SUMMARY

Five orchid species comprising of four terrestrials (*Dactylorhiza hatageria, Eulophia dabia, Habenaria commelinifolia, Malaxis acuminata*) and an epiphytic (*Saccolabium papillosum*) of biological significance and/or endangered, were included under the scope of present studies. With the aim to develop an efficient micropropagation system, the attempts were made to test the asymbiotic and symbiotic germination response of the mature seeds from fully ripened dehisced capsules (pods) to study and compare the various morphogenetic processes during the development of seedlings. The mycorrhizal endophytes were isolated from the roots of all the studied taxa for the purpose of symbiotic germination and to test the specific requirement for the endophyte by the mature embryos for their *in vitro* germination response and their subsequent development leading to seedling formation. The endophytes were also identified based on their morphological and growth characteristics *in vitro*. The utility of the pseudobulb segments for raising asymbiotic (control) and symbiotic plantlets in one of the species (*Malaxis acuminata*) was also tested.

Invariably all the studied genera have an endotrophic and intracellular fungus in the roots of their adult plants. The meristematic root tips, stems, leaves, subterranean tubers and rhizomes were fungus free excepting *M. acuminata* where the fungus tend to infect the pseudobulbs depicting thereby a modification in the trophic niche of this fungal endophytes in this species.

The mode of fungal entry was through epiblema cells as well as epiblema hairs in *H. commelinifolia, M. acuminata*; it was through only epiblema in *E. dabia* and *S. papillosum* while in *D. hatageria* it was through epiblema hairs. Generally the fungal entry was mediated through the passage cells. A variable extent of radial infection was
observed while it was deep upto the innermost layer of cortex adjoining endodermis in *Eulophia dabia*, it was checked in the innermost 1-2 cortical layers in *Dactylorhiza hatageria*, while 2-3 layers of innermost cortex in *Habenaria commelinifolia* and *Malaxis acuminata* and 5-6 innermost cortical layers in *Saccolabium papillosum*. The vascular bundles remained free of infection in all of them. The cortex was differentiated into host cell zones and digestion zones. While the cells of host cell zone undergo enlargement along with hypertrophy of the nucleus; the latter often get enmeshed in the fungal network of 'pelotons'. The digestion cells were involved in digestion of the fungus wherein the fungal clumps diminish in size and ultimately left as cell wall materials. A tolypophagus mode of fungal digestion was observed in all these photosynthetic orchids wherein fungus continuously colonize the cells in the form of pelotons-meshwork while the latter are continuously digested by the host cells. Occasional presence of two fungal clumps in the same cell as in *D. hatageria, E. dabia,* and *H. commelinifolia* shows a bigeneric/repeated mode of infection in them.

The fungal endophytes isolated from the plants were invariably *Rhizoctonia* anamorphs as identified by their colony growth characteristics on a variety of media viz. PDA, OMA and WA and the morphological features of vegetative hyphae and moniliods. The fungal endophytes were identified as *Ceratorhiza goodyera-repentinis* (Constantin and Dufour) Moore; (Basionym: *Rhizoctonia goodyera-repentinis* Constantin and Dufour) for *D. hatageria*; *Ceratorhiza ramicola* (Weber and Roberts) Moore; Basionym: *Rhizoctonia ramicola* Weber and Roberts for *E. dabia*; *Ceratorhiza pernacatena* (Zelmer and Currah) for *H. commelinifolia*; *Rhizoctonia endophytica* H.K. Saksena & Vaartaja for *M. acuminata* and *Moniliopsis anomala* Burgeff ex Currah (= *Rhizoctonia anomala* Burgeff) for *S. papillosum*. 
All the present taxa responded positively for asymbiotic germination *in vitro* thereby successfully bypassing the fungal requirements of germination. Presently, the germination potential of mature seeds (12-22wap) from fully ripened pods (capsules) was accessed. The germination response and the various morphogenetic changes were greatly affected by the chemical stimulus in the nutrient pool. It was also mediated by the photoperiod, a 12/12 hrs; D/L photoperiod appeared to be stimulatory for germination. The development of the seedlings was protocorm mediated; this sequential development of the mature seeds into spherules and protocorms was followed by leaf and root differentiation. The protocorm multiplication, an inherent trait of orchids was selectively expressed depending upon the chemical stimulus in the present cultures. The chlorophyll development was pre-protocorm phenomenon in *Eulophia dabia* and *Saccolabium papillosum* and it was post-protocorm phenomenon in *Habenaria commelinifolia* and *Dactylorhiza hatageria* while the protocorms invariably developed chlorophyll at the time of their differentiation in *Malaxis acuminata*.

Symbiotically, *D. hatageria*, *E. dabia* and *H. commelinifolia* responded for germination while the former species also showed non-specific requirement of the endophyte for germination, however, the formation of complete seedlings in this taxa could be achieved only when the host endophyte was used. The fungal entry was mediated through the suspensor end of the embryo. The fungal pelotons were formed in the host cells time and again and vary rapid digestion occurs, which thus shows that fungus is parasitized by the orchid host. The chlorophyll development was a pre-protocorm phenomenon in *H. commelinifolia* while it is post-protocorm phenomenon for *E. dabia* and *D. hatageria*. The effect of light for germination appeared to vary
with the species. In general the germination rates and the various morphogenetic events were considerably accelerated than their asymbiotic counterparts and the seedling obtained therein were more healthy, robust, and pathogen resistant. In case of *Malaxis acuminata* the pseudobulb nodal segments were co-cultured with the fugal endophyte and interestingly the plantlets obtained were better in their health status then their controls suggesting thereby that these segments can serve as good explants for symbiotic experiments.

The *ex vitro* transfer of the asymbiotically raised seedlings required elaborate acclimatization procedure; moreover their establishment in the green house depended upon the nature of the substratum and requirement of the fungal endophyte accounting for their low survival rates. However, the symbiotically raised seedlings/plantlets were less demanding and can easily be transferred to the green house conditions. The fungal back-up in their roots helps in their easy recruitment in the substratum and hence responsible for their high survival rates. Moreover the development of tubers and their subsequent growth as in *Habenaria commelinifolia* suggests a good efficacy of the symbiotic methods in micropropagation programmes.