DISCUSSION
The orchids are known to produce a large number of minute and non-endospermic seeds with more or less undifferentiated mass of embryonal cells enclosed within a transparent seed coat, and the present taxa are no exception. The rudimentary state of an orchid embryo has been attributed to the suppressed development of endosperm (Veyret, 1974) but a direct correlation between the extent of endosperm development and differentiation of embryos is far from confirmed. Incidentally, Sletilla hyacinthina, B. striata, Epidendrum vitellinum, Platyclinie glumacea, Polystachya microbambusa and Sobralia sacrantha embryos are the only known exceptions with a rudimentary cotyledon (cf. Arditti, 1967; Veyret, 1974).

**Germination**

The orchids require a suitable fungus for germination in nature, the fungus is believed to convert the complex carbohydrates into simpler forms required by the germinating seeds (cf. Arditti, 1967). They can, however, germinate in vitro without the intricacies of host-fungus relationship, if provided with an appropriate nutrition (Knudson, 1921, 1922, 1925). The asymbiotic technique of germination has added new dimensions in orchid culture and it has been effectively utilised to germinate elite species and hybrids including those which are not easy to obtain in quantities (Mitra, 1986).
Unfortunately, however, the mature seeds from dehisced capsules, germinate poorly due probably to the development of dormancy factors and/or accumulation of certain inhibitory substances (Burgeff, 1954; Withner, 1955). The immature seeds, on the other hand, are capable of normal development, when cultured, prior to their being fully ripe (cf. Arditti, 1967) and their importance in micropropagating a large variety of orchids including the hard to germinate ones, first assessed by Withner (1953), is being increasingly realized (Arditti et al., 1981, 1982a,b; Katiyar et al., 1987; Linden, 1980; Mathews and Rao, 1980; McIntyre et al., 1972; Raghuvanshi et al., 1985; Rao and Avadhani, 1963; Sagawa, 1963; Sagawa and Velmayer, 1966; Sauleta, 1965; Sharma and Vij, 1985, 1986; Teo and Teo, 1976; Velmayer and Sagawa, 1967; Vij and Pathak, 1988c,d; Vij et al., 1981, 1985, 1987b, 1988a,b,d; Yam and Weatherhead, 1988; Yang et al., 1989). According to Linden (1980), the immature seeds germinate better than the mature ones because of their metabolically awake embryos and the ability of their distended testa cells to readily facilitate moisture uptake from the medium. The technique of germinating immature seeds in fact involves embryo culture and is popularly known as 'green pod culture'. Its main advantages are that:

- the immature seeds, free from dormancy and/or inhibitory substances, germinate readily;
- the embryos are saved from the harmful effects of sterilizing agent since only the capsules ('pods') need to be surface sterilized, and
- the time lapse between pollination and seed sowing is significantly reduced.

However, as all the seeds in a 'pod' have to be used in a single sowing, there is a risk of failure, should the capsules be harvested at a wrong developmental stage (Teo, 1978).

Of the 16 species of commercially viable and/or threatened orchids, representing both terrestrial and epiphytic/or lithophytic habits, included under the scope of present studies, fourteen were successfully subjected to green pod culture technique. In some species, attempts were also made to test the germination potential of mature seeds. The germination response, in general, varied with the habit, developmental stage of the seeds (wap), and the nutrient pool. In what follows some of the salient features of the investigation are discussed in light of the available information in literature.

Terrestrial taxa:

In *Herminium lanceum*, the mature seeds invariably failed to germinate, whereas the immature ones germinated readily in KC medium and its various modifications with growth adjuncts, thereby indicating their wide nutritional amplitude. Incidentally, the germination response was significantly improved in media enriched with P/YE/CH/U, in this species. The immature seeds, on the other hand, either failed to
germinate as in *Dactylorhiza harteni* or their germination remained arrested at the spherule/or protocorm stage as in *Diplomorhiza hirauta* and *Goodyera biflora* unless the medium was selectively supplemented with growth adjuncts. The growth hormones invoked initiation of germination process, but seedling differentiation was, in general, obligatory to the use of organic growth supplements in these species, suggesting thereby their variable requirements for growth adjuncts during different stages of germination. A perusal of literature reveals that *Dactylorhiza, Habeneria,* and *Ophrys* seeds germinate to form hairy protocorms even in distilled water but their subsequent growth remains arrested unless these are transferred to a carbohydrate regime (Stoutamire, 1974). Since in the present cultures, sucrose was invariably used as a carbohydrate source and germination was obligatory to the presence of growth hormones and organic growth supplements (source of nitrogenous compounds) in the medium, it is reasonable to believe that exogenous supply of growth hormones and nitrogenous compounds play an important role in their germination and subsequent seedling development and as a corollary thereof, in other terrestrial orchids as well.

According to Yam and Arditti (1990), many terrestrial genera are difficult to germinate and these difficulties have often been attributed to the development of inhibitory factors in their embryos (Stoutamire, 1974) and/or the persistent nature of epidermis of the inner integument of
their seeds which impede hydration (Veyret, 1959). Presently, as Dactylorhiza hatagirea, Diplomeris hirsuta and Goodyera biflora seeds swelled readily through imbibition, when sown on culture medium, it appears that germination inhibitory factors, if any, are developed in their embryos.

According to Stoutamire (1974), the protocorms in the ground growing taxa of open grass land, and well drained or seasonally dry soils, are non-chlorophyllous. The chlorophyll development, however, varied with the species in the present cultures, it developed prior to (Diplomeris hirsuta) or 2 (Hermium lanceum), 6 (Goodyera biflora) and 20 (Dactylorhiza hatagirea) weeks after the development of protocorms. Since all these species inhabit well drained shady and/or open grass lands, it is worthwhile to consider that chlorophyll development in the protocorms, is a genetic attribute. The extent of its development, however, varied with the medium composition.

Dactylorhiza hatagirea protocorms/seedlings copiously released brownish exudates into the medium, these necrosed and ultimately perished. Similar observations, were earlier attributed to improper nutrition in Dactylorhiza, Gymnadenia, Ophrye, Platanthera and Satyrium cultures (Stoutamire, 1974). Incidentally, repeated subculture and ultimate transfer to the community pots, proved useful in saving Dactylorhiza hatagirea protocorms from necrosis.
Epiphytic taxa:

The immature seeds, in general, germinated readily and better than the mature ones. These, however, failed to germinate as in *Kingidium tenaxiae*, or germinated poorly as in *Cymbidium lowianum* and *C. maestralii*, unless the medium was suitably supplemented with growth adjuncts (see Tables XIII, XVI, XXVIII).

Interestingly, the epiphytic taxa germinated better than their terrestrial counterparts due probably to their simpler requirements and amenability to a wider nutritional amplitude. Such a behaviour of the epiphytes seems to be correlated with their genetic and ecological stability. The nutritional complexity of terrestrial taxa, on the other hand, may be attributed to their inherent adaptability to highly competitive habitat.

Perusal of literature reveals that though the placental tissue is capable of producing protocorm mediated plantlets in orchids (Mitra, 1985), very young and mature ovules don't form good explants in orchids, due to dormancy, pH, inhibitory and other metabolic factors (Withner, 1953, 1955). The fertilized ovules, from a few wk-old unripe capsules, are, however, capable of germinating *in vitro* (Ito, 1955, 1961; Mitra, 1986; Sagawa and Vaimayer, 1968; Teo and Teo, 1976; Tauchiya, 1954a, b, 1958; Vaimayer and Sagawa, 1967; Vij et al., 1981). Several authors have tried to assess the time lapse between pollination and germination of orchid ovules *in vitro*. 


with a view to find the earliest stage for their successful culture. According to Rao (1977) the shortest time period at which the orchid ovules can be germinated, in vitro, varies between 40-85 days after pollination depending upon the species. Dorchila pulcherrima, however, represents an exception, where the ovules from pollinated ovaries germinated immediately after getting fertilized in vitro (Yasugi, 1984).

Presently, the germination potential of immature seeds could not be assessed at different stages of development (wap), instead their germination response was randomly investigated, 0-20 wks prior to dispersal. Significantly, the seeds procured about 20 or more wks prior to dispersal, as in Cymbidium lowianum and C. mastersii, germinated poorly than those procured 8-10 wks before dispersal. The germination response was also impaired in seeds procured less than 8 wks prior to dispersal, suggesting thereby the operation of dormancy and other inhibitory factors during very early and later stages of seed development. According to Arditti et al. (1982b), the immature seeds respond best, when collected after 1/2 or 1/3 of the total time they take to mature, and almost similar observations were made later in several Indian species by Vij and Pathak (unpublished). According to the latter authors, the earliest stage at which the orchid seeds (embryos) attain viability and are capable of normal development varies with the genera and species and their ability to germinate is directly correlated with their procurement time (wap) and the total time (wap) required to
reach maturity.

The development of the orchid seedlings from germinating seeds is usually protocorm mediated and similar observations were made in most of the present cultures. In *Cymbidium lowianum*, however, the development of protocorms into seedlings was invariably associated with an intervening rhizomatous phase. Development of rhizomatous structures, prior to organogenesis, in this and the related species *Cymbidium viridescens* and *C. farreri* (Champagnat et al., 1968); *Cremastos appendiculata*, *Eupholidium maculatum*, *Geodorum pictum* (Stoutamire, 1974); *Eulophia yushulana* (Weatherhead et al., 1986); *Cymbidium macrorhizum* (Vij and Pathak, 1988d) and *Eulophia daban* and *E. hormusii* (Arora, 1990) may be a genetic attribute. Incidentally, protocorm multiplication, an inherent trait of orchids, was selectively expressed depending upon the chemical stimulus, in the present cultures and this potential (a delayed expression of polyembryony) appears to have an adaptive significance leading to species perpetuation in this group of plants.

It has already been indicated that orchid germination, in nature, is dependent upon a suitable association (symbiotic) with a fungal endophyte. Their transformation from a heterotrophic to autotrophic system in the early stages of development is quite an unusual aspect of differentiation, since a reverse situation prevails in most of the other symbiotic systems. The stage at which the germinating
entities tend to acquire chlorophyll (turns autotrophic?) was observed to vary with the species and the nutrient regime in the present cultures. The chlorophyll development was a pre-protocorm formation phenomenon in Bulbophyllum umbellatum, Coelogynae barbata, Cymbidium abernianum and Stanhopea maduxiana, whereas it followed protocorm development in Cymbidium louianum and C. mastersii. The stage of its development (pre-or post-protocorm) was, however, directly correlated with the quality and combination of the growth adjuncts in the medium. Though, it is difficult to suggest that the chlorophyll development is definitely associated with a functional change from heterotrophic to autotrophic mode of nutrition, the present studies are tempting enough to consider that its initial development during germination is directly governed by the genetic and/or nutritional factors.

**Culture medium**

A vast variety of culture media have been devised on more or less empirical basis for orchid germination (Arditti, 1967; Arditti et al., 1982b; Withner, 1959). Most of these media are species specific and differ from one another in the quality and quantity of major and minor salts (Arditti et al., 1982b). A universal medium to satisfy the requirements of orchids is, however, still to be formulated, since much is not known about their minimum mineral nutrition. Presently, the green pod (embryo) cultures were raised and maintained in
either of the 2 chemically defined media M and/or KC.

Excepting *Dactylorhiza hattori* and *Kinidium taenialis*, the embryos germinated successfully, in all the present species when cultured in the basal medium. The germination frequency and subsequent seedling development was, however, variously affected in the additional presence of organic growth supplements (source of reduced nitrogen) due probably to their variable requirements for nitrogenous compounds. In *Dactylorhiza hattori*, germination was obligatory to the presence of growth hormones in the medium and the seedlings differentiated only when the medium was supplemented with YE+KN, suggesting thereby their varied nutrient requirements during different stages of germination leading to seedling development. The growth adjuncts (P), similarly, proved obligatory for *Kinidium taenialis* germination, but organogenetic response, in the germinating entities, was dependent upon the additional presence of GA₃ and KN, in the combination.

The significance of nitrogen nutrition is well established in orchid germination and it is believed to be provided by mycorrhizal fungus in nature (Magrou, 1944). However, the importance of an appropriate nitrogen source (ammonium and/or nitrate, individually or in combination) during in vitro germination has been variously assessed.

Ammonium ions proved definitely superior for *Cymbidium*, and slightly better for *Cattleya*, seeds, whereas nitrate ions favoured better germination in *Vanda tricolor*.
(Curtis and Spoerl, 1943). According to Lugo (1955), ammonium nitrogen was promotory and nitrate nitrogen inhibitory during *Vanilla planifolia* germination. Similar results were subsequently obtained in *Cattleya labiata* (Raghavan and Torrey, 1963, 1984) and *Orchis laxiflora* (Mead and Bulard, 1979). In *Bletilla striata* ammonium nitrogen was useful for germination (Ichihashi and Yamashita, 1977) and its low concentration promotory for root growth, irrespective of the level of nitrate ions in the medium (Ichihashi, 1979). Burgeff (1936) reported better utility of ammonium ions during *Raphiopedilum* germination and generalized that nitrate ions are best utilized by the epiphytic (autotrophic), and ammonium ions are necessary for the growth and development of ground growing (heterotrophic and saprophytic), species, when grown in light. According to Raghavan (1976), several species are unable to utilize nitrate during early stages of germination, whereas the ability of the others to utilize it, is associated with the development of nitrate reductase due probably to co-evolution of the biochemical and morphological differentiation in orchids. Mitra (1987) suggested that the ammonium salts promote growth during early stages of germination and protocorm development whereas nitrate ions are required during organogenesis and seedling development, and maintained that the requirement for an appropriate nitrogen source differs with the genetic constitution of the species, its stage of development and the nutrient pool. However, Withner (1959) emphasized the utility of both nitrate and
ammonium ions in orchid cultures and the requirement of both these ions was later confirmed in Dendrobium phalaenopsis (Gandawidjaja, 1980) and Vanda hybrids (Mathews and Rao, 1980). Presently also most of the species germinated readily in M and KC media, which contain both the ammonium and nitrate ions, due probably to the utility of both these nitrogen sources during germination. According to Arditti (1967, 1977a), nitrogen in general, promotes seed germination and subsequent seedling growth at low concentrations and proves detrimental to these processes at heavier doses and its requirement depends upon, and/or is related to, other media components. Whatever be the exact nitrogen requirements of the present species, the author seems to agree with Mitra (1987) that variable patterns of differentiation and growth under similar set of nutritional environment may be a genetic attribute and calls for a detailed biochemical investigation on orchid nutrition. M medium which is essentially a modification of KC medium from which it differs in the quality of phosphates, minor ions and vitamins, favoured better germination and seedling growth in Bulbophyllum umbellatum, Coelogyne viscosa and Erica javanica. However, a similar germination response of Bulbophyllum sikkimensis, Coelogyne viscosa, C. rigida and Cymbidium eburneum in KC medium, and the ability of Coelogyne viscosa to germinate with almost equal frequencies in both M and KC media, suggests that orchid seeds and seedlings can perhaps adapt to a variety of combinations and concentrations of
The vitamins promote tissue and organ growth \textit{in vitro} (Bonner, 1937; Schopfer, 1943) and their absence in the medium leads to deficiency symptoms in the seedlings (Arditti, 1963; Arditti and Harrison, 1977; Anderson, 1967; Ernst \textit{et al.}, 1970; Kano, 1968; Noggle and Wynd, 1943; Prasad and Mitra, 1975; Rao and Avadhani, 1963). In this connection, it is worthwhile to mention that the present species germinated well in KC and M media, both of which contain a variety of vitamins. In nature, they or their precursors are believed to be provided by the fungal partner of the orchids (Arditti and Ernst, 1984). The exact vitamin requirement of orchid seeds and seedlings \textit{in vitro} is, however, difficult to assess since a variety of these are present in agar or sugar, both of which are important constituent of medium (cf. Arditti, 1967; Arditti and Ernst, 1984).

\textbf{Effects of growth hormones on germination, protocorm development and seedling differentiation}

The growth hormones proved obligatory for germination in \textit{Dactylorhiza hatagirea} and were selectively required during different morphogenetic changes leading to seedling development in this and two other species \textit{Cymbidium lowianum} and \textit{Diplomeria hirsuta}. In other species, their effects on the germination frequency, protocorm development and growth and differentiation of seedlings, however, varied with their
quality and combination, and the genetic constitution and physiological age of the seeds (embryos). Almost similar conclusions that the effects of the hormones vary with their concentrations and medium composition, culture conditions and the genetic constitution, physiological age and requirement of explants were arrived at by Arditti and Ernst (1984). In what follows the present results are discussed in relation to the literature reports.

**Auxins**

According to Hayes (1969), the orchid seeds are low in auxin contents and the transport of exogenous nutrition, in these, during germination in vivo, is, perhaps, stimulated by the auxins produced by their fungal partner in the mycorrhizal association. Arditti (1977b) showed the benign effect of an exogenous supply of auxins in several orchids including *Laelia* and similar results were obtained by many subsequent workers (cf. Arditti and Ernst, 1984). Presently, these hormones variously affected the cultures and some of their effects are discussed as follows:

**IAA**

With IAA in the medium, the germination frequency was either enhanced (*Bulbophyllum sikkimensis, Coelogyne viscosa, Cymbidium lowianum, Dendrobium versatifulum, Diplomeria hirata, Goodyera biflora, Herminium lanceum, Stanhopea maduixiana*) or impaired (*Cymbidium mastersii*) or it remained
unaffected \textit{(Bulbophyllum umbellatum, Coelogyne barbata, C. rigidum, Cymbidium aburneum, Eria javanica)}. It favoured protocorm multiplication in \textit{Coelogyne viscosa, Cymbidium aburneum} and \textit{Eria javanica}, early leaf organogenesis in \textit{Bulbophyllum sikkimense, Coelogyne barbata, Cymbidium aburneum, Dendrobium venatrifolium, Eria javanica, Stanhopea maduxiana}, and rhizogenesis and seedling development in \textit{Bulbophyllum sikkimense, Coelogyne barbata, C. rigidum, Cymbidium aburneum, Dendrobium venatrifolium, Eria javanica, Herbiniun lanceum} and \textit{Stanhopea maduxiana}. It, however, failed to induce organogenesis in \textit{Cymbidium lourianum, Dactylorhiza hatagirea, Diplomera hiruta} and \textit{Goodyera biflora}.

Almost similar effects of an exogenous supply of this auxin are known in literature. It was slightly inhibitory during germination in \textit{Dendrobium} but enhanced the germination frequency in \textit{Lealicattleya} (Kano, 1965) and \textit{Phalaenopsis} (Ernst, 1967). It supported better germination and accelerated protocorm development in \textit{Rhynchoastylis retusa, Saccobalamium calcicolare} and \textit{Vanda testacea} (Vij et al., 1981). IAA inhibited germination in \textit{Vanda Miss Joaquim} (Goh, 1971) and \textit{Dendrobium noble} (Miyazaki and Nagaematsu, 1965) but proved somewhat effective during seedling development in the former species (Rao and Avadhani, 1963). Its benign effect was shown during germination and seedling development in \textit{Pachystoma senile} (Vij et al., 1985). The protocorms showed an accelerated development in \textit{Cattleya} (Withner, 1951), \textit{Miltonia} and
Odontoglossum (Hayes, 1969), and they multiplied in Cymbidium cultures (Fonnesbech, 1972), in its presence. The survival rate and growth of Cattleya seedlings was significantly enhanced in IAA enriched medium (Meyer, 1945; Boesmann, 1962). However, in Rhynchostylis retusa the protocorms showed a progressive decrease in size with a corresponding increase in the level of IAA in the medium (Sood, 1984). IAA, however, proved ineffective in Coeloglossum viride, Dactylorhiza purpurella, Goodyera repens and Platanthera biflora seed cultures (Hadley, 1970).

**IBA**

Like IAA, the effects of IBA varied with the species. The germination was either promoted (Bulbophyllum sikkimense, Coelogynae viscosa, Cymbidium lowianum, Dendrobium veratrifolium, Diplomeris hirauta, Goodyera biflora, Harminium lanceum, Stanhopea maduxiana) or it remained unaffected (Bulbophyllum umbellatum, Coelogynae rigida, Cymbidium aburnum, Eria javanica), in its presence. It favoured protocorm multiplication (Coelogynae viscosa, Cymbidium aburnum, Eria javanica) and early development of leaf (Bulbophyllum sikkimense, Coelogynae barbata, Dendrobium veratrifolium, Eria javanica, Harminium lanceum, Stanhopea maduxiana) and root (Bulbophyllum sikkimense, B. umbellatum, Coelogynae barbata, C. rigida).
Fris javanica, Hemanium lanceum, Stanhopea maduxiana). It failed to induce organogenesis in Cymbidium lowianum, Diplomeris hiruta and Goodyera biflora.

IBA is similarly known to have promoted germination in Cattleya wernerii (Meyer and Pelloux, 1948), Cattleya, Cymbidium, Cypripedium, Oncidium, Phalaenopsis and Vanilla (Hegarty, 1955), and protocorm growth in Dendrobium (Pages, 1971). Germination was best achieved (Agapanthepilosum, Vij and Malhotra, 1988), and the protocorm multiplied rapidly (Eulophia debiai Sharma and Vij, 1988) in IBA supplemented combinations. IBA proved stimulatory to seedling growth at lower concentrations and inhibitory at higher ones in Vanilla planifolia (Withner, 1951). It was slightly effective in promoting seedling growth in Cattleya (Withner, 1951) but induced stunted shoot growth in Pachystoma senile seedlings (Vij et al., 1985). IBA proved ineffective during germination in Dendrobium and Brassolaeliocattleya (Kano, 1965) and some other orchid species (Burgeff, 1936).

2, 4-D

The germination frequency was variously affect in
combinations containing 2,4-D. It was slightly improved (Bulbophyllum sikkimensis, Cymbidium eburneum, G. lowianum, Eria javanica, Goodyera biflora) or impaired (Bulbophyllum umbellatum, Cymbidium mastersii, Diplomeris hirsuta, Herminium lanceum, Stanhopea maduxiana). The organogenesis was delayed (Coelogynura barbata, C. rigidia, Eria javanica) or remained suppressed (Bulbophyllum sikkimensis, B. umbellatum, Cymbidium eburneum, G. lowianum, G. mastersii, Dendrobium verrucifolium, Herminium lanceum, Stanhopea maduxiana) in its presence. This auxin, however, proved ineffective in Dactylorhiza hatagirea, Diplomeris hirsuta and Goodyera biflora cultures.

Germination inhibitory effect of 2,4-D was, earlier reported by Goh (1971) in Vanda Miss Joaquina and by Sharma and Tandon (1986) in Coelogynura punctulata. 2,4-D inhibited growth and development of Cymbidium protocorms (Fonnesbech, 1972), and even at low concentration (0.5 mg/l), it caused death of protocorms unless used with KN or BAP in Cattleya and Cymbidium cultures (Kusumoto, 1978). It induced non-organogenetic (Bulbophyllum leopardinum, Dendrobium genotypica, D. bicameralum, D. denuaduana, Vanda bicolor) or rhizogenic (Luisia zeylanica) seed callus (Arora, 1990). Bose and Mukerjee (1976) recorded its promotory effect on protocorm development in Cymbidium gigantum. 2,4-D favoured protocorm callusing in Cymbidium aloifolium (Bopaiah and Jorapur, 1986) but suppressed chlorophyll synthesis in Cymbidium cultures (Raghavan, 1976);
Cymbidium aburneum, Dendrobium moschatum, Rhynchostylis retusa, Thunia alba and Vanda parviflora (Sood, 1984); Dendrobium amoenum, D. chrysanthum, Luisia zeylanica and Schoenorchis gemmatum (Arora, 1990).

The above mentioned data suggest that 2,4-D, in general, is not suited for germination as it impairs the chlorophyll development and induces unorganised growth in protocorms (non-organogenetic callus) in many species. Similar views were expressed by Mitra (1986) about the utility of this auxin in orchid cultures. According to Shamia (1986), 2-4, D also promotes chromosomal instability in the cultures.

NAA

NAA variously improved (Bulbophyllum sikkimense, Coelogynae viscosa, Cymbidium lowianum, Dendrobium veratrifolium, Goodyera biflora, Herminium lanceum), or impaired (Bulbophyllum umbellatum, Coelogynae barbata, Cymbidium mastersii, Diplomeris hirsuta, Stanhopea madusiana) the germination frequency. It induced protocorm multiplication in Coelogynae viscosa, Cymbidium aburneum and Eria javanica. It promoted early differentiation of leaf and root primordia in Cymbidium aburneum and the seedlings showed an accelerated development in its presence. On the other hand, it delayed organogenesis in Coelogynae barbata, C. rigida, C. viscosa and Eria javanica and proved inhibitory to organogenesis in Bulbophyllum sikkimense, B. umbellatum, Cymbidium mastersii, Dendrobium
variatrilium, Herminium lanceum and Stanhopea maduxiana.

Literature studies revealed that the effects of NAA vary with the species in orchids. It enhanced germination in Cattleya (Meyer and Pelloux, 1948), Bletilla, Cattleya and Cymbidium (Straus and Reisinger, 1976) and Paphiopedilum hybrids (Flames, 1978), besides improving protocorm growth and differentiation in Vanda hybrids (Mathew and Rao, 1980). The seedlings growth in Bletilla, Cattleya and Cymbidium (Straus and Reisinger, 1976) and Epidendrum nocturnum (Yates and Curtis, 1949) was promoted in its presence. It was, however, only slightly effective in promoting growth in Cattleya seedlings (Withner, 1951). According to Arora (1990), NAA favoured protocorm proliferation (Dendrobium chrysanthem, Eulophia hormeii) and organogenesis (Sulphurium leopardinum, Dendrobium denneanum, D. heterocarpum, D. normale, D. primulinum, Eulophia debia).

Perusal of literature reveals that the effective concentration of NAA varies from species to species. In Coelogynae punctulata, it favoured better seedling development, when used at 0.1 mg/l (Sharma and Tandon, 1986), whereas in Cymbidium virescens, it supported better development of rhizome when used at concentrations varying from 0.1 - 10 mg/l (Saw, 1969). Optimal results were obtained by using NAA at 0.1 mg/l in Cattleya (Ichihashi and Kako, 1973) and at 1.25 mg/l in Vanda (Payawal and de Guzman, 1972). NAA proved useful, at 0.5 - 2.5 mg/l for Pachyypamos senile germination.
but its higher dose proved lethal (Sood, 1984). Root and shoot growth was promoted at low level, and inhibited at high level, of NAA, in Cymbidium (Fonnesbech, 1972) and Cattleya and Dendrobium (Vajrabhaya and Vajrabhaya, 1976) cultures.

Since the present experiments were designed to assess the comparative effects of different hormones at 1 mg l⁻¹, the most effective conc. of NAA in different species could not be analysed. However, further experiments are suggested in this direction.

Gibberellins:

$GA_3$

The effects of $GA_3$ also varied with the species. The germination was improved (*Bulbophyllum sikkimense, Coelogyne viscosa, Cymbidium luvianum, Dendrobium veratrifolium*), impaired (*Bulbophyllum umbellatum, Coelogyne barbata, Cymbidium mastersii, Stanhopea maduxiana*) or it remained unaffected (*Coelogyne rigida, Cymbidium aburnum, Diplomeria hireuta, Fria javanica, Goodyera biflora*) in its presence. The protocorms were more or less elongated and the leaves somewhat stiolated in $GA_3$ enriched nutrition in accord with the similar effects of this growth regulator in several orchid species (cf. Arditti and Ernst, 1984). Incidentally, $GA_3$ also induced accelerated morphogenetic changes leading to seedling development in several species including *Cymbidium aburnum, Dendrobium veratrifolium* and *Fria javanica*.

The germination and protocorm formation in
Cattleya, Cypripedium, Cymbidium, and Odontoglossum were accelerated in the medium containing GA$_3$, but chlorophyll development and subsequent differentiation eluded the protocorms (Humphreys, 1958). GA$_3$ promoted rapid growth in Cattleya seedlings (Blowers, 1958) but it proved inhibitory for the purpose in Phalaenopsis (Hyatt, 1965). In Orchis purpurella, it favoured better survival rate of protocorms and promoted shoot elongation (Hadley and Harvais, 1968). Similarly leaf and root growth was promoted in Cymbidium (Bose and Mukherjee, 1976; Fannesbech, 1972) and ground growing European orchids (Harbeck, 1963) in its presence. According to Kano (1965), GA$_3$ proved inhibitory for germination in Brassolaeliocattleya, and root development in Dendrobium cultures. This growth hormone, however, failed to induce positive effect during germination of the ground growing Cypripedium calceolus, Dactylorhiza maculata, Epipactis helleborine and Listera ovata (Van Waez and Debergh, 1986).

According to Arditti and Ernst (1984), exogenous supply of gibberellins usually proves detrimental as the orchid seedlings are capable of synthesizing their gibberellin requirements and have a limited ability to deactivate the hormone. The germination impairing effect of this hormone, in the above listed species, may thus be correlated with its supraoptimal level (endogenous and exogenous) in the cultures. Since GA$_3$ was invariably used at 1 mg l$^{-1}$ in the present
cultures, further studies are suggested to analyse the optimum requirement of GA$_3$ in different species by varying its concentration in the medium. Incidentally, the effects of GA$_3$ varied with its concentration in the medium in Coelogyne punctulata cultures (Sharma and Tandon, 1986), root and shoot showed slightly enhanced development in medium containing 0.5 - 1.0 mg l$^{-1}$ of GA$_3$, whereas rhizogenesis eluded the cultures, when gibberellin was used at 1-10 mg l$^{-1}$.

Cytokinins:

KN

The cytokinins are most important growth regulators required during germination in terrestrial orchids (Harvais, 1982). Since several of mycorrhizal fungi, other than those associated with orchids, produce cytokinins, Arditti and Ernst (1984) assumed that orchid mycorrhizae also produce cytokinins. According to them, orchid seeds are more sensitive to higher cytokinin levels than the protocorms, suggesting, thereby, that they have a low or no requirement for cytokinins. Incidentally, KN was used at uniform concentration of 1 mg l$^{-1}$ in the present cultures.

Like other growth hormones, its effects on germination and seedling development varied with the species. The germination frequency was improved (Bulbophyllum sikkimensis, Coelogyne viscosa, Cymbidium lowianum, Dendrobium verrucifolium, Herminium lanceum, Stanhopea maduxiana), impaired (Bulbophyllum
umbellatum, Cymbidium mastersii) or it remained unaffected (Coelogyne barbata, C. rigid a, Cymbidium eburneum, Diplomeris hirsuta, Eria javanica, Goodyera biflora) when KN was used in the medium. The cytokinin induced protocorm multiplication in Cymbidium eburneum and favoured early leaf (Coelogyne barbata, Cymbidium eburneum, Dendrobium veratricifolium, Stanhopea maduxiana) or root (Coelogyne barbata, Dendrobium veratricifolium, Herminium lanceum, Stanhopea maduxiana) differentiation. Dendrobium veratricifolium, Herminium lanceum and Stanhopea maduxiana seedlings also showed accelerated development when cultured in KN enriched medium.

Variable effects of KN are also reported in literature. It promoted germination in Cypripedium calceolus (Borries, 1969) and Coelogyne punctulata (Sharma and Tandon, 1986), but proved inhibitory during the process in Vanda Miss Joaquim (Rao and Avadhani, 1963), and Dendrobium hybrid (Kano, 1965). The germination in Coelogyne viride, Dactylorhiza purpurella and Platanthera bifolia was impaired, but the protocorms showed better growth, in KN enriched medium (Hadley, 1970). KN sustained normal protocorm development in Cymbidium (Kusumoto, 1978) and promoted their multiplication in Vanda (Payaval and de Guzman, 1972) and Cymbidium cultivars (Fonnasbech, 1972). It had a pronounced effect on growth and development of seedlings in Cattleya (Pierik and Steegman, 1972), Coelogyne punctulata (Sharma and Tandon, 1986), Cypripedium reginae (Harvais, 1982) and Orchis purpurella.
However, Kt proved ineffective during Goodyera repens germination (Hadley, 1970) and detrimentally affected rhizogenesis in Leucocattleya (Kano, 1965).

KN and Auxins/or Gibberellins

Ever since Skoog and Miller (1957) proposed that the organ differentiation in plants is regulated by an interplay of auxins and cytokinins, the use of these hormones, in combination, is being increasingly realized for developing a medium for a new plant type. In this connection, it is worthwhile to mention that the exogenous requirement for the hormones depends upon the endogenous level in the plant system which varies with the tissue, plant type, and the phase of plant growth. In order to assess their combined effects, KN was used with auxin (IAA, IBA, 2,4-D, NAA)/or Gibberellin (GA$_3$) in the medium.

KN and IAA

A synergistic action of KN and IAA favoured better germination (Coelogynae barbata, $C$. rigida, Cymbidium lowianum, Dendrobium verrucosum, Herminium lancastre) and early organogenesis (Bulbophyllum umbellatum, Era javanica). The organogenetic processes were somewhat retarded in Coelogyne barbata, Cymbidium eburneum and Stanhopea maduxiana, when KN and IAA were used together, in the medium, and the combination proved ineffective for inducing organogenesis in Dactylorhiza hatagirea, Diplomeris hiraeuta and Goodyera biflora. Incidentally,
IAA alleviated the delayed organogenetic effect of KN in *Bulbophyllum sikkimense* but was rendered ineffective by the cytokinin in *Cymbidium mastersii* cultures.

Similarly, a combination of these hormones has yielded discordant results in earlier experiments. The combination proved inhibitory for germination in *Dendrobium* and rhizogenesis in *Laeliocattleya* (Kano, 1965), and significantly impaired the germination of *Dactylorhiza* (Hadley, 1970) and *Aerides multiflorum*, *Eria spicata* and *Vanda cristata* (Pathak, 1989b). The combination, however, supported better differentiation in *Dendrobium crepidatum*, *Eulophia hormusii*, *Saccolabium calceolare* and *S. papillosum* cultures (Sood, 1984).

KN and IBA

Almost similar results were obtained when IBA was used with KN in the medium. A synergistic action of the growth hormones induced better germination (*Coelogynae barbata*, *Dendrobium veratrifolium*, *Herminium lanceum*) and advanced organogenesis leading to seedling development (*Bulbophyllum umbellatum*, *Eria javanica*). However, development of both leaf and root, as in *Coelogynae barbata* and *Cymbidium eburneum*, or/root alone, as in *Dendrobium veratrifolium* and *Stanhopea maduxiana*, was significantly delayed when cultured in IBA and KN enriched medium. IBA successfully alleviated the harmful effect of KN during organogenesis in *Bulbophyllum sikkimense*. Likewise, the morphogenetic changes in the seeds
leading to seedling development were beneficially or
detrimentally affected in several Indian species including
Aerides multiflorum, Cymbidium aloifolium, C. macrochizion,
C. pendulum, Dendrobium chrysanthum, Eria spicata, Luisia
teretifolia, Luisia trichorhiza, Rhynchosbyta retusa and
Vanda cristata (Pathak, 1989b), and Bulbophyllum leopoldinum,
Dendrobium chrysanthum, D. decneanum, D. heterocarpum,
D. primulinum, Phaius tankervilliae, Arundina graminifolia,
Luisia reyalina, Shoenorchis gnammatum (Arora, 1993), when
IBA was used with KN in the medium. A synergistic action of
the hormones promoted protocorm multiplication in Aerides
multiflorum, and Luisia trichorhiza (Pathak, 1989b).

KN and 2,4-D

A combination containing KN and 2,4-D, favoured
enhanced germination (Cymbidium louianum, Herminium lanceum),
protocorm multiplication (Cymbidium abunnon and Herminium
lanceum), and early organogenesis (Bulbophyllum alkimense,
Herminium lanceum). The combination, however, impaired
germination in Coelogyne barbata and Stanhopea maduxiana and
proved inhibitory during organogenesis in Coelogyne rigida,
C. viscosa and Eria javanica, unless used with AC.

The toxic effect of 2,4-D, during protocorm growth,
was alleviated in the presence of KN (Kusumoto, 1978). The
combination, however, favoured the development of non-
organogenetic seed callus in Coelogyne nitida, Dendrobium
According to Pathak (1989b), the combination, though it proved inhibitory to germination in several species and shoot development in *Cymbidium aloifolium* cultures, yet it favoured protocorm multiplication in *Fria spicata*.

**KN and NAA**

KN, when used in combination with NAA, counteracted the organogenesis suppressing effect of the auxin (*Bulbophyllum sikkimense* and *Herminium lanceum*) or proved ineffective (*Bulbophyllum umbellatum, Dendrobium veratrifolium* and *Stanhopea maduxiana*). Interestingly, these hormones antagonised the inhibitory effect of each other during organogenesis in *Cymbidium mastersii*. The combination, however, was inhibitory to organogenesis in *Coelogyn urigida, Cymbidium ohburnum*, and *Fria javanica*. Earlier, these growth regulators were successfully used for better seedling growth in *Cyripedium reginae* (Harvais, 1982), *Coelogyn punctulata* (Sharma and Tandon, 1986) and *Cymbidium pendulum, Dendrobium chrysanthum* and *Luisia trichorhiza* (Pathak, 1989b). Arora (1990) also indicated their benign effect during initial germination in *Arundina graminifolia, Bulbophyllum leopardinum, Dendrobium heterocarpay* and *Thunia alba*, and leaf (*Bulbophyllum leopardinum, Dendrobium normale, Thunia alba*) and root (*Bulbophyllum leopardinum, Dendrobium heterocarpay, Luisia revlanica*) development. These data suggest a species specific utility of these growth regulators.
together in the medium, significantly enhanced the germination frequency and advanced organogenetic processes and subsequent seedling development in *Herminium lanceum*. These growth regulators were also effectively used for inducing organogenesis in germinating entities in *Kingidium tasmale*. KN, however, proved antagonistic to GA₃ in *Eria javanica*.

**Effects of Organic growth supplements on germination, protocorm formation and seedling development**

As already indicated, the nutrient requirements of orchids vary during germination. While some of these germinate in simple basal media, the nutritional complexities of other can be satisfied by using P, YE, CH, U, coconut water, banana extract etc. in the medium (Arditti and Ernst, 1984).

Presently, the effect of four of the organic growth supplements (P, YE, CH, U) was tested on germination and subsequent seedling development, in the investigated species. P, YE, and CH, were each used at 1 gm l⁻¹, whereas U was used at 25 mg l⁻¹ in the medium. The salient features of the investigations are discussed as follows:
proved obligatory for germination in *Kinogidium taenialis*, and for inducing organogenesis in *Goodyera biflora*. It improved germination frequency in *Bulbophyllum sikkimense*, *Coelogyne barbata*, *C. viscosa*, *Cybidium lowianum*, *C. mastersii*, *Dendrobium veratrifolium*, *Herminium lanceum* and *Stanhopea maduxiana*, induced protocorm multiplication in *Bulbophyllum sikkimense*, *Coelogyne barbata*, *C. rigida*, and *Cybidium aburneum*, and advanced organogenesis in *Bulbophyllum sikkimense*, *B. umbellatum*, *Coelogyne barbata*, *C. rigida*, *Cybidium aburneum*, *C. mastersii*, *Dendrobium veratrifolium*, *Eria javanica* and *Stanhopea maduxiana*. The above mentioned effects were, in general, more pronounced in its combination with KN. This organic supplement, however, failed to induce organogenesis in *Dactylorhiza hatagirea* protocorms. It also failed to invoke organogenetic response in *Kinogidium taenialis* protocorms unless used with KN and GA₃; however, it could not alleviate the inhibitory effect of IBA/or NAA during germination in this species. *Goodyera biflora* protocorms, on the other hand, multiplied very rapidly on P-supplemented medium enriched with KN and auxins (IAA, IBA, IAA)/or GA₃.

Ever since Lami (1927) reported a rapid growth and development of *Phalaenopsis* and *Vanda* seedlings in P enriched medium, this organic growth supplement has been successfully used to germinate several orchid species. It was obligatory for germination in *Spiranthes cornua* (Stoutamire, 1954) and
favoured better germination in *Vanilla* (Bouriquet, 1947),
*Cypripedium* (Liddell, 1953), European terrestrial species
(Harbeck, 1963), *Brassavola nodosa*, *Brassolaeliocattleya*,
*Cymbidium* hybrid, *Cymbidium viridescens*, *Dendrobium* hybrid,
*Paphiopedilum callosum* and *P. insigne* (Kano, 1965), *Calanthe*
*malyca* (Krishna Mohan and Jorapur, 1984), and *Acampe praemorsa*
(Krishna Mohan and Jorapur, 1986). The seedlings of *Paphiopedilum*,
*Phaius* and *Vanda* (Curtis, 1947), and *Cattleya* (Mariat, 1948)
grew well in its presence. The benign effects of this organic
growth supplement were also reported in *Aerides multiflorum*,
*Rhynchostylis retusa*, *Saccocalbus calceolae* and *Vanda testacea*
(Vij et al., 1981), and *Paphiopedilum* (Fast, 1971; Flames, 1978)
cultures. It also promoted development and differentiation of
protocorms in *Eulophia dabia* (Sharma and Vij, 1986) and *Vanda*
hybrids (Mathews and Rao, 1980). P was useful during rhizome
growth in *Cymbidium lowianum* (Sood, 1984) but it impaired
germination in *Dactylorhiza maculata* (Van Wass and Debergh,
1986) and *Thunia alba* (Arora, 1990). On the other hand, this
organic growth supplement was ineffective during early stages
of germination in *Cattleya*, *Dendrobium* and *Vanda* (Morel, 1974)
and when used in combination with banana, apple/or potato
juice, it favoured protocorm multiplication but proved inhibi-
tory for seedling growth in *Cymbidium* (Kusumoto and Furukawa,
1977).

P is a well known source of reduced nitrogen and it
contains a number of growth promoting substances. Its
discordant effects as noted above may thus be directly correlated with nitrogen requirement of the explants, their endogenous level of growth promoters and the medium composition.

YE:

YE is an extract of autolysed yeast in water and it contains vitamins and other substances which are usually required by tissues. It is an important source of reduced nitrogen and has been effectively used in germination and proliferation in many orchid species (Mitra, 1986). In the present cultures, its effects varied with the species. It favoured better germination in most of the species including Bulbophyllum sikkimense, Coelogyne barbata, C. viscosa, Cymbidium lowianum, Dendrobium veratrifolium, Herminium lanceum and Stanhopea maduxiana, whereas germination was invariably impaired in Cymbidium mastersii when cultured in YE supplemented medium. Additional use of KN impaired germination response in Coelogyne barbata, Dendrobiium veratrifolium and Stanhopea maduxiana. YE favoured protocorm multiplication in Bulbophyllum sikkimense, Coelogyne barbata and Cymbidium eburneum. The organogenetic processes leading to seedling development were accelerated (Bulbophyllum sikkimense, B. umbellatum, Coelogyne barbata, C. rigid, C. viscosa, Dendrobium veratrifolium, Eria javanica, Herminium lanceum, and Stanhopea maduxiana) or suppressed (Cymbidium eburneum and C. mastersii) in its presence.
Almost similar discordant effects of YE are known in orchid literature. It favoured enhanced germination in *Goodenora repens* (Downie, 1943), *Cyripedium* (Liddell, 1953), *Cymbidium masterii* (Prasad and Mitra, 1975) and *Orchis laxiflora* (Mead and Bulard, 1979), but proved inhibitory during germination in *Brassolaeliocattleya*, *Dendrobium* hybrid, *Laeliocattleya* (Kano, 1965) and *Aerides multiflorum* (Vij et al., 1981). *Cymbidium* protocorms showed an accelerated development but organogenesis in these was significantly retarded when cultured in YE enriched medium (Kusumoto, 1978). Almost similar results were obtained in *Rhynchostylis retusa* and *Vanda tessaceae* (Vij et al., 1981). YE is also known to have invoked protocorm multiplication in several orchid species (Arora, 1990; Curtis, 1947; Mariat, 1948; Pathak, 1989b; Prasad and Mitra, 1975; Flamee, 1978; Mathews and Rao, 1980) but it proved somewhat inhibitory in *Vanda Miss Joaquim* (Rao and Avadhani, 1983). YE, though proved as a best additive for *Serapias parviflora* and *S. orientalis* cultures (Voth, 1976), was intolerant in *Cyripedium reginae* and other Canadian native species (Harvais, 1973, 1974). According to Arditti (1977b), the quality of YE varies with the mode and batch of its preparation and the writer is inclined to agree with him (Arditti) that the variable effects of this organic growth supplement, as listed above may be directly correlated with its quality. Incidentally, YE contains a variety of vitamins, amino acids, heavy metals and many other substances and it
may be difficult to determine its functional components in orchid culture. Almost similar views were expressed earlier about this organic growth supplement by Arditti (1967).

CH

CH was beneficially employed to improve germination frequency and induce early initiation of organogenetic processes in most of the present cultures. It, however, proved inhibitory for organogenesis in Bulbophyllum sikkimensis, Cymbidium eburneum and C. mastersii.

In Coleogyne rigidula, KN accelerated the early organogenetic effect of CH. The cytokinin however, impaired germination (Dendrobium veratrifolium and Herminium lanceum) and differentiation and development of seedlings (Coleogyne barbata, Dendrobium veratrifolium, Eria javanica, Stanhopea maduxiana) when used with the organic growth supplement. Incidentally, KN proved obligatory (Bulbophyllum sikkimensis)/or inhibitory (Bulbophyllum umbellatum) for organogenesis in CH supplemented medium.

CH is a complex mixture of casein, amino acids and other relatively simple substances. Its composition varying with the extent of hydrolysis of the nitrogen substances in the casein, seems to account for its variable effects during germination in orchids. It favoured better germination in Dactylorhiza purpurea (Harvais, 1972) and Aerides multiflorum, Rhynchochilus retusa, Saccolabium calceolare and Vanda.
teat aces, (Vij et al., 1981), however, its continuous supply, according to the latter authors (Vij et al.) retarded the development of Vandaceous seedlings. *Cymbidium mastersii* germinated well to produce healthy protocorms in CH enriched medium (Prasad and Mitra, 1975) and *Galeola septentrionalis* protocorms showed better growth in the presence of this organic growth supplement (Nakamura et al., 1975). While the effect of CH was not pronounced in *Vanda Miss Joaquim* (Rao and Avadhani, 1963), and *Dactylochiza maculata* (Van Waes and Debergh, 1986), it significantly advanced rhizogenesis in *Orchis laxiflora* (Mead and Bulard, 1979) and favoured growth and differentiation in *Eulophia daba* protocorms (Sharma and Vij, 1986). In *Spathoglottis plicata*, CH was useful during germination, but it detrimentally affected the seedling growth (Chennaveeraiah and Patil, 1975). According to Arora (1990), it favoured protocorm multiplication (*Eulophia daba, E. horminum*) and accelerated seedling growth (*Dendrobium amoenum, D. bicamaratus, D. denudans, D. heterocarum, Eulophia daba, Luisea zeylanica, Shoenorchis gemmata*). These data indicate that the effects of CH vary with the species, and the development stages of the germinating entities.

It impaired germination (*Rulhophyllum umbellatum, Cymbidium mastersii*), Chlorophyll development (*Rulhophyllum umbellatum, R. sikkimensis*) and delayed subsequent organogenesis.
(Coelogyne rigid, Cymbidium mastersii). However, in Bulbophyllum sikkimense, Coelogyne barbata, Cymbidium lowianum, Dactylorhiza hatagirea, Dendrobium veratrifolium, Hemionitis lanceum and Stanhopea maduixian, it enhanced germination. Protocorm multiplication was also observed in Coelogyne barbata and Cymbidium aburneum cultures, in its presence.

Additional use of KN, in U enriched combinations, impaired germination (Coelogyne barbata, Dendrobium veratrifolium) and organogenesis (Cymbidium aburneum, Dendrobium veratrifolium, Stanhopea maduixian). However, it proved obligatory for inducing organogenesis in Bulbophyllum sikkimense and accelerated the process in Eria javanica. Incidentally, the chlorophyll development was, in general, impaired in U supplemented medium. Almost similar results were earlier obtained in several species by Sood (1984), who voiced against the use of U during initial stages of seed germination.

Perusal of literature reveals that U proved promotory (Cattleya, Laeliocattleya, Vanda) or inhibitory (Dendrobium, Phalaenopsis) for the growth of orchid embryos (cf. Mitra, 1986). According to Lugo (1955), Vanilla planifolia seeds germinate only in weak solution of inorganic nitrogen but can tolerate higher concentrations of organic nitrogen when supplied as Urea (U). U proved inhibitory for initial stages of germination in Dendrobium heterocarpum and Eulophia debia and accounted for delayed organogenesis in Dendrobium bicameratum and Shoelorchia
gemmatum, when used at 50 mg/l (Arora, 1990). According to Vij et al. (1981) the effect of U varies with the species in epiphytic taxa, it favoured germination and protocorm development in Rhynchostylis retusa and Saccorhiza calycolor but proved inhibitory for growth in Aerides multiflorum and Vanda parviflora. In Rhynchostylis retusa, lower concentration of U (25 mg/l) favour protocorm callusing whereas its higher concentrations (50-100 mg/l) promote organogenesis. Presently, this organic growth supplements was uniformly used at 25 mg/l, and further experiments are suggested to assess the effect of this organic growth supplement by varying its concentration in the medium.

Regeneration

Tissue culture (micropropagation) techniques are highly advantageous than the conventional methods of clonal propagation, as a large number of plants can be produced from a single individual, in a relatively less time and space. Infact micropropagation is the only commercially viable method of cloning orchids and it has revolutionized the orchid industry. Moral (1960) cultured excised Cymbidium shoot-tips and observed that instead of developing into a plantlet, they developed into a mass of protocorm like bodies (PLBs), each capable of further proliferations upon segmentation and subculture. Marston and Veraural (1967) estimated that if subcultured repeatedly and successfully, a single protocorm would generate
about four million Cymbidium plants in a year. However, as the shoot meristem culture involves the sacrifice of the mother plant in monopodial orchids, the emphasis has shifted towards exploring the possible utility of alternate but equally effective explants (cf. Arditti, 1977a, b), but the efforts are not commensurate with the large size of orchid family. Presently, the utility of explants obtained from protocorm, pseudobulb, rhizome, leaf, and root was assessed in some of the species and some of the pertinent results are discussed as follows:

Protocorm:

Morel (1963) for the first time, demonstrated the utility of protocorm slices in rapid cloning of Cymbidium, and the technique, has been effectively used to micropropagate several species and hybrids including Aerides multiflorum, Rhynchostylis retusa, Saccocalinium calceolare and Vanda parviflora (Vij et al., 1981); Cattleya, Odontoglossum and Paphiopedilum (Morel, 1974); Cymbidium ensifolium and C. goeringii (Wang, 1988, Wang et al., 1988); Dactylorhiza maculata (Gruenschneider, 1973) and Doritaenopsis (Amaki and Haruzo, 1989).

In the present studies, the response of Cymbidium aburnum protocorm segments varied with the quality and combination of growth adjuncts in the nutrition pool (MS medium). The explants callused when subjected to either of 2,4-D and NAA or KN regimes, but they directly generated
shoot buds under the collective stimulus of KN and NAA. NAA was earlier shown to be obligatory for proliferations in *Cymbidium ensifolium* protocorm slices, but the response varied with the nature of the medium and level of NAA used (Wang, 1988). A higher concentration of the auxin (5 mg l\(^{-1}\)) favoured PLBs mediated development in agar-gelled medium, whereas its low level (0.2 mg l\(^{-1}\)), in liquid medium, promoted direct shoot development. According to Asaki and Haruzo (1989) the regeneration pathway is significantly affected by the explant source, the basal explants generate shoot buds and the apical ones PLBs. In the present cultures, however, the regeneration pathway in the explants, irrespective of their source, was dependent upon the nature and combination of the growth adjuncts in the nutrient pool. The explants callused when cultured under either of 2,4-D and NAA/or KN regimes, these generated PLBs in the additional presence of AC/organic growth supplements (CH/u) and shoot buds when cultured in medium enriched with NAA+KN.

**Pseudobulbs**

*Coelogyna rigidia* and *C. viscosa* pseudobulb segments, procured from axenic seedlings, proliferated selectively depending upon the quality and combination of growth adjuncts in the medium. According to Shimasaki and Uemoto (1987a), shoot formation was directly induced in the pseudobulb explants by both auxin and cytokinin treatment in the epiphytic *Cymbidium*, *C. dayanum* whereas the explants developed rhizomes,
when treated with higher concentrations of auxins (2,4-D, NAA) in the terrestrial ones (C. karan and C. goerincli).

In Coelogyne rigida, the basal explants responded by generating non-organogenetic callus in medium containing (BAP+IAA)/or direct shoot buds in the one containing (IAA+KN)/(YE+AC).

In C. viscosa, on the other hand, AC, was obligatory for the development and rooting of shoot buds. NAA favoured PLBs and/or callus development, whereas 2,4-D invariably promoted the development of non-organogenetic callus. The extent and mode of regeneration, likewise, varied with the quality of organic growth supplements in the medium in this species. P induced multiple PLBs in 25% basal explants. However, additional use of AC in the combination proved useful in enhancing the per cent response of the explants and modifying the regeneration pathway from PLBs to shoot buds. YE induced direct development of shoot buds and when used with AC, it favoured PLB generation. Shoot buds were similarly obtained when YE was replaced by either of U and CH in the medium.

The pseudobulb segments were used to propagate Arundina, Cattleya, Cymbidium, Miltonia, Phalaenopsis (Morel, 1964b, 1970), Cymbidium (Shimazaki and Uemoto, 1987a), Cattleya (Vajrabhaya, 1978), Dendrobium (Vij and Sood, 1982; Vij and Pathak, 1989b), Eria and Pholidota (Pathak, 1989b) and Thunia (Arora, 1990) species. The growth regulators, NAA and KN, individually/or in combination proved effective in activating

Though cytological studies could not be made in the present regenerants, cytological uniformity in the pseudobulb segment raised plants in *Dendrobium chrysanthum, Eria spicata* and *Pholidota articulata* (Pathak, 1989b) suggests that pseudobulbs can be effectively used for cloning desired genotypes in orchids.

**Rhizome:**

Literature studies reveal that rhizome segments have been successfully used for propagating *Cattleya* (Reinert and Mohr, 1967), *Cymbidium goeringii* and *C. pumilum* (Ueda and Torikata, 1972), *C. kanran* (Kim et al., 1988); *C. kanran* and *C. goeringii* (Shimasaki and Uemoto, 1987b); *C. macrorhizum* (Pathak, 1989b), and *Eulophia horaeifolia* (Arora, 1990).

Presently, these developed axillary buds in *Cymbidium louianum*. The per cent response of the explants and the number of buds generated therein, however, varied with the quality and/or combination of the growth adjuncts. A synergistic action of KN with IAA/NAA accounted for the production of multiple buds and a similar tendency was also observed when the medium was supplemented with P and AC. The buds, so
developed failed to differentiate in combination containing IAA+KN whereas in the ones containing NAA+KN, and P+AC, these differentiated into rhizomatous bodies with distinct nodes and internodes. Development of aerial shoots, however, remained elusive.

Proliferation inducing effect of the growth adjuncts has also been indicated in rhizome segment cultures in Cattleya (Reinert and Mohr, 1967), Spatheglottis plicata (Bapat and Narayanaswami, 1977), Cymbidium kanran (Kim et al., 1988), Eulophia hormusjii (Vij et al., 1989) and Cymbidium macrorhizon (Pathak, 1989b). Incidentally, the rhizome induced callus failed to differentiate PLBs and/or shoot buds in Spatheglottis plicata (Bapat and Narayanaswami, 1977). Ueda and Torikata (1972), however, used L-arginine for inducing differentiation in Cymbidium goeringii and C. pumilum cultures. According to Shimasaki and Uemoto (1987b), higher auxin/cytokinin ratio enhanced rapid growth of the cultured rhizomes whereas, a lower ratio resulted in the formation of shoots. According to Kim et al. (1988), rhizome propagation and subsequent differentiation of shoots and roots therefrom varied with the quantity and quality of the growth hormones in the medium. However, in the present investigation the growth regulators were used at uniform concentration of 1 mg l⁻¹ and the proliferating rhizomes invariably failed to develop roots and aerial shoots.
Leaf:

The potential of leaf tissue to produce protocorm like bodies (PLBs) in juvenile Cymbidium leaves in vitro (Wimber, 1963, 1965) opened up new possibilities in orchid regeneration since a large number of identical clones can be raised from a single leaf through direct or callus mediated organogenesis (Arditti, 1977a). About sixty species and hybrids from different phylectic groups and representing diverse habits and habitats have so far responded to leaf segment cultures (cf. Vij and Pathak, 1990).

Presently, the explants from plants grown outdoor, copiously released brownish exudates, lost their chlorophyll and perished within 8-10 wks without showing any regeneration response. Whereas, when procured from axenic seedlings, they proliferated along their cut ends in Coelogyne rigid and Cymbidium mastersii. Such a differential response of the mature and juvenile leaves to regenerate under identical nutritional conditions seems to be correlated with their source and physiological age as suggested by Vij et al. (1984, 1986).

Incidentally, the proliferations were obligatory to the use of growth adjuncts into the medium and per cent response of the explants, and their ability to generate PLBs/or shoot buds varied with the species and the nutritional regime. The regeneration was induced via PLBs (Coelogyne rigid) or via both PLBs and shoot bud formation.
generate PLBs directly in Cymbidium (William, 1962, 1965),
Phalaenopsis (Tanaka and Sakanishi, 1977, 1980), and
Rhynchochlaena retusa (Vij et al., 1984) or through callusing in
Aranda and Dendrobium (Manorama et al., 1984), and Vanda
(Mathews and Rao, 1985) cultures. Occasional development of
shoot buds was also observed in Neofinetia falcata and
Papilionanthe teres (Vij and Pathak, 1990). The foliar
explants, in orchids, develop PLBs in solid medium but these
variously yield callus masses in liquid cultures (cf. Vij et al.,
1985) but the present observation of the development of PLBs/
shoot buds, in agar-gelled medium, hint at the role of growth
adjuncts in guiding the regeneration pathway. In this
connection, it is worthwhile to mention that YE proved
obligatory for inducing PLB generation in Costavviva rigida.
The role of growth adjuncts in regeneration, in leaf explants,
is also well documented in literature (Abdul Karim and Hairani,
1990; Arditti, 1977a,b; Manorama et al., 1984; Mathews and
Rao, 1985; Tanaka and Sakanishi, 1977, 1980; Tanaka et al.,

Roots:
The ability of the roots to regenerate shoot primordia
in Neottia nidus-avis (Irmisch, 1853) and plantlets in
Phalaenopsis stuartiana (Scully, 1971; Veitch, 1891)

'in vivo' suggested their possible utility for propagating orchids. Since then several species including Aerides criocarum, Cephalanthera rubra, Epidendrum microphyllum, Habenaria repens, Isotria verticillata, Listera cordata, Neottia nidus-avis, Phalaenopsis schilleriana, P. deliciosa, Pogonia ophioglossoides, Taeniophyllum proliferum and T. reiwaanii have been reported to generate buds/or shoots/or callus on their roots in nature (cf. Churchill et al., 1972).

Seechey (1970) advocated that chlorophyllous aerial roots can be used for micropropagation purposes in orchids. Ever since Champagnat (1971) propagated Neottia nidus-avis from root tips in vitro, several authors have attempted to test the regeneration potential of the orchid roots. The root explants grew in length but failed to regenerate in Vanda (Goh, 1970) and Epidendrum (Churchill et al., 1972). The root explants from axenic seedlings of Epidendrum St'Brianianus regenerated through callus mediated organogenesis (Stewart and Sutton, 1978). More recently, the regeneration potential of root explants has been successfully attempted in several species and hybrids of diverse phylogenetic affinities and these include Aerides multiflorum, Cymbidium pendulum, Papilionanthe teresa, Vanda cristata, V. testacea (Pathak, 1989b); Catastetum hybrid (Cataetum trullis x Cataetum berthrand; Kerbauy, 1984a); Cataetum pileatum (Kraus and Monteiro, 1989); Cyrtopodium (Sanchez, 1988); Oncidium varicosum (Kerbauy, 1984b);
Presently the regeneration potential of root explants was tested in 13 species but a positive response was obtained only in *Cymbidium mastersii*. The explants from proximal root ends generated PLBs and/or shoot buds along their cut surfaces whereas, the ones from the apical ends showed extended growth in the presence of certain selective growth adjunct(s) in MS medium (See Table XX). Perusal of literature reveals that except for *Cattleya pileatum* (Kraus and Kerbany, 1987; Kraus and Monteiro, 1989), *Neottia nivicola* (Champagnat, 1971) and *Vanilla planifolia* (Philip and Nainar, 1988; Philip and Padikkala, 1989), where root apical meristem is directly involved in regeneration process, in all the other species tested so far, including the present one, the apical meristem whenever responds, shows an extended root growth. Earlier Philip and Nainar (1988) stressed the significance of endo- and exo-genous level of auxin in controlling the transformation of root into shoot. The supra-optimal level of auxin, according to them (Philip and Nainar) favours the development of root meristem and its lower level leads to the development of shoot meristem. According to Pilot and Elliott (1981), the
endogenous level of auxin in the roots increases from suboptimal to supra-optimal level with age. A comparative analysis of *Vanilla planifolia* root-tip extracts have shown that the tips from older aerial roots have higher auxin content than their juvenile counterparts (Philip and Padikkala, 1989). The inability of explants from mature roots, in the present species, may thus be correlated with the increased endogenous level of auxin. Experimental confirmation is, however, suggested in this direction. It is, however, worthwhile to mention that the importance of the juvenility, genetic constitution and physiological age of the explant besides the medium composition were considered as important factors for regeneration of *Rhynchosystylis retusa* roots *in vitro* (Sood and Vij, 1986; Vij et al., 1987a).

A perusal of the Table XXX, which summarizes the list of number of orchids so far regenerated through root segments *in vitro*, indicates that the regeneration response of the roots is widespread in orchids and may infact be an inherent trait of the family which can be 'turned on' by providing a suitable nutritional regime. Incidentally, the physiological age of the explants which is an important parameter determining their regeneration potential, diminishes with the root maturity due probably to an increased endogenous level of auxins (?).
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Orchids</th>
<th>Regeneration part</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Neottia</td>
<td>Root tip</td>
<td>Champagnat, 1971</td>
</tr>
<tr>
<td>2.</td>
<td>Phalaenopsis amabilis</td>
<td>Root tip</td>
<td>Tanaka et al., 1976</td>
</tr>
<tr>
<td>3.</td>
<td>Epidendrum O'brienianum</td>
<td>Root tip</td>
<td>Stewart and Button, 1978</td>
</tr>
<tr>
<td>4.</td>
<td>Catasetum hybrid</td>
<td>Root tip</td>
<td>Kerbauy, 1984a</td>
</tr>
<tr>
<td></td>
<td>(Catasetum trullae Lindl. x Catasetum Berthrand)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Oncidium varicosum</td>
<td>Root tip</td>
<td>Kerbauy, 1984b</td>
</tr>
<tr>
<td>6.</td>
<td>Rhynchostylis retusa</td>
<td>Root tip/cut end</td>
<td>Sood and Vij, 1985; Vij et al., 1987a</td>
</tr>
<tr>
<td>7.</td>
<td>Rhynchostylis retusa and Vanda hybrid</td>
<td>Cut end</td>
<td>Chaturvedi and Sharma, 1986</td>
</tr>
<tr>
<td>9.</td>
<td>Cyrtopodium</td>
<td>Root tip/cut end</td>
<td>Sanchez, 1988</td>
</tr>
<tr>
<td>10.</td>
<td>Phalaenopsis</td>
<td>Root tip/cut end</td>
<td>Yoneda and Momose, 1988</td>
</tr>
<tr>
<td>11.</td>
<td>Aerides multiflorum</td>
<td>Root tip/cut end</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Cymbidium pendulum</td>
<td>Root tip/cut end</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Papilionanthe teres</td>
<td>Cut end</td>
<td>Pathak, 1989b</td>
</tr>
<tr>
<td>14.</td>
<td>Vanda cristata</td>
<td>Cut end</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>V. testacea</td>
<td>Root tip/cut end</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>Vanilla planifolia</td>
<td>Root tip</td>
<td>Philip and Padikkala, 1989</td>
</tr>
<tr>
<td>18.</td>
<td>Vanda bicolor</td>
<td>Cut end</td>
<td>Arora, 1990</td>
</tr>
<tr>
<td>19.</td>
<td>Cymbidium mastersii</td>
<td>Cut end</td>
<td>Present study</td>
</tr>
</tbody>
</table>
Brownish exudates, released by the explants (particularly from terrestrial taxa), hampered the growth in present cultures. Such exudates are frequently encountered in orchid cultures and have been variously identified as phenolics and/or alkaloids (cf. Ernst, 1974). The phenolics, released from *Cattleya marista*, have been isolated and identified as eucomic acid, tyramine, hydrosuconic acid and dopamine (Ishi et al., 1976, 1979).

Activated charcoal, an active adsorbant, was effectively used to check the release of phenolic exudates in the medium and save the present cultures from their harmful effects. Similarly, the adverse effects of brownish exudates on germination and subsequent growth were successfully alleviated by using AC in *Cymbidium macrophizon* (Vij and Pathak, 1988d), and several other orchid cultures (cf. Arditti and Ernst, 1984; Ichihashi and Kako, 1977; Vij et al., 1988d; Weatherhead and Harberd, 1980; Weatherhead et al., 1986).

Significantly, besides checking the release of phenolic exudates, this organic adsorbant (AC) favoured better germination, protocorm formation and organogenesis in *Coelogyne viscosa* and *Dendrobium verratifolium*. It also alleviated the callus inducing effect of 2,4-D in *Bulbophyllum umbellatum*, *Coelogyne viscosa*, and *Dendrobium verratifolium*, and NAA in *Bulbophyllum umbellatum*, *Dendrobium verratifolium* and *Herminium lanceum*. Its presence was obligatory for
organogenesis in combinations containing 2,4-D (Cymbidium eburneum, Stanhopea maduxiana), and NAA (Cymbidium mastersii, Stanhopea maduxiana). Bulbophyllum umbellatum, Coelogynopsis rigidula, C. viscosa and Cymbidium louianum protocorms multiplied rapidly when cultured in AC enriched medium. Even the regeneration potential of protocorm (Cymbidium eburneum), pseudobulb (Coelogynopsis rigidula, C. viscosa), rhizome (Cymbidium louianum) and leaf and root (Cymbidium mastersii) explants was obligatory to the incorporation of AC in the medium. AC remarkably enhanced the root and shoot development in Paphiopedilum (Ernst, 1974); Eulophia yushaniana (Weatherhead et al., 1986); Habenaria edgeworthii, H. intermedia and H. latilabris (Vij et al., 1988); Phalaenopsis and Paphiopedilum (cf. Arditti and Ernst, 1984) and Pleione (Weatherhead and Harberd, 1980) seedlings.

The benign effect of AC has been attributed to its ability to aerate the medium (Arditti, 1979, Ernst, 1975; Nair et al., 1989; Yam et al., 1989), absorb the light (Klein and Bopp, 1971; Proskauer and Berman, 1973) and enhance availability of energy quantum to per unit of plant material (Werckmeister, 1970a,b), and adsorb ethylene (Ernst, 1974) and other growth inhibitors present in agar and released by the tissues themselves (Weatherhead et al., 1986) and through the dehydration of sucrose during autoclaving (5-hydroxymethyl furfuryl). Since in the present experiments, the harmful effect of certain growth adjuncts was successfully alleviated
by using AC, it appears that these and other potential growth inhibitors (present in the agar/or, released by the explants themselves) are probably adsorbed by AC. Similar adsorptive potential of AC has been indicated earlier (Arditti et al., 1982b; Krikorian, 1988; Nair et al., 1989; Yam et al., 1989).

According to Yam et al. (1989), AC does not adsorb all growth inhibitory substances in the medium, and if it does, the substance(s) in question is(are) probably not required and/or is (are) required only at the level(s) which remain in the media following the adsorption.