RESULTS AND DISCUSSION

The orchid seeds are dust or flour like and vary in size, shape and colour in different species. The colour may be white, cream, blue green, brown, red or orange. Diversity of seed shape is considerable and five basic forms are recognised (Clifford and Smith, 1969). The seeds are unique in several respects, they are exceedingly small (80-130 um wide and 470-560 um long) produced in large numbers. Each seed contains an embryo composed of 8 to 100 cells with endosperm undeveloped or completely lacking (Savina, 1974). Orchid embryo consists of undifferentiated mostly isodiametric cells with dense granulated cytoplasm and conspicuous nuclei.

For the 3 different species of Cymbidium namely C. aloifolium, C. giganteum, and C. eburneum, five different media namely Knudson 'C' (1946), Vacin & Went (1949); Burgeff's (1936); Murashige and Skoog (1962) and Fonnesbech's (1972 a,b), were tried (Tables 34-38), in order to select best out of them. In case of Eulophia nuda var. purpurea only 4 different media namely Knudson 'C' (1946); Vacin & Went (1949), Murashige & Skoog (1962), and Burgeff's (1936) media were tried. All these media were variously supplemented with growth regulators and vitamins along with complex additives besides the control. On the basis of observations,
modified Knudson 'C' medium (1946) was finally chosen for
*C. aloifolium, C. giganteum* and *E. nuda var. purpurea* seeds
whereas Fonnesbech's (1972a, b) medium was found suitable for
*C. eburneum* seeds. Prasad and Mitra (1975) have succeeded in
culturing *C. mastersii* seeds in Knudson 'C' medium, but failed
to culture the seeds of *C. eburneum* in the same medium. This
infers that, the medium for individual species of *Cymbidium*
is specific in nature. The effects of individual media are
represented graphically.

The protocorms obtained were again transferred to
fresh medium containing the growth regulators (Table Nos. 39-40)
to study the development of seedlings. The presence of
inorganic salts in the basal medium was noticed to play a
vital role in seed germination and seedling growth. Prasad
and Mitra (1975) have demonstrated this in their study on the
nutrient requirements for germination of seeds and development
of protocorms and seedlings of *Cymbidium*, in aseptic cultures.
This could be studied by slightly altering the composition
and proportion of the original medium. Though the technique
is time consuming it was found necessary to standardize the
composition of the additives from individual species to get
maximum results.

After the inoculation of the seeds to various media
like Knudson 'C', Vacin & Went, Murashige & Skoog, Burgeff's
and Fonnesbech's media, the flasks were kept under observation once in two days to note the progress of seed germination, the time taken for the greening of the seeds and protocorm development (Table Nos. 43-46). The results are recorded. The initiation of germination was found enhanced in Knudson 'C' medium for C. aloifolium C. giganteum and E. nuda var purpurea seeds whereas it is in Fonnesbech's medium in case of C. eburneum seeds.

The complex organic mixtures were found to influence the seed germination and growth of orchid seedlings. These include complex mixtures like coconut water, banana pulp, peptone, casein hydrolysate yeast extract etc. In the present study, however, only coconut water, peptone, casein hydrolysate and banana pulp were used. The concentration of these supplements and their individual effect on the germination of seeds and development of seedlings were recorded (Table Nos. 47 - 54). According to Past (1973), addition of peptone (2g/l) and the milk of unripe coconut (20-50 ml/l) to the culture medium stimulates seed germination. Withner (1959) reports that, the addition of coconut water to orchid seed germination medium, enhances the seedling growth. Valmayor (1974) investigated that coconut water and banana pulp as additives to the media enhances seed germination and seedling growth. Different orchid groups possessed different optimal
nutrient requirements for seed germination and seedling development. According to Chua (1978), when the amount of coconut water increased from 0 to 450 ml/l there was an increase in CO$_2$ concentration in the culture vessels and a decrease in seedling growth. Levels above 150 ml/l appear to be excessive. Krishna Mohan and Jorapur (1984) showed that, the addition of peptone alone to the Vacin and Went medium increased the percentage of seed germination in case of Calanthe masuca. The presence of banana pulp provides better nourishment for the growing seedlings. Banana as the complex additive, was found to stimulate seedling growth which hinders early germination. Therefore, in our opinion it should only be added to the medium that would be used to transplant seedlings. However, in the present study, it was observed that peptone (500-2000 mg/l); coconut water (100 - 150 ml/l); Casein hydrolysate (500 - 2000 mg/l) and Banana pulp (50 - 200 g/l) individually stimulates the percentage of seed germination and seedling growth in all the cases.

The presence of particular vitamins and specific growth regulators may probably play a vital role in germination of seeds (Joseph Arditti, 1982). However, in the present investigation the role played by individual vitamins and growth regulators were carefully studied and recorded (Table 45 - 54). The role of auxin (NAA), vitamins
(pyridoxine HCl, Nicotinic acid, Thiamine HCl, Folic acid, Riboflavin and Biotin), Cytokinin (Kinetin) and amino acid (Glycine), is quite interesting to note. In case of Cymbidium aloifolium it is found that the combination of Glycine (0.5 ppm), NAA (0.2 ppm), Pyridoxine HCl (1.0 ppm), Nicotinic acid (1.0 ppm) Thiamine HCl (2.0 ppm), Folic acid (0.2 ppm), Riboflavin (0.2 ppm) Biotin (0.2, 0.5 ppm) and Kinetin (0.2 ppm), helped in the initiation of seed germination and development of protocorms (Fig. 54). The presence of glycine (0.2 ppm), NAA (2.0 ppm), Pyridoxine HCl (2.0 ppm), Nicotinic acid (2.0 ppm), Thiamine HCl (2.0 ppm), Folic acid (2.0 ppm), Riboflavin (2.0 ppm), Biotin (2.0 ppm) and Kinetin (2.0 ppm) in the basal medium promoted the growth of roots, height of the plantlets and in the increase of number of leaves during the development of seedlings (Figs. 56-73). In case of C. eburneum the combination of Glycine (0.2 ppm), NAA (1.9 ppm), Pyridoxine HCl (1.0 ppm), Nicotinic acid (0.5 ppm) Thiamine HCl (2.0 ppm), Folic acid (0.5 ppm), Riboflavin (0.2 ppm) Biotin (0.2 ppm) and Kinetin (0.5 ppm), helped in the initial seed germination and development of protocorm (Fig. 92 ). The presence of Glycine (2.0 ppm), NAA (2.0 ppm), Pyridoxine HCl (1.0, 2.0 ppm) Nicotinic acid (2.0 ppm), Thiamine HCl (2.0 ppm), Folic acid (2.0 ppm) Riboflavin (2.0 ppm), Biotin (1.0, 2.0 ppm), Kinetin (2.0 ppm) in the basal medium have helped in the growth of shoots roots and in the increase of leaf numbers (Figs. 93-102). In case of C. giganteum, the
combination of Glycine (0.2 ppm), NAA (1.0 ppm), Pyridoxine HCl (1.0 ppm), Nicotinic acid (0.5 ppm), Thiamine HCl (2.0 ppm), Folic acid (0.5 ppm), Riboflavin (0.2 ppm) Biotin (0.2 ppm) and Kinetic (0.2 ppm), in the basal medium helped in the initial seed germination and protocorm development (Fig. 81'). The presence of Glycine (2.0 ppm), NAA (2.0 ppm), Pyridoxine HCl (2.0 ppm), Nicotinic acid (2.0 ppm), Thiamine HCl (2.0 ppm), Folic acid (2.0 ppm), Riboflavin (2.0 ppm), Biotin (2.0 ppm), and Kinetin (2.0 ppm) in the basal medium helped in the growth of the plantlet, development of the roots and increase in the number of leaves, (Figs. 82-85) so also in case of E. nuda var. purpurea the combination of Glycine (0.2 ppm), NAA (0.2 ppm), Pyridoxine HCl (1.0, 2.0 ppm), Nicotinic acid (0.5 ppm), Thiamine HCl (1.0 ppm), Folic acid (0.5 ppm), Riboflavin (0.2 ppm), Biotin (0.2 ppm) and Kinetin (1.0 ppm) have initiated the seed germination and protocorm development (Fig. 106). The presence of Glycine (2.0 ppm), NAA (2.0 ppm), Pyridoxine HCl (2.0 ppm), Nicotinic acid (2.0 ppm), Thiamine HCl (2.0 ppm), Folic acid (2.0 ppm), Riboflavin (2.0 ppm), Biotin (2.0 ppm) and Kinetin (2.0 ppm) in the basal medium, have helped in the development of the tuber, growth of the shoot and root (Figs. 107-115). All these results infer that, the requirement of the growth regulators in the initial stages of seed germination and during the development of seedlings,
depends upon the individual plant species. The vitamin requirement is specific to the individual species of orchids during its growth.

Most of the terrestrial orchids pose difficulties regarding seed germination (Hasegawa et al., 1976; Hibino et al., 1978; Ichuhashi and Yamashita, 1977; Kano, 1965, 1968; Mi, 1978; Suzuki and Abe, 1977). This is because the germination levels are low and seedling development is found to be very poor, when compared with that of the epiphytes.

In case of E. nuda var. purpurea, the protocorms developed started bulging in majority of the cases forming tubers (Fig.103-106). However, the subsequent development showed variation regarding the growth and development. The tubers obtained were transferred to fresh media (Table 37) supplemented with various growth regulators. The observation revealed that, addition of these growth regulators and other supplements like complex additives really helped in the seedling development (Figs.107-115). The concentration of all these growth regulators and complex additives, also plays a vital role in the seed germination and seedling growth. This is also true in case of all the 3 species of Cymbidium studied (Table Nos. 47-54). It is thus evident that the nutrient requirements of the three stages of the plant namely, the seed, the protocorm and the seedling, are
not the same. With the onset of germination of seeds they gradually become more and more autotrophic. When the seedlings become autotrophic they need especially major and minor inorganic salts for their metabolism and growth. Their need for exogenous supply of organic compounds decreases (Prasad and Mitra, 1975). On the basis of all these observations, it is found that the combination of the supplements which helped in the enhancement of seed germination and seedling development (particularly concentrations as recorded in the Tables), would result in the best development of seedlings. These developed seedlings could be finally transferred to the new environment, i.e., sterilized fern pieces and moss in the earthen pots, where they are now growing successfully (Figs. 72, 73, 87, 102, & 117).