PREFACE

Indian sub-continent is well represented by orchid flora which constitute an integral part of country's wonderful natural heritage. The orchid wealth of Indian region, especially the sub-Himalayan belt, is gradually dwindling. Barring a few, most orchid species are in danger of extinction due to large scale commercial collection and habitat destruction as well. Besides conventional *ex situ* and *in situ* conservation approaches, the potential of recent biotechnological methods needs to be fully exploited.

Tissue culture is the foundation on which all biotechnology rests and it is the best known method for propagation of rare and endangered plant species in general and orchids in particular. In the present dissertation, methods have been developed and standardized for *in vitro* mass propagation of a few critically endangered orchids of North Eastern Himalayas.

Although the present work is an unified attempt on *in vitro* germination of seeds and induction and nutrition of PLB/callus and plant regeneration of three orchids, for the sake of convenience the project report is divided into five self-contained chapters under three general parts. The first part containing two chapters has been ***in vitro* germination and growth of seeds**, while the part two consisting of a lone chapter is devoted to **development of a rapid mass propagation protocol** for the endangered orchids concerned. The third and last part of the thesis, namely, **"Hardening and artificial seed technology"** has been elaborated in chapter four and five respectively.

First chapter deals with ' **Effect of media and organic additives on initiation of germination and growth of seeds under *in vitro* conditions in *Arundina* (Blume), *Dendrobium* (Swartz) and *Geodorum* (Jackson),'** wherein, a systematic and extensive investigation has been undertaken to find out suitable media for initiation of seed germination and growth, development and regeneration of PLBs/ calluses in *Arundina graminifolia, Dendrobium moschatum* and *Geodorum densiflorum.*

During the last few years tissue culture techniques have been extensively exploited for rapid and large scale propagation of orchids. For successful realization of the potentials of these techniques it needs a systematic and critical examination of

the role of growth regulators on seed germination and seedling development of orchids. Second chapter deals with ' **Effect of growth regulators on seed germination; growth, development and regeneration of PLBs/ callus** in the representatives of these genera.

Plant tissue culture, the most commercially successful area of plant biotechnology, has introduced an exciting new phase into plant propagation and breeding. Orchid is the first horticultural plant cloned by tissue cultural methods on a commercial scale. Third chapter deals with **'Stem-disc culture --- development of a rapid mass propagation protocol for *Arundina, Dendrobium* and *Geodorum*'**, wherein, success was attained in the development of a rapid regeneration method using thin sections of stems from *in vitro* grown plants of *Arundina graminifolia,* and *Geodorum densiflorum*. These orchids were once very common in the forests of North Eastern Himalayas but commercial exploitation and expansion of cultivated lands have threatened their existence.

Orchids are healthy competitors in the international cut flower market. The small proportion (<5%) of the total international cut flower trade currently occupied by orchid highlights their potential to take even the greater share of the market. There is an active market for micropropagated orchid plantlets which are produced at a rate of 11 million units per year.

Recent advances in plant tissue culture techniques have revolutionised the plant tissue culture industry in general and orchid industry in particular. But constraints of labour and other expenses like nutrient medium, hardening and delivery of *in vitro* raised plants are yet to overcome. Fourth chapter entitled **'Hardening of *in vitro* regenerated plantlets in *Arundina* and *Geodorum*'** was undertaken to find out a suitable process for hardening the *in vitro* raised plantlets of *Arundina graminifolia* and *Geodorum densiflorum* by using the conventional method as well as by **artificial seed technology (Chapter 5).** The investigation has been successful in development of a suitable method for acclimatization and field transfer of the *in vitro* raised plantlets in two critically endangered orchids of North Eastern Himalayas.

The artificial seed technology is an exciting and rapidly growing area of research in plant cell and tissue culture. Production of artificial seeds has unravelled new vistas in plant biotechnology. The commercial application of plant tissue culture technology has been successfully demonstrated. However, constraints of high labour and other expenses like hardening and delivery of tissue cultured plants remain to be overcome. In this context artificial seed technology assumes greater importance because of low cost and high volume propagation system.

Fifth and last chapter of the project deals with **'Artificial Seed Technology -- development of a protocol in *Arundina* and *Geodorum* -- two critically endangered terrestrial orchids'**, wherein, successful production and regeneration of artificial seeds have been possible by encapsulating the PLBs of *A. graminifolia* and *G. densiflorum*, a pair of terrestrial endangered orchids of North Eastern Himalayas with a view to conservation of them. Many facets of artificial seed production by encapsulating PLBs have been extensively investigated with an ultimate view to conservation of the two orchids.

The development of a protocol for the two endangered North Eastern Himalayan orchids may be an useful addition to the *in vivo* germination and regeneration of plantlets for storage and transportation of precious and costly orchid hybrids and for conservation of elite and endangered orchid germplasm. The judicious and intelligent coupling of artificial seed technology with that of microcomputer in achieving automated encapsulation would tremendously increase the efficiency of encapsulation and production of artificial seeds and revolutionize the current concept of commercial micropropagation in the next millenium.