Germination of embryo/seeds of 10 rare and endangered orchid sps found in N. E. India were tried in established culture media viz. Kc (Knudson's-C, 1936), N\textsubscript{3}f (Burgeff, 1936), VW (Vacin and Went, 1949), and Nit (Nitsch and Nitsch, 1969). The earliest possible stage of developing embryos for inducing protocorm in 4 basic media were also determined. Immature embryos below 4 months did not respond for the induction of protocorms in all the sps tested, except \textit{S. plicata} and \textit{P. nanas} Hook. Best response with respect to protocorm induction was observed when 4-month old capsules of \textit{A. odoratum} Lour, 5-month old capsules of \textit{D. farmeri} Paxt., \textit{D. premulinum} Ldl., \textit{R. retusa} and \textit{V. coerulea} Griff. were cultured in VW medium; whereas, 5-month old embryos of \textit{C. aloifolium} (Lind.) SW and \textit{C. iridioides} D. Don and 3 months old capsules of \textit{P. nanas} Hook, and \textit{S. plicata} showed best response when cultured in Nit medium. Similarly, N\textsubscript{3}f medium was found to be suitable for culturing 5 months old \textit{P. fairianum} (Lindl.) Stein embryos, rather than culturing relatively mature seeds. The suitable age of the embryos alongwith appropriate media components were the key factors for maximum protocorm induction frequencies in these orchid sps.

Effect of growth hormones (IAA, NAA, Kin, BAP) and vitamin combinations (nicotinic acid, thiamine HCl, pyridoxin HCl, folic acid, biotin)
and complex additives (CW, BE, PE, YE, P) were also studied by supplementing in standard medium for improving protocorm induction and subsequent seedling growth. VW medium supplemented with 20% CW, 5% BE and 5% PE and the same supplements in Nut medium were found to be the best for maximum protocorm induction, and seedling growth in the case of A. odoratum and P. nanas, respectively. Similarly, in the case of R. retusa Bl., and V. coerulea Griff. a synergistic combination of CW (20%), BE (5%) and PE (5%) alongwith vitamins (5 mg/l nicotinic acid, 0.5 mg/l thiamine HCl, 0.5 mg/l pyridoxin HCl, 0.5 mg/l folic acid, 0.05 mg/l biotin) in VW medium and the same supplements in Nut medium in the case of P. fairieanum (Lindl.) were found to be most suitable for maximum induction of protocorms and for subsequent seedling growth. Healthy and vigorous growth of seedlings could be maintained in the same medium. For C. aloifolium SW. Nut medium containing 0.5 mg/l IAA + 0.1 mg/l Kin; for C. iridioides D. Don. the same medium containing 1 mg/l NAA + 0.5 mg/l BAP, and for S. plicata Bl., the same medium alongwith 0.5 mg/l NAA + 0.1 mg/l BAP were found to be the most suitable so far as the protocorm induction and seedling growth were concerned. In other complex additive and growth hormones supplemented cultures, protocorm induction and seedling growth were considerably delayed. Thus, the study revealed that orchid embryos require certain additives alongwith defined media for supporting maximum protocorm induction and optimum seedling growth.
The effect of different carbon sources (glucose, fructose, sucrose, maltose, starch) on protocorm induction and seedling growth was also studied in 5 orchid sps (*A. odoratum*, *C. aloifolium*, *D. premulinum*, *P. nanas*, *R. retusa*, *V. coerulea*). The revealed that sucrose at 20 or 30 g/l concentration in respective media (*VW* and *Nit* media) were required for maximum protocorm induction and optimum growth of seedlings. However, for *C. aloifolium* SW., inclusion of 30 g/l fructose in *Nit* medium was proved to be most suitable for as protocorm induction and seedling growth were concern. Fructose and glucose were found to be intermediate in supporting protocorm induction and supporting seedling growth in other orchid sps studied. Maltose in the studied concentration was found to be less suitable than glucose in the other orchid sps studied. On the other hand, starch in the studied concentrations did not support protocorm growth in any of the 5 orchid sps studied.

Axillary buds of the nodal segment of *V. pilifera* Holtt. developed into shoots directly after 8 weeks in supplemented MS medium containing 2.0 mg/l IAA + 0.1 mg/l BAP. The developing shoots could be cut into smaller fragments and subcultured in the same medium, for induction of new shoots from the nodes. When, these shoots were transferred to another medium containing 0.5 mg/l NAA, induction of roots were observed from the node. Micro-propagation of *V. pilifera* Holtt. is, therefore, possible using this method.

Axillary buds (procured from shoots and pseudobulbs) of 5 orchid sps were cultured in 4 standard media viz., MS (Murashige and Skoog, 1965),
SH (Schenk and Hildbrandt, 1972), H (Heller's, 1971) and Nit. Axillary buds in these studies showed the induction of PLB's via callusing in all the sps. Shoot buds of *A. odoratum* Lour. cultured in H medium supplemented with 4.0 mg/l 2, 4-D + 2.0 mg/l BAP + 15% CW + 2 g/l peptone was proved to be the most suitable for induction of PLB's to plantlets. Whereas, in the case of *C. aloifolium* SW., optimal combination of 4.0 mg/l IAA + 2.5 mg/l BAP + 10% CW; for *C. iridioides* D. Don. optimal concentration of 3.5 mg/l IAA + 2.5 mg/l BAP + 20% CW and for *R. retusa* Bl. an optimal concentration of 3.0 mg/l IAA + 2.5 mg/l Kin + 20% CW supplied to MS medium were found to be the best with respect to early induction of PLB's to plantlets. Similarly, buds of *S. plicata* Bl. cultured in Nit medium supplemented with 3.0 mg/l IAA + 2.0 mg/l Kin was found to be the most suitable for induction of PLB's via callusing. Subculturing the PLB's in the same medium differentiated to leaves and roots after minimum (12-20 weeks) duration. However, the duration required for the induction of PLB's to plantlets differed with the concentration of auxin-cytokinin used in the present investigations. The plantlets could be produced in mass-scale through these methods.

Juvenile leaf explants of a few orchid sps were cultured in 4 defined media (MS, SH, H, Nit) to study their callus inducing potentialities. Leaf sections of 5 orchids sps responded favourably in selective modifications in MS and H media for the induction of callus. Leaf explants of 4 orchids sps (*A. odoratum* Lour., *C. iridioides* D. Don, *D. premulinum* Ldl., *R. retusa* Bl.)
required 3.0 mg/l 2, 4-D and 2.0 mg/l BAP in H medium for induction of calli in minimum 10-15 days duration. Whereas, same auxin-cytokinin combinations in MS medium was found to be optimum for the induction of calli from V. coerulea Griff. leaves.

Regeneration of calli derived from leaf explants of 5 orchid spp have also been studied. The optimum supplements for induction of PLB's were determined. Calli of A. odoratum Lour., regenerated to plantlet via PLB's in H medium containing 1.5 mg/l NAA + 2.0 mg/l Kin. Calli of C. aloifolium SW. grown in H medium alongwith 1.5 mg/l 2, 4-D and 1.0 mg/l BAP was found to be the most suitable for initiation of PLB's to plantlet. Similarly, for C. iridioides, calli grown in H medium supplemented with 0.5 mg/l NAA + 1.0 mg/l Kin, for D. premulium 0.5 mg/l NAA + 2.0 mg/l Kin, and for R. retusa 1.5 mg/l BAP + 200 ml/1CW were found to be the best so far as initiation plantlets from PLB's. Moreover, MS medium containing 0.5 mg/l NAA + 2.0 mg/l Kin was found to be the most suitable supplements for faster induction of leaves and roots from PLB's in the above mentioned orchid spp.

Experiments were carried out to observe the effect of different light source on the induction and subsequent growth of calli from leaf explants in A. odoratum Lour., C. aloifolium SW. R. retusa Bl. and V. coerulea Griff. The callus induction percentage in red light incubated leaf explant was significantly higher compared to white light in A. odoratum Lour. and R. retusa Bl., whereas the values for induction time and average fresh weight was not found to statistically significant. In the case of D. premulium Ldl. different light
sources were found to have no effect in the studied parameters. Thus, except for increasing frequency of callus induction in certain orchid sps when cultured under red light, the different light sources studied have no effect on the other parameters studied. However, yellow and green light were found to have adverse effect on callus induction frequency, and its subsequent growth in *A. odoratum* Lour., *C. aloifolium* SW., *D. premulinum* Ldl., *R. retusa* Bl., and *V. coerulea* Griff.

Calli induction potentialities of root explants in a few orchid sps were also studied. Root explants of *A. odoratum* Lour. cultured in H medium supplemented with 4.0 mg/l 2, 4-D and 2.0 mg/l BAP was found to be the best for the induction of PLB's and its differentiation of leaves and roots. MS medium supplemented with 2.5 mg/l NAA + 2.0 mg/l BAP was found to be the most suitable for induction of PLB's and their regeneration in *C. aloifolium*. Similarly, for *C. iridioides*, H medium along with 3.0 mg/l Kin and for *R. retusa* same medium containing 4.0 mg/l IAA + 2.5 mg/l Kin were found to be the most suitable for earliest PLB initiation and its sequential differentiation. Explants cultured in Nit medium supplemented with 3.5 mg/l 2, 4-D + 2.5 mg/l Kin were most suitable for *S. plicata* for PLB formation and their regeneration to plantlets.

A comparative study was carried out to determine the nitrate reductase (NR) activity in the leaves of *in vitro* and *in vivo* grown 1 year old seedlings. Four epiphytic orchid sps viz., *A. odoratum* Lour., *C. aloifolium* SW., *R. retusa* Bl., *V. coerulea* Griff. and one terrestrial orchid sps *S. plicata* Bl. were
used. The plantlet raised through embryo culture in either VW or Nit media were utilized. The maximum NR activity was observed in the leaves collected from in vitro plantlets of A. odoratum Lour, R. retusa Bl., S. plicata Bl. and V. coerulea Griff., whereas NR activity of the remaining two orchid sps (C. aloifolium SW and D. premulinum Ldl.) was found to be maximum in the leaves of in vivo grown plantlets. Thus, it appears that NR activity in orchid leaves do not depend upon nature, habit or habitat of the plant where the orchid species grow, but probably on certain other factors present in the leaves of the plants.