INTRODUCTION

One of the largest families of the flowering plants, Orchidaceae constitutes about 40 per cent of the monocotyledonous taxa. Undoubtedly called the gems of mountain flora, the orchids bear some mystic and intriguing botanical features including zygomorphic flowers with well developed gynostegium, compound pollen, elaborate perianth and resupinated ovary, microscopic and non-endospermic seeds with highly reduced embryos and their dependence upon a suitable mycorrhizal association for germination in nature. Essentially outbreeders, the orchids are well adapted for insect and bird pollinations. Due to weakly developed barriers of reproductive isolation, hybrids are easily formed at both the inter- and intra-generic levels and their number far exceeds that of natural species (cf. Vij, 1995).

With their greatest concentration in tropics, the orchids are world wide in distribution (except Antarctica). Their numerical strength, in terms of species, has been variously assessed between 17,000 and 35,000. However, Atwood (1986) places the total number of orchid species at 19,128. The orchids grow as epiphytes, terrestrials and lithophytes; terrestrial habit is primitive in them. The orchids are still in an active state of speciation and provide an excellent material for understanding the process of evolution (Dressier and Dodson, 1960).

Unfortunately, the orchids do not form a dominant vegetation anywhere in the world. The orchid seed, in general, is a very simple structure weighing between 6.3 and 0.3 micrograms, between 470µ - 560µ in length, and 80µ -120µ in width (Abraham and Vatsala, 1981). Rasmussen (1995) reported that the orchid seeds are among the smallest known in the plant kingdom and Hirt (1906) used the term scobiform for
these seeds, i.e. usually having large volume to weight on account of the inflated air filled testa. Although the orchid seeds are produced in large numbers (1,300 – 4,000,000) per capsule; lack of metabolic machinery and functional endosperm, and requirement of a suitable fungal association are the factors responsible for their poor germination in nature (0.2% - 0.3%). Besides this, indiscreet exploitation of natural resources by human agencies and occurrence of dreadful natural calamities are major factors accounting for Rare/Endangered/Threatened (RET) status of several of their species. Nearly 200 million plants including orchids are destroyed every year through agricultural land clearing alone (Beckner, 1979). Infact, the entire Orchidaceae family figures prominently in the Red Data Book prepared by International Union for Conservation of Nature and Natural resources (IUCN, 1991). Nearly 10% of Indian orchids are threatened of survival. The number of endangered terrestrial orchids has been significantly higher in temperate and alpine zones, than that of endangered epiphytic ones in the tropical and sub tropical zones (cf. Vij, 1995).

Many countries have already developed list of such dwindling plants so as to conserve them. While the protection of orchid habitat and resuming the plants from degraded ones by cultivating them in Orchidaria /Botanical gardens are important strategies for orchid conservation, classification of elite varieties and their performance evaluation in foster homes are prerequisites for establishing an orchid based floriculture. Efforts in this direction are, however, quite meagre in India. The Convention on International Trade in Endangered Species (CITES) of Wild Flora and Fauna, in 1975, suggested a regulation of international trade in organisms threatened with extinction in wild and a ban on their collection from wild. Unfortunately, orchid piracy continues unabated and it is expected to continue as long as these plants enjoy an economic and commercial status. Thus, in order to save their natural populations from commercial pressure, mass propagation is an important strategy (Stewart, 1989). Mass propagation through conventional methods has, however, lost its significance due to their less productivity. The tissue culture techniques which allow rapid and round the year mass propagation of desired genotypes has, on the other hand, emerged as an important tool for conservation and commercialization of this important group of plants.
Eversince, Kundson (1922) succeeded in bypassing the fungal requirement of *Cattleya* seeds, during germination *in vitro*, by providing a suitable carbohydrate stimulus, the technique of asymbiotic germination has been positively tested in a variety of species and hybrids from diverse climates (Arditti *et al*., 1982; Vij and Pathak, 1988c; Yam and Weatherhead, 1988). The ability of orchid seeds to germinate prior to reaching maturity (Withner, 1943) has also added new dimensions to commercial cultivation of these plants; the time gap between pollination and seed sowing can be significantly reduced (Rao and Avadhani, 1964; Vij *et al*., 1981, 1985, 1987, 1988a, 1988b, Devi *et al*., 1997; Nath *et al*., 1991; Pathak *et al*., 1992, 1999; Malemnganba *et al*., 1994; Nagaraju and Parthasarthy, 1994; Hazarika and Sarma; 1995; Pyati and Murty, 1995; Vij *et al*., 1995; Kondo *et al*., 1997; Lawrence, *et al*., 1997; Leroux *et al*., 1997; Prakash *et al*., 1997; Pathak *et al*., 1999; Vij and Kher, 1997; Alouffa *et al*., 1998; Vij and Sharma, 1999). However, as orchids are outbreeders, raising them through seed/embryo culture is a disadvantageous proposition in cut-flower industry where pure-lines are required. Consequently, efforts have also been made to develop appropriate multiplication system to maintain genetic purity of regenerants.

Rotor (1949) for the first time used *Phalaenopsis* floral-stalk cuttings for the purpose. Morel (1960), however, developed a technique for raising virus free *Cymbidium* clones *in vitro* using shoot apical meristem. The technique has been widely accepted but it has a limited utility in monopodial orchids, since it requires the sacrifice of mother plant. Attempts have, therefore, been made to devise an equally effective multiplication system using organs whose excision is not detrimental to the survival of the mother plant. The regeneration potential of explants sourced from leaves (Churchill *et al*., 1973; Loh *et al*., 1975; Allenberg, 1976; Vij *et al*., 1984, 1986 b, 1994 b; Chaturvedi and Sharma, 1986; Seeni, 1988; Vij and Pathak, 1988a, 1990; Abdul Karim and Hairani, 1990; Yam and Weatherhead, 1991a; Seeni and Latha, 1992; Nayak *et al*., 1997; Kher *et al*., 1999; Sharma *et al*., 1999), roots (Beechey, 1970; Champagnat, 1971; Scully, 1971; Sood and Vij 1986; Vij *et al*., 1987; Vij and Pathak, 1988 b, Philips and Padikkala, 1989; Yam and Weatherhead, 1991 b; Vij, 1993, 1994 b), stem cuttings (Sagawa, 1961; Scully, 1966; Mosich *et al*., 1974; Vij *et
al., 1994 a; Ganesh et al., 1996; Vij et al., 1999; Gupta and Vij, 1999), floral-stalks (Kotomori and Murashige, 1965; Intuwong and Sagawa, 1973; Sagawa and Kunisaki, 1982; Singh and Prakash, 1984; Vij et al., 1986 a; Lim-Ho and Lee, 1987; Kaur and Vij, 1995; Vij et al., 1997 b), pseudobulbs (Vajrabhaya, 1978; Vij and Sood, 1982; Vij and Pathak, 1989; Vij and Dhiman, 1997 a; Sharma et al., 1999 b), rhizomes (Ueda and Torikata, 1972; Bapat and Narayanaswami, 1977; Vij et al., 1989; Lee, 1989; Sheelavantmath et al., 1999), and tubers (Champangnat and Morel, 1972; Morel, 1974) has been positively tested. Even the fobar peels have been used to micropropagate Rhynchostylis retusa and Vanda coerulea (Vij and Kaur, 1992, 1997; Vij, 1994 a). Tran Thanh Van and Trinh (1978) successfully induced direct flowering in the excised dermal tissues of Nicotiana tabacum. This potential is, however, yet to be tested in orchids.

Growth and morphogenesis of plant tissues in vitro are largely governed by the composition of the culture medium. Infact, all the cells of the plants have same general needs which could be characterized as: structural elements (carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, magnesium); bio-catalytical elements (the elements forming bio-catalysts such as iron, copper, manganese, and cobalt); indispensible elements (potassium, calcium, boron, molybdenum); and stimulating elements (sodium, chlorine, cobolt, iodine, nickel, bromine), and a balanced nutrient is one which provides most of these elements in a required amount for the normal growth of plant (cf. Srivastava, 1999). To satisfy the nutritional requirements of orchids in vitro, a variety of culture media [N_{3}F (Burgeff, 1936); K'C' (Knudson, 1946); VW (Vacin and Went, 1949); MS (Murashige and Skoog, 1962); M (Mitra et al., 1976)] have been devised on more or less empirical basis. While most of them are species specific, a medium capable of satisfying the nutritional complexities of a large number of taxa is yet to be devised (Vij, 1995).

Synthetic/Artificial ‘seed’ technology has added new dimensions to the development of an efficient delivery system for the propagules, since it is a low cost and high volume propagation system (Redenbaugh et al., 1987). Besides this, the synthetic ‘seeds’ provide a significant alternative to perpetual maintenance of living specimens for conservation and preservation of desired germplasm. Synthetic ‘seeds’ have so far
been prepared in about 20 orchid species (Singh, 1991; Vij et al., 1992; Corrie and Tandon, 1993; Malemnganba et al., 1996; Deka et al., 1997; Nagaraj and Prakash, 1997).

The regenerative response of an explant in vitro is influenced by genomic constitution and physiological state of the donor plant, culture medium composition and endogenous and exogenous level of hormones. The exogenous requirement for growth regulators depends on the endogenous levels in the plant system which varies with the plant tissue type and the phase of plant growth. Generally, a higher cytokinin : auxin ratio promotes shoot formation and higher auxin : cytokinin ratio promotes root formation (Skoog and Miller, 1957); sometimes cytokinin alone is enough for optimal multiplication (Lane, 1979; cf. Bhojwani and Razdhan, 1983; Garland and Stoltz, 1981). In tissue culture, it is essential to have a homogenous cell population which is stable genetically and physiologically. However, cytological heterogeneity in cultures may arise due to cumulative effect of variability contributed by parent plant (Torrey, 1967) and culture conditions (Butcher et al., 1975). Usually, long term cultures show loss of morphogenetic potentiality and high degree of irregularity in the frequency of differentiation indicating thereby some relationship between chromosomal aberrations and plant regeneration (cf. Bhojwani and Razdhan, 1983). Consequently, it becomes necessary to evaluate the cytological status of the regenerants to ensure trueness to the parent type. Incidentally, some attempts have already been made to test the cytological fidelity of regenerants in vitro (Sood, 1984; Kaur, 1988; Pathak, 1989).

Field establishment and distant transport of tissue culture derived plants involve sudden exposure to harsher climate, as a result many of the propagules succumb to these changes. They need to be hardened prior to their lab to land transfer.

India is one of the major orchid habitats of the world; nearly 1,141 species in 166 genera (Satish Kumar and Manilal, 1994) dwell in the country with Himalayas as their main centre and others scattered in North – Eastern, Peninsular regions (on the main land) and Andamans (off shores). In general, the terrestrial orchids are common in North Western India and the epiphytic ones in North Eastern India. A high degree of
endemism in India (288 species; Jain, 1986) suggests that the country has been one of the important centres of orchid speciation.

Incidentally, the importance of orchids has long been recognized. In India, the earliest reference to orchids dates back to 'Vedic period' and they are frequently mentioned in ancient sanskrit treatises including 'Nighantus' and 'Amarkosha' by Sushrutu. The compilation 'Sushruta Samhita' and 'Charaka Samhita' (600 –200 B.C.) also contain reference to these plants. Many of them have been collected earlier not for their flowers but for the aphrodisiac properties of their tubers (Lawler, 1984). In fact, they continue to be used in the local systems of medicine to cure dermal problems (Dendrobium alpestre), malignancy (Dendrobium nobile), rheumatism (Acampe papillosa), dysentry (Bletilla campanulata, Dactylorhiza hatagirea), miscarriage (Eulophia flaccida), nervous disorders (Cymbidium elegans; Cypripedium pubescens; Epipactis latifolia) etc. (Lawler, 1984; Suresh Kumar et al., 1994). De Clercq (1994) has reported the utility of Cymbidium hybrid, Epipactis helleborine and Liparis ovata in anti-AIDS medicine while Rastogi and Dhawan (1990) have reported anti-viral and anti-cancerous properties of Vanda parviflora. Their therapeutic values could be related to a variety of phytoconstituents including alkaloids, flavonoids, terpenes, carbohydrates and glycosides (Vij, 1995). Incidentally, Habenaria edgeworthii, H. intermedia, Malaxis muscifera, and Ephemerantha macraei are important constituents of a rejuvenating drug (Chyavanprash). The essence 'Vanillin' obtained from the unripe fruits of Vanilla planifolia is, however, the most important commercial produce of orchids. The utility of Cymbidium aloifolium to monitor radiation levels in the environment has also been indicated. More recently, orchids have emerged as leaders in floriculture and account for multimillion dollar cut-flower industry in several European and Asian countries due to incredible range of diversity in size, shape and colour of their flowers. Cymbidium ranks among top ten floriculturally important plants and accounts for 2.7% of total cut-flower production (Chadha, 1994).

Despite the fact that the beauty and utility of Indian orchids is being increasingly realized, their commercial potential on the sound scientific lines has remained unexploited in the country due mainly to the lack of awareness, proper planting
material and cultivation protocols, and of course, different national priorities (Vij, 1998). Incidentally, some attempts have already been made to conserve and propagate this group of plants at several major orchid research centres [Panjab University, Chandigarh (Vij and Sood, 1982; Vij and Pathak, 1989, Vij and Kaur, 1992, Pathak et al., 1999), Arunachal Pradesh Forest Department, Itanagar (Rao and Ahmed, 1997; Sinha and Hegde, 1997); Indian Institute of Horticultural Research, Bangalore (Prakash, 1994; Prakash et al., 1997); Tropical Botanic Garden and Research Institute, Trivandrum (William Decruse et al., 1994; Radha et al., 1994; Menon et al., 1994; Suresh Kumar and Seeni, 1994; Seeni and Bejoy, 1997); Kerala Agricultural University, Vellanikkara (Rajeevan, 1994, 1997); North Eastern Hill University, Shillong (Joy et al., 1994; Kumaria et al., 1994; Sharma and Chauhan, 1994; Tandon, 1997)] however, the attempts are not as yet commensurate with the dimensions of the problem. The fact that orchids are more vulnerable to extinction than other plant groups is apparent from the list of 150 rare and endangered Indian species (Vij, 1995).

It is with this background that the present studies were conducted to develop an efficient propagation system for some economically important and/or endangered orchid taxa [Bulbophyllum careyanum (Hook.) Spreng., Cymbidium hybrid (‘Great Waltz x Valley Flower’), Dendrobium chrysotoxum Lindl., Pholidota articulata Lindl., Rhynchostylis gigantea (Lindl.) Ridl., Vanda coerulea Griff., and V. cristata (Wall.) Lindl.] through asymbiotic seeds germination and regeneration of various explants from different plant organs. Efficacy of different media and role of phytohormones was also assessed to identify appropriate nutritional regime. Attempts were made to study the cytological status of the propagules and the utility of synthetic ‘seeds’ as an efficient storage and delivery system.