

## ORCHIDS: THE WORLD'S MOST WONDROUS PLANTS

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### ABSTRACT

Orchids are among the most advanced flowering plants, showing an incredible range of diversity in shape, size, and colour of their flowers. They command a high demand and price for their beautiful and long-lasting flowers. Commercially, these plants are propagated using tissue culture techniques and form the basis of multi-million dollar cut-flower industries in many countries.

### INTRODUCTION

As one talks of orchids, one is reminded of distinctive or incredible form of flower, colour, beauty, fragrance, and what not. They belong to the family *Orchidaceae* which is one of the largest and highly advanced family among the flowering plants with about 25,000 to 35,000 species in over 800 genera (Chadha, 1992). Orchids are distributed all over the world excepting Antarctica. They show an incredible range of diversity in shape, size, and colour of their flowers, and are highly valued for the bewitching beauty and long lasting quality of these which extends up to 6-10 wks. Orchids have been attracting floriculturists since time immemorial due to their fads, fancies, and fashions, and this has led to 'Orchid Mania' throughout the world. These are often considered to be luxury flowers, probably because many varieties are highly priced and difficult to cultivate. The orchids are expensive and orchid industry in the world is well established, and has further scope for enlargement.

These wondrous and beautiful plants were earlier thought to be parasites growing on trees. The British naturalist, Charles Darwin, assumed that these plants drew their nutrition from taller forest hosts on which they grew. But now it is proved beyond doubt that orchids can prepare their own food by photosynthesis and they use their hosts merely for anchorage. They can withstand many abnormalities and endure drought and frost. This gives one ample opportunity to experiment with them and learn about them through trial and error.

Based on their varying habits, orchids are classified into saprophytes, terrestrials, and epiphytes. Majority of the orchids are generally found perched on tree trunks,

e.g., species of *Aerides*, *Chiloschista*, *Coelogyne*, *Cymbidium*, *Dendrobium*, *Pleione*, and *Vanda*.

The world's richest areas, so far as the orchids are concerned, are Columbia and Indo-Malaysian region from the Himalayas to New Guinea. Orchids are well represented in India by about 1150 species belonging to 164 genera (Sathish Kumar and Manilal, 1994). The north-eastern region of the country alone harbours around 650 orchid species (Kataki *et al.*, 1984). The orchids in the region are fast depleting from their natural habitats due to deforestation and over-exploitation for commercial purposes. The people of the region are fond of growing orchids besides other flowering plants. As a result, the orchids are collected indiscriminately in large numbers from nature and sold in market place, and also house to house. Therefore, there is a need for both conservation and sustainable utilization of orchids. Some of the rare and extinct or possibly endangered orchid species are listed in Table 1.

Table 1. Some important rare and endangered orchids of the world

Rare Orchids	Endangered Orchids
<i>Anoectochilus crispus</i> Lindl.	<i>Acriopsis harae</i> Tuyama
<i>A. sikkinensis</i> King & Pantl.	<i>Agrostophyllum myrianthum</i> King & Pantl.
<i>Bulbophyllum leopardinum</i> Lindl.	<i>Anoectochilus clarkei</i> (Hook. f.) Scidnf. & Sm.
<i>B. leptanthum</i> Hook. f.	<i>A. tetrapterus</i> Hook. f.
<i>B. lobbii</i> Lindl.	<i>Bulbophyllum andersoni</i> (Hook. f.) J. J. Sm
<i>Bulleyia yunnanensis</i> Schltr	<i>B. conchiferum</i> Reichb. f. .
<i>Calanthe alpina</i> Hook. f.	<i>Calanthe chloreuca</i> Lindl.
<i>C. herbacea</i> Lindl.	<i>C. densiflora</i> Lindl.
<i>Cheirostylis griffithii</i> Lindl.	<i>Coelogyne treutleri</i> Hook. f.
<i>Coelogyne treutleri</i> Hook. f.	<i>Cymbidium simonsianum</i> King & Pantl.
<i>Cymbidium eburneum</i> Lindl.	<i>Dendrobium bolboflorum</i> Falc.
<i>C. giganteum</i> Wall.	<i>D. miserum</i> Reichb. f.
<i>Dendrobium crystallinum</i> Reichb. f.	<i>Diplomeris pulchella</i> D. Don
<i>D. infundibulum</i> Lindl.	<i>Eria pudica</i> Ridl.
<i>D. wardianum</i> Warner	<i>Eulophia macrorhizon</i> Hook. f.
<i>Eria carinata</i> Lindl.	<i>Galeola altissima</i> (Bl.) Reichb. f.
<i>Eulophia mannii</i> Hook. f.	<i>Habenaria cumminsiana</i> King & Pantl.
<i>Galeola cathcartii</i> Hook. f.	<i>Oberonia lobulata</i> King & Pantl.
<i>Hermidium orbiculare</i> Hook. f.	<i>Orchis puberula</i> King & Pantl.
<i>Paphiopedilum fairrieanum</i> (Lindl.) Stein	<i>Paphiopedilum spicerianum</i> (Reichb. f.) Pfitz.
<i>Pleione hookeriana</i> (Lindl.) B.S. Williams	<i>Phalaenopsis mastersii</i> King & Pantl.
<i>Thunia marshalliana</i> Rolfe	<i>Tainia khasiana</i> King & Pantl.
<i>Vanda coerulea</i> Griff. ex Lindl.	

In addition to their commercial value, orchids are of considerable importance in medicines, food, and perfumes. Some orchids have been reported to have antibacterial activity. Propagation of a vast majority of orchids is through seeds rather than vegetative means. The capsules ('pods') formed are few in number, as most of the flowers are not pollinated in nature. Although the number of seeds in ripening capsules is quite high, only less than 1% of the seeds germinate in their natural habitats, because a specific mycorrhizal fungus is required for their germination (Rao, 1977).

It was in 1909 that root-infecting fungus helpful in orchid seed germination was isolated for the first time (Bernard, 1909). It opened the way for the development of *in vitro* culture techniques in orchids. An American Botanist, Prof. Knudson, clarified many important points regarding formation of seedlings and development of different organs. This resulted in successful germination of orchid seeds *in vitro* without fungal associations in early twenties of the last century. Knudson (1951) suggested a medium, which provided balanced organic and inorganic nutrition for the developing seedlings. To date, a large number of media have been found suitable for germination and growth of orchid seedlings. Some of the important media used for orchid culture are given in Table 2.

With the discovery that orchid seeds germinate following fungal infection, only a practical seed germination method utilizing fungi was developed. It was the so-called symbiotic method, but asymbiotic method of germinating orchid seeds became popular after Knudson's discovery of essential nutrient requirements of orchids.

*In vitro* orchid seed germination requires the acquisition of certain skills and knowledge. Orchid capsules are first surface sterilized with suitable sterilizing agents and the seeds are scooped out from these under aseptic conditions; the seeds are germinated on a suitable medium under controlled conditions of temperature, light, and humidity. The response of orchid seeds to physico-chemical factors differs from species to species (Arditti and Ernst, 1984; Bhuyan and Deka, 1999; Kumaria and Tandon, 1991; Ramsay, 1997; Pathak *et al.*, 1992; Sharma and Tandon, 1987; Szendark and Read, 1996; Vij and Pathak, 1988; Vij *et al.*, 1981, 2000a; Yam and Weatherhead, 1988). Some additives like coconut milk, banana homogenate, apple juice, etc. have been shown to stimulate seed germination and seedling growth. Orchid seeds is considered to have germinated when the embryo swells, bulges out of bursted seed coat, enlarges, and develops into protocorms (Fig. 1A). This stage is accompanied by the formation of epidermal hairs within a few days. Thereafter, development of seedling follows (Fig. 1B). Growth and development continues after transflasking. Both semi-solid and liquid media are used for the germination of orchids *in vitro*.

Table 2. Composition of different media used for orchid multiplication

Constituents	Media composition (mg <sup>-1</sup> )					
	Curtis (1943)	Modified Kaudson C (1946)	Nitsch (1969)	Mitra <i>et al.</i> (1976)	Vacin and Went (1949)	
1	2	3	4	5	6	
<i>Inorganic</i>						
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	350	100	-	200	-	
KNO <sub>3</sub>	-	180	950	180	535	
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	-	100	-	100	500	
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	-	-	-	150	-	
KH <sub>2</sub> PO <sub>4</sub>	120	-	68	-	250	
MgSO <sub>4</sub> ·7H <sub>2</sub> O	260	250	185	250	250	
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	-	-	-	-	200	
NH <sub>4</sub> PO <sub>4</sub>	220	-	720	-	-	
CaCl <sub>2</sub>	-	-	166	-	-	
MnSO <sub>4</sub> ·H <sub>2</sub> O	22.5	-	-	-	-	
MnSO <sub>4</sub> ·4H <sub>2</sub> O	-	0.075	25	-	7.5	
KI	0.83	0.8	-	0.05	-	
MnCl <sub>2</sub> ·4H <sub>2</sub> O	-	-	-	0.40	-	
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	1.0	-	10.0	0.05	-	
H <sub>3</sub> BO <sub>3</sub>	6.2	6.2	10.0	0.6	-	
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.03	0.025	0.025	0.05	-	
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.25	0.25	0.25	0.05	-	

1	2	3	4	5	6
	-	-	-	0.05	-
$\text{Co}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$	-	-	-	-	-
ZnCl <sub>2</sub>	3.93	-	-	3.9	-
$\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$	0.025	0.025	-	-	-
AlCl <sub>3</sub>	0.03	-	-	-	-
$\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$	5.53	25.0	27.8	16.71	-
$\text{Na}_2\text{EDTA} \cdot 2 \text{H}_2\text{O}$	-	74.6	37.3	22.35	-
Ferric Citrate	-	-	-	-	28.0
$\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$	1.0	-	-	-	-
NiCl <sub>2</sub>	0.03	-	-	-	-
Organic					
Inositol	0.1	-	100	-	-
Nicotinic acid	1.0	-	5.0	1.25	-
Pyridoxine HCl	1.0	0.3	0.5	0.3	-
Thiamine HCl	1.0	0.3	0.5	0.3	-
Glycine	-	-	2.0	-	-
Folic Acid	1.0	-	0.5	0.3	-
Biotin	1.0	-	0.5	0.05	-
Riboflavin	-	0.3	-	0.05	-
Glucose	10,000	-	-	-	-
Sucrose	-	20,000	20,000	20,000	20,000
Agar	14 g	9 g	9 g	9 g	9 g

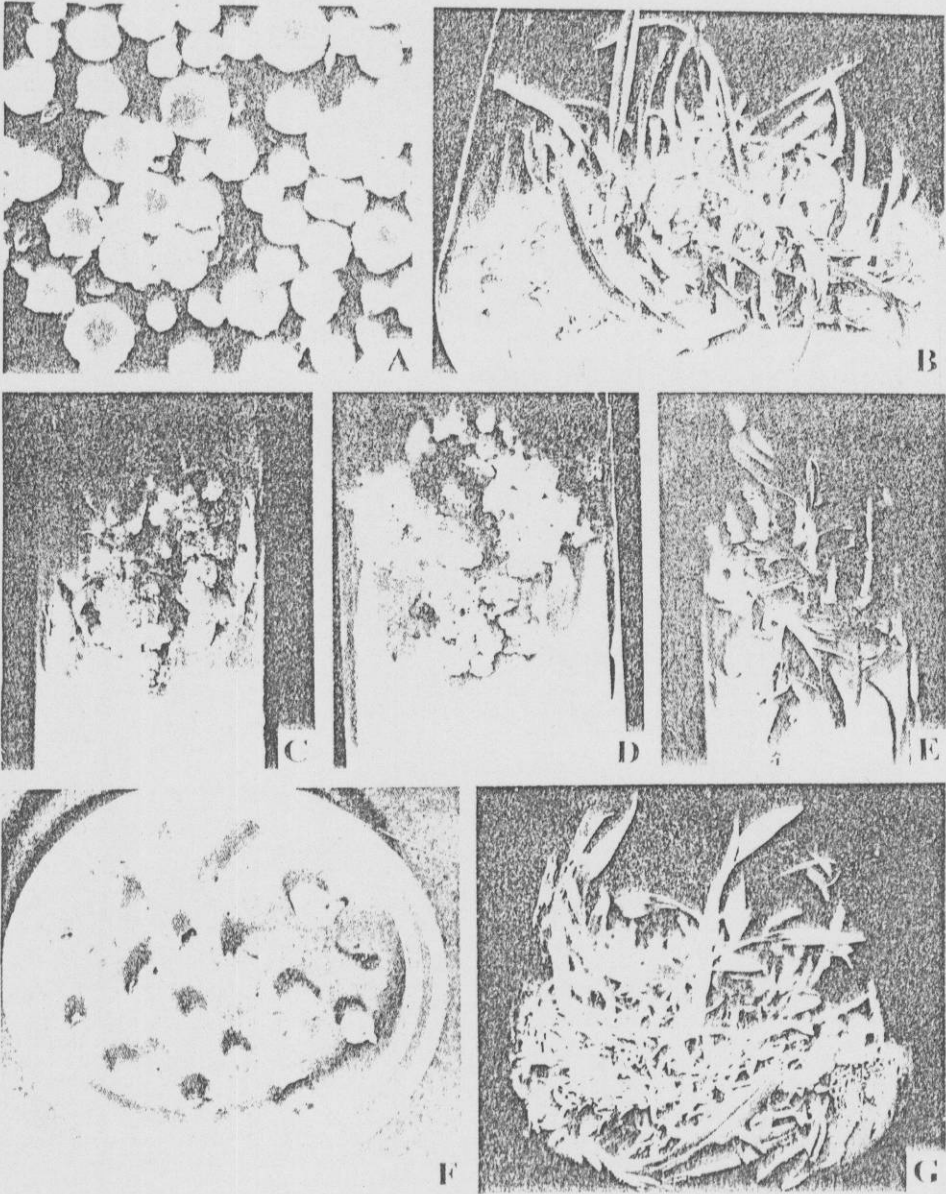


Fig. 1 A-G: A-B. *Cymbidium giganteum*: A, Protocorms; B, Development of protocorms into seedlings; C-G. *Dendrobium fimbriatum* var. *oculatum*: C, Formation of protocorm-like bodies (PLBs) on shoot tip explant; D, Plantlets developing from PLBs; E, Complete plantlets with well developed shoots and roots; F, Germinating artificial seeds; G, Development of healthy plantlets ready for transfer to the greenhouse.

Clonal propagation of orchids using tissue culture techniques is gaining wide importance in orchid industry. As orchids are heterozygous and their vegetative propagation is extremely slow, the commercial application of tissue culture techniques for rapid clonal propagation of some hybrids is of great importance. It is particularly valuable in perpetuating clones of special merit. One of the major breakthroughs in Orchidology was the method of clonal propagation suggested by the late French Botanist, George M. Morel in 1964 who observed the formation of protocorm-like bodies (PLBs) from less than 1 mm long *Cymbidium* shoot tips cultured *in vitro*. The PLBs, on sub-culturing, multiply and subsequently develop into virus-free plantlets (Fig. 1C-E, G). Hybrids of *Aranda*, *Ascocenda*, *Cattleya*, *Cymbidium*, *Dendrobium*, *Oncidium*, *Phalaenopsis*, *Renanthera*, *Renantanda*, and *Vanda* have been successfully mericloned using this procedure. In addition to commercial propagation, tissue culture of orchids has emerged as a valuable tool for basic research in plant sciences and an effective method of saving many species from extinction.

Different explants used for orchid micropropagation include leaves, roots, rhizomes, shoot meristems, axillary buds, inflorescences, etc. (Arditti and Ernst, 1993; Chang and Chang, 1998; George and Ravishanker, 1997; Kostenyuk *et al.*, 1999; Kumaria and Tandon, 1994; Tanaka, 1987; Teng *et al.*, 1997; Van *et al.*, 1999; Vij and Kaur, 1999; Vij and Pathak, 1990; Vij *et al.*, 1989, 1997, 2000b; Wang, 1988). Explants are first washed thoroughly in water and then rinsed in 70-80% alcohol for about 5-7 sec. These are surface sterilized using suitable sterilizing agents such as sodium/calcium hypochlorite or mercuric chloride under aseptic conditions and rinsed with sterilized distilled water 4-5 times to remove the sterilizing agent completely. The explant is cut into small segments and placed on the nutrient medium, the composition of which varies from one species to another. In case of meristem culture, the meristem is carefully excised under aseptic conditions and placed on the medium. The cultures are incubated under temperature of  $25 \pm 2^\circ\text{C}$  and light intensity of 2000-3000 lux for 8-10 hrs a day. A schematic representation of *in vitro* multiplication of orchids is shown in Figure 2.

The incorporation of certain additives and growth regulators in the nutrition medium has proved to be beneficial for tissue culture of orchids (Arditti and Ernst, 1993). The interacting effects of cytokinins and auxins on shoot-root balance in orchids are also well documented by many workers. Vitamins such as ascorbic acid, biotin, folic acid, inositol, and many others have a differential effect on orchids and their development.

There are also reports of production of orchid plantlets through the germination of artificial seeds (Corrie and Tandon, 1993; Datta *et al.*, 1999; Khor *et al.*, 1998; Sharma *et al.*, 1992; Vij *et al.*, 1992, 1993) (Fig. 1F). Artificial seed technology is an exciting and rapidly growing area of research in orchids wherein

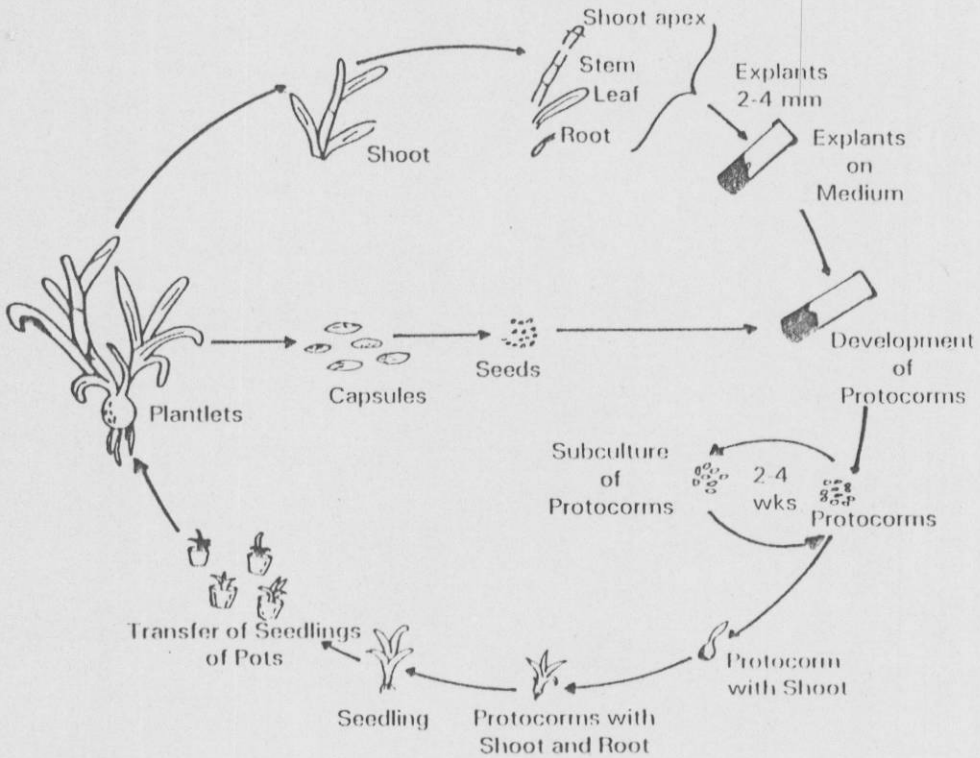


Fig. 2. A schematic representation of *in vitro* multiplication of orchids.

the propagules are encapsulated in an alginate matrix. The technology not only helps in easy handling and transplantation of plantlets but can also be used for conservation of endangered and desirable genotypes. The embryo preservation by vitrification has also been reported to be a promising technique for cryopreservation of orchids (Ishikawa *et al.*, 1997). Most recently, transformation systems have been established in order to achieve the desirable traits in orchids, which are otherwise difficult or impossible to obtain using conventional techniques (Belarmino and Mii, 2000; Knapp *et al.*, 2000). More detailed studies on the transformation efficiency are now in progress to have orchids with morphological and economically important traits such as flower colour and size, and resistance to insects and diseases.

Orchids are grown commercially in a large number of countries. USA is the largest importer of orchids followed by Japan, Germany, France, Italy, Europe, and the Netherlands. Nearly one million blooms of *Cymbidium* and *Cattleya* are exported to Sydney each year. In England, Cooksbridge, Kingsteignaton, and Slough are the places where orchids are grown on a large scale. In France, it is estimated that the annual value of plants produced ranges from US \$ 800,000 to 1 million and that

of flowers is around US \$ 0.5-0.75 million. Singapore, Malaysia, and Thailand are the main exporters of orchids among the South-East Asian countries. Thailand alone exports orchid spikes worth US \$ 30 million, followed by Singapore with US \$ 16 million. Countries like Indonesia, Burma and Malaysia are also entering the market. Recently, in our country some private companies have started work on mass multiplication of some native orchids. However, India's floriculture industry is still in its infancy. The current market for floriculture products in India is estimated at about US \$ 80 million, of which US \$ 32 million is contributed by modern cut flowers. In India, the orchids have naturalized in profusion, whereas in several countries they are grown as the agricultural cash crops.

The miniature orchids are a great attraction to many enthusiasts. Both ordinary and exotic orchid hybrid varieties are gaining importance for scientific and commercial purposes. No wonder, the orchids are the most wondrous of all the plants of the World !

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