

P R E F A C E

The word 'Orchid' brings to one's mind some vision of the distinctive or incredible form of flower, of colour, of beauty, of fragrance and what not. Taxonomically, they represent the most highly evolved family among monocotyledons; with approximately 750 genera with 18,000 species. Orchids are highly specialized in a number of ways. It is distributed in all continents except the Antarctica.

Orchids are commercially grown in many countries. The cut flower trade includes the hybrids of the genera like Arachnis, Cattleya, Cymbidium, Dendrobium, Epidendrum, Oncidium, Phalaenopsis, Renanthera and Vanda. Many countries are now involved in export and import of orchid cutflowers as well as orchid plants.

In many valuable orchids vegetative propagation by bulb is a slow process requiring an average of 5-10 years to obtain a good sized plants. Now a days orchid growers are successfully using tissue culture for rapid and masspropagation of orchids.

Plant tissue culture has opened a number of possibilities and the literature has accumulated rapidly over the last 30 years. The application of tissue culture techniques to orchids and the benefits derived, such as production of quality plants in large quantities by clonal multiplication, establishment of hybrid plants, improvement orchid trade and industry, is now wellknown.

The pioneering work of Bernard (1909) is important in the development of in vitro culture techniques of orchids. He successfully isolated the root-infecting fungi helpful in orchid seed germination. When sterilized seeds were grown in association with the fungus, the percentage of germination improved considerably. The importance of balanced nutrition in maintaining a stable relationship between host and endophyte was explained by many workers.

Subsequently, the work of Knudson clarified many important points regarding formation of seedlings and organogenesis. His work showed, for the first time, that germination of orchid seeds was possible in vitro without fungal association. The medium proposed by Vacin & Went (1949) is widely used for germination of hybrid seeds.

Occasionally a particular recipe is proposed for a given genus or species, and improvised by the addition of various growth adjuncts to the medium, such as coconut milk, tomato juice, banana extract, different fruit juices, fish emulsion, beef extract and even beer.

The present dissertation presents a comprehensive developmental biology and tissue culture studies in some commercially important orchids. The Chapter 1 of the dissertation has been devoted to a systematic and intensive studies with respect to in vitro seed germination and growth in Cymbidium hybrids and Aerides multiflorum Roxb. The development and standardization of efficient in vitro culture techniques for germination of immature seeds and callus induction from germinated seeds is important for successful application of tissue culture techniques. Very little work has been done on the nutrient requirements for germination of seeds and development of protocorms and seedlings of Cymbidium in aseptic cultures. Information is completely lacking in this direction in Aerides. Most of the tissue culture studies on orchids have been performed with the objective of micropropagation or clonal multiplication. No investigation, however, have exploited the potentiality of immature seeds from green capsules as explant source for induction of Plbs.

Tissue culture of orchids has been primarily concerned with plant regeneration and has yielded variable results with each species examined. This perhaps reflects the different genotype used and the variety of culture media and other experimental conditions adopted. The main objective of these investigations have been clonal multiplication of orchids having commercial importance with not attempts to investigate in details the growth and development of serially propagated callus. Information is available on the initiation, growth and organogenesis of callus derived from various explant sources in other plant materials. Lacunae existing in this direction in orchids warrant justification of undertaking systematic study with respect to initiation, growth and differentiation of callus derived from suitable explants in some commercially important members of Orchidaceae. The Chapter 2 of the present dissertation deals with the growth pattern of callus tissues in Cymbidium hybrids and Aerides multiflorum Roxb. The objective of the present study was to investigate the performance of callus derived from immature seeds in different culture media including a newly defined one and to select a suitable medium for optimal growth and development of callus in the orchids selected for the purpose.

Early investigations have emphasized the importance of certain vitamins and growth factors for development of plants including orchids. The use of amino acids and their derivatives as nitrogen source has been tried by some workers. Most of the amino acids tested in the experiments either inhibited or did not favour growth. Most of the early investigations have been on nutritional requirements especially utilization of different vitamins and amino acids in germination and growth of orchid seeds. Different growth hormones have been commonly used in orchid culture and their effects are variable with orchid species. The present investigation was directed at elucidating the effect of incorporating different growth substances, vitamins and amino acid alone or in combination into the culture medium. Chapter 3 of the thesis is concerned with effect of hormones, vitamins and amino acids on the growth of callus derived from Cymbidium hybrids and Aerides multiflorum Roxb.

Previous cytological studies on orchids are mostly concentrated on genera of horticultural importance. Cytological studies had been mainly confined to the chromosome counts only without any detailed analysis of other parameters of the chromosomes. In spite of what little data accumulated on cytology of different members of Orchidaceae, certain

aspects of the cytology of some of the important genera are yet to be investigated. In spite of the horticultural importance and commercial value, the cytological information on orchids is rather inadequate. In view of the scanty work on orchids especially on Cymbidium species and the importance of karyotype analysis in elucidating phylogenetic relatedness, the present investigation was undertaken which includes a detailed chromosome analysis in commercially important hybrid Cymbidium and a wild taxon of Aerides. For a proper assessment of in vitro cytological situation it seems to be justified to undertake a detailed cytological investigation on the experimental materials under in vivo conditions. The taxa included in the present scheme of work are Cymbidium 'Christmas Beauty' and Aerides multiflorum Roxb. The Chapter 4 presents cytology of Cymbidium hybrid and Aerides multiflorum Roxb under in vivo condition.

Various degree of success has been met with in studies relating to initiation, growth and regeneration of callus tissues in some Cymbidium including successful induction of callus and regeneration using shoot apical meristem as an explant source in Cymbidium 'Shallow' from this laboratory.

Tissue cultural studies has been undertaken in Aerides multiflorum for the first time in the present scheme of work. Cytological investigations on the plant tissues in vitro carried out in a number of materials had led to the realization that chromosomal instability of the cultivated somatic cells is a phenomenon of common occurrence in tissue and cell cultures. The study of callus cytology have gained much significance in view of the fact that the ability of a callus for growth, organogenesis and regeneration is greatly dependent on the chromosomal constitution of the callus. It seems to be worthwhile to undertake a comprehensive investigation on the cytology and organogenesis of some orchids under in vitro conditions and one of the precise objectives of the present undertaking is a screening of cytological situation in regenerated organ especially roots differentiated in vitro. Differentiation is a complex developmental phenomenon and elucidation of the regulatory component that induce and control the process at various levels of cellular organization is still fragmentary in nature. Tissue culture has emerged as a new approach to the understanding of the nature of differentiation in recent times. Chapter 4 of the thesis is concerned with the cytology and developmental morphology of Cymbidium

hybrids and Aerides multiflorum Roxb. under in vitro conditions.

Similar studies as presented in Chapter 1 have been extended in the materials Dendrobium pieradii Roxb. and Vanda teres Lindl. and form the content of Chapter 6.