above 60 Gy were found to be lethal to callus and PLBs. The irradiated protocorms above 60 Gy also failed to regenerate into shoots and roots. Based on the above observations, doses upto 60 Gy were selected for irradiation.

4.9.1 Irradiation of protocorms

Seventeen hybrids were subjected to irradiation. The development of irradiated protocorms into seedling differed in different doses. Significant variations occurred in the time taken to reach each stage in culture. A general observation is that irradiation reduced the height of the plants and as the dose increased the height was also reduced accordingly. Higher doses of irradiation also

Table 49. PLB formation from the *in vitro* leaf of New Pink x Pink Tips seedlings

Medium - ¼ MS + 1.5% sucrose

Growth hormone concentration		Cultures developing PLBs (%)	Time taken for PLB development (days)
BAP (mg l^{-1})	NAA (mg l^{-1})		
5	1	Nil	-
5	2	Nil	-
10	1	5.00	45.00
10	2	10.00	45.00
20	1	25.00	40.00
20	2	30.00	42.00
25	1	50.00	36.00
25	2	60.00	30.00

Table 50. Plantlet development from the *in vitro* leaf PLBs

Medium - ¼ MS + 1.5% sucrose

Growth hormone concentration		Cultures developing PLBs (%)	Time taken for PLB development (days)
BAP (mg l^{-1})	NAA (mg l^{-1})		
5	1	5	1
5	2	5	2
10	1	5	3
10	2	5	2

caused smaller and broader leaves with dark green colour. The rate of growth became slow at the higher doses of irradiation (Plate 25). A slight increase in the number of leaves and shoots was also observed in the high doses. Percentage of survival was also reduced in the irradiated ones. The data pertaining to the variations are presented under the following heads.

4.9.1.1 Pramott-II x Emma White

Eventhough irradiation has decreased the survival percentage (except in 20 Gy dose), there was no significant variation among the different doses (Table 51). The non-irradiated cultures gave 66.67 per cent survival and the lowest survival was that of 40 Gy (50.00%).

Days to first leaf production was minimum in non-irradiated protocorms (32.33), which was significantly superior to all others (Table 52). Maximum time taken for first leaf production was 65 days it was in 60 Gy.

No significant difference was noticed between the non-irradiated protocorms and those irradiated with 10 Gy with regard to days for shoot and root formation (65.00 days). Maximum time for shoot and root formation as well as for planting out was observed in the irradiation dose of 60 Gy (128.00 and 433.33 days, respectively).

Eventhough a slightly higher number of shoots and number of leaves were noticed in irradiated cultures, there was no significant difference.

4.9.1.2 Sonia 28 x Emma White

Data on the changes due to irradiation are presented in Table 52. Significant difference was not observed in the survival percentage of irradiated and non-irradiated ones. The minimum time for first leaf production was taken by the unirradiated (33.67 days) which was significantly superior to all others. Maximum time was taken by 60 Gy (77.33 days). Maximum time for shoot and root

Table 51. Effect of irradiation on the growth of seedlings of Pramott-II x Emma White

Irradiated material - Protocorm

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for different-iation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	66.67	32.33	65.00	2.67	5.00	268.80	Normal seedlings with thick dark green leaves
10	58.00	38.33	65.00	3.00	6.00	285.00	Dark green leaves and seedling were of same size as of nonirradiated
20	75.00	44.33	86.00	4.00	5.00	305.00	Protocorms were darker and larger than those of nonirradiated and 10 Gy. But plants were of same size as that of nonirridated one leaf was found to be broader than others
30	58.33	50.00	85.67	3.00	4.00	346.67	Dark green leaves and plants shorter than those of 20 Gy
40	50.00	53.33	89.00	3.67	5.00	370.00	Some leaves were narrow and some leaves broad. Some of them had light green colour also. Plants were of similar size as 30 Gy
50	58.88	61.00	108.00	3.67	6.30	403.30	Leaves were closely arranged in comparison to others. Leaves narrow and dark green. Stout, short and compact stem
60	58.00	65.00	128.00	3.00	6.33	433.33	Maroon lines noticed on some shoots after planting
CD (0.05) SEm±	NS	5.55 1.83	11.07 3.65	NS	NS	22.04 7.26	Leaves were compactly arranged, thick and broad and dark green. Stem thick and short

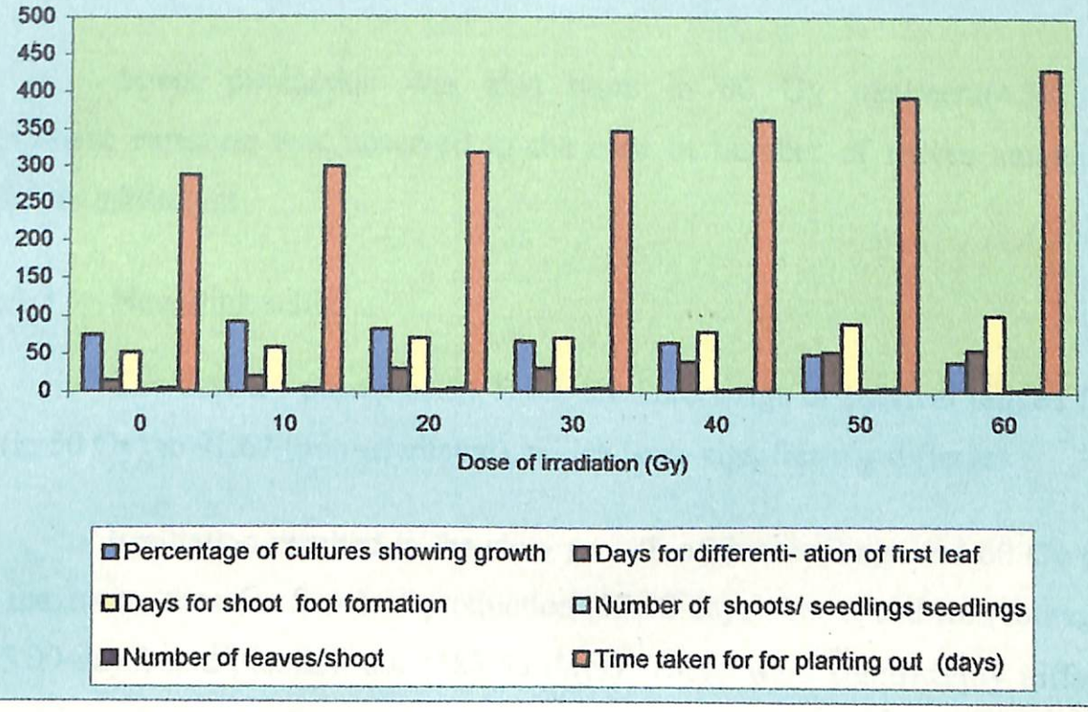
Table 52. Effect of irradiation on the growth of seedlings of Sonia-28 x Emma White

Irradiated material - Protocorm

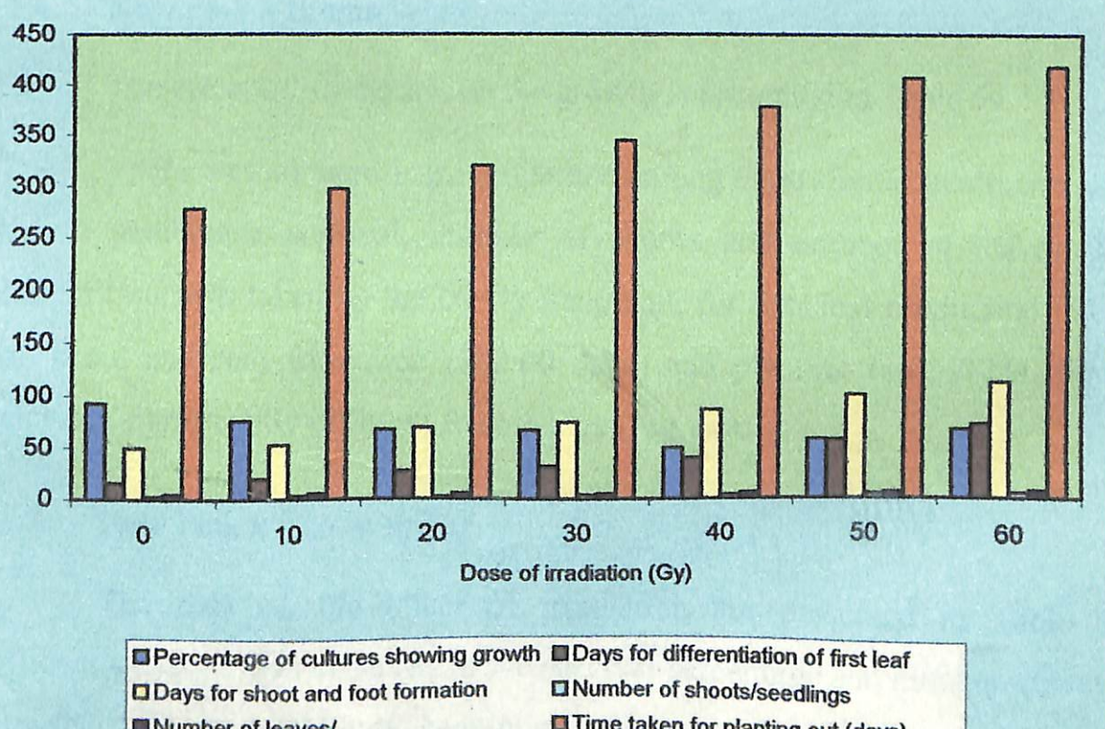
Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	66.00	33.67	82.00	2.00	6.00	275.00	Narrow leaves normal plants. Thick leaves, root growth normal. Maroon colouration on stem and leaf margin after planting out.
10	50.00	42.00	94.00	1.67	5.33	293.00	Some plants had broad leaves and some narrow leaves. Normal sized plants.
20	75.00	49.00	102.00	2.67	6.00	302.67	Thick leaves and thick roots of same length (2.5 cm) prominent veins on leaves. Light green leaves in some plants.
30	58.00	58.00	108.00	3.00	6.00	308.00	Dark green, light green and pale yellow leaves noticed. Broader leaves and small plants.
40	50.00	61.00	115.00	3.00	5.67	323.00	Very short stem and broad leaves. Roots also small and less in number.
50	58.00	71.00	120.00	4.00	7.00	334.00	Dark green, light green and pale yellow leaves observed. Very small and stout stem plants, less in number rest were very small (0.5-1.0 cm) sized shoots only. No uniform growth of plants. Plants were similar in these irradiated materials.
60	60.00	77.33	125.00	4.33	7.00	342.00	
CD (0.05) SEm±	NS	7.55 2.49	8.49 2.80	1.36 0.45	NS	10.89 3.59	

Fig. 6. Effect of irradiation on the growth of seedlings

a) New Pink x Emma White



b) New Pink x Candy Stripe



formation as well as for planting out was for 60 Gy (125 and 342 days, respectively). These were significantly low for the unirradiated protocorms (82.00 and 275.00 days, respectively).

Shoot production was also more in 60 Gy treatment(4.33). No significant variation was observed in the case of number of leaves among the different treatments.

4.9.1.3 New Pink selfed

The data are presented in Table 53. Percentage of survival ranged from 41 (in 50 Gy) to 91.67 (non-irradiated), which were significantly different.

Irradiation resulted in the slow growth of the seedlings and 60 Gy took the maximum time for first leaf production (53.00 days), shoot and root formation (125.00 days) and planting out (388.33 days). These were significantly different from others. Eventhough a slight increase in the number of shoots and leaves was noticed in higher doses, it was not significant .

4.9.1.4 New Pink x Emma White

The effect of irradiation on the growth is presented in Table 54.

There was no significant difference among the different treatments with regard to percentage survival, number of shoots and number of leaves. But maximum time was taken by the 60 Gy treatment, for first leaf production (57.00 days), shoot and root formation (102.00 days) and planting out (432.00 days), which were significantly different from others (Fig. 6a).

4.9.1.5 New Pink x Candy stripe

The data on the effect of irradiation are presented in Table 55. Significant variation was observed in the survival percentage and number of leaves among the different treatments. Maximum time was taken by the 60 Gy treated

Table 53. Effect of irradiation on the growth of seedlings of New Pink selfed seeds

Irradiated material - Protocorm

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for different-iation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	91.67	17.00	51.00	3.00	5.33	288.00	Normal plant (size -3 cm). Green, thick leaves (4 x 0.5 cm) roots many and elongated.
10	67.00	23.00	59.00	2.67	6.00	298.00	Plant size - 2.8-3 cm, green leaves (4 x 0.8 cm). Root growth also normal.
20	67.00	28.00	79.33	3.33	6.00	307.00	Larger protocorms compared to all others. Plants were of normal size and leaf larger than unirradiated.
30	66.67	38.67	91.00	3.00	5.00	329.00	Opposite arrangement of leaves seen in some plants. Dark green leaves, wavy margin in some leaves normal.
40	42.00	46.00	106.00	3.00	6.33	356.67	Plants were small, thick and with closely arranged dark green leaves. Roots very less (4-5 Nos).
50	41.00	50.00	118.00	2.33	6.00	382.00	Dark green, thick leaves, some plants were large, others were very small (0.5-0.8 cm).
60	58.00	53.00	125.00	3.00	7.00	388.33	Very slow growth, short plants with small broad leaves, and root growth restricted very few plants others very small (0.8-1 cm).
CD (0.05) SEm±	30.21 9.96	4.16 1.37	9.04 2.98	NS	NS	9.07 2.99	

Table 54. Effect of irradiation on the growth of seedlings of New Pink x Emma White

Irradiated material - Protocorm

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ Seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	75.00	15.00	52.00	2.00	5.00	288.00	Plants (size - 3.5 cm) with dark green leaves (3.5 x 0.6 cm). Roots 10 in numbers and 2.5 cm long.
10	92.00	21.00	59.00	3.00	5.33	300.00	Plant size was smaller than that of unirradiated, some leaves were larger and some smaller, roots similar to that of normal.
20	83.00	30.67	72.00	3.00	5.00	319.33	Plants very much similar to those of 10 Gy
30	67.00	32.00	72.00	2.67	5.00	349.00	Plants smaller than normal and 20 Gy, more number of roots observed. Leaves small.
40	66.00	41.00	80.00	3.00	5.33	365.00	Plants small, with normal sized leaves. Dark green and light green leaves observed.
50	50.00	54.00	91.00	3.67	5.00	395.00	Short plants, thick dark green leaves root growth normal. Number of plants very less.
60	40.00	57.00	102.00	3.00	6.33	432.00	Broad leaves broader than length. Root growth very less. Thick and short stem only very few plants, others not grown up. Growth was not uniform.
CD (0.05) SEm±	NS	6.89 2.27	8.07 2.66	NS	NS	15.89 5.24	

protocorms, for first leaf production (72.00 days), shoot and root formation (113.00 days) as well as for planting out (418.00 days). Maximum number of shoots was observed in 60 Gy treatment. No significant difference could be noticed with respect to the number of leaves in different treatments (Fig. 6b).

4.9.1.6 Irradiation of other protocorms

Apart from the above crosses, irradiation was done at the protocorm stage in New Pink selfed, Emma White x Pink Tips, Emma White selfed, Candy Stripe x New Pink, Candy Stripe selfed, Hieng Beauty x Sonia 28, Hieng Beauty x Emma White and Hieng Beauty x New Pink. The effect of irradiation in all these crosses are presented below.

All the protocorms, which were irradiated, showed the same trend of growth with only variations in the number of days taken for reaching each stage (Table 56 -67). Maximum survival was with the non-irradiated protocorms, which ranged from 66.67 to 100.00 per cent, according to the crosses. Time taken for first leaf production was maximum for the 60 Gy irradiation (44 days to 77 days). Shoot and root formation in 60 Gy took the maximum time, which ranged from 72 days to 142 days in different crosses and were significantly different. Maximum time for planting out of seedlings was also with the 60 Gy treated seedlings, which recorded a range of 325.00 to 435.00 days in different crosses, with significant variations from others.

A slight increase in the number of shoots and number of leaves, according to the increase in the dose of irradiation was found, but it was not significant.

4.9.2 Irradiation of PLBs

The effect of irradiation on the growth of PLBs from New Pink x Pink Tips is presented in Table 68.

Table 55. Effect of irradiation on the growth of seedlings of New Pink x Candy Stripe

Irradiated material - Protocorm

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	92.00	16.00	49.33	3.00	5.00	277.00	Plants of normal size (4-4.5 cm), green leaf (size 3.5 x 0.5 cm) long roots (3.5 cm), 8-10 in numbers.
10	75.00	20.00	52.00	3.33	5.33	297.00	Dark green leaves, shoots and roots normal as those of unirradiated.
20	66.67	26.67	69.00	2.33	5.33	320.00	Leaf smaller than normal, roots were more in number (14-15 Nos.)
30	67.00	32.00	74.33	4.00	5.00	345.00	Short stem and small leaves. Roots also small and less in number.
40	50.00	40.00	87.00	4.00	6.00	378.00	Leaf and stem were of normal size. Two small leaves with wavy margin also observed. Maroon lines on the leaf margin after planting.
50	58.00	57.67	102.00	4.33	6.00	407.00	Leaf breadth was more than length and dark green short plants with closely arranged leaves.
60	67.00	72.00	113.00	5.00	7.00	418.00	Very small leaves and short thick stem. Internode very much reduced root size and number very less. Number of plants very less compared to all others and growth not uniform.
CD (0.05) SEm±	NS	4.79 1.58	5.72 1.88	0.75 0.25	NS	18.02 5.94	

Table 56. Effect of irradiation on the growth of seedlings of New Pink x Pink Tips

Irradiated material - Protocorm

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	92.00	17.67	50.00	3.67	4.67	322.00	Normal plants of 4 cm height and 4-5 leaves. Roots were of normal size 3 cm leaf and 8-10 in numbers.
10	75.00	23.00	53.33	3.67	5.00	309.00	Plants were of same size as those of unirradiated. Some leaves were larger than normal.
20	67.00	28.00	65.00	4.00	5.00	322.00	Thick leaves and small roots (2.2 cm) and plant size same as that of normal.
30	50.00	37.33	74.00	5.00	5.33	342.00	Thick stem, more number of roots, some leaves broad and some were narrow, plant size smaller than normal.
40	66.67	52.00	88.00	4.00	5.33	367.00	Four leaves had a crinkled appearance with wavy margin and they were of smaller size than normal. Plants were smaller than 30 Gy.
50	41.67	67.00	99.00	5.00	6.00	403.33	Plant number very less. Thick and small shoots, leaves dark green, and also light green in some plants, closely arranged leaves, aerial roots seen, opposite arrangement of leaves.
60	33.00	77.00	103.00	5.33	7.00	435.00	Plants small and same size as those of 50 Gy. Number of plants very less. Others are very very small (0.5-0.3 cm).
CD (0.05)	21.35	6.49	5.25	1.09	NS	26.27	
SEm ±	7.04	2.14	1.73	0.36		8.66	

Table 57. Effect of irradiation on the growth of seedlings of Emma White x Pink Tips

Irradiated material - Protocorm

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	92.00	32.00	60.00	2.33	4.00	334.00	Normal shoots (3.5 cm), large leaves (4x0.8 cm). Root number 10, with a length of 3 cm, healthy plants with green leaves.
10	83.33	40.00	75.00	3.00	4.33	341.00	Roots were thicker and larger. Dark green leaves. Plant height - 3 cm, leaves smaller than unirradiated (3.5 x 0.8 cm)
20	91.67	43.33	84.00	3.00	6.00	354.00	Thick leaves and shoots, dark green large leaves, plant size same as that of unirradiated.
30	66.00	47.00	84.33	4.00	5.00	369.33	Thicker stems, roots smaller than normal, leaves were thick, dark green and broad. Roots normal size but less in number.
40	75.00	55.00	93.00	3.33	5.00	384.00	Plant was shorter than 30 Gy (2.5 cm). Leaves broader than length and closely arranged on the stem. Three plants were seemed to be larger than normal with dark green leaves.
50	50.00	61.33	97.00	4.00	5.00	390.00	Plants very short, slight maroon colouration noticed on some of the leaves.
60	58.00	67.00	98.00	3.67	6.00	401.33	Roots were very small and thick, stem width was also more than others compact stem, narrow leaves. Stunted growth. Five plants only survived.
CD (0.05) SEm±	NS	4.64 1.53	5.64 1.86	NS	NS	9.34 3.08	

Table 58. Effect of irradiation on the growth of seedlings of Emma White selfed

Irradiated material - Protocorm

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	92.00	27.00	67.00	3.67	4.67	254.00	Healthy seedlings (2.5-3 cm height) normal leaf (size - 3.2 x 0.8 cm). Roots were large (3-3.5 cm).
10	83.33	31.00	72.00	4.00	4.00	270.00	Shoots and roots were very small (2.5 cm). Leaves were dark green, very few had pale green leaves also.
20	75.00	32.00	76.00	4.00	4.67	275.00	Leaves were broader than 10 Gy other features were same as those of 10 Gy.
30	83.00	40.67	82.33	2.67	3.33	292.00	Root prouction was very less (4-5 Nos.). But strong and healthy and thicker than that of others, leaf dark green.
40	67.00	43.00	88.67	3.00	5.00	295.00	Dark green leaves, stem short and thick and closely arranged leaves on stem. Root production normal.
50	58.00	48.00	93.00	3.00	5.00	329.00	More thick leaves noticed. Plant size was smaller, root production was normal.
60	67.00	53.00	97.00	2.33	6.33	345.00	Very short, compact and short seedlings, but some leaves were broader, and some were light green coloured. Majority of leaves dark green and broad. Only half of the established protocorms grew upto plants.
CD (0.05) SEm±	NS	4.37 1.44	3.58 1.18	1.15 0.38	NS	15.53 5.12	

Table 59. Effect of irradiation on the growth of seedlings of Emma White x Sonia-28

Irradiated material - Protocorm

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	100.00	28.67	44.00	3.00	3.67	285.00	Large healthy plants, 3-4 leaved, with 5-6 roots, leaves large (3.5 x 0.8 cm) and roots elongated (3-4 cm). Green leaves.
10	100.00	29.00	53.00	3.00	3.67	288.00	Plants were similar to that of unirradiated, pale green leaves in 5 plants.
20	100.00	32.00	58.00	2.67	4.00	317.00	Dark green leaves, plants were of same size as those of unirradiated. Roots smaller.
30	92.00	36.00	58.33	4.00	4.33	344.00	Thicker shoots, roots and leaves had a wavy margin. Slow growth noticed. Aerial roots noticed.
40	83.33	41.00	62.00	3.33	5.00	361.67	Closely arranged leaves, compact stem, dark green and thick leaves, one of the leaves had a pale green colour and crinkled appearance.
50	100.00	46.67	70.00	3.00	7.00	383.00	Thick dark green leaves, closely packed on compact stem, thick and healthy stems. Good root growth, narrow leaves in one plant.
60	83.00	46.00	77.00	2.33	6.00	395.00	Leaves in general were dark green and broad, but very few leaves were narrow and tapering. Plant number very less and elongated with a pale colouration. Root growth was less.
CD (0.05) SEm±	NS	3.85 1.27	5.16 1.70	NS	NS	22.99 7.58	

Table 60. Effect of irradiation on the growth of seedlings of Emma White x Banyat Pink

Irradiated material - Protocorm

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	100.00	30.67	53.00	3.00	4.00	266.00	Normal sized plants (2.5-3 cm) with normal leaves (3.4 x 0.5 cm), more number of roots (15-17 nos. of 4 cm length) thick, green leaves.
10	100.00	32.00	54.00	3.00	5.00	269.00	Shoots smaller (2.5 cm) , leaves larger (5 to 5.5 cm x 0.5 cm), roots were of normal size (4 cm) but less in number (10 No.)
20	100.00	34.00	57.00	3.00	5.00	274.33	Root length was more compared to all others. Dark green, elongated leaves, stem of normal height as that of unirradiated
30	92.00	39.00	64.00	2.67	4.33	291.00	Smaller stems with large leaves (6.5 cm x 0.6 cm) with normal number of roots of smaller size (2.5 cm)
40	91.67	39.00	66.33	3.33	5.00	298.00	Only a very few plants got established. Large sized leaves (5.5 x 0.6 cm), small stems and about 30 numbers of roots seen, thick roots and leaves, dark green leaves
50	83.33	45.33	73.00	4.00	6.00	314.00	Number of plants very less, very small stem (1.5 cm) and large leaves (5.6 x 0.8 cm) yellow colouration in 4 leaves. Compact, short, stout stem.
60	75.00	46.00	73.00	3.67	6.33	321.00	Compact and very short plants, roots 15 nos. Broader and thick, dark green leaves, closely arranged. Establishment in the field very poor.
CD (0.05) SEm±	16.56 5.46	5.43 1.79	6.13 2.02	NS	NS	7.76 2.56	

Table 61. Effect of irradiation on the growth of seedlings of Pink Tips selfed

Irradiated material - Protocorms

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	100.00	30.00	67.00	2.33	5.67	290.00	Small plants, broad leaves of smaller size long roots which were 5-6 in number.
10	97.00	31.00	77.00	2.00	5.33	300.00	Very long, thin roots, elongated leaves, very thick and dark green, small plants. Plants smaller than unirradiated.
20	92.00	32.00	90.33	3.00	5.00	303.33	Elongated narrow, pale green leaves, plant height normal as that of unirradiated, leaves closely arranged.
30	75.00	34.00	100.00	3.00	5.00	313.00	Elongated leaves, stout thick stem, shorter stem, leaves were closely arranged and pale green in some plants. Roots more in number and elongated.
40	93.33	38.00	110.00	4.00	5.00	350.00	Plants similar to those of 30 Gy.
50	78.00	41.00	130.00	4.00	6.00	384.00	A pale yellow colouration along the margin of the leaf seen in 4 plants, many roots (15 nos.) of almost equal size. Leaves smaller than those of 40 Gy.
60	75.00	44.00	142.00	3.33	6.00	407.00	Compact, very small and thick stem. Leaves were broader and closely arranged. Roots normal size and a few in number. Internodal length very less, Grown up plants very less in number.
CD (0.05) SEm±	18.52 2.94	6.55 2.16	7.80 2.57	NS	NS	9.68 7.10	

Table 62. Effect of irradiation on the growth of seedlings of Candy Stripe x Hieng Beauty

Irradiated material - Protocorms

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	83.33	34.33	57.33	2.33	4.00	307.00	Healthy seedlings with very good growth of shoots and roots.
10	91.67	42.00	75.67	3.00	4.33	323.33	Leaves were broader than those of unirriadiated plant size same as that of unirradiated with two exceptions (small)
20	66.67	43.00	90.00	2.67	5.00	328.00	Short stem, small leaves and thick roots in some plants. Roots were longer than those of 10 Gy.
30	75.00	44.33	100.67	3.00	5.67	347.00	Compact plants with broad leaves, leaf margin of most of the plants wavy, some leaves have maroon margin also.
40	75.00	49.00	100.00	3.00	5.33	370.00	Plants similar to those of 30 Gy and no variation observed.
50	66.67	56.00	107.00	3.33	6.00	388.00	Plants were shorter than 40 Gy. Pale coloured leaves and broader leaves observed in some plants. Thick stem than others.
60	58.33	62.00	112.00	3.67	6.67	397.00	Leaves of some plants were narrow and some broad. Maroon lines on stem and leaf margin after planting. Small, compact, short shoots with dark green leaves. In general. 25 per cent of the established protocorms grew to plants.
CD (0.05) SEm±	NS	3.43 1.13	6.56 2.16	NS	NS	12.28 4.05	

Table 63. Effect of irradiation on the growth of seedlings of Candy stripe x New Pink

Irradiated material - Protocorms

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	66.67	24.33	52.67	2.33	4.00	278.33	Small plants with closely arranged leaves, leaves were thick, dark green and narrow.
10	41.87	27.67	65.00	1.67	2.67	286.00	Narrow leaves, stem same size as that of unirradiated. Some leaves were light green and some dark green.
20	42.00	33.00	72.67	1.67	4.33	288.33	Plants were shorter than that of unirradiated, leaves were thick and broad.
30	25.00	36.67	74.33	2.00	3.33	293.00	Small plants, maroon lines on some leaves after planting, but not prominent. Leaves were broader than length, thick and dark green.
40	20.00	40.00	73.00	2.00	5.00	300.00	Short plants, growth was very poor. Roots were small and less in number than 10 Gy, 20 Gy, 30 Gy. Maroon lines on the margin of leaves of some plants after planting.
50	28.00	41.00	75.00	2.67	4.67	305.00	Plants similar to those of 40 Gy but very less in number.
60	33.00	47.00	81.67	4.00	5.67	325.00	Very slow growth, short, thick and stout plants with small leaves. Length and breadth of leaves were less. Roots also very less in number, opposite arrangement of leaves observed on the shoots, crinkled leaves also seen in some of the plants. Very few plants were obtained.
CD (0.05) SEm±	NS	5.61 1.85	7.31 2.41	1.09 0.36	NS	13.83 4.56	

Table 64. Effect of irradiation on the growth of seedlings of Candy Stripe selfed

Irradiated material - Protocorms

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	83.33	27.33	67.67	2.67	5.00	293.00	Normal plants of 4 cm height and with 5-6 leaves of size 3.5 x 0.5 cm, green leaves. Roots - 8 Nos.
10	75.00	28.00	71.00	3.00	4.33	296.33	Plants were similar to that of unirradiated but with smaller green leaves.
20	66.67	35.00	77.67	2.33	4.00	310.00	Leaves were thick and darker than 10 Gy, smaller and broder leaves. Plants were little smaller.
30	58.00	42.67	87.67	3.00	5.00	331.67	Leaf breadth was more than leaf length in most of the plants. Thick dark green leaves. Plants smaller than 20 Gy.
40	50.00	53.00	87.33	2.00	5.00	365.00	Plants of similar size as those of 30 Gy but dark green leaves.
50	53.00	59.00	97.67	3.00	6.33	398.00	Broad and small leaves in 2 flasks. Roots more in number than 20 Gy, 30 Gy, 40 Gy but smaller roots.
60	41.67	71.00	105.67	4.00	7.00	424.00	Thick darker leaves of very small size in three plants. Others were broad leaved and of normal length. Roots medium sized. Short, compact stem with more shoots. Very few plants obtained
CD (0.05) SEm±	NS	6.43 2.12	7.34 2.42	NS		14.29 6.72	

Table 65. Effect of irradiation on the growth of seedlings of Hieng Beauty x Sonia-28

Irradiated material - Protocorms

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	92.00	33.67	76.00	2.67	4.33	296.67	Slow growth, plants were of normal size. Normal leaves and roots. Maroon lines on the margin of leaves after planting.
10	100.00	36.00	84.67	2.00	4.00	305.00	Plants were shorter than unirradiated. Leaves were thick and narrow and dark green.
20	91.67	38.00	93.00	3.67	4.00	317.00	Dark green thick, broad leaves in most of the plants but a few had narrow leaves also. Root growth normal.
30	92.00	41.00	97.67	3.00	4.00	322.67	Two plants had boarder leaves (boarder than length) than of normal size. Prominent veins and wavy margin on leaf of some of the plants.
40	83.00	51.00	114.33	2.67	4.00	330.00	Very slow growth, healthy, compact, stout plants, thicker than those of 10 Gy, 20 Gy and 30 Gy. Dark green as well as light green leaves observed.
50	66.67	54.00	115.00	3.00	6.00	342.00	Broad leaves closely packed on short stem, margin of leaves maroon colouration. Normal roots.
60	75.00	61.00	118.00	3.00	6.33	355.00	Plant size similar to that of 50 Gy, maroon lines on leaf margin in some plants. Narrow leaves and broad leaved plants observed. Thick and small roots.
CD (0.05) SEm±	NS	5.07 1.67	7.37 2.43	NS	NS	10.25 3.38	

Table 66. Effect of irradiation on the growth of seedlings of Hieng Beauty x Emma White

Irradiated material - Protocorms

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	92.00	29.67	60.00	2.33	3.67	283.00	Very small plants, smaller and broader, green leaves, elongated and thick roots.
10	75.00	30.00	65.00	3.00	3.00	279.00	Plants similar to those of unirradiated.
20	83.00	34.67	68.00	3.00	4.00	295.00	Very small stout seedlings, with dark green thick leaves. Plants were almost same as those of unirradiated. Leaves were closely arranged.
30	64.00	39.00	72.33	3.67	6.00	294.00	Thick stem, broad leaves, broader than length, in two plants. Dark green thick leaves.
40	66.67	40.00	75.00	3.33	5.00	308.00	One plant was larger and others same size as those of unirradiated. Leaves larger and dark green.
50	75.00	45.00	83.00	4.00	6.00	368.00	Wavy margin noticed for leaves of some plants. Leaves were smaller than those of 40 Gy.
60	68.00	43.00	85.67	4.00	6.33	402.00	Protocoms enlarged more and were of larger size than others and were dark green. Leaves dark green. Stout and compact stem, with closely arranged broad thick leaves. Roots were more in number. Colour variation (pale colour) observed in leaves of two plants.
CD (0.05) SEm±	NS	5.76 1.90	5.37 1.77	NS	NS	22.02 7.26	

Table 67. Effect of irradiation on the growth of seedlings of Hieng Beauty x New Pink

Irradiated material - Protocorms

Dose of irradiation (Gy)	Percentage of cultures showing growth	Days for differenti-ation of first leaf	Days for shoot and foot formation	Number of shoots/ seedlings	Number of leaves/ shoot	Time taken for planting out (days)	Remarks
0	91.67	17.67	53.00	2.00	5.00	286.57	Leaves were green and thick. Normal seedlings, roots were long and more in number (12 Nos.). Broad leaves noticed.
10	83.00	31.00	73.00	2.00	6.00	297.67	Thick and dark green leaves of same size as those of unirradiated, plants a little smaller, with opposite leaves, normal roots.
20	92.00	30.00	89.00	2.67	5.00	264.00	Dark green broader leaves as smaller shoots. Two leaves were found twisted. Normal roots.
30	58.00	40.00	91.00	2.33	5.00	315.00	Some pale yellow leaves observed along with dark green leaves. Broad and thick leaves, stem similar to that of 20 Gy.
40	68.00	43.00	101.00	2.00	5.67	315.00	Not much difference observed between plants of 30 Gy and 40 Gy except some broader leaves.
50	66.67	47.00	107.67	3.00	6.33	348.00	Small thick dark green leaves, plants were smaller than those of 40 Gy. Very closely arranged leaves. Roots smaller, thicker and less in number.
60	75.00	50.67	114.00	3.00	6.00	383.00	Plants similar to those of 50 Gy, but smaller. Dark, thick green leaves. Uniform sized and smaller roots. Some leaves were broader than length. Closely arranged leaves. Only 50 per cent of the established protocorms grew to plants.
CD (0.05) SEm±	NS	5.64 1.86	5.67 1.87	NS	NS	35.67 11.76	

As the dose increased the survival percentage decreased, reaching to zero in 50 Gy and 60 Gy. The maximum survival was with the non-irradiated (50%). Number of shoots and number of leaves did not vary much among the treatments. The time taken for planting out varied from 295.00 days in non-irradiated to 360.00 days in 40 Gy treated PLBs. The size of the plants became reduced in the irradiation treatments and plantlet recovery was very less in all the treatments.

Irradiation details of the PLBs of Sonia 28 x Candy Stripe are presented in Table 69.

Least survival was observed in 20 Gy treatment (5%) followed by 30 Gy, 50 Gy and 60 Gy (10%, each). In the non-irradiated PLBs, the survival percentage was 40 per cent. There was not much difference between the treatments with respect to number of shoots and number of leaves. The non-irradiated plantlets took 300.00 days for planting out while the plantlets of 60 Gy treatment took 410.00 days. Size reduction in plantlets was also noticed in the irradiated ones. Number of plantlets recovered was very less in all the treatments.

4.9.3 Irradiation of callus

Effect of irradiation on the callus of Emma White selfed seeds (Table 70), showed that as the irradiation dose increased from zero to 60 Gy, the regeneration percentage got decreased, and no regeneration was obtained at the highest dose of 60 Gy. The non-irradiated and those irradiated with 10 Gy had 60 per cent regeneration. Not much variation was observed among different treatments with regard to number of shoots and number of leaves. For plantlet formation it took 300.00 days for non-irradiated callus and 400.00 days for 60 Gy irradiation. The number of plants obtained from non-irradiated and irradiated calli was very low, with a maximum of three plants.

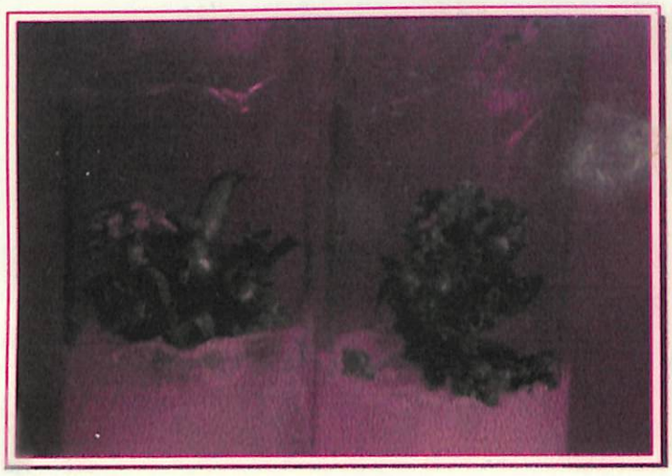
Table 68. Effect of irradiation on the growth of PLBs from *in vitro* leaf of New Pink x Pink Tips seedlings

Irradiation dose (Gy)	Percentage cultures showing growth	Number of shoots	Number of leaves	Time taken for plantlet formation	Remarks
0	50.00	2	5	295	Normal plantlets - 2 Nos.
10	25.00	2	4	300	Smaller plantlets - 2 Nos
20	10.33	3	5	330	Very small plantlets 3 in number with broader leaves
30	10.00	3	6	350	Small plantlet - only one obtained with elongated leaves and elongated roots
40	5.00	2	4	360	Very small plantlet with small narrow leaves. Root production very less
50	0	-	-	-	
60	0	-	-	-	

Table 69. Effect of irradiation on the growth of PLBs from *in vitro* leaf of Sonia-28 x Candy Stripe

Irradiation dose (Gy)	Percentage cultures showing growth	Number of shoots	Number of leaves	Time taken for plantlet formation	Remarks
0	40	2	5	300	Only 2 plantlets obtained with normal size
10	20	2	5	325	Two small plantlets with large leaves and large roots
20	5	2	4	358	Broad leaves with a few number of roots - one plantlet only
30	10	3	4	385	One small plantlet only obtained with broad leaves and more number of roots
40	20	2	5	390	Two plantlets with smaller leaves and less number of roots
50	10	2	6	405	One plantlet only with large broad leaf and small roots
60	10	2	5	410	Thicker leaves and very small plantlets - 2 Nos. Roots smaller and less in number

Plate - 25 Effect of irradiation on seedling growth



(a) Non irradiated (left) and irradiated at 20 Gy (right)

(b) Irradiated at 30Gy (left) non irradiated (right)

(c) Irradiated at 60 Gy (left) and non irradiated (right)

(d) Irradiated at 30 Gy

(e) Irradiated at 20 Gy

Irradiation details of the callus from Sonia 28 x Hieng Beauty are presented in Table 71.

Maximum regeneration was observed with the non-irradiated and 40 Gy irradiated calli (40%, each). The lowest regeneration was with 60 Gy (10%). Comparatively lesser number of shoots and a slight increase in the number of leaves were observed with higher doses of irradiation. The time for planting out increased with the dose of irradiation, reaching to 420.00 days for 60 Gy. The number of plantlets obtained from each treatment was very less. A slow growth rate and reduced height was noticed for the plants irradiated with higher doses.

4.10 Planting out and hardening

The seedlings, which reached plant out stage were taken out of the tubes. They were washed thoroughly in running water to remove all the traces of agar and treated with a fungicide solution (Bavistin 0.05% + Indofil - M-45 0.05%), for 10 minutes. These were planted to the community pots filled with the media after taking initial observations.

The medium consisted of brick pieces and charcoal pieces in equal proportion filled in small mud pots and plastic pots having many holes. Small pieces of coconut husk were also used in the media, when planting out was done during summer days. Media were also treated with the fungicide before planting. Support was also provided to the plants in the media using small pegs (Plate 26).

The pots containing the seedlings were kept under shade and watered regularly to maintain humidity. Immediately after planting a polythene covering was also given besides the shade net. Once the plants got established this was removed. After this, nutrient sprayings were also given regularly.

Survival percentages of different hybrids are presented in Table 72.

Table 70. Effect of irradiation on the growth of callus (Emma White selfed) from protocorms

Irradiation dose (Gy)	Culture showing regeneration (%)	Number of shoots	Number of leaves	Total days taken for plantlet formation	Remarks
0	60.00	3.00	4.00	300.00	Only 3 plants were obtained
10	60.00	4.00	5.00	325.00	2 plantlets obtained with normal size
20	30.00	4.00	6.00	355.00	2 smaller plantlets with broad leaves
30	25.00	4.00	6.00	360.00	Very small plantlets - 2 Nos with broad leaves
40	25.00	3.00	6.00	390.00	Only one plantlet very small and with small leaves
50	15.00	4.00	5.00	400.00	Only one plantlet with small and dark green leaves. Root production very less.
60	-	-	-	-	

Table 71. Effect of irradiation on the growth of callus from Sonia-28 x Hieng Beauty protocorms

Irradiation dose (Gy)	Culture showing regeneration (%)	Number of shoots	Number of leaves	Total days taken for plantlet formation	Remarks
0	40.00	4	5	325.00	Plantlets smaller compared to the normal seedlings leaves normal size. Plantlets 4 Nos. only
10	20.00	3	5	340.00	Small plantlets - 2 Nos. Almost similar to that of unirradiated.
20	30.00	3	4	368.00	Small plantlets - 3 Nos. broad leaves and large leaves. Wavy leaf margin also noticed.
30	30.00	2	6	370.00	Four plantlets obtained with smaller leaves and short stem. Roots normal.
40	40.00	2	6	390.00	Four plantlets obtained with smaller leaves and short stem. Roots normal.
50	20.00	3	5	398.00	Very small plantlets - 2 Nos. Broad small leaves as thick stem.
60	40.00	2	6	420.00	Very small stout stem with closely arranged leaves. Small and less number of roots only one plantlet obtained. Field establishment not obtained.

Table 72. Field establishment of hybrid seedlings

Hybrids	Survival percentage	Hybrids	Survival percentage
Emma White x Pink Tips	70.50	Emma White x New Pink	75.00
Sonia-28 x Candy Stripe	40.00	New Pink - Selfed	94.00
Sabine x Pink Tips	67.00	Candy Stripe x Hieng Beauty (30 Gy)	38.00
Emma White x Sonia-28	71.00	Emma White x Banyat Pink (10 Gy)	58.00
Emma White x Banyat Pink (30 Gy)	83.00	Banyat Pink selfed	75.00
Pink Tips selfed	90.00	Sonia 28 x Emma White (10 Gy)	93.00
Candy Stripe x Pink Tips	71.00	Emma White x Sabine	87.00
New Pink x Banyat Pink	33.00	Banyat Pink x Pink Tips	50.00
Sabine selfed	40.00	Sonia-28 x Pink Tips	79.00
Sabine x Sakura Pink	29.00	Candy Stripe x Pink Tips	73.00
Pramott-II x Candy Stripe	25.00	Sakura Pink x Emma White	90.00
Sakura Pink x Candy Stripe	80.00	Pink Tips x New Pink	92.00
Pink Tips x Candy Stripe	43.00	Pink Tips x *D. crumenatum*	17.00
Hieng Beauty x Candy Stripe	20.00	Hieng Beauty x Banyat Pink	29.00
New Pink x Sonia-28	73.00	Sakura Pink x Pink Tips	54.00
Candy Stripe selfed (10 Gy)	44.00	Emma White x Pink Tips	84.00
Sonia-28 (selfed)	91.00	New Pink x Pink Tips	92.00
New Pink x Emma White	91.00	Pramott-II x Banyat Pink	33.00
Banyat Pink x Hieng Beauty	71.00	Pink Tips x Sonia-28	87.00
Banyat Pink x Sonia-28	60.00	Pink Tips x Emma White	93.00
Candy Stripe x New Pink	64.00	New Pink x *D. chrysanthum*	14.00

The percentage of survival in the field varied in different hybrids and it ranged from a minimum of 14 percent (New Pink x *D. chrysanthum*) to a maximum of 94 percent (New Pink selfed). Field establishment was below 50 per cent for Sonia-28 x Candy Stripe, New Pink x Banyat Pink, Sabine selfed, Sabine x Sakura Pink, Pramott-II x Candy Stripe, Pink Tips x Candy Stripe, Hieng Beauty x Banyat Pink, Candy Stripe selfed, Pramott-II x Banyat Pink and New Pink x *D. chrysanthum*.

4.11 Nutrient regulation in the seedlings

Data on the effect of different concentration of the nutrients on the growth of the seedlings are presented in Table 73.

Maximum number of shoots (5.50) was observed for the treatments 30:10:10 green care at 0.2 per cent as well as 17:17:17 NPK complex at 0.1 per cent level each given as alternate day sprayings. This was followed by 30:10:10 Green care (0.1 percent) + 17:17:17 NPK complex (0.1%) sprayed on alternate days (5.00) (Fig. 7).

The lowest number of shoots 3.00 and 3.17 was obtained for the treatments involving 30:10:10 Green care 0.10 per cent and MS stock solution (1/10 strength), each sprayed once a week. 17:17:17 NPK complex (0.1%) sprayed at weekly intervals also gave lesser number of shoots (3.50).

Number of leaves was maximum (10.33) for 30:10:10 Green care (0.1%) sprayed on alternate days followed by Green care 30:10:10 (0.2%) sprayed on alternate days (9 leaves). The treatments T_5 and T_{13}, each, produced 8 leaves. The least number of leaves, 5.17, was observed in T_3 and T_{11}.

The number of shoots also increased in the treatments involving Green care 30:10:10 at both 0.1 and 0.2 per cent concentrations sprayed on alternate days (Table 74). Spraying of 17:17:17 NPK complex (0.1%) at alternate days also

Table 73. Mean number of shoots in *Dendrobium* as influenced by different nutrient solutions

Treatments	Number of shoots at monthly intervals										
	1	2	3	4	5	6	7	8	9	10	11
T_1	2.17	2.33	2.33	2.50	2.83	3.17	3.67	3.50	3.50	4.00	4.17
T_2	2.33	2.33	2.33	2.17	4.00	3.17	3.17	3.33	3.33	3.50	3.67
T_3	2.17	2.17	2.17	2.17	2.33	2.67	3.00	3.00	3.17	3.17	3.17
T_4	2.00	2.33	2.50	2.83	3.00	3.17	3.33	3.50	3.67	4.00	4.33
T_5	2.00	2.00	2.00	2.17	2.17	2.67	2.83	3.00	3.17	3.33	3.50
T_6	2.00	2.00	2.00	2.17	2.50	2.67	2.83	3.00	3.00	3.00	3.00
T_7	2.33	3.00	3.17	3.67	3.83	3.83	4.33	4.83	5.17	5.17	5.50
T_8	2.83	3.00	3.17	3.33	3.50	3.50	3.67	3.83	3.67	3.67	4.00
T_9	2.83	2.83	2.83	3.00	3.00	3.00	3.17	3.17	3.83	4.00	4.00
T_{10}	3.33	3.33	3.50	3.83	4.00	4.33	4.50	4.50	4.83	5.33	5.50
T_{11}	2.50	2.50	2.83	2.83	3.17	3.50	3.67	3.83	4.00	4.17	4.17
T_{12}	2.33	2.33	2.33	2.50	2.50	2.83	2.83	3.17	3.50	3.50	3.50
T_{13}	2.83	3.17	3.67	3.67	3.67	4.50	4.67	4.83	5.33	5.33	5.00

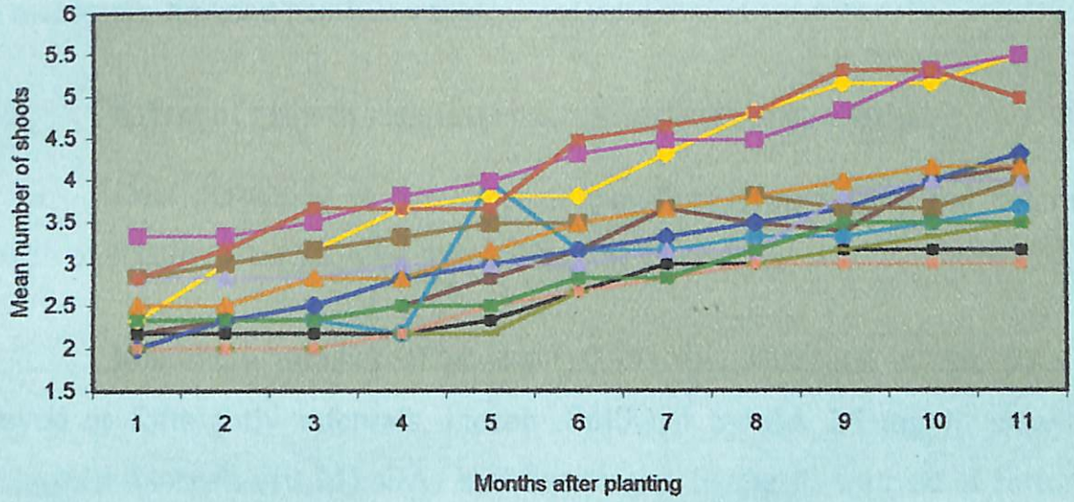
T_1 - MS stock at 1/10 strength - alternate day
T_2 - MS stock at 1/10 strength - twice/week
T_3 - MS stock at 1/10 strength - once/week
T_4 - 30:10:10 green care at 0.1 per cent - alternate day
T_5 - 30:10:10 green care at 0.1 per cent - twice/week
T_6 - 30:10:10 green care at 0.1 per cent - once/week
T_7 - 30:10:10 green care at 0.2 per cent - alternate day
T_8 - 30:10:10 green care at 0.2 per cent - twice/week
T_9 - 30:10:10 green care at 0.2 per cent - once/week
T_{10} - 17:17:17 NPK complex at 0.1 per cent - alternate day
T_{11} - 17:17:17 NPK complex at 0.1 per cent - alternate day
T_{12} - 17:17:17 NPK complex at 0.1 per cent - once/week
T_{13} - 30:10:10 green care (0.1%) + 17:17:17 NPK complex (0.1%) - alternate day

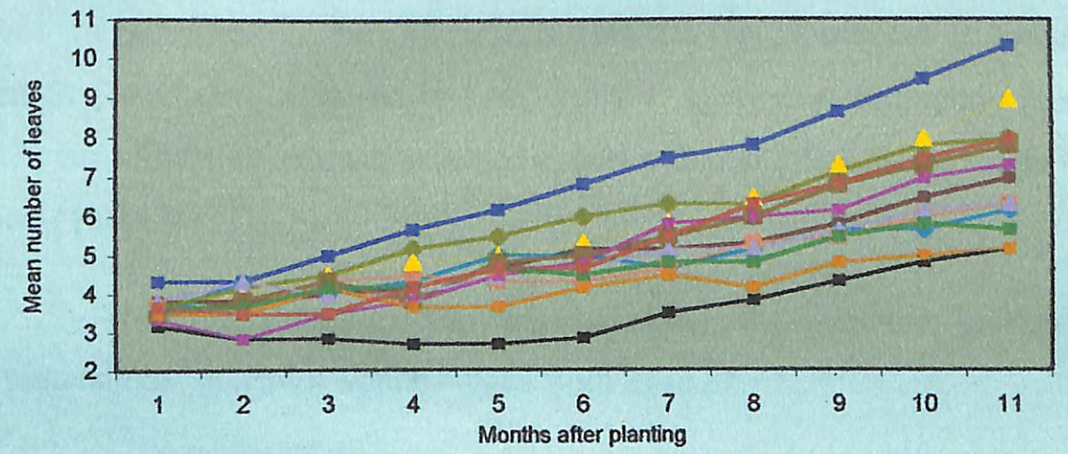
Table 74. Mean number of leaves in *Dendrobium* as influenced by different nutrient solutions

Treatments	Number of leaves at monthly intervals										
	1	2	3	4	5	6	7	8	9	10	11
T_1	3.83	3.83	4.00	4.17	4.67	5.17	5.17	5.33	5.83	6.50	7.00
T_2	3.50	4.33	4.00	4.33	5.00	5.00	4.67	5.17	5.67	5.67	6.17
T_3	3.17	2.83	2.83	2.67	2.67	2.83	3.50	3.83	4.33	4.83	5.17
T_4	4.33	4.33	5.00	5.67	6.17	6.83	7.50	7.83	8.67	9.50	10.33
T_5	3.67	4.17	4.50	5.17	5.50	6.00	6.33	6.33	7.17	7.83	8.00
T_6	3.67	3.67	4.33	4.50	4.33	4.33	4.67	5.33	5.50	6.00	6.33
T_7	3.83	4.00	4.50	4.83	5.00	5.33	5.83	6.50	7.33	8.00	9.00
T_8	3.50	3.83	4.33	3.83	4.83	5.00	5.50	6.00	6.83	7.33	7.83
T_9	3.83	4.33	4.00	4.00	4.50	4.67	5.17	5.17	5.67	6.17	6.33
T_{10}	3.33	2.83	3.50	3.83	4.50	4.83	5.83	6.00	6.17	7.00	7.33
T_{11}	3.50	3.50	4.17	3.67	3.67	4.17	4.50	4.17	4.83	5.00	5.17
T_{12}	3.67	3.67	4.17	4.17	4.67	4.50	4.83	4.83	5.50	5.83	5.67
T_{13}	3.67	3.50	3.50	4.17	4.67	4.67	5.50	6.33	6.85	7.50	8.00

T_1 - MS stock at 1/10 strength - alternate day
T_2 - MS stock at 1/10 strength - twice/week
T_3 - MS stock at 1/10 strength - once/week
T_4 - 30:10:10 green care at 0.1 per cent - alternate day
T_5 - 30:10:10 green care at 0.1 per cent - twice/week
T_6 - 30:10:10 green care at 0.1 per cent - once/week
T_7 - 30:10:10 green care at 0.2 per cent - alternate day
T_8 - 30:10:10 green care at 0.2 per cent - twice/week
T_9 - 30:10:10 green care at 0.2 per cent - once/week
T_{10} - 17:17:17 NPK complex at 0.1 per cent - alternate day
T_{11} - 17:17:17 NPK complex at 0.1 per cent - alternate day
T_{12} - 17:17:17 NPK complex at 0.1 per cent - once/week
T_{13} - 30:10:10 green care (0.1%) + 17:17:17 NPK complex (0.1%) - alternate day

Fig. 7. Growth of *Dendrobium* hybrids as influenced by different nutrient solutions





- MS stock at 1/10 strength - alternate day
- MS stock at 1/10 strength - twice/week
- MS stock at 1/10 strength - once/week
- 30:10:10 green care at 0.1 per cent - alternate day
- 30:10:10 green care at 0.1 per cent - twice/week
- 30:10:10 green care at 0.1 per cent - once/week
- 30:10:10 green care at 0.2 per cent - alternate day
- 30:10:10 green care at 0.2 per cent - twice/week
- 30:10:10 green care at 0.2 per cent - once/week
- 17:17:17 NPK complex at 0.1 per cent - alternate day
- 17:17:17 NPK complex at 0.1 per cent – twice/week
- 17:17:17 NPK complex (0.1 per cent) – once/week
- 30:10:10 green care (0.1 per cent) + 17:17:17 NPK complex (0.1 per cent) - alternate day

showed a good increase in growth. Rate of growth in the number of shoots was very less in T_1 and T_{12}. With regard to the rate of increase in the number of leaves the maximum rate and positive effect was observed in T_4 and T_7.

4.12 Effect of growth regulators on growth of the seedlings

Data pertaining to the effect of growth regulators GA_3 and BA on the growth of seedlings are presented in Tables 75, 76 and 77.

Maximum number of shoots (10.75) was observed in BA 50 mg l^{-1} sprayed at fortnightly intervals, closely followed by BA 25 mg l^{-1} sprayed at fortnightly intervals (10.25). GA_3 at 5 mg l^{-1} and 10 mg l^{-1} sprayed at fortnightly intervals could not influence the number of shoots (6.25, each).

Tallest shoot was observed in GA_3 10 mg l^{-1} sprayed at fortnightly intervals (16.25 cm), followed by GA_3 5 mg l^{-1} sprayed at fortnightly intervals (15.75 cm). All the other treatments had almost a similar effect on the height of the shoots (Table 76) (Fig. 8).

Not much difference was observed among the treatments in increasing the total number of leaves, which ranged from 21 to 27.

Flowering was also observed in two of the treatment plants. The flowered plants were from the treatments GA_3 10 mg l^{-1} and GA_3 5 mg l^{-1} sprayed at fortnightly intervals.

4.13 Field evaluation of hybrid seedlings

Vegetative characters were recorded for 26 hybrids in the field at four months' intervals and data on the growth performance are presented in Tables 78 to 103.

Growth parameters like number of shoots, height of each shoot, number of leaves as well as length and breadth of largest leaf were observed at an interval

Table 75. Number of shoots in *Dendrobium* hybrids as influenced by GA_3 and BA

Treatments	Mean number of shoots at monthly intervals										
	Initial	1	2	3	4	5	6	7	8	9	10
Control	4.00	4.00	4.50	4.50	5.25	5.00	5.25	5.50	5.50	5.75	5.75
GA_3 - 5 mg l^{-1} fortnightly interval	4.00	4.25	4.50	4.50	4.75	5.50	5.50	5.75	5.75	6.00	6.25
GA_3 - 5 mg l^{-1} monthly interval	4.25	4.50	4.50	4.50	5.25	5.00	5.50	5.75	5.75	5.75	6.00
GA_3 - 10 mg l^{-1} fortnightly interval	4.00	4.25	4.25	4.50	4.50	4.75	4.75	5.00	5.50	5.50	6.25
GA_3 - 10 mg l^{-1} monthly interval	4.00	4.25	4.25	4.50	4.75	5.25	5.50	5.50	5.25	5.75	6.50
BA - 25 mg l^{-1} fortnightly interval	4.00	4.75	5.25	6.00	6.75	7.25	8.00	8.50	9.25	10.00	10.25
BA - 25 mg l^{-1} monthly interval	4.25	4.75	5.00	5.50	6.25	7.00	7.50	8.00	8.50	8.75	9.25
BA - 50 mg l^{-1} fortnightly interval	4.50	5.00	5.75	6.25	7.00	7.25	8.25	9.00	9.50	10.25	10.75
BA - 50 mg l^{-1} monthly interval	4.00	4.50	5.00	5.75	6.25	7.00	7.25	7.50	8.00	8.25	9.00

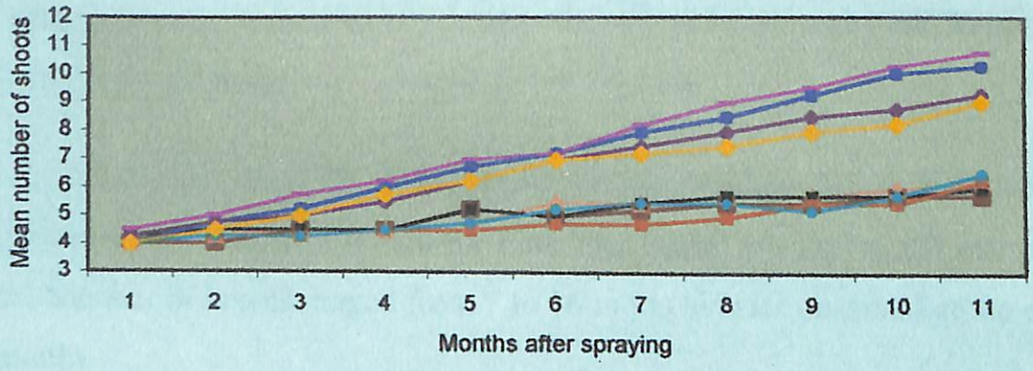
Table 76. Height of shoots of *Dendrobium* hybrids as influenced by GA_3 and BA

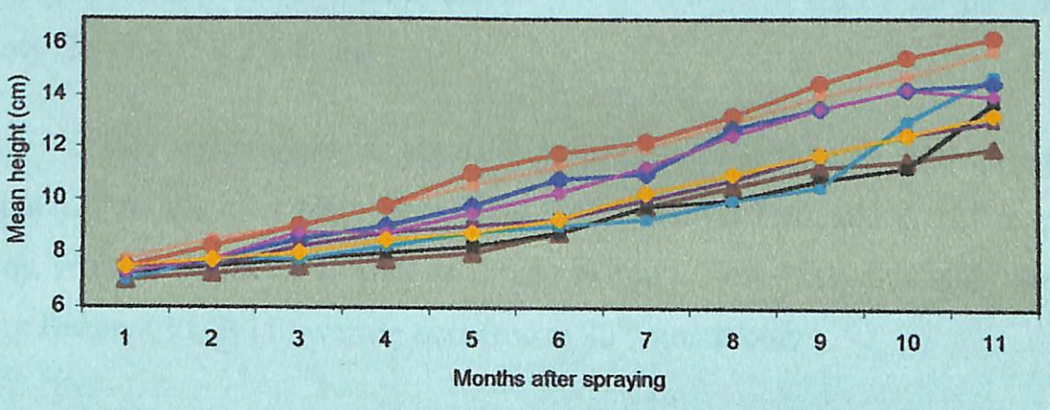
Treatments	Mean height (cm) at monthly intervals										
	Initial	1	2	3	4	5	6	7	8	9	10
Control	7.00	7.25	7.50	7.75	8.00	8.75	9.75	10.50	11.25	11.50	12.00
GA_3 - 5 mg l^{-1} fortnightly interval	7.75	8.50	9.00	9.75	10.50	11.25	12.00	13.00	14.00	14.75	15.75
GA_3 - 5 mg l^{-1} monthly interval	7.25	7.50	7.75	8.00	8.25	8.75	9.75	10.00	10.75	11.25	13.75
GA_3 - 10 mg l^{-1} fortnightly interval	7.50	8.25	9.00	9.75	11.0	11.75	12.25	13.25	14.50	15.50	16.25
GA_3 - 10 mg l^{-1} monthly interval	7.00	7.75	7.75	8.25	8.75	9.00	9.25	10.00	10.50	13.00	14.75
BA - 25 mg l^{-1} fortnightly interval	7.50	7.75	8.50	9.00	9.75	10.75	11.0	12.75	13.50	14.25	14.50
BA - 25 mg l^{-1} monthly interval	7.50	7.50	8.25	8.75	9.00	9.25	10.00	10.75	11.75	12.50	13.00
BA - 50 mg l^{-1} fortnightly interval	7.25	7.75	8.75	8.75	9.50	10.25	11.25	12.50	13.50	14.25	14.00
BA - 50 mg l^{-1} monthly interval	7.50	7.75	8.00	8.50	8.75	9.25	10.25	11.0	11.75	12.50	13.25

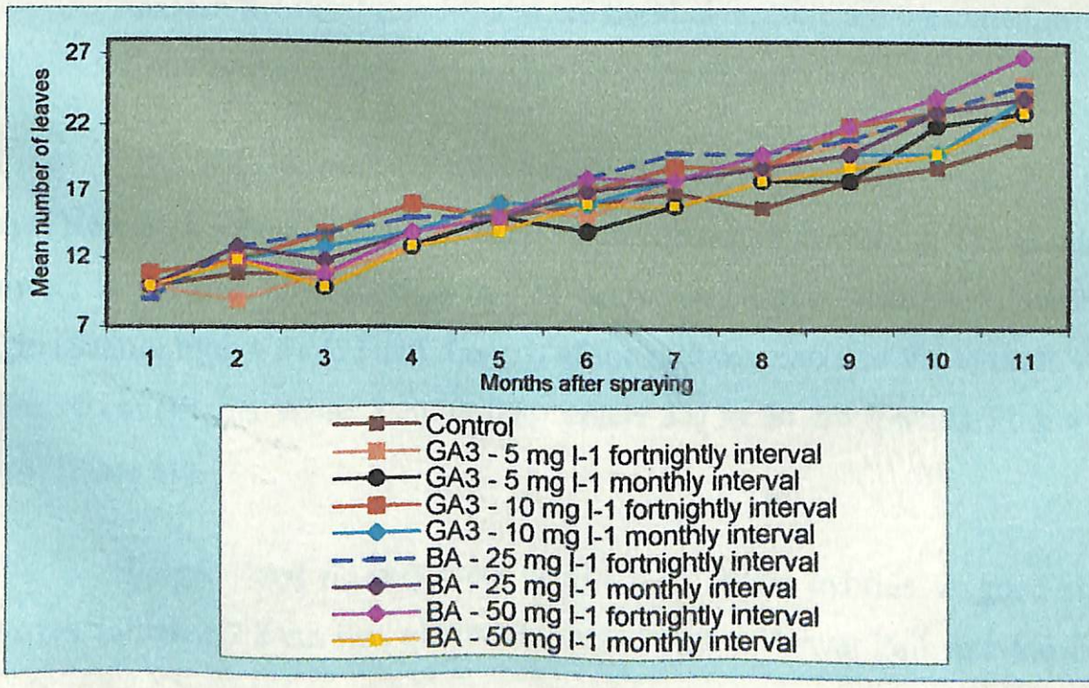
Table 77. Number of leaves in *Dendrobium* hybrids as influenced by GA_3 and BA

Treatments	Mean number of leaves at monthly intervals										
	Initial	1	2	3	4	5	6	7	8	9	10
Control	10	11	11	13	15	16	17	16	18	19	21
GA_3 - 5 mg l^{-1} fortnightly interval	10	9	11	13	16	15	18	19	21	23	25
GA_3 - 5 mg l^{-1} monthly interval	10	12	10	13	15	14	16	18	18	22	23
GA_3 - 10 mg l^{-1} fortnightly interval	11	12	14	16	15	17	19	19	22	23	24
GA_3 - 10 mg l^{-1} monthly interval	10	12	13	14	16	16	18	19	20	20	24
BA - 25 mg l^{-1} fortnightly interval	9	13	14	15	15	18	20	20	21	23	25
BA - 25 mg l^{-1} monthly interval	10	13	12	14	15	17	18	19	20	23	24
BA - 50 mg l^{-1} fortnightly interval	10	12	11	14	15	18	18	20	22	24	27
BA - 50 mg l^{-1} monthly interval	10	12	10	13	14	16	16	18	19	20	23

Fig. 8. Growth of *Dendrobium* hybrids as influenced by GA_3 and BA







of 4 months. An average of 4-5 shoots were found in all the hybrids at about one year after planting. Emma White x Banyat Pink (30 Gy) and Pink Tips selfed (10 Gy) had a maximum of 7 shoots at the 16th month.

A maximum of 22.00 cm height was observed in Banyat Pink x Hieng Beauty and a minimum of 7.60 cm for Pink Tips selfed (10 Gy) at the end of 12th month. Number of leaves ranged from 7 to 16 in the hybrids observed at the end of 12th month.

Size of the leaves was maximum in New Pink x Banyat Pink (15.5 x 2.3 cm) at the end of 12th month and it was minimum in Candy Stripe x Hieng Beauty (30 Gy) (7.8 x 1.8 cm).

Flowering could be obtained in some of the hybrids. Flowering was seen at 12th month after planting in New Pink x Banyat Pink and in 13th month in Emma White x Sonia-28 as well as Emma White x New Pink. In Candy Stripe x Hieng Beauty (30 Gy) flowering occurred at 20th month only.

4.13.1 Flowering in hybrids

sixteen hybrids flowered and the floral characters are presented in Table 104. Variations could be observed in the floral characters.

The hybrids took 32 days (Sonia-28 x Banyat Pink) (Plate 27) to 41 days (New Pink x Banyat Pink) from spike emergence to flowering. The number of flowers in the first inflorescence ranged from two (Emma White x Sonia-28) to eight (Sakura Pink x Pink Tips). Length of the inflorescences of the hybrids varied from 10 cm (Emma White x Sonia-28) (Plate 33) to 36 cm (Sakura Pink x Pink Tips) (Plate 31).

Larger sized flowers were also noticed in the hybrids. A good size of flowers 8.0 cm x 7.8 cm was observed in New Pink x Banyat Pink and the size of

flowers in Banyat Pink x Hieng Beauty was 8.0 cm x 7.2 cm. Smaller sized flowers of 6.0 cm x 6.0 cm was found in Candy Stripe x Hieng Beauty (30 Gy).

Sakura Pink x Pink Tips had the maximum internodal length between the first two flowers (4.0 cm) and the internodal length between the apical flowers was maximum (2.5 cm) for Candy Stripe x Pink Tips.

The time taken for wilting of all florets ranged from 32 days (Banyat Pink x Hieng Beauty and Emma White x Sonia-28) to 47 days (Emma White x New Pink) (Plate 29). The colour of the flowers is also described.

Pink Tips selfed 10Gy (Plate 35),New Pink x Pink Tips (Plate 30) and Emma White x Sabine (Plate 34) are also described.

Table 78. Growth performance of Emma White x Sonia-28 seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	2	2.5	2.2	-	-	-	-	-	7	4.2	0.7	Roots - 12 Length - 3.3 cm
4	3	4.8	4.2	0.8	-	-	-	-	8	7.2	1.0	
8	3	7.5	7.0	2.0	-	-	-	-	10	10.5	1.3	Dark green thin leaves
12	4	10.2	9.8	4.3	1.0	-	-	-	12	14.3	1.6	Flowering at 13th month
16												

Table 79. Growth performance of Emma White x New Pink seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	2	2.5	1.5	-	-	-	-	-	4	3.5	0.7	Roots - 14 Length 1.8 cm
4	3	5.0	2.2	1.0	-	-	-	-	6	7.0	1.8	
8	3	8.8	3.2	2.0	-	-	-	-	7	12.2	2.0	
12	5	13.5	6.5	3.5	2.1	2.0	-	-	9	17.0	2.3	
16												Flowering at 13th month

Table 80. Growth performance of New Pink x Sonia-28 seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	4	3.8	2.5	0.5	0.5	-	-	-	8	2.8	0.5	Roots - 11 Length of roots - 4.5 cm
4	4	6.6	4.2	1.9	2.0	-	-	-	11	5.3	1.5	
8	4	9.5	7.0	3.5	3.4	-	-	-	13	9.8	2.1	Leaves light green
12	5	13.00	9.8	5.7	5.8	1.0	-	-	14	14.0	2.8	
16												

Table 81. Growth performance of Emma White x Banyat Pink (30 Gy) seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	4	1.8	2.0	1.2	0.5	-	-	-	12	3.6	0.6	Roots - 28 Length - 3.5 cm
4	4	4.2	2.9	2.0	1.0	-	-	-	12	7.8	1.6	A silver line at the margin of leaves
8	5	6.2	4.2	3.2	2.3	0.8	-	-	13	9.8	2.7	
12	6	7.7	5.9	4.1	3.3	2.2	1.3	-	14	11.4	2.8	
16	7	9.0	7.0	5.5	4.5	3.5	2.5	2.5	15	13.0	3.0	Thick growth

Table 82. Growth performance of Candy Stripe selfed (10Gy) seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	2	2.8	2.5	-	-	-	-	-	5	3.5	0.7	Roots - 8 Length - 3 cm
4	3	4.9	4.0	1.0	-	-	-	-	7	5.5	1.2	
8	3	7.6	7.0	2.8	-	-	-	-	9	8.0	1.7	Thin stem, maroon lines on margin and midrib of leaves and on stem.
12	3	9.5	8.9	4.5	-	-	-	-	10	11.0	2.0	
16												

Table 83. Growth performance of Candy Stripe x Hieng Beauty (30 Gy) seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	2	3.0	2.5	-	-	-	-	-	4	2.8	0.5	Roots - 15 Length of roots - 1.8 cm
4	3	4.5	3.1	0.8	-	-	-	-	5	3.4	0.8	
8	4	5.8	4.8	1.7	0.5	-	-	-	8	5.0	1.2	
12	4	8.0	5.5	3.0	1.5	-	-	-	7	7.8	1.8	
16	4	11.2	8.0	5.2	3.7	-	-	-	9	9.5	2.4	Flowering at 20th month

Table 84. Growth performance of New Pink selfed seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	3	2.6	2.0	1.5	-	-	-	-	16	3.4	0.4	Roots - 14 Root length - 2.5 cm
4	3	4.0	4.0	3.0	-	-	-	-	17	5.5	0.7	Very thin plants, long leaves, wide stem (5.5 cm width).
8	4	5.5	4.9	4.0	1.0	-	-	-	16	7.8	1.1	
12	4	7.9	6.2	5.8	2.0	-	-	-	16	10.5	1.5	
14	5	10.0	7.9	7.3	3.3	0.8	-	-	18	12.0	1.8	Flowering in 16th month

Table 85. Growth performance of Banyat Pink x Sonia-28 seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	2	2.8	2.8	-	-	-	-	-	8	1.8	0.4	Roots-9 Length of roots - 3.2 cm
4	3	4.6	4.3	0.5	-	-	-	-	8	3.9	1.0	
8	3	7.8	6.5	1.3	-	-	-	-	9	7.2	1.8	
12	4	10.4	9.2	3.4	1.0	-	-	-	11	10.7	2.4	
14	5	12.2	10.8	4.0	1.8	0.6	-	-	11	12.0	2.6	Flowering in 14th month

Table 86. Growth performance of Emma White x Banyat Pink (10 Gy) seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	2	3.0	2.3	-	-	-	-	-	5	3.5	0.5	Roots - 10 Length of roots - 2.6 cm
4	3	4.6	3.0	1.0	-	-	-	-	6	5.2	0.9	
8	3	6.6	4.8	2.1	-	-	-	-	6	8.0	1.4	
12	3	9.0	6.5	3.5	-	-	-	-	7	11.0	1.8	Stout stem, light green and thin narrow leaves.
16												Flowering in 16th month

Table 87. Growth performance of Candy Stripe x New Pink seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	4	2.9	2.5	0.4	0.6	-	-	-	11	3.3	0.5	Roots - 13 Length of roots - 2.4 cm
4	4	3.9	3.6	1.1	1.5	-	-	-	11	5.2	1.0	
8	4	5.8	5.8	2.3	2.8	-	-	-	13	8.0	1.3	
12	5	8.0	7.8	4.0	4.2	1.0	-	-	14	10.3	1.8	Maroon lines on leaves and stems.
16	5	10.1	10.0	6.3	5.7	1.8	-	-	16	12.8	2.0	

Table 88. Growth performance of Banyat Pink selfed seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	4	3.5	2.5	0.8	0.5	-	-	-	7	4.4	1.0	Roots - 12 Length of root - 3.5 cm
4	4	4.9	3.8	1.5	1.3	-	-	-	10	7.2	1.7	
8	4	6.6	5.9	2.7	2.1	-	-	-	12	9.8	2.2	
12	4	8.8	7.8	4.5	3.0	-	-	-	12	12.5	2.8	
16												Flowering in 16th month

Table 89. Growth performance of Pink Tips selfed seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	1	3.0	-	-	-	-	-	-	5	3.8	0.5	Roots - 8 Length of roots - 4.2 cm
4	3	4.9	1.8	1.5	-	-	-	-	7	5.0	0.9	Thin shoots maroon lines on the margin of leaves.
8	4	7.2	3.4	3.0	0.8	-	-	-	11	6.9	1.7	
12	5	9.8	6.1	4.8	1.7	0.5	-	-	15	9.2	2.3	
16	5	13.3	9.0	6.5	3.8	1.0	-	-	14	11.5	3.0	Flowering in 16th month

Table 90. Growth performance of Emma White x Banyat Pink (30 Gy) seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	2	3.0	2.3	-	-	-	-	-	5	3.5	0.7	Roots - 17 Length of roots - 2.4 cm
4	3	6.3	3.8	1.5	-	-	-	-	9	6.8	1.4	
8	4	10.5	5.6	2.7	1.2	-	-	-	13	10.8	2.5	
12	4	15.0	7.5	4.2	2.1	-	-	-	15	15.0	3.0	
16												Flowering in 16th month

Table 91. Growth performance of Sonia-28 selfed seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	2	3.8	3.0	-	-	-	-	-	9	3.5	0.5	Roots - 6 Length of roots - 2.8 cm
4	3	6.4	5.8	0.8	-	-	-	-	11	5.8	1.2	
8	4	10.8	9.1	2.8	0.7	-	-	-	12	9.8	2.0	Thick, stout stem with light green immature leaves,
12	4	13.5	11.8	4.3	2.0	-	-	-	14	12.3	2.3	
16												

Table 92. Growth performance of Emma White x Pink Tips seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	2	4.0	1.5	-	-	-	-	-	6	3.5	0.5	Roots - 12 Length of roots - 3.8 cm
4	3	6.8	2.4	2	-	-	-	-	7	6.5	1.2	Light green leaves, maroon lines on stem
8	3	9.8	4.1	2.8	-	-	-	-	9	10.0	2.1	
12	4	13.0	6.0	3.8	1.5	-	-	-	11	13.5	2.6	
14	4	15.4	6.5	4.3	1.9	-	-	-	11	15.0	3.0	Flowering in 16th month

Table 93. Growth performance of Sonia-28 x Emma White (10 Gy) seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	2	3.5	2.3	-	-	-	-	-	7	4.2	0.5	Roots -8 Length - 4.2 cm
4	3	5.0	4.0	1.0	-	-	-	-	6	7.0	1.3	
8	3	6.8	5.3	2.8	-	-	-	-	6	9.5	2.0	
12	3	9.0	7.5	4.5	-	-	-	-	7	12.0	2.4	Maroon lines on stem and leaf margin
16	4	13.0	10.0	6.0	1.0	-	-	-	8	13.5	2.6	

Table 94. Growth performance of Banyat Pink x Hieng beauty seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	2	4.2	2.5	-	-	-	-	-	6	3.0	0.5	Roots - 10 Length - 3.8 cm
4	3	7.5	4.9	1.0	-	-	-	-	7	4.2	1.2	
8	3	12.5	8.0	2.8	1.0	-	-	-	8	7.5	2.3	
12	4	17.5	11.5	3.6	2.8	-	-	-	10	10.2	3.0	
14	4	22.0	13.0	5.0	4.0	-	-	-	12	13.0	3.4	Flowering at 14th month

Table 95. Growth performance of Pramott II x Candy Stripe seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	4	2.5	1.5	0.5	0.5	-	-	-	10	4.4	1.0	Roots - 9 Length - 3.2 cm
4	3	6.2	3.0	2.1	-	-	-	-	10	6.5	1.4	
8	3	10.0	4.8	4.3	-	-	-	-	11	9.8	1.9	
12	3	14.0	7.2	6.1	-	-	-	-	11	11.5	2.4	
14	3	16.0	8.5	7.3	-	-	-	-	12	12.5	2.8	Flowering at 14 month

Table 96. Growth performance of Candy Stripe x Pink Tips seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	4	3.5	2.5	0.5	0.2	-	-	-	10	4.0	1.0	Roots - 18 Length of roots - 3.5 cm
4	4	6.8	5.1	1.2	1.6	-	-	-	10	5.8	1.4	
8	4	10.2	8.7	2.0	3.1	-	-	-	11	7.2	1.9	
12	5	14.5	12.5	3.7	5.8	1.0	-	-	12	10.8	2.3	
16	5	18.8	15.9	6.6	8.2	2.0	-	-	12	14.0	2.8	Flowerin in 16th month

Table 97. Growth performance of Banyat Pink x Pink Tips seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	3	3.0	10.7	1.6	-	-	-	-	7	3.0	0.5	Roots - 8 Nos. Length - 3 cm
4	3	5.2	2.1	2.6	-	-	-	-	8	4.5	1.0	Light green leaves
8	4	8.5	2.7	3.8	0.5	-	-	-	12	7.6	1.6	
12	5	12.0	4.2	5.2	1.0	1.5	-	-	15	10.1	2.1	
16	5	15.0	7.0	8.0	2.0	3.0	-	-	17	13.0	2.5	Flowering in 16th month

Table 98. Growth performance of Emma White x Sabine seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	3	3.2	1.8	0.5	-	-	-	-	7	3.0	0.5	Roots - 15 Length - 3 cm
4	3	5.8	3.0	1.7	-	-	-	-	8	4.5	1.0	
8	4	8.2	5.2	4.0	1.0	-	-	-	10	7.2	1.4	
12	4	11.4	8.0	6.8	2.5	-	-	-	11	9.8	1.6	
16	5	14	11.8	9.5	5.0	0.8	-	-	13	13.2	2.1	Flowering in 16th month

Table 99. Growth performance of Emma White x Pink Tips seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	3	3.1	0.7	0.5	-	-	-	-	7	3.2	0.6	Roots - 10 Length - 3 cm
4	4	4.4	1.7	1.0	0.5	-	-	-	7	5.5	1.0	
8	4	7.6	3.2	2.0	2.3	-	-	-	9	8.0	1.7	
12	5	11.2	6.8	3.3	3.8	1.0	-	-	10	10.8	2.2	
16	5	15.0	10.0	5.0	6.0	4.0	-	-	12	12.5	2.6	Flowering in 16th month

Table 100. Growth performance of Sakura Pink x Pink Tips seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	4	3.3	1.0	0.5	0.5	-	-	-	6	3.5	0.5	Roots - 11 Root length - 3.5 cm
4	4	5.6	3.0	1.0	1.3	-	-	-	8	6.2	1.0	
8	4	9.7	5.7	3.2	3.6	-	-	-	8	8.5	1.7	Light green leaves
12	5	13.0	7.9	5.3	6.0	1.0	-	-	10	10.5	2.3	
16	5	16.5	10.0	7.6	8.0	2.5	-	-	12	13.5	3.0	Flowering in 14th month

Table 101. Growth performance of New Pink x Banyat Pink seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	2	4.2	2.0	1.8	-	-	-	-	6	4.3	1.0	Roots - 13 Length - 3 cm
4	3	8.0	2.9	2.7	-	-	-	-	8	8.0	1.5	Thin, light green leaves
8	3	12.5	4.6	4.5	-	-	-	-	8	11.8	2.0	
12	4	17.0	8.0	8.5	0.8	-	-	-	9	15.5	2.3	Flowering in 12th month
16												

Table 102. Growth performance of Pink Tips selfed (10 Gy) seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	3	2.8	1.7	1.5	-	-	-	-	6	4.0	0.9	Roots - 10 Length - 3.5 cm
4	4	4.0	3.3	2.8	1.5	-	-	-	7	5.2	1.2	Maroon colour very much prominent on margin of leaf and stem
8	6	6.3	5.8	5.0	3.8	1.2	1.0	-	8	6.8	1.8	
12	7	7.6	7.5	7.2	7.0	2.8	2.0	1.0	10	8.5	2.4	
14	7	8	7.9	7.6	7.8	3.4	2.5	1.8		10.5	2.5	Flowering in 14th month

Table 103. Growth performance of New Pink x Pink Tips seedlings

Months after planting	Number of shoots	Height of each shoot (cm)							No. of leaves	Length of largest leaf (cm)	Breadth of largest leaf (cm)	Remarks
		1	2	3	4	5	6	7				
0	4	2.8	1.0	1.0	0.5	-	-	-	6	2.7	0.5	Roots - 11 Length - 3 cm
4	3	5.1	2.3	2.0	-	-	-	-	7	5.5	1.0	
8	4	8.3	3.1	3.0	0.8	-	-	-	8	8.3	2.0	Light green leaves, maroon lines on stem.
12	4	12.0	4.0	3.8	1.5	-	-	-	10	12.0	2.4	
16	4	15.0	4.8	4.5	2.3	-	-	-	10	14.0	2.8	Flowering in 16th month

Table 104. Floral characters of some of the hybrids in the field

Hybrids	Time taken from spike emergence to flowering (days)	No. of flowers per spike	Length of spike (cm)	Size of flowers		Internodal length		Days for opening of all florets	Days for wilting of all florets	Colour of flowers
				Length (cm)	Breadth (cm)	Basal (cm)	Apical (cm)			
Banyat Pink xHieng Beauty	38	4	12.5	8.0	7.2	1.2	1.1	8	32	Pink petals, sepals and lip, petals double size of sepals. Towards the base of lip and sepals white colour, lip deeper pink. Pink dots on anther cap
Pink Tips selfed	38	4	18.0	6.5	6.5	2.8	1.8	8	42	Light pink sepals, petals and lip
Sonia-28 x Banyat Pink	32	6	23.5	7.0	6.6	2.0	1.0	13	41	Deep purple petals and sepals,. lip is deep pink. Petals more than double size of sepals. Petals almost covering the sepals. Towards the base of petals, sepals and lips white colouration.
Candy Stripe x Hieng Beauty (30 Gy)	35	3	15	6.0	6.0	2.5	1.5	5	44	Petals larger than sepals but not double size. Petals sepals and lip are light pruple coloured. Towards base of all, a white colour seen and yellowish white towards base of lip.
Emma White x New Pink	36	4	13.5	6.7	6.5	2.6	2.0	10	47	Petals double the size of sepals, full pink in sepals and petals lip deep pink the petals. Towards base of flower greenish white colour seen. Light green colouration at the tip of sepals.

Contd.

Table 104. Continued

Hybrids	Time taken f Rom spike emergence to flowering (days)	No. of flowers per spike	Length of spike (cm)	Size of flowers		Internodal length		Days for opening of all florets	Days for wilting of all florets	Colour of flowers
				Length (cm)	Breadth (cm)	Basal (cm)	Apical (cm)			
Emma White x Sonia-28	35	2	10.0	7.0	6.5	1.2	-	4	32	Light pink petals and very light pink sepals. Petals larger than sepals, deep pink lip and towards base of the flower greenish white colour. Margin of the petals and lip wavy. Separate petals and sepals. Lip perpendicular to the flower and not bending downwards.
New Pink x Banyat Pink	41	4	27.5	8.0	7.8	2.0	1.5	9	39	Sepals and petals pink and white., deep pink for lip and base of lip yellowish white. A white line through the center of the sepals, pink dots on anther cap.
Emma White x Banyat Pink (30 Gy)	38	6	22.0	7.0	7.0	3.5	1.7	11	39	Separate petals and sepals. Sepals full light pink and petals full pink. Lip deep pink and base yellowish green. Petal almost double size of sepal.
Pink Tips selfed (10 Gy)	35	4	16.2	7.2	7.2	2.5	2.0	8	32	Dark maroon colour for inflorescence stalk and flower buds. Light pink petals, sepals and lip. sepals and petals of almost the same size.

Contd.

Table 104. Continued

Hybrids	Time taken from spike emergence to flowering (days)	No. of flowers per spike	Length of spike (cm)	Size of flowers		Internodal length		Days for opening of all florets	Days for wilting of all florets	Colour of flowers
				Length (cm)	Breadth (cm)	Basal (cm)	Apical (cm)			
Sakura Pink x Pink Tips	37	8	36	7.5	7.2	4.0	1.5	17	46	Light pink Petals, sepals and lip, a little deep pink. Base of lip light yellow. Separate petals and sepals.
Candy Stripe x Pink Tips	39	6	27	7.5	7.5	2.9	2.5	14	40	Light pink petals and sepals,. very light pink and white towards the base. Lip deep pink and base greenish white. Separate petals and sepals and petals double size of sepals.
Emma White x Sabine	38	6	27	6.8	6.8	2.9	2.2	11	40	Sepals, petals and lip are pink and white towards the base. Sepals and petals of equal size.
Emma White x Pink Tips	35	6	29	6.4	6.4	3.0	1.9	12	44	Very light pink sepals, petals and lips. Sepals and petals of equal size.
New Pink x Pink Tips	40	8	32	7.4	7.4	3.1	1.8	16	47	Purple and white flowers, lighter than that of New Pink. Petals larger than sepals.
New Pink selfed	43	7	25.3	7.5	7.3	3.0	1.8	12	49	Purple and white sepals, petals and lip. Separate petals and sepals.
Banyat Pink x Pink Tips	38	4	12.5	8.0	7.2	2.7	2.1	8	32	Large and deep purple petals, sepals smaller than petals with purple colour and with purple lip. Large flowers.

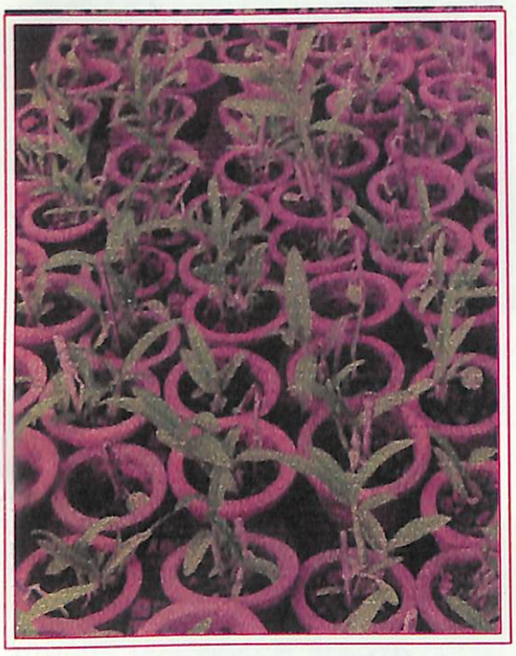


Plate - 26 Field view of the hybrid seedlings

Plate - 27 Sonia 28 x Banyat Pink

Plate - 29 Emma White x New Pink

Plate - 28 Banyat Pink x Pink Tips

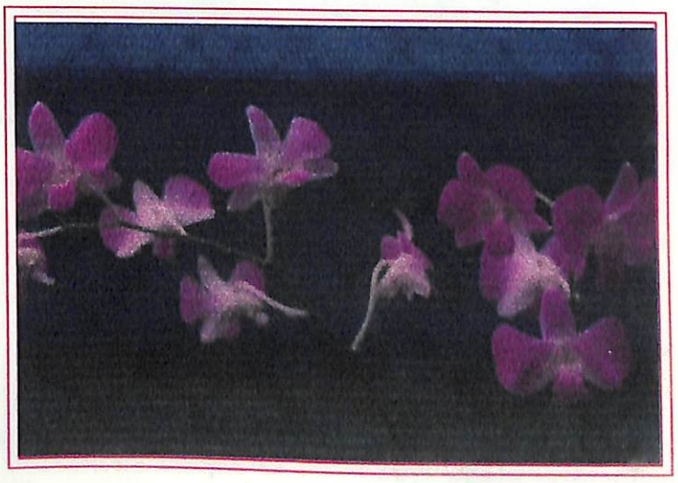


Plate - 30 New Pink x Pink Tips

Plate - 31 Sakura Pink x Pink Tips

Plate - 32 Emma White x Sonia 28

Plate - 33 Banyat Pink x Hieng Beauty

Plate - 34 Emma White x Sabine

Plate - 35 Pink Tips selfed (10 Gy)


DISCUSSION

DISCUSSION

Orchids, a unique plant group, are very interesting for their floral complexities and diversity of their long lasting and colourful flowers. Their unmarked ornamental value makes them supreme among the floricultural plants. Orchids are the integral part of India's wonderful heritage. Despite the fact that a large number of natural and artificial hybrids have been reported both at interspecific and intergeneric levels (Abraham and Vatsala, 1981), orchid breeding has remained in its infancy in India.

Dendrobium is a very complex and extremely large genus widely used in the commercial cut flower production in Kerala. Most of the hybrids grown in Kerala are exotic. Hybrids produced by employing plants that are found to perform well under our conditions, are expected to have better adaptability here.

The present study therefore, is undertaken to collect information on varietal characters, correlations among characters, floral biology, pollen and pollination studies, refinement of medium for the best growth of *in vitro* seedlings, *in vitro* mutagenesis and evaluation of the field performance of hybrids for improved flower size, flower number, spike length, new colour combinations etc. This chapter deals with the brief discussion on the findings in the right of the work already done.

Continuous breeding and selection have produced hybrids of high quality. Effectiveness of selections based on phenotypic performance can be more useful and reliable only if selection is based on heritability estimates. The magnitude of variation and the heritability estimate are the important parameters along which selection is usually made.

5.1 Vegetative and floral characters

5.1.1 Vegetative characters

The varieties differed significantly with respect to vegetative characters.

New Pink recorded the maximum number of shoots, viz., 9.00, followed by Hieng Beauty. The least number was possessed by Emma White, Sakura Pink and Pramott-II (5.67).

Tallest varieties in this study were Sonia 28 (30.17 cm), New Pink (29.07 cm) and Hieng Beauty (27.30 cm). All others had equal height. Sakura Pink possessed the least height (22.17 cm).

Maximum number of leaves was recorded for Candy Stripe (33.33) and Sakura Pink had the lowest number (21.33).

Maximum leaf length was recorded for Hieng Beauty, Emma White and New Pink (15.17 cm, each) and minimum for Sabine (12.90 cm). Emma White had the maximum breadth of leaf (4.80 cm) followed by Candy Stripe (4.50 cm). Minimum breadth was recorded for Sakura Pink (3.07 cm).

Candy Stripe, New Pink and Sabine took maximum number of days from spike emergence to flowering (40.00 days, each) and it was the least for Sakura Pink (31.33 days).

Dendrobium, being the second largest genus having over 1300 species (Baker and Baker, 1996), the variations expressed by the species are seen in the variety also. These differences are important in breeding because they contribute to the yield and other desirable attributes also.

5.1.2 Floral characters

The differences expressed by the parents are manifested in the progeny also.

Candy Stripe had the maximum number of flowers per spike (11.67) and Sabine as well Banyat Pink had the least number (5.67). New Pink had the maximum size of flowers (8.27 cm) and Sakura Pink the minimum (5.60 cm). Sonia 28 had the longest spikes (35.60 cm) and Banyat Pink had the shortest (22.83 cm).

Maximum time for opening of all florets was taken by Candy Stripe (19.33 days) and minimum by Banyat Pink (10.60.days). The flowers lasted for a maximum of 57 days in Sonia 28 and for a minimum of 39.33 days in Banyat Pink. New Pink had the maximum vase life (18.33 days) and Candy Stripe the minimum (13 days).

Candy Stripe had the maximum internodal length between first two flowers (3.93 cm) and Sabine had the maximum internodal length between last two flowers (2.50 cm). Minimum of these observations was for Sakura Pink (2.60 cm) and Banyat Pink (1.60 cm). Though the differences are, in general, significant among the varieties, the usefulness of these differences also depends on economic considerations.

Year round flowering occurred in the hybrids Hieng Beauty, Emma White, Sonia 28, Candy Stripe and New Pink.

5.2 Variation and correlation studies

5.2.1 Variability and heritability

The magnitude of variation of genotypes in relation to environmental influence is to be assessed and the available variability can be partitioned into genetic and non genetic components.

Phenotypic coefficient of variation was higher than genotypic coefficient of variation in general, in this study, indicating the influence of environment. The high coefficient of variation observed for number of flowers per

spike favours selection for this character. The number of flowers per spike is a character which decides the marketability of the spike and hence is of great importance in cut flowers like orchids. Higher the number of flowers, higher the cut flower value. Studies conducted by Rehman *et al.* (1993) in the orchids of West Bengal also detected high variability for flowers per inflorescence. Days for opening of all florets is another important trait in orchids, and higher variability in this character facilitates selection. Further the high variability observed for number of shoots is important, since it contributes to number of spikes. This can be exploited efficiently and effectively for bringing considerable improvement through selection.

Assessing the heritability is valuable in any plant breeding programme, since it provides basis for selection on phenotypic performance. According to Burton (1952), GCV alone is not a correct measure of heritable variance and it should be considered together with heritability to get a correct picture of the amount of success to be expected from selection. In the present study, heritability was of moderate to high magnitude for most of the characters. The breadth of flower and length of flower which together constitutes the size of flower exhibited highest heritability. Size of florets is one of the most important trait which enhances the cut flower value and in turn the market demand of orchids. High heritability for flower size was obtained by Rehman *et al.* (1993) while studying the genetics of some of the West Bengal orchids.

5.2.2 Association of characters

In a cut flower the economic characters are number of flowers (spikes) produced, flower size and the vase life. These are generally complex in nature and influenced by many plant characters through different physiobiochemical mechanisms. Improvement of the above mentioned economic characters is possible, only by knowing the association of various characters.

The present study revealed significant and positive association between flowers per spike and other characters, viz., mean height of shoots, total number of leaves, length of inflorescence, days for opening of all florets and internodal length.

Genotypic correlation coefficients were higher than phenotypic correlation coefficients. Long inflorescence is a desirable attribute for cut flowers. In the present study, the mean height of shoots was highly correlated with length of inflorescence. Thus selection for taller shoots will help in obtaining longer inflorescence, similarly selection for more number of leaves can result in longer inflorescence.

Nagayoshi *et al.* (1996) also reported positive correlations between many characteristics like length of stem and length of leaf, leaf length and leaf number, leaf length and flower number, length and width of leaf and stem diameter as well as leaf number and flower number in *Habenaria radiata*.

The increased number of leaves would have contributed to higher rate of photosynthesis which in turn would have resulted in longer inflorescence. Like wise, flowers per spike, is the deciding factor for the price of spike. This also showed high positive correlation with total number of leaves. So management practices for increasing the number of leaves will in turn facilitate the production of longer inflorescence with more number of flowers, thus contributing to the market value of spikes.

Another important criterion for cut flower selection is the size of the flower and it is seen that length of flowers and breadth of flowers were highly correlated.

5.2.3 Genotypic path coefficient analysis

The direct and indirect effects of fifteen characters on number of flower per spike showed that the days from spike emergence to first flower opening had

the maximum direct effect. It was followed by breadth of flower, internodal length between first two flowers, days for opening of all flowers, vase life and length of inflorescence. The direct selection for the characters, viz., days from spike emergence to first flower opening, days for opening of all flowers and length of inflorescence would be beneficial for crop improvement, since these characters also registered positive correlation coefficients. The duration of flowering registered high positive direct effect in gladiolus also (Misra and Saini, 1990 and Jisha, 1999). Negative direct effect on flowers per spike was for leaf breadth, length of flower, number of shoots, internodal length between last two flowers and days for wilting of all flowers. The direct effect on number of shoots was negative eventhough their correlation with flowers per spike was positive. This could be due to its positive indirect effect through other characters like days from spike emergence to first flower opening, breadth of flowers, vase life and internodal length of first flower. The positive correlation observed in the case of leaf breadth, although it had high direct negative effect could be due to high positive indirect effect for days from spike emergence to first flower opening. Like wise, the days for wilting of all flowers which showed negative direct effect, exhibited positive correlation coefficients presumably due to the high positive indirect effect of days from spike emergence to first flower opening. In general, the high positive indirect effects of days from spike emergence to first flower opening and breadth of flower were responsible for the positive correlation with number of flowers shown by many characters.

5.3 Floral morphology

Dendrobium flowers occur in an array of colours ranging from creamy white to deep purple. Significant variations occur in the size and shape of flowers in ceratobium and phalaenanthe types. The flowers are zygomorphic, having three sepals and three petals of which the third one is highly modified called the lip or labellum. The male and female reproductive organs are fused to form the column or gynostemium. All the flowers were found to be borne with the labellum

uppermost which is three lobed. The buds became respinate just before or during flower opening, by a twisting of the pedicel in conformity with the reports of Nyman *et al.* (1984) and Abraham and Vatsala (1981). The resupination of the uppermost labellum was also reported by Varghese (1995).

5.4 Floral Biology

For any successful hybridisation programme, knowledge of the floral biology, pollen and pollination studies should form the basis. Identification of compatible parents is also a pre-requisite for successful hibridisation.

5.4.1 Anthesis

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

Anthesis occurred in the day time with a peak between 9.00 am and 11.00 am. Varghese (1995) also reported a peak time of flower opening between 9.00 am and 10.00 am in dendrobium varieties. A similar report of flower opening in the morning was reported by Christensen (1992) in *Stelis argentia*.

It took 1-2 days for the complete opening of each flower.

5.4.2 Stigma receptivity

In the present study with *Dendrobium* varieties, the stigma remained receptive throughout the day after anthesis but the total period of stigma receptivity varied in different hybrids. This is in close confirmation with the findings of Devi and Deka (1992) that the stigma remained receptive throughout the day after anthesis in *Dendrobium amoenum, Spathoglottis plicata* and *Aerides odoratum*.

In general, the receptivity period was much longer than some of the important cross pollinated crops. Stigma receptivity was found to be maximum

between second and fifth day after anthesis, although the stigma remained receptive from the day of anthesis to the tenth day (in New Pink). Devi and Deka (1992) also observed that the stigma remained receptive upto five days after anthesis in *Dendrobium amoenum,* although the flowers retained freshness for a longer period.

5.5 Pollen studies

5.5.1 Pollen production

Significant variation was observed among the different hybrids for pollen production. It ranged from 2720 to 13120 per pollinium. Such variation in pollen production has been reported in different hybrids of *Dendrobium* by Varghese (1995). This is also in confirmity with the reports of Nair *et al.* (1964) that the relative quantity of pollens produced per flower per anther varies from variety to variety even within a species.

5.5.2 Pollen morphology

The pollen grains in the family Orchidaceae are found in agglutinated 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 Sheehan and Sheehan (1979). The pollen grains were found in tetrads. This is in conformity with the reports of Abraham and Vatsala (1981) that the pollen grains of orchids exist as tetrads, held together by elastic threads of tapetal origin. Das amd Ghoshal (1988) also reported the tetrad nature of pollen in *Dendrobium* species.

5.5.3 Pollen size

Large sized pollen grains were observed in Sabine, New Pink (47.46 μ) and the smallest pollen grains in *D. moschatum* (18.68 μ).

5.5.4 Pollen fertility

Pollen fertility was found to be maximum (91.93%) for New Pink followed by Emma White (86.09%) and Pink Tips (82.85%). Varghese (1995) also observed high pollen fertility in New Pink and Kasem White.

Pollen fertility was comparatively low in the *Dendrobium* species tested which also had the small sized pollens. A direct relationship between the size and fertility in the *Dendrobium* hybrids was also reported by Varghese (1995).

5.5.5 Pollen germination

Maximum germination of pollen grains was obtained with 2 per cent sucrose + 1 per cent agar (80.33%) followed by 2 per cent sucrose + 2 per cent agar (78.00%). This is in close confirmity with the findings of Varghese (1995) that 2 per cent sucrose + 1 per cent agar was a suitable medium for germination of *Dendrobium* pollen. The influence of agar in culture medium for germination was also reported by Vilasini *et al.* (1966) in *Hibiscus rosa-sinensis*.

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

There was considerable difference among the varieties with respect to pollen germination. Emma White had maximum germination (80.63%), followed by New Pink (75.00%) and minimum was in *Dendrobium pierardii* (14.22%). Fertility assessed by germination tests recorded almost the same trend as shown by the acetocarmine tests.

A common observation is that the wild species of *Dendrobium* have comparatively small sized pollens, less pollen output and low fertility as evidenced from the results. Varghese et al. (1997) also observed that smaller pollen grains

showed a low fertility and had a low rate of production per pollinium in some *Dendrobium* hybrids.

5.6 Compatibility studies

5.6.1 Self compatibility

In the present study, ten *Dendrobium* varieties, namely, Candy Stripe, Emma White, Hieng Beauty, New Pink, Pink Tips, Pramott-II, Sabine, Sakura Pink, Sonia-28 and Banyat Pink were selected and selfed. All were found to be self compatible, except Hieng Beauty. Johansen (1990) observed that certain species of *Dendrobium* did not produce seed pods after selfing and that shrivelling and yellowing of the ovary were noticed. Cent per cent pod set was observed in Pink Tips, Emma White and New Pink on selfing and it was only 40 per cent in Sabine. A fairly high percentage of pod set was obtained upon selfing *D. crepidatum*, *D. primulinus* and *D. transparens* but self incompatibility was found in *D. chrysotoxum* and *D. pierardii* (Das and Ghoshal, 1988).

5.6.2 Cross compability

The crossability profiles of all the ten varieties were described. Of the different hybrids, Emma White had the maximum compatible crosses and hence can be regarded as a good female parent. This is in close confirmation with the findings of Varghese (1995) who suggested Emma White and Hieng Beauty as good female parents. Maximum incompatible crosses were observed on Sabine.

Emma White, New Pink and Pink Tips, could also perform well as good male parents giving successful crosses. Of the six species tried as male parents, *D. fimbriatum* gave maximum cross compatibility followed by *D. moschatum*.

Among the different crosses, some of the reciprocal crosses were not found successful. Unilateral incompatibility between species of the genus *Dendrobium* and between species of different genera, viz., *Dendrobium*, *Spathoglottis*, *Phaius*, *Coelogyne* and *Rhyncostylis* have been reported by Devi and

Deka (1994). Failure of the reciprocal cross to set seeds may 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 Wimber, 1959). They also 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 infertility in polyploids to pairing abnormalities during meiosis where there has been an addition of one or more complete or incomplete chromosome sets. According to Devi and Deka (1994), the incompatibility shown among the intergeneric and interspecific orchid crosses may be due to genetic imbalance. Das and Ghoshal (1988) also reported unsuccessful crosses in some *Dendrobium* species due to unequal chromosome numbers.

5.7 Post pollination changes

In the present study, eventhough the ovaries swelled due to pollination stimulus, in some crosses further development was not seen. This may be due to certain post fertilization barriers or the ovaries might have swelled due to stimulatory parthenocarpy (Varghese, 1995).

The hybrid pods, in certain cases turned yellow and bursted soon at an early immature stage or abscised. Thus it seems that ovaries swelled parthenocarpically due to 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.

The time taken for culturing of pods or maturity of embryos varied from 90 days to 135 days. Nagashima (1982) reported a period of 115-120 days for maturation of embryos in *Cymbidium goeringii.*

5.8 Green pod culture

The unique nature of orchid seeds with undifferentiated embryos and suppressed endosperm formation necessitates symbiotic or *in vitro* germination. Hence enhancement of germination and production of more number of healthy seedlings become necessary by media manipulation under aseptic conditions.

Green pod culture was reported to be a major advancement in increasing the germination of orchid seeds and reducing the flowering time (Tsuchiya, 1954). Studies have shown that seeds from unripe capsules can have high germination rates and may be preferable because they can be collected before dehiscence with no seed loss (Rao, 1974; Arditti *et al.*, 1981; Henrich *et al.*, 1981 and Linden, 1992).

Green pod culture also 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, Stenberg and Kane, 1998). Successful embryo culture has been reported for embryos shortly after fertilization (Valmayer and Sagawa, 1967).

5.8.1 Standardisation of the age of the pod

Cent per cent germination could be obtained when 100 day old pods were used, followed by 90 days and 110 days old pods. Successful culture of the pods of age 85 days to 110 days was reported by Varghese (1995). Highest germination was obtained from the seeds when harvested with the embryos almost mature. Eventhough there was variation, germination could be obtained with the pods of age 70 days (80.00%) to 150 days (33.33%).

Mitra (1986) also reported that seeds obtained from green pods after 8-12 weeks of anthesis, germinated readily in a large number of wild species. Matured seeds were hard to germinate due to dormancy factors and the critical stage when seeds pass on to the dormant stage, should be identified.

Nagashima (1993) reported that germination was poor and took longer time in seeds collected when the embryos were at pre-tetrad or intermediary stages. This may be the reason for the difference in germination percentages in pods of different maturity. Tomita and Tomita (1997) also reported the relationship between seed maturity and germination in *Cypripedium* and found that the capsules ripened 14-16 weeks after pollination.

Stancato *et al.* (1998) could obtain high germination percentage in seeds of four months capsule compared to five months capsule in *Laelia purpurata*. Yam and Weatherhead (1988) also reported the high germination of immature seeds in *Pholidota* and Sharma (1998) in *Vanda coerulea*.

According to Yam and Weatherhead (1988), immature seeds taken from green capsules proved to be the suitable starting material for germination of the majority of orchid species. The use of unripe capsule is often preferable not only for ease of sterilization but also for higher germination percentages. Sharma (1998) has also reported the poor germination of bursted capsules compared to the unbursted ones in *Vanda*.

5.8.2 Standardisation of surface sterilization

Surface sterilization of the explant is a vital step in *in vitro* culturing. Chemicals, their concentrations and duration of treatments are the factors determining the success. In the present study, significant difference could be noticed among the surface sterilization treatments in controlling the contamination. Maximum survival percentage was obtained by treating with mercuric chloride (0.1%) for one minute followed by flaming after a dip in 70 per cent alcohol. The

treatment involving flaming after a dip in alcohol alone also gave good success. Luks and Shevchonke (1977), could obtain 100 per cent germination and vigorous seedlings when *Bletilla* capsules were sterilized with alcohol and inoculated.

Present results are also in conformity with the reports of successful use of alcohol for surface sterilization (Hazarika and Sarma (1995), Pyate and Murthy (1995), Tomita and Tomita (1997), Stancato *et al.* (1998), Stenberg and Kane (1998), Yam and Weatherhead (1988), and Sharma (1998), Devi *et al.* (1998).

5.8.3 Stages of germination

Orchid seeds are unique in having an unorganised embryo and no functional endosperm. During the seed germination after greening, the embryo forms a tuberous or swollen structure called protocorm. This phenomenon has been reported by many scientists from earlier periods (Bernard, 1909, Arditti and Bills, 1965 and Singh, 1993).

When leaves were produced the protocorms become autotrophic in nature. After the two leaf stage, the protocorms and rhizoids lose their nutritive function and the real roots were formed (Singh, 1993).

The protocorm formation in *Dendrobium ovatum* seeds was reported in 7-10 weeks, during which time the colour varied from yellow to light green and dark green (Pyati and Murthy, 1995).

5.8.4 Embryo culture of the hybrids

The earliest stage at which embryos can be cultured successfully, varies with the genus, species, hybrid and local conditions and it should be determined experimentally (Arditti, 1982).

Differences in the harvesting time were observed among the different hybrids of the crosses in the present study, which ranged from 90 to 135 days.

Hegde (1984) also observed that the time taken for maturity of pods depends on the habit of the species crossed.

5.8.5 Culture details of the hybrids

The *in vitro* germination response of the hybrid embryos differed in different hybrids. Some of the seeds did not germinate and certain other seeds germinated but did not reach the seedling stage.

The least germination percentage was recorded for New Pink x *Dendrobium crumenatum*, Sonia-28 x *D. crumenatum* and Banyat Pink x *D. chrysanthum* . High percentage of germination was recorded for the seeds of New Pink x Emma White, Emma White x New Pink, New Pink x Pink Tips, Pink Tips x Sonia-28, Pink Tips x Emma White and Banyat Pink x New Pink. Out of the selfed seeds, Pink Tips and Sonia 28 gave good germination. From the present study, it is evident that seeds of different crosses made on the same female parent as well as their selfed seeds behaved differently in culture with respect to germination, time taken for greening, protocorm formation, leaf development, shoot and root formation as well as plantlet formation.

Since the seeds of the crosses on the same female parent behaved differently in culture, no relationships could be worked out between the hybrids in respect of the time taken for seedling development. This could be due to the highly heterozygous nature of orchids. In some of the crosses, eventhough germination was observed further development to seedlings was stopped at different stages. This was noticed in Candy Stripe x Banyat Pink, Candy Stripe x Emma White, Candy Stripe x *D. moschatum*, Emma White x *D. moschatum*, Hieng Beauty x *D. fimbriatum*, New Pink x *D. crumenatum*, New Pink x *D. fimbriatum*, Sabine x *D. densiflorum*, Sabine x *D. pierardii*, Sakura Pink x *D. moschatum* and Banyat Pink x *D. chrysanthum*.

No germination was observed in Candy Stripe x Emma White, Candy Stripe x Pramott-II, Candy Stripe x *D. chrysanthum*, Emma White x *D. chrysanthum*, Hieng Beauty x *D. pierardii*, Pramott-II x Pink Tips, Sabine x Emma White, Sakura Pink x *D. fimbriatum*, Sonia-28 x *D. pierardii*, Sonia-28 x *D. fimbriatum* and Banyat Pink x *D. densiflorum*.

This could be due to the absence of functional embryos, eventhough seeds seemed to be normal.

5.8.6 Refinement of culture media

5.8.6.1 Effect of basal media

According to Singh (1993), Vacin and Went and Knudson-C media are the most commonly used media for orchid germination. Arditti (1982) also reported Knudson-C as the best medium for the germination of dendrobium seeds. In the present study germination was found to be maximum in Vacin and Went, Knudson-C and MS ½ strength media. The days taken for greening and protocorm formation were also less in these three meida.

The higher germination of orchid seeds in Vacin and Went medium was reported by Devi *et al.* (1990 and 1998). The simple low salt medium (Modified VW) used by Sagawa and Shoji (1967), which gave high rate of protocorm proliferation and plantlet growth was found to be desirable and commercially profitable for seed germination and seedling growth (Prakash *et al.*, 1996).

Although MS medium has been used successfully in orchid tissue culture, (Arditti, 1977), it is not generally used on orchid seeds, especially during the initial stages, because of its high nutrient concentrations (Stenberg and Kane, 1998). Knudson-C medium had been successfully used for orchid seed germination in many instances (Bose and Mukherjee, 1974; Rubluo *et al.*, 1989).

Pyati and Murthy (1995) could get high germination of *Dendrobium ovatum* in VW and Knudson-C media. They also reported the greening of seeds regardless of the medium composition.

Height of the seedlings and length of the longest leaf were significantly higher in VW, Knudson-C, ½ strength MS and ¼ strength MS. However the leaf number and root number did not differ significantly among the different media. This is in conformity with the findings of Stenberg and Kane (1998) that with respect to leaf number, KC and MS media behaved similarly. Dark protocorms observed in KC medium were also reported by Stenberg and Kane (1998) and Sharma and Chauhan (1995).

5.8.6.2 Effect of kinetin and IBA on seed germination and seedling growth

The growth hormones kinetin and IBA, each at 1 mg l^{-1}, gave total germination of seeds. The promotory effects of plant growth regulators such as kinetin and NAA on seed germination and protocorm development in orchids have been reported by Kano (1965) as well as Mathews and Rao (1980).

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 involve germination by satisfying their nutritional complexities.

In the present study treatment involving kinetin at 8 mg l^{-1}, along with IBA 4 mg l^{-1} took minimum days for first leaf production (25.33 days) and for shoot and root formation (43.00 days).

Regarding the seedling parameters like height, leaf number, number of roots, root length and leaf length, kinetin, BAP, NAA and IBA were found to have profound influence. In general BAP or kinetin at 4 mg l^{-1} along with NAA or IBA at 4 mg l^{-1} were found to be effective. Reports on NAA stimulating germination

and seedling growth in several genera like *Cattleya* (Withner, 1951) and *Vanda* (Mathews and Rao, 1980) are available. Lee *et al.* (1993) have reported better shoot production in *Dendrobium moniliforme* in a medium containing IBA and kinetin. It has also been reported that IBA and NAA enhance seed germination and seedling growth in a large number of orchid species (Singh, 1993).

The interaction influence of cytokinin (Kinetin) and auxin (IBA/NAA) on seed germination and seedling growth of *Dendrobium* was reported by Hazarika and Sarma (1995) as well as Vij *et al.*, (1981).

It is an established fact that cytokinins and auxins help in cell division and promote cell expansion (Horgan, 1984 and Bandurski and Nonhebel, 1984). The ratio of auxins to cytokinins has been shown to affect root and shoot formation in many plant cell cultures.

5.8.6.3 Effect of kinetin, BAP and adenine on seedling growth

Adenine at 6 mg l^{-1} produced maximum height (3.63 cm), maximum number of leaves (6.13) and maximum leaf length (3.03 cm) in comparison with BAP and kinetin. Adenine and adenine sulphate are known as cytokinin synergists (Nitsch et al., 1967). They increase the activity of cytokinins like BAP or kinetin. Adenine is a nitrogenous base of DNA which is widely used in tissue culture medium for its growth regulatory effects. Davis et al, (1977) reported the possible role of adenine in enhancing apical dominance.

According to Bhaskar (1996) a combination of Adenine 8 mg l^{-1} and BA 16 mg l^{-1} recorded maximum number of leaves in *Phalaenopsis*.

5.8.6.4 Effect of sucrose on seed germination and seedling development

Maximum and early germination was obtained with 3.0 per cent sucrose. The time taken for greening and protocorm formation was also minimum at this level. But the time taken for shoot and root formation was minimum with

0.5 per cent sucrose. Different sucrose levels could not make any significant difference on the height and number of leaves. But 0.5-1.0 per cent sucrose gave significantly higher values for the length of leaf, number of leaves, number of roots and length of roots.

Sucrose is an important carbon source used for the embryo culture of orchids. It supports initial germination and subsequent growth. Its effect depends on concentration.

Eventhough higher sucrose concentration is needed during the initial stages of seed germination and growth, it can be reduced during the subsequent growth periods. This is in confirmation with the report that organogenesis is promoted at suboptimal concentrations while protocorm proliferation is enhanced by supra optimal concentrations (Arditti, 1979). According to Sing (1993), orchid seedlings require no exogenous supply of sugars and as such the quantity can be drastically reduced. Hinnen et al. (1989) reported the favourable effect of 1.5 per cent sucrose on the differentiation of excised tissues and organs of several crops.

5.8.6.5 Effect of charcoal on germination and further growth

Significant difference could not be obtained with regard to the germination percentage and time taken for greening in different charcoal concentrations.

Different seedling parameters like height, number of leaves, leaf length, number of roots and root length were found to increase at the charcoal concentrations of 0.5-1.0 g l^{-1}.

Charcoal has been used as a purifying and decolorising compound for liquids since 18^{th} century.

Charcoal has the capacity to adsorb hormones, vitamins and some toxic chemicals (Fridborg *et al.*, 1978). Hence it should be used carefully in media,

which contain these components. Its concentration should be such that it should not adsorb enough of the compounds to cause inhibition of growth, which indicate that the hormones and vitamins are needed only at the levels, which remain in the media following the adsorption. Hence addition of excessive concentration should be avoided. In the present study 0.5-1.0 g l^{-1} was found to be the optimum concentration to get maximum growth.

The beneficial effects of charcoal are due to many reasons. Charcoal absorbs phytotoxic metabolites, which may be released by the tissues (Yam *et al.*, 1990). It is also likely that the charcoal provides added aeration which could improve growth. Excellent growth of *Paphiopedilum* seedlings was observed in a medium containing 0.2 per cent charcoal compared to that without charcoal (Ernst, 1975).

5.8.6.6 Effect of media supplements

In the present study enriching the nutrient media with adjuncts or organic compounds like coconut water, peptone and banana pulp have given good responses. The seedling characters like height, leaf number, leaf length, root number and root length were found to be maximum in the media supplemented with coconut water at 100-150 ml l^{-1} and peptone 500-1000 mg l^{-1}.

Promotary effect of complex additives, on the seedling growth is on record in orchid cultures. Sheehan (1983) found that the addition of coconut water in the nutrient medium enhanced seedling growth. Singh and Prakash (1985) have confirmed the beneficial effect of coconut water (150 ml) in embryo culture of orchids. Improvement in seed germination and seedling development of *Vanda coerulea* with the addition of coconut water was reported by Devi *et al.* (1998).

According to Arditti (1982), banana pulp may enhance the orchid seedling growth. Ernst (1982) described a medium containing charcoal and ripe

banana suitable for seedling growth of *Paphiopedilum*. Yam and Weatherhead (1988) also successfully used banana pulp for orchid seed culture.

According to Sudeep (1994), number of shoots, length of shoot and number of leaves were improved with banana pulp @ 5 to 10 per cent or coconut water 5 to 10 per cent along with NAA 2 ppm and BA 5 ppm in *Dendrobium* tissue culture.

Devi and Deka (1994) reported that the average fresh weight and average length of leaves and roots were maximum in peptone supplemented medium, followed by that in coconut water supplemented medium. Improvement in the growth of orchid seedlings after adding peptone has been reported in *Vanda* (Mathews and Rao, 1980), *Dendrobium monile* (Chung *et al.*, 1981) and *Bletilla striata* (Chung *et al.*, 1983).

Peptone is an organic compound which is composed of vitamins, amino acids and other compounds. Arditti (1977) and Churchill (1972) suggested that orchid roots specifically require peptone which is a rich source of amino acids. In *Dendrobium crepidatum* and *D. pierardii*, peptone at 2 g l^{-1} favoured vegetative growth from floral buds (Vij et al., 1991).

In the undefined additives like coconut water, cytokinin like substances were reported (Strauss and Rodney, 1960). The stimulatory properties of coconut water were partly due to the presence of zeatin riboside (Dodds and Roberts, 1987). According to Shantz and Steward (1952), coconut water also contains a number of cell division factors and free amino acids. Hence all these findings confirm the present result of the beneficial effects of coconut water, peptone and banana pulp on the growth of orchid seedlings.

5.8.7 Callusing

Maximum callusing from seeds was obtained in 4 mg l^{-1} NAA + 2 mg l^{-1} 2,4-D and with the addition of kinetin 2 mg l^{-1}. The immature condition of seeds as

well as undifferentiated embryos may be the probable factors for the callusing of the seeds (Vij *et al.*, 1981). The production of callus by the addition of 2,4-D has been reported by Mitra (1986). The presence of auxin in 2,4-D enhances cell division and hence callus is formed.

5.8.8 Production of PLBs

PLBs were formed from *in vitro* leaves in a medium containing 25 mg l^{-1} BA + 2 mg l^{-1} NAA. Production of PLBs in a medium containing NAA was reported by Soediono (1983). Bhaskar (1996) could obtain cent per cent success in PLB formation from *in vitro* leaf, in a medium with 25 mg l^{-1} BA + 10 mg l^{-1} adenine + 1 mg l^{-1} NAA.

5.9 *In vitro* mutagenesis

It may be desirable to introduce variants deliberately in the hope of obtaining a few, perhaps spectacular types in ornamental crops like orchids. The basic requirement must be to obtain genetic diversity from which specially desired qualities could be selected. One of the techniques for imparting genetic diversity is through mutation.

Investigations have contributed much to the induction of variation *in vitro* (Nickel, 1973) and the results with orchids are promising (Wimber and Vancott, 1967, Chaichareon, 1973, Sanguthai and Sagawa, 1973, Sanguthai *et al.*, 1973). It has been reported that tissue culture greatly facilitates the application of mutagenesis to tissues or cells (Nickel, 1973).

Ionising radiation has been found to be good mutagen. It can interact with the cells to produce a genetic effect in the immediate vicinity of its ionisation track (Muller, 1954). The more pentrating irradiation, the more effective it is, in inducing mutations.

Tolerance of tissues to radiation varies greatly depending on kind and stage of growth. Dosage of gamma rays ranging from 2000 to 4000 R at a rate of 900 R per hour seen to be optimum for the purpose of inducing mutation in *Cymbidium* protocorm (Harn, 1970). Older ones and sliced protocorms are even more sensitive. However, whole protocorm treatments are preferable (Harn, 1970).

Dendrobium mericlones 2.0 to 2.5 cm size can tolerate gamma radiation up to a strength of 5000 R without much loss of viability. Plants irradiated with higher doses did not produce new growth in later years. Some of the irradiated *Dendrobium* Pompadour produced flowers with modifications in shape or colour or both (Vajrabhaya, 1977).

In the present study, gamma rays up to 60 Gy have been successfully used to irradiate under *in vitro* conditions in *Dendrobium* protocorms. According to Vajrabhaya (1977) *Dendrobium* mericlones can tolerate gamma radiation up to 5000 R.

Seventeen hybrids were irradiated and the germination was found to be decreasing as the dose increased. At higher doses the growth was found to be slow, taking more time to reach each stage of development. Mazumder and Bhowmik (1997) in *Spathoglottis plicata* and Vajrabhaya (1977) in *Dendrobium* also reported similar results. The irradiated plants also had less height compared to normal plants. But the leaves became broader and thicker by irradiation and a slight increase in the number of shoots and leaves was also seen in the irradiated seedlings. The same trend was noticed in all the hybrids.

According to Vajrabhaya (1977) radiation may also cause phenotypic changes owing to developmental abnormality. White Sim carnation, for example, could produce red flowers after irradiation (Sagawa and Mehlquist, 1957). Such variation, which is not a somatic mutation but a mechanical damage can also occur

in orchid clone (Vajrabhaya, 1977). The purplish colouration noticed in some of the leaves of irradiated hybrids may be attributed to this reason.

Wilson (1993) reported a delayed bud break in rose by gamma radiation which confirms the slow growth in the present study, with higher doses of irradiation.

In the irradiated callus 60 Gy had a lethal effect and in PLBs, doses of 50 Gy and 60 Gy were lethal. All other responses in PLBs and callus were similar to that obtained in protocorms.

EMS induced mutants in lady's slipper orchid was reported by Arora and Jana (1980).

5.10 Planting out and hardening

The seedlings which reached plant out stage were thoroughly washed and treated with fungicide. These were planted in media consisting of brick pieces and charcoal pieces in equal proportion. In summer months pieces of coconut husks were also added to the media. High humidity was maintained in the surrounding. According to Wainwright (1988), the plants were very poorly adapted to resist low RH, higher light levels and more variable temperature prevailing outside.

The survival percentage in different hybrids varied considerably and the range was 14 per cent (New Pink x *D. chrysanthum*) to 94 per cent (New Pink selfed).

The successful planting out of the *Dendrobium chrysanthum* (brick, charcoal, tree fern, bark pieces, leaf mould and dry sphagnum in 1:1:1:1:1:2 ratio) and *Paphiopedilum spicerianum* (leaf mould, perlite, vermiculite and dry sphagnum in 1:1:1:2 ratio) was reported by Sharma and Chauhan (1995). According to Hazarika and Sarma (1995), after transplanting of the seedlings of

Dendrobium transparens (FYM, chopped fern roots, leaf mould and charcoal at the ratio of 2:1:2:1), the rate of survival was found to be 90 to 95 per cent.

Anderson (1980) reported the need for thorough washing of the plantlets to remove the traces of nutrient media and sterilizing the potting mixture for eliminating the fungal infection. Soaking of media in disinfectant before planting was also reported by Kim *et al.* (1988).

The media used for planting out should provide good support to the plants, supply water and ensure good drainage and aeration around the plants since they are epiphytic in nature. Here the medium selected had brick pieces and charcoal pieces which satisfies the above requirements. The use of charcoal as a successful medium was also confirmed by Seeni and Latha (1990), Kumar (1992), Singh (1993). Lakshmidevi (1992) recommended brick and charcoal pieces in equal proportion as a potting medium for *Dendrobium fimbriatum* and *D. moschatum* seedlings. Sudeep (1994) observed coconut husk as a good potting medium for *Dendrobium* plantlets.

5.11 Effect of nutrients on seedling growth

Dendrobium, being epiphytic in nature, are grown on large media components. Most of these components are devoid of any nutrients and they cannot hold nutrients. Hence regular schedule of fertilizing in liquid form is highly essential. In the initial stages of growth, more nitrogen is needed for shoot and leaf production. Hence the plants sprayed with 30:10:10 (NPK) showed good growth with more number of shoots and leaves.

The growth pattern in *Dendrobium* seedlings in terms of number of shoots and number of leaves at different nutrient levels clearly indicated that Green care 30:10:10 (NPK) at 0.1-0.2 per cent concentration sprayed at alternate days was the best for getting good growth of seedlings. 17:17:17 complex sprayed on

alternate days also gave good results. Green care, which is a commercial formulation, contains major elements as well as the minor elements.

Many scientists have reported improvement of growth and flowering in orchids by the application of nutrients. Spraying N, P and K in the ratio of 20:20:20 and 10:30:20 at weekly intervals was recommended for orchids under South Indian condition by Abraham and Vatsala (1981). An effective spray of 17:17:17 NPK complex was recommended for *Cymbidium traceanum* by Sobhana and Rajeevan (1995). Excellent results were also reported in orchids by using inorganic fertilizer mixtures containing equal proportions of N, P and K (Singh, 1992).

5.12 Effect of growth regulators on seedling growth

Naturally occurring hormones play an important role during the process of plant growth and development. Exogenous application of growth regulators may induce or promote flowering, prevent or delay it (Yadav and Bose, 1989).

In the present study, maximum number of shoots was observed with BA 50 mg l^{-1} and 25 mg l^{-1}, sprayed at fortnightly intervals. Maximum height of shoots was obtained in GA_3 treated plants. GA_3 induces cell elongation and thus shoot elongation (Jones and Mac Millan, 1984) and hence the effect may be due to this.

Cytokinins have a property of induction of more number of shoots and leaves. The production of multiple inflorescence by cytokinins (BA) and the regulation of flowering by gibberellins were reported in orchids (Yadav and Bose, 1989). The promotory effect of GA on flowering was also reported by Jones and Mac Millan (1984).

5.13 Field evaluation of hybrid seedlings

The hybrids were evaluated in the field for getting superior vegetative characters like number of shoots, height of shoots, leaf size and number of leaves

as well as floral characters like flower size, flower number, spike length and new colour combinations.

Evaluation of growth performance of the hybrids in the field indicated that, although there was a progressive increase in the vegetative characters, different hybrids performed differently in the field. Variations could be observed in the vegetative characters like height of shoots, length of leaf and number of leaves. Slight variation in the leaf colour and stem colour was also noticed in some of the hybrids.

Number of shoots, one year after planting varied from four to five. Height of shoots was maximum for Banyat Pink x Sakura Pink (17.5 cm) followed by New Pink x Banyat Pink (17.0 cm). Among the hybrids, number of leaves was highest for Banyat Pink x Pink Tips. The length of largest leaf was maximum for Emma White x New Pink followed by New Pink x Banyat Pink. Breadth of largest leaf was highest in Banyat Pink x Sakura Pink and Sonia-28 x Hieng Beauty (3.0 cm, each). Results are indicative of the superiority of New Pink and Banyat Pink as parents for imparting good vegetative characters. Evaluation is being continued for consistent results.

Flowering was also noticed in some of the hybrids. First flowering was observed 12 months after planting in the hybrid New Pink x Banyat Pink followed by Emma White x Sonia-28 and Emma White x New Pink (13^{th} month). The other crossess which flowered early were Banyat Pink x Hieng Beauty and Sakura Pink x Pink Tips. Hence for imparting earliness, parents like New Pink, Banyat Pink and Emma White are superior.

Since evaluation was made on the first and second inflorescences, floral characters like number of flowers and length of the inflorescence were less and are not indicative of their actual performance. But large sized flowers were noticed in New Pink x Banyat Pink (8.0 x 7.8 cm) and Banyat Pink x Hieng Beauty (8.0 x

7.2 cm). Superiority of New Pink and Banyat Pink in improving floral size is seen here. Promising colour variations were also noticed in some of the hybrid flowers.

The evaluation is being continued and confirmatory results may be obtained only after attaining stability in flowering.

Based on the informations gathered from the present investigation, following further lines of work are suggested.

1) Improving the popular commercial cut flower varieties of *Dendrobium* with respect to one or two desirable characters. Species indigenous to Western Ghats should also be tried.
2) Breeding to impart dwarfness, fragrance etc. by crossing the desirable wild species like *Dendrobium canaliculatum* and *Dendrobium crumenatum.*
3) Polyploidy breeding and mutation breeding can be attempted to exploit further variability.
4) The pollen pistil interaction and incompatibility problems are to be studied.


SUMMARY

SUMMARY

The present investigations on improvement of *Dendrobium* through hybridsation and *in vitro* mutagenesis was conducted in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, during 1996-1999.

Ten varieties and six species of *Dendrobium* were included for the trial. All the varieties showed significant variations among themselves for the characters studied. New Pink had the maximum number of shoots (9 Nos.), leaf length (15.17 cm), maximum sized flowers (8.27 cm x 7.9 cm) and maximum vase life (18.33 days).

Number of flowers was maximum for Candy Stripe (11.67 Nos.). Candy stripe had more leaf breadth (4.50 cm) and maximum leaf number (33.33) also. Maximum number of days for opening of all florets was taken by Candy Stripe (19.33 days) followed by Pink Tips and Sonia 28 (18.33 and 18 days, respectively).

The flowers lasted for maximum number of days in Sonia 28 and Pink Tips (57 and 56.33 days respectively) and it took only 39.33 days for the wilting of all florets in Banyat Pink.

Vase life ranged from 13 days (Candy Stripe) to a maximum of 18.33 days in New Pink. Year round flowering occurred in the varieties Hieng Beauty, Emma White, Sonia 28, Candy Stripe and New Pink. Pink Tips and Sabine had maximum flowering during April to November.

In general, PCV was slightly higher than GCV for all the characters. Both PCV and GCV were highest for number of flowers per spike (PCV = 26.39

and GCV = 23.55), followed by days for opening of all florets (PCV = 21.64, GCV = 19.64) and number of shoots (PCV = 20.79, GCV = 15.38).

The heritability was of moderate to high magnitude, in general. The characters which showed maximum heritability were breadth of flower (97.0), length of flower (96.9) and breadth of leaf (95.6). Heritability was the lowest for number of shoots (54.7).

The characters having significant correlation with number of flowers per spike were mean height of shoots, total number of leaves, length of inflorescence, days for opening of all florets and inter nodal length (first flower). All the characters showed positive correlation. Days for opening of all florets registered the highest positive correlation (rg = 0.924, rp = 0.838).

Genotypic correlation coefficients were higher than phenotypic correlation coefficients. Highest positive correlation among all the characters was observed between length of flowers and breadth of flowers (rg = 0.969), rp = 0.949). Number of shoots had highest positive correlation with breadth of flowers (rg = 0.746, rp = 0.582). Mean height of shoots was highly correlated with length of inflorescence (rg = 0.961, rp = 0.763). Total number of leaves showed highest positive correlation with length of inflorescence (rg = 0.833, rp = 0.709), followed by number of flowers per spike and days for opening of all florets. Days from spike emergence to first flower opening registered significant positive correlation with average breadth of leaves and number of shoots. Length of flower showed highest positive correlation with breadth of flower (rg = 0.969, rp = 0.949), followed by internodal length between last two flowers (rg = 0.728, rp = 0.612).

The direct and indirect effects of fifteen component characters on the number of florets per spike indicated that days from spike emergence to first flower opening had the highest positive direct effect (1.596) followed by breadth of

flower (0.815). Other characters, which had positive direct effect were days for opening of all florets (0.450), length of inflorescence (0.291), total number of leaves (0.070) and average length of leaves (0.010). But length of flower, average breadth of leaves, number of shoots and height of shoots exhibited a negative direct effect (-1.151, -1.419, -0.659, -0.004, respectively).

The indirect effect of all characters through number of shoots was negative. Length of flower had highest positive indirect effect through breadth of flower (0.790) and days from spike emergence to first flower opening (0.715).

The flower is built on a very simple pattern of three outer sepals and three inner petals. The three sepals are alike and of the inner petals, lateral two are similar and to a little broader than the sepals. The third petal is highly modified and enlarged and is called the 'lip' or 'labellum'.

The gynostemium or column 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 pollinium. The pollinium lie in a cavity covered by a fringed cap. There are two pairs of pollinia in dendrobium. Just below the anther, is the stigma which is separated by a partition structure called rostellum. The stigma is a shiny depression filled with extremely sticky fluid.

In all the *Dendrobium* varieties flower opening occurred during the day time. The time of opening started from 7.30 am and lasted upto 2.30 pm which varied in different flowers of a variety. It took almost a day for the complete opening of the flowers depending on the variety.

In Hieng Beauty, Emma White, Candy Stripe and Sakura Pink, the flowers of the inflorescence opened almost uniformly at an interval of 24 hours while those of New Pink, Sonia-28, Pramott-II, Pink Tips, Sabine and Banyat Pink,

took almost two days to complete the anthesis of each flower bud. The longevity of each flower on the plant was for 35 to 45 days.

Irrespective of the time of the day at which pollination was carried out, successful pod set could be obtained from 8 am to 5 pm.

Maximum stigma receptivity period as observed by pod set was for New Pink (3^{rd} to 10^{th} day after anthesis) followed by Pink Tips (2^{nd} to 8^{th} day after anthesis). All the hybrids were found to have receptive stigma from second day of anthesis.

Out of the six *Dendrobium* species, *D. fimbriatum* gave the maximum cross compatibility when used as male parent.

Pollen production was the lowest in *Dendrobium pierardii* (2720) and maximum pollen production was in Emma White ((13120). New Pink also had a substantial pollen out put (11040). The pollen grains were found as spherical to rectangular in shape and were found to exist as tetrads. Pollen size ranged from 18.68 μ in *D. moschatum,* to 47.46 μ in Sabine.

Pollen fertility was found to be maximum (91.93%) in New Pink followed by Emma White (86.09%) and Pink Tips (82.85%). Fertility was the lowest in *D. chrysanthum* (46.77%). Emma White had the maximum germination percentage (80.63%) and the minimum was that of *D. pierardii* (14.22%).

Out of the ten varieties, only Hieng Beauty was found to be self incompatible.

Slight variation was observed in the time taken for the maturity of the pods for culturing and it varied from 95 days in Candy Stripe to 120 days in Pink Tips.

In certain crosses, ovary swelling was observed but it was fallen after 30 days before reaching maturity. Crosses of Pramott-II x Sonia-28, as well as Hieng Beauty x Sabine, fall in this category.

Emma White had the maximum cross compatibility and it was found compatible with all the nine varieties and four species. Its incompatibility was noticed with *D. densiflorum.* Maximum incompatible crosses were observed on Sabine. Emma White, New Pink and Pink Tips were good male parents. These three varieties gave 100 per cent pod set when crossed to the all other varieties.

In the unsuccessful crosses, either the flower remained intact, retaining its freshness or the flowers faded and abscised 2-3 days after pollination. In the case of successful crosses ovary remained as the part of the spike and gradually enlarged to form the pod, whereas the petals and sepals withered.

Among the surface sterilization treatments of the pod, maximum survival percentage was obtained with the combination in which the pod was kept in mercuric chloride (0.1%) for one minute and flamed after a dip in 70 per cent alcohol.

Maximum seed germination was obtained with 90, 100 and 110 days old pods.

After about 15-20 days of inoculation, greening of the seeds was noticed. The germinated seeds got bulged and swollen structures (protocorms) were formed in 45-60 days. The protocorms increased in size and from these, leaves were produced in about 75-90 days. Shoot and root formation occurred in 100-120 days.

The minimum germination percentage was observed with New Pink x *D.crumenatum*, Sonia 28 x *D.crumenatum* and Banyat Pink x *D. chrysanthum.* High per cent of germination was noticed in New Pink x Emma White, Emma

White x New Pink, New Pink x Pink Tips, Pink Tips x Sonia 28, Pink Tips x Emma White, Banyat Pink x New Pink.

Pink Tips selfed and Sonia 28 selfed gave the highest germination. The days taken for each stage in culture varied significantly among the hybrids.

Emma White selfed took the least number of days (253 days) for planting out and Sonia 28 x *D.crumenatum* took maximum days (385 days) for planting to the field.

Out of the different basal media tried cent per cent survival through germination was obtained for Vacin and Went medium which was significantly superior to MS medium at its full strength (85% germination).

But significant difference could not be observed in germination in different media like KC and MS at its (half strength and one fourth strength).

Among the 20 treatments involving kinetin, BAP and IBA, days taken for first leaf production was minimum (25.33 days) for the treatment involving kinetin 8 mg l^{-1} and IBA 4 mg l^{-1} followed by kinetin 8 mg l^{-1} + IBA 2 mg l^{-1}.

Significant difference was noticed among the media with both the growth hormones and the media with only kinetin. Maximum number of days was taken for greening in the medium without any growth regulators.

MS at ¼th strength, KC and VW showed better response in respect of seedling growth. Full germination was obtained with kinetin 1 mg l^{-1} + IBA 1 mg l^{-1}, kinetin 2 mg l^{-1} + IBA 1 mg l^{-1} as well as kinetin 2 mg l^{-1} + IBA 2 mg l^{-1}.

Early germination, protocorm formation as well as shoot and root formation were observed in the medium containing kinetin/BAP at 4 mg l^{-1} to 8 mg l^{-1} and IBA/NAA at 2 mg l^{-1} to 6 mg l^{-1}. These media also gave higher values

for seedling characters like height, leaf number, leaf length, root number and root length.

Adenine at 6 mg l^{-1} and 8 mg l^{-1} gave the maximum height, maximum number of leaves and the longest leaf.

Eventhough maximum germination was obtained with 3 per cent sucrose in the medium, it took the maximum days for shoot and root development (101.33 days).

The time taken for greening and protocorm development were minimum (26.67 days and 45.33 days, respectively) with 3 per cent level, which were significantly different from those at 0.5 per cent level.

Minimum time for shoot and root formation was taken by the cultures in the medium with 0.5 per cent sucrose (88.67 days) which was significantly different from all others.

Maximum height (3.00 cm) and number of leaves (5.67) were obtained with the sucrose concentration of 0.5 per cent and the minimum with 3 per cent sucrose.

The longest leaves were produced at 0.5 per cent sucrose level (3.6 cm) which was significantly superior to that with 3 per cent sucrose (3.2 cm).

Medium with 3 per cent sucrose gave the least value in respect of height (2.63 cm), number of leaves (4.67), length of leaf (3.20 cm), number of roots (4.60) and length of roots (3.17 cm).

Time taken for greening was not much affected by the concentration of charcoal in the culture medium, eventhough 0.5 per cent charcoal gave the minimum number of days (26.33) for greening.

Significant difference could be obtained among the treatments with respect to the time taken for protocorm development. There was no significant difference between the treatments with 2 per cent charcoal and without charcoal in respect of time taken for protocorm development (52.00 and 55.00 days, respectively).

Height of the shoots was maximum with 1.5 per cent of charcoal but significant difference was not observed between the charcoal levels of 0.5, 1.0, 1.5 and 2.0 per cent with respect to height. Longest leaves were produced with 1.0 per cent charcoal (2.77 cm).

Minimum time of 13.67 days was taken for first leaf production in coconut water 150 ml l^{-1} followed by coconut water 100 ml l^{-1} (14.00 days).

Maximum time (16.00 days) was taken for the first leaf production in banana pulp 20 mg l^{-1}.

Effect of various media supplements namely peptone, coconut water and banana pulp on the growth of the seedlings indicated a maximum height of 3.20 cm in the treatment involving coconut water 150 ml l^{-1}. Peptone at 1000 mg l^{-1} could increase the height to 3.03 cm and coconut water at 100 ml l^{-1} as well as at 200 ml l^{-1}, increased the height to 2.7 cm each. Coconut water at 150 ml gave maximum number of leaves (5.93) followed by peptone 1000 mg l^{-1} (5.73).

Coconut water 150 ml l^{-1} and peptone 1000 mg l^{-1} produced maximum length of leaves (3.30 cm and 3.19 cm respectively).

Protocorms, callus induced from protocorms and PLBs from *in vitro* leaf were subjected to irradiation by gamma rays under *in vitro* conditions. Doses above 60 Gy were found to be lethal to callus and PLBs. The irradiated protocorms above 60 Gy also failed to regenerate into shoots and roots.

Seventeen hybrids were subjected to irradiation. The development of irradiated protocorms into seedling differed in different doses. Significant variations occurred in the time taken for each stage in culture. A general observation is that irradiation reduced the height of the plants and as the dose increased the reduction in height also was more. Higher doses of irradiation also caused smaller and broader leaves with dark green colour. The rate of growth became slow in the higher doses of irradiation. A slight increase in the number of leaves and shoots was also observed in the high doses. Percentage of survival was also reduced in the irradiated ones.

The seedlings, which reached plant out stage were taken out, treated with a fungicide, Indofil - M-45 (0.05%), for 20 minutes and planted to the community pots.

The medium consisted of brick pieces and charcoal pieces in equal proportion.

The pots containing the seedlings were kept under shade and watered regularly to maintain humidity.

The percentage of survival in the field varied in different hybrids and it ranged from a minimum of 14 percent (New Pink x *D. chrysanthum*) to a maximum of 94 percent (New Pink selfed).

The effect of different concentration of the nutrients on the growth of the seedlings indicated maximum number of shoots (5.50) was observed for the treatments 30:10:10 green care at 0.2 per cent as well as 17:17:17 NPK complex at 0.1 per cent level each given as alternate day sprayings.

Number of leaves was maximum (10.33) for 30:10:10 Green care (0.1%) sprayed on alternate days. The number of shoots also increased in the

treatments involving Green care 30:10:10 at both 0.1 and 0.2 per cent concentrations sprayed on alternate days.

Regarding the effect of growth regulators GA and BA on the growth of seedlings, maximum number of shoots (10.75) was observed in BA 50 mg l^{-1} sprayed at fortnightly intervals, closely followed by BA 25 mg l^{-1} sprayed at forntightly intervals (10.25). Tallest shoot was observed in GA 10 mg l^{-1} sprayed at fortnightly intervals (16.25 cm), followed by GA 5 mg l^{-1} sprayed at fortnightly intervals (15.75 cm).

Growth parameters like number of shoots, height of each shoot, number of leaves as well as length and breadth of largest leaf were observed at an interval of 4 months. An average of 4-5 shoots were found in all the hybrids at about one year after planting. Emma White x Banyat Pink (30 Gy) and Pink Tips selfed (10 Gy) had a maximum of 7 shoots at the 16th month.

A maximum of 22.00 cm height was observed in Banyat Pink x Hieng Beauty and a minimum of 7.6 cm for Pink Tips selfed (10 Gy) at the end of 12th month. Flowering was seen at 12th month after planting in New Pink x Banyat Pink and in 13th month in Emma White x Sonia-28 as well as Emma White x New Pink.

The number of flowers in the first inflorescence ranged from two (Emma White x Sonia 28) to eight (Sakura Pink x Pink Tips). Length of the inflorescences of the hybrids varied from 10 cm (Emma White x Sonia-28) to 36 cm (Sakura Pink x Pink Tips).

A good size of flowers 8.0 cm x 7.8 cm was observed in New Pink x Banyat Pink and the size of flowers in Banyat Pink x Hieng Beauty was 8.0 cm x 7.2 cm.

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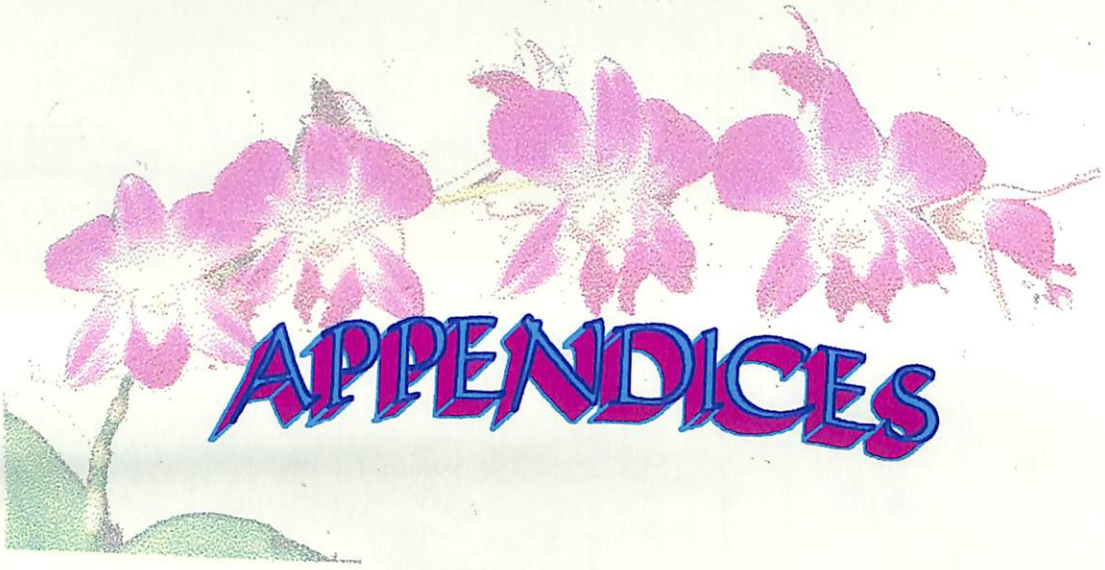
APPENDICES

APPENDIX-I

Composition of Murashige and Skoog (1962) medium

Constituents	Quantity	Volume made up	Volume pipetted
Solution A			
Ammonium nitrate	16.5 g	1000 ml	100 ml
Potassium nitrate	19.0 g		
Magnesium sulphate	3.7 g		
Potassium dihydrogen phosphate	1.7 g		
Solution B			
Calcium chloride	4.4 g	500 ml	50 ml
Solution C			
Boric acid	0.62 g	100 ml	1 ml
Manganese sulphate	2.23 g		
Zinc sulphate	0.86 g		
Potassium iodide	0.083 g		
Sodium molybdate	0.025 g		
Solution D			
Ferrus sulphate	2.78 g	500 ml	5 ml
Sodium EDTA	3.74 g		
Solution E			
Cobalt chloride	0.025 g	1000 ml	1 ml
Copper sulphate	0.025 g		
Solution F			
Nicotinic acid	50 mg	100 ml	1 ml
Pyridoxine HCl	50 mg		
Thiamine HCl	10 mg		
Glycine HCl	200 mg		
Sucrose	30.00 g		
Myo-inositol	100.00 mg		
Agar	6.00 g		
pH	5.8-6.0		

APPENDIX-II
Composition of Vacin and Went (1949) medium

Constituents	Quantity/litre
Ammonium sulphate	500 mg
Magnesium sulphate	250 mg
Manganese sulphate	7.5 mg
Potassium nitrate	525 mg
Potassium dihydrogen phosphate	250 mg
Dicalcium phosphate	200 mg
Tricalcium phosphate	200 mg
Ferric tartarate	28 mg
Sucrose	20 g
Agar	8 g
pH	5.5-6.0

APPENDIX-III
Composition of Knudson-C (1946) medium

Constituents	Quantity/litre
Calcium nitrate	1 g
Potassium dihydrogen phosphate	250 mg
Magnesium sulphate	250 mg
Ammonium sulphate	500 mg
Ferrous sulphate	25 mg
Manganese sulphate	7.5 mg
Sucrose	20 g
Agar	8 g
pH	5.0 - 6.0

APPENDIX-IV

Abstract of Analysis of variance for pollen characters

Character	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Pollen germination percentage	15	32	1409.20	79.23
Pollen fertility percentage	15	32	384.37	115.82
Pollen diameter	15	32	526.50	46.95
Pollen production	15	32	45871966.67	1508750.00

APPENDIX-V

Abstract of Analysis of variance for green pod culture of Banyat Pink crosses

Character	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Germination percentage	6	14	3978.97	113.24
Days for greening	6	14	48.64	4.43
Days for protocorm development	6	14	279.19	41.57
Days for first leaf production	6	14	342.49	16.67
Days for shoot and root formation	5	12	139.69	15.22
Days for planting out	5	12	362.22	37.50

APPENDIX-VI

Abstract of Analysis of variance for green pod culture of Sakura Pink crosses

Character	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Germination percentage	8	18	2035.42	96.30
Days for greening	8	18	30.70	4.78
Days for protocorm development	8	18	51.32	7.70
Days for first leaf production	7	16	98.57	63.41
Days for shoot and root formation	7	16	63.88	21.54
Days for planting out	7	16	297.47	51.04

APPENDIX-VII

Abstract of Analysis of variance for green pod culture of Hieng Beauty crosses

Character	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Germination percentage	8	18	2566.67	85.19
Days for greening	8	18	192.01	11.19
Days for protocorm development	7	16	403.76	8.29
Days for first leaf production	6	14	794.52	8.48
Days for shoot and root formation	6	14	844.71	9.33
Days for planting out	6	14	96.43	57.14

APPENDIX-VIII

Abstract of Analysis of variance for green pod culture of Emma White crosses

Character	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Germination percentage	12	26	2496.05	102.56
Days for greening	12	26	447.34	8.64
Days for protocorm development	12	26	415.75	9.82
Days for first leaf production	10	22	230.69	14.09
Days for shoot and root formation	10	22	422.21	16.27
Days for planting out	10	22	949.70	34.09

APPENDIX-IX

Abstract of Analysis of variance for green pod culture of Pink Tips crosses

Character	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Germination percentage	11	24	3366.41	111.11
Days for greening	11	24	35.35	5.67
Days for protocorm development	11	24	70.09	12.25
Days for first leaf production	10	22	72.23	14.94
Days for shoot and root formation	10	22	155.83	14.79
Days for planting out	9	20	2023.06	53.33

APPENDIX-X

Abstract of Analysis of variance for green pod culture of Sabine crosses

Character	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Germination percentage	7	16	2068.00	80.21
Days for greening	7	16	104.64	4.21
Days for protocorm development	6	14	352.32	19.38
Days for first leaf production	5	12	104.72	32.83
Days for shoot and root formation	5	12	425.39	26.89
Days for planting out	5	12	1860.00	83.33

APPENDIX-XI

Abstract of Analysis of variance for green pod culture of Sonia 28 crosses

Character	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Germination percentage	10	22	2769.09	135.61
Days for greening	10	22	186.62	6.88
Days for protocorm development	10	22	322.63	21.88
Days for first leaf production	10	22	545.67	21.15
Days for shoot and root formation	10	22	584.29	15.97
Days for planting out	10	22	3259.70	50.18

APPENDIX-XII

Abstract of Analysis of variance for green pod culture of Pramott-11 crosses

Character	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Germination percentage	8	18	2733.33	113.99
Days for greening	8	18	145.90	7.15
Days for protocorm development	8	18	598.29	23.07
Days for first leaf production	8	18	289.82	22.85
Days for shoot and root formation	8	18	636.17	42.82
Days for planting out	8	18	1321.57	26.48

APPENDIX-XIII

Abstract of Analysis of variance for green pod culture of New Pink crosses

	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Germination percentage	11	24	3596.97	72.22
Days for greening	11	24	18.13	8.39
Days for protocorm development	10	22	94.62	98.33
Days for first leaf production	9	20	95.12	16.53
Days for shoot and root formation	9	20	52.31	14.1
Days for planting out	9	20	1155.65	271.67

APPENDIX- XIV

Abstract of Analysis of variance for green pod culture of Candy Stripe crosses

Character	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Germination percentage	8	18	3384.26	84.26
Days for greening	8	18	150.95	7.22
Days for protocorm development	8	18	329.12	5.30
Days for first leaf production	7	16	233.66	13.33
Days for shoot and root formation	6	14	481.49	34.14
Days for planting out	4	10	4165.00	50.00

APPENDIX – XV

Abstract of analysis of variance for different treatments under *in vitro* conditions.

Character	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Effect of pod age				
Germination percentage	8	18	1741.20	71.30
Days to greening	8	18	69.57	4.56
Effect of basal media				
Germination percentage	4	10	98.33	55.00
Days for greening	4	10	16.33	4.00
Days for protocorm development	4	10	13.90	2.13
Seedling height	4	10	0.49	0.04
Leaf number	4	10	1.57	0.53
Leaf length	4	10	0.18	0.03
Root number	4	10	0.23	0.27
Root length	4	10	0.85	0.03
Effect of Growth regulators				
a) Effect of Kinetin and IBA				
Germination percentage	6	14	310.11	64.29
Days for greening	6	14	32.11	5.10
Days for first leaf production	19	40	9.04	3.72
Days for shoot and root formation	11	24	28.33	5.92
b) Effect of BA and NAA				
Height	19	40	0.26	0.10
Leaf number	19	40	1.45	0.51
Leaf length	19	40	0.55	0.09
Root number	19	40	1.76	0.36
Root length	19	40	1.03	0.11
c) Effect of Kinetin, BA and IBA				
Days to shoot and root formation	19	40	29.77	4.38
Height	19	40	0.36	0.10
Leaf number	19	40	1.64	0.49
Leaf length	19	40	0.11	0.06
Root number	19	40	2.15	0.66
Root length	19	40	1.21	0.11
d) Effect of Kinetin, BA and Adenine				
Height	11	24	1.45	0.03
Leaf number	11	24	4.48	0.56
Leaf length	11	24	0.79	0.04
e) Effect of sucrose levels				
Germination percentage	3	8	407.64	68.75
Days for greening	3	8	20.31	2.92
Days for protocorm development	3	8	39.33	2.08

Contd.

APPENDIX – XV continued

Days for shoot and root formation	3	8	82.22	1.92
Height	3	8	0.08	0.06
Leaf number	3	8	0.53	0.67
Leaf length	3	8	0.10	0.01
Root number	3	8	2.15	0.30
Root length	3	8	0.57	0.07
f) Effect of charcoal levels				
Germination percentage	4	10	10.0	48.33
Days for greening	4	10	5.23	4.47
Days for protocorm development	4	10	64.67	9.13
Days for shoot and root formation	4	10	94.33	2.47
Height	4	10	0.16	0.03
Leaf number	4	10	1.43	0.67
Leaf length	4	10	0.12	0.12
Root number	4	10	1.67	0.17
Root length	4	10	0.45	0.13
g) Effect of peptone, coconut water and banana pulp				
Days for first leaf production	10	22	2.02	4.42
Days for shoot and root formation	10	22	4.72	6.67
Height	10	22	0.34	0.04
Leaf number	10	22	0.98	0.58
Leaf length	10	22	0.32	0.04
Root number	10	22	2.67	0.50
Root length	10	22	0.63	0.08

APPENDIX – XVI continued

Character	New Pink selfed				New Pink x Pink Tips			
	Degrees of freedom		Mean square		Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error	Treatment	Error	Treatment	Error
Percentage growth	6	14	892.86	297.62	6	14	1230.16	148.81
Days for differentiation of first leaf	6	14	584.08	5.62	6	14	1551.56	13.76
Days for shoot and root formation	6	14	2451.16	26.71	6	14	1345.49	9.00
Number of shoots/seedling	6	14	0.43	0.43	6	14	1.16	0.38
Number of leaves/shoot	5	14	0.98	1.52	5	14	2.10	0.91
Days for planting out	5	14	4925.19	26.86	5	14	6712.30	225.00

Character	New Pink x Candy Stripe				Pramott-II x Emma White			
	Degrees of freedom		Mean square		Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error	Treatment	Error	Treatment	Error
Percentage growth	6	14	515.87	238.10	6	14	188.49	238.10
Days for differentiation of first leaf	6	14	1267.94	7.52	6	14	416.43	10.05
Days for shoot and root formation	6	14	1730.52	10.62	6	14	1538.83	40.00
Number of shoots/seedling	6	14	2.33	0.19	6	14	0.83	0.38
Number of leaves/shoot	5	14	1.83	0.71	5	14	2.19	1.29
Days for planting out	5	14	8910.32	105.95	5	14	11454.76	158.33

Contd.

APPENDIX – XVI continued

Character	New Pink x Emma White				Candy Stripe selfed			
	Degrees of freedom		Mean square		Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error	Treatment	Error	Treatment	Error
Percentage growth	6	14	585.32	505.95	6	14	615.08	238.10
Days for differentiation of first leaf	6	14	759.76	15.48	6	14	795.21	13.52
Days for shoot and root formation	6	14	903.98	21.24	6	14	574.30	17.57
Number of shoots/seedling	6	14	0.52	0.48	6	14	1.10	0.48
Number of leaves/shoot	5	14	0.67	0.91	5	14	3.41	1.81
Days for planting out	5	14	8082.33	82.33	5	14	7893.65	66.67

Character	Candy Stripe x New Pink				Candy Stripe x Hieng Beauty			
	Degrees of freedom		Mean square		Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error	Treatment	Error	Treatment	Error
Percentage growth	6	14	722.22	277.38	6	14	376.98	416.67
Days for differentiation of first leaf	6	14	186.22	10.24	6	14	259.08	3.87
Days for shoot and root formation	6	14	258.38	17.43	6	14	1124.98	14.05
Number of shoots/seedling	6	14	1.08	0.38	6	14	0.60	0.38
Number of leaves/shoot	5	14	3.08	3.52	5	14	2.89	1.24
Days for planting out	5	14	713.76	62.33	5	14	3498.16	49.14

Contd.

APPENDIX – XVI continued

Character	Hieng Beauty x Emma White				Hieng Beauty x Sonia-28			
	Degrees of freedom		Mean square		Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error	Treatment	Error	Treatment	Error
Percentage growth	6	14	277.78	595.24	6	14	396.83	327.38
Days for differentiation of first leaf	6	14	108.38	10.86	6	14	322.56	8.33
Days for shoot and root formation	6	14	262.41	9.38	6	14	806.16	17.67
Number of shoots/seedling	6	14	1.11	0.57	6	14	0.60	0.38
Number of leaves/shoot	5	14	3.76	2.48	5	14	3.05	1.57
Days for planting out	5	14	6697.08	158.05	5	14	1231.71	34.19

Character	Emma White x Pink Tips				Pink Tips selfed			
	Degrees of freedom		Mean square		Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error	Treatment	Error	Treatment	Error
Percentage growth	6	14	793.65	416.67	6	14	343.65	111.91
Days for differentiation of first leaf	6	14	443.00	7.05	6	14	84.56	13.95
Days for shoot and root formation	6	14	543.08	10.33	6	14	2196.30	19.86
Number of shoots/seedling	6	14	1.19	0.62	6	14	1.44	0.52
Number of leaves/shoot	5	14	0.89	1.19	5	14	0.87	2.14
Days for planting out	5	14	1940.22	28.52	5	14	6209.52	151.19

Contd.

APPENDIX- XVI

Abstract of Analysis of variance for the effect of irradiation

Character	Emma White x Banyat Pink				Emma White x Sonia-28			
	Degrees of freedom		Mean square		Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error	Treatment	Error	Treatment	Error
Percentage growth	6	14	277.78	89.29	6	14	188.49	178.57
Days for differentiation of first leaf	6	14	113.64	9.57	6	14	174.44	4.81
Days for shoot and root formation	6	14	211.65	12.19	6	14	345.22	8.67
Number of shoots/seedling	6	14	0.44	0.52	6	14	0.60	0.38
Number of leaves/shoot	5	14	2.16	2.29	5	14	4.19	1.91
Days for planting out	5	14	1444.94	19.67	5	14	4259.21	172.38

Character	Emma White selfed				Hieng Beauty x New Pink			
	Degrees of freedom		Mean square		Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error	Treatment	Error	Treatment	Error
Percentage growth	6	14	416.67	535.71	6	14	515.87	267.86
Days for differentiation of first leaf	6	14	280.44	6.24	6	14	395.08	10.38
Days for shoot and root formation	6	14	377.10	4.19	6	14	1343.87	10.52
Number of shoots/seedling	6	14	1.30	0.43	6	14	0.30	0.38
Number of leaves/shoot	5	14	2.75	2.67	5	14	1.00	2.48
Days for planting out	5	14	3188.49	78.57	5	14	4740.32	415.00

Contd.

APPENDIX – XVI continued

Character	Sonia-28 x Emma White				Sonia-28 x Pink Tips			
	Degrees of freedom		Mean square		Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error	Treatment	Error	Treatment	Error
Percentage growth	6	14	257.94	297.62	6	14	337.30	535.71
Days for differentiation of first leaf	6	14	719.30	18.57	6	14	222.19	7.33
Days for shoot and root formation	6	14	677.67	23.48	6	14	617.76	9.48
Number of shoots/seedling	6	14	2.19	0.62	6	14	0.71	0.62
Number of leaves/shoot	5	14	1.30	1.57	5	14	2.16	1.57
Days for planting out	5	14	1645.43	38.71	5	14	1464.05	51.86

Character	Sonia-28 x Hieng Beauty			
	Degrees of freedom		Mean square	
	Treatment	Error	Treatment	Error
Percentage growth	6	14	99.21	178.57
Days for differentiation of first leaf	6	14	141.27	10.05
Days for shoot and root formation	6	14	497.54	22.43
Number of shoots/seedling	6	14	1.00	0.76
Number of leaves/shoot	5	14	1.27	4.67
Days for planting out	5	14	2194.08	66.38

APPENDIX – XVII

Composition of Greencare 30:10:10

Constituents	Quantity
Total nitrogen	30%
Available phosphoric acid	10%
Soluble potash	10%
Iron EDTA	0.33%
Boron	200 ppm
Copper EDTA	700 ppm
Manganese EDTA	500 ppm
Zinc EDTA	720 ppm
Molydenum	5 ppm
Magnesium EDTA	500 ppm
Vitamin B_1	100 ppm

IMPROVEMENT OF *Dendrobium* THROUGH HYBRIDISATION AND *In vitro* MUTAGENESIS

By
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ABSTRACT OF THE THESIS

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requirement for the degree of

Doctor of Philosophy in Horticulture

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ABSTRACT

The present investigations on improvement of *Dendrobium* through hybridsation and *in vitro* mutagenesis were conducted in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, during 1996-1999.

Ten varieties and six species of *Dendrobium* were included for the trial. All the varieties showed significant variations among themselves for the characters studied. New Pink had the highest number of shoots (9 Nos.), maximum leaf length (15.17 cm), largest flowers (8.27 cm x 7.9 cm) and longest vase life (18.33 days). Number of flowers was maximum for Candy Stripe (11.67 Nos.)

Anthesis in *Dendrobium* was observed between 7.30 am and 2.30 pm and the stigma receptivity period ranged from second to 10^{th} day of flower opening, in different varieties.

Pollen out put ranged from 2720 (*Dendrobium pierardii*) to 13120 (Emma White). Pollen grains were found as agglutinated masses called pollinia and each flower had two pollinia.

Sabine had the maximum pollen size (47.46 µ). Pollen fertility of the hybrids ranged from 46.77 per cent (*D. chrysanthum*) to 91.93 per cent (New Pink). Maximum pollen germination occurred in two per cent sucrose + 1 per cent agar (80.33%) as well as in 2 per cent sucrose + 2 per cent agar (78.00%). Emma White had the maximum pollen germination (80.63%).

Heritability was of moderate to high magnitude. Genotypic correlation coefficients were found to be higher than phenotypic correlation coefficients, indicating lesser environmental effects on the characters.

The characters having significant positive correlation with number of flowers per spike were mean height of the shoots, total number of leaves, length of inflorescence, days for opening of all florets and internodal length of first two florets.

All the varieties, except Hieng Beauty, were self compatible. Emma White had the maximum cross compatibility as the female parent and Sabine had the least. Emma White, New Pink and Pink Tips performed as good male parents. Out of the six species tried, *D. fimbriatum* gave the maximum cross compatibility.

When green pod culture was employed, 90-110 days old pods gave the best results in culture. Mercuric chloride treatment (0.1%) for one minute, followed by alcohol dip and flaming was found to be the best surface sterilization method for pods.

Germination percentage of hybrid seeds was minimum in New Pink x *Dendrobium crumenatum* and maximum in New Pink x Emma White. Pink Tips selfed seeds gave 100 per cent germination. Maximum for planting out was taken by Sonia-28 x *D. crumenatum* seedlings (385 days) and minimum by Emma White selfed seedlings (253 days).

Growth hormones like kinetin and IBA, each at 1 mg l^{-1} and 2 mg l^{-1}, gave better germination. Early germination, protocorm formation as well as shoot and root production were observed in the medium containing kinetin/BAP at 4 mg l^{-1} to 8 mg l^{-1} and IBA/NAA at 2 mg l^{-1} to 6 mg l^{-1}. These media also gave higher values for seedling characters like height, number of leaves, leaf length, number of roots and root length.

With regard to the different sucrose levels, that at 0.5 per cent gave best seedling growth. Charcoal at 0.5 per cent gave early germination and early shoot and root formation and at 1.0 per cent level gave best seedling growth.

Regarding the effect of media supplements, coconut water at 150 ml l^{-1} and peptone 1000 mg l^{-1} gave best seedling growth. Adenine at 4-6 mg l^{-1} also had a favourable effect on seedling characters.

Callus formation was favoured by NAA 4 mg l^{-1} + 2,4-D - 2 mg l^{-1} along with kinetin 2 mg l^{-1}. Formation of PLBs from *in vitro* leaf was obtained in a medium containing BAP 25 mg l^{-1} + NAA 2 mg l^{-1}.

Protocorms, PLBs and callus were subjected to *in vitro* mutagenesis using gamma rays up to 60 Gy. As the dose of irradiation increased the height was reduced. Higher doses of irradiation produced smaller and broader leaves with dark green colour. Survival rate was found to decrease in the irradiated materials. At 60 Gy the PLB and callus did not survive.

Planting out of different hybrid seedlings was successfully carried out in the field. Equal proportion of charcoal pieces and brick pieces was used in the medium. Survival of the hybrids ranged from 14 per cent in New Pink x *D. chrysanthum* to a maximum of 94 per cent in New Pink selfed. The seedlings were maintained by regular nutrient and pesticide sprayings.

Out of the different nutrient solutions tried 30:10:10 NPK mixture (0.1 - 0.2%) sprayed on alternate days gave best results in terms of number of shoots and number of leaves.

Regarding the effect of growth hormones, BA at 50 mg l^{-1} at fortnightly intervals gave maximum number of shoots and leaves, followed by BA 25 mg l^{-1}. Maximum height of the seedlings was obtained with GA 10 mg l^{-1} sprayed at fortnightly intervals.

Field evaluation of the hybrids revealed variations in the growth and floral characters.