Biodiversity is the variety and differences among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part. This includes genetic diversity within the species and of ecosystems. Thus, in essence, biodiversity represents all life. Biological diversity is the very basis of human survival and economic well-being as it provides food, clothing, shelter, medicine, biomass, energy and industrial raw materials, and yet there remains a great deal or waiting to be discovered for human use. It is estimated that the species diversity living in our planet is about 5 to 50 million, out of which only 1,435,662 species have been described so far (Wilson, 1988). But, the rich biodiversity of the planet is under siege due to various factors. The human population has witnessed a three-fold increase in the last century and the rate of fossil fuel consumption has increased by 12-fold during the period, by which the carrying capacity of earth would saturate by the middle of this 21st century (Myers, 1990). According to IUCN (IUCN, 2000, Red List of Threatened Species, Switzerland: The World Conservation Union), plant species are declining in south and central America, Central and West Africa and Southeast Asia.
Malaysia has the most threatened species (681) followed by Indonesia (384), and so on. Globally, the number of threatened plants listed is 5,611, but this is based on an assessment of only 4% of the world’s described plants, which suggest that the percentage of threatened plants may be much higher. The recent report of IUCN brings out a list of 34 biodiversity hotspot regions of the world which indicates an alarming situation the world is faced with, in terms of biodiversity resource vis-a-vis future of mankind. Biodiversity hotspots are geographical regions which are extremely rich in species, have high endemism, and are under constant threat. The 34 biodiversity hotspots by region are: 4 in North and Central America (California floristic province, Caribbean Island, Madrean pine-oak woodlands, Meso-America); 5 in South America (Atlantic Forest, Cerrado, Chilean Winter Rainfall-Valdivian Forest, Tumbes-Choco-Magdalena, Tropical Andes); 4 in Europe and Central Asia (Caucasus, Irano-Antolian, Mediterranean, Mountains of Central Asia); 9 in Africa (Cape Floristic Region, Coastal forest of eastern Africa, Eastern Afromontane, Guinean Forest of West Africa, Horn of Africa, Coastal Forest of Eastern Africa, Madagascar and the Indian Ocean Islands, Maputaland-Pondoland-Albany, Succulent Karoo); 13 in Asia-Pacific (East Melanesian Island, Himalaya, Indo-Burma, Japan, Mountains of Southwest China, New Caledonia, New Zealand, Philippines, Polynesia-Micronesia, Southwest Australia, Sundaland, Wallacea, Western Ghats and Sri Lanka).

India is one of the 17 mega biodiversity countries, and has 26 recognized endemic centres that accounts for nearly a third of the flowering plants, though it constitute only 2.4% of land mass. Also, it is a host of 3 biodiversity hotspots viz: Himalayas, Indo-Burma, and Western Ghats and Sri Lanka. It is not only rich in biological diversity but is also an important centre of origin of agri-biodiversity. The endemism of Indian biodiversity is impressively high with about 33% of the country’s
recorded flora being endemic to the country and are concentrated mainly in the North-East India, Western Ghats, North-West Himalayas and Andaman and Nicobar islands. India has a total of 89,451 animal species accounting for 7.31% of the faunal species in the world and the flora accounts for 10.78% of the global total (MoEF 1999). But according to State of Forest report 1999 (FSI, 2000), the forest cover in India is losing at an alarming rate coupled with various factors, which poses greater threat to the rich biodiversity of the country. The main causes of habitat loss are agricultural activities, extraction (including mining, logging and harvesting) and unplanned developmental works. However, according to Wood et al., (2000) the underlying causes of biodiversity loss are poverty, macroeconomic policies, international trade factors, policy failures, poor environmental law/weak enforcement, unsustainable development projects and lack of local control over resources.

North-East India is a centre of megabiodiversity and is equally rich in flora and fauna and contains more than one-third of the country’s total biodiversity. It lies between 22° 9' - 29° 6' N latitude and 89° 7' E longitude. North-East India is the most important floristic region owing to its rich biodiversity and inhabits some botanic rarities. It is known for its diverse and most extensive lush forest cover and species composition, but is one of the major regions facing severe deforestation. The region is one of the 18 hottest hotspots of the world, having at least 7,500 flowering plants out of which 750-800 are orchids, 58 bamboos, 64 citrus, 28 conifers, 500 mosses, 700 ferns and 728 lichen species. The region is considered a meeting region of temperate east Himalayan flora, paleo-arctic flora of Tibetan highland and wet evergreen flora of South East Asia and Yunnan, forming a bowl of biodiversity. The region host a number of botanical curiosities like Sapria himalayana, Nepenthes khasiana and saprophytic orchids like species of Epipogium and Galeola and primitive angiospermic plants like Exbucklandia,
Manglietia, Holboellia, etc. The rich presence of ancient plants like Magnolia, Michelia, Camellia, Rhododendron, orchids etc. in the region indicates that evolutionary development in wild and cultivated plants are continuously taking place (Chowdhery, 2001). Takhtajan (1969) named the region “The cradle of flowering plants” which is one of the richest and most interesting floristic regions of India, with orchids forming a prominent feature of the vegetation.

The region of North-East India is blessed with almost all types of vegetation and has a number of ‘sacred groves or forest’. It is estimated that out of 1229 species of orchids known from India, about 750 to 800 species are found in North-East region of the country (Chowdhery, 1998; Deb et al., 2003; Deb and Imchen, 2008; Hynniewta et al., 2000; Kataki, 1986; King and Pantling, 1898; Kumar and Manilal, 1994; Pradhan, 1979). A comparative analysis of distribution of orchid species within the region shows that maximum diversity is found in Arunachal Pradesh followed by Sikkim and the lowest in Tripura. The region has the highest number of monotypic orchid genera while large number of saprophytic orchid species belonging to the genera Aphyllorchis, Cymbidium, Epipogium, Eulophia, Galeola, Gastrodia, Stereosandra, etc are also present. On the other hand, due to wide altitudinal variation and topographical features supported by favourable climatic conditions, the state is endowed with a rich floristic biodiversity including huge number of orchid species. The habitats of orchids are classified on the basis of different vegetation and forest types. They are: tropical moist evergreen and deciduous forest type (100- 1000 m), subtropical evergreen and semi evergreen forest type (1000-2000 m), temperate and sub temperate forest type (2000-3500 m) and alpine zone (3500-5000 m).

The family Orchidaceae is one of the largest and the most evolved family among the flowering plants with a worldwide distribution comprising about 25,000-35,000
species in some 800 genera (Chowdhery, 2001; Deb et al., 2003; Deb and Imchen, 2008). More new species are being added every year. Orchids are distributed all over the world excepting Antarctica and are found in almost every colour except black. In Australia, two species i.e., *Rhizanthella gardneri* and *R. slateri* are found in underground condition and are extremely rare. They exhibit an incredible range of diversity in shape, size, and colour of their flowers, and are highly valued for their beauty. These wondrous and beautiful plants have been attracting floriculturists since time immemorial and have led to ‘Orchid Mania’ throughout the world. They command a high market value due to their beautiful and long-lasting flowers. In addition to their commercial value, orchids are of considerable importance in medicines, food, and perfumes, while some are reported to have antibacterial activity (Deb et al., 2009).

Based on their varying habits, orchids are classified into saprophytes (without leaves), terrestrial and epiphytes (stem/pseudobulb with leaves). Majority of the orchids are epiphytes and generally found perched on tree trunks. Some grow as terrestrial on land, as lithophytes on rock/stones and as saprophytes on decaying organic materials. Roots help them in anchoring with the substratum of their habitat. The smallest orchid measures only 1 (one) mm across (*Bulbophyllum globuliforme*), while *Vanilla* species can climb the tallest tree in the forest and can be up to 20 m long whereas, *Grammatophyllum speciosum* is the biggest orchid in the world. Orchids are differentiated not only by their flowers but also by their leaves and roots. Orchids are further divided into two types on the basis of vegetative structure and its growth: *Monopodial orchids* which do not have rhizomes or pseudobulbs but grow from single vegetative apex continuously season after season eg: *Aerides, Rhynchostylis, and Vanda*, and *Sympodial orchids* which have a number of vegetative apices situated in the rhizomes, e.g.: *Coelogyne, Dendrobium, Bulbophyllum*. The rhizomes/bulb/pseudobulbs
act as reserved organ and help the orchids to combat the extreme drought conditions faced by epiphytic orchids. Orchids stand distinct in having velamenous roots; zygomorphic flowers with well-developed gynostegium, compound pollen, elaborate perianth and resupinate ovaries; and microscopic and non-endospermic seeds with undifferentiated (reduced) embryos. The orchids are adapted to insect pollination; their flowers flaunt a variety of temptations, i.e. bright colours, a safe landing platform in the form of labellum (lip), a nourishing drink, tantalizing odours and even sex to the pollinators (insects).

Orchids are known to mankind for the last several centuries for their beautiful and attractive flowers and as medicinal plants. It was Theophrastus, a Greek Philosopher, who has mentioned in his writing “Enquiry into Plants”. ‘For the Chinese’ who have been growing orchids for the last 500 years, it is the symbol of Scholar—unassuming, enduring and ascetic. It also stood as a symbol of love, beauty, grace, nobility and elegance in a woman. “Paint bamboo when you are angry, orchids when you are happy” is a well-known Chinese saying. Orchids have adapted themselves to extremes of the environmental conditions producing thereby great variations in vegetative forms and one may often find it difficult to identify them as orchids if they are not flowering. Many orchid flowers resemble in shape of a slipper (Paphiopedilum, Cypripedium), dancing girl (Oncidium, Renanthera), moth (Phalaenopsis), spider (Brassia), scorpion (Arachnis), bee (Ophrys), pineapple orchid (Dendrobium densiflorum), etc. Orchids are popular worldwide due to their marvellous flower architecture and the spectrum of colours, and comprise one of the most successful groups of plants— the Orchidaceae. (370-285 BC) (Chowdhery, 1998).

The North-East region of India is rich in biodiversity, which has played an important role in the economy of the region from ancient times. Unfortunately the plant
Orchids are an important ornamental crop in floriculture industry due to their beautiful foliage, colourful and fragrant flowers of varying shapes, and long vase life of cut flowers. The amenability of these plants to hybridization has been successfully exploited by man to raise novel and striking hybrids in his horticultural pursuits (Vij, 2002, Deb, 2010). This fascinating feature has placed them at the top most position in aesthetic world.

Genetic resources of the region in general and orchid diversity in particular are fast depleting due to indiscriminate felling of forest trees including ground vegetations for ‘slash and burn/shifting cultivation’ together with ruthless exploitation of plants for trade and unplanned human activities. In the recent years, extinction has been the destiny of a great number of plant species including several unique and irreplaceable varieties, while many await a similar fate. Some of the rare and endemic, threatened, endangered orchids of the region are Arachnis flos-aeris, A. labrosa, Aerides odorata, Anoectochilus crispus, Bulbophyllum rotschialdianum, Calanthe ciciliae, Ceratostylis himalaica, Cleisostoma appendicatum, C. filiforme, C. racimeferum, Coelogyne hitendrae, C. suaveolens, C. griffithii, Cymbidium aloifolium, Cymbidium iridioides, C. tigrimum, Dendrobium aggregatum, D. chrysotoxum, D. densiflorum, D. devonianum, D. moschatum, D. nobile, D. williamsonii, Eria alba, Liparis bituberculata, Oberonia clarkii, O. denculata, O. orbicularis, Panisia apiculata, Paphiopedilum hirsutissimum, Peristylus mannii, Pholidota griffithii, Renanthera im schootiana, Taenia latifolia, T. viridi-fusca, Vanda bicolor, V. coerulea, and many more.

Orchids are an important ornamental crop in floriculture industry due to their beautiful foliage, colourful and fragrant flowers of varying shapes, and long vase life of cut flowers. The amenability of these plants to hybridization has been successfully exploited by man to raise novel and striking hybrids in his horticultural pursuits (Vij, 2002, Deb, 2010). This fascinating feature has placed them at the top most position in aesthetic world. This group of plants is highly valued both in the national as well as in the international markets. However, unlike the south Asian countries, India has not been able to make inroads into this multibillion dollar business despite rich natural wealth of orchid diversity (Kumar and Manilal, 1994). So, commercial orchid growing is primarily in the hands of hobbyist and nurserymen, who collect orchids from naturally grown
population, to meet their national and international commitments, adding to conservation related problems (Chadha, 1992).

Although, one of the largest families among flowering plants, the orchids are also probably among the most seriously threatened group of plants. Their vulnerability stems from two factors: the first being their highly specialized nature of germination and growth in association with a specific fungus and pollinator insects, and second being the attractive and beautiful flower of many species, making them so-sought after by man. In recent years though biotechnological means have been adopted for their multiplication in mass scale and a ban has been imposed for their wild collection for trade under Convention on International Trades in Endangered Species (CITES) regulations, through wild life conservation laws, still there are activities of collections from the wild. In developing countries like India, shifting cultivation and continual expansion of agricultural land coupled with deforestation for developmental activities have been a major threat to these plants. Each orchid species is adapted to life in a specialized environment. Because of their specialized requirements many orchids are very restricted in distribution and endemism is high in many cases (Hegde and Sinha, 2002). Habitat destruction and disturbances coupled with lack of ecological awareness of people in general have driven some of the orchids from their natural niches to near extinction. Destruction and fragmentation of forest causes decrease in pollinator population. This results in the low frequency of pollinators visiting the orchid flowers. Nearly 98 per cent of flowering individuals fail to set fruits under natural conditions due to lack of pollinators (Calvo, 1993). Mass propagation using conventional and tissue culture techniques thus seem to be the only strategy to commercialize orchids and conserve their natural populations from collection pressures (Vij, 2002).
The orchid seeds are exceedingly small and non-endospermous with undifferentiated embryos and produced in large numbers and their germination in nature depends upon a suitable association with a mycorrhizal fungus to provide an essential physico-chemical stimulus for initiating germination (Harley, 1959). Ever since Knudson (1922) demonstrated the possibility of bypassing the fungal requirement of orchid seeds during germination in vitro, asymbiotic seed germination has been accepted as an important tool for propagating orchids (Arditti et al., 1982). The orchids are primarily sexual but they also reproduce and propagate by vegetative means as well through seeds. The rate of vegetative propagation (i.e. keikis, back-bulbs, division of shoots etc.) is very slow in many orchid species and seed germination in nature is also very poor (~0.2-0.3%) because of their poorly organized and lack of an appropriate metabolic machinery to utilize their own lipidaceous food reserves, and require a fungal stimulus for germination in nature. The asymbiotic germination potential of fertilized ovules (seeds) has been positively tested in several commercially viable and or threatened Indian taxa (Vij, 2002). But, not all the orchids need the same nutrient composition and response of orchid seeds to physio-chemical factors differs from species to species.

Plant Tissue Culture and Mass Multiplication and Conservation of Rare and Threatened Orchids

Orchids which were earlier thought to be parasites growing on trees are in fact the most advanced group of flowering plants. The orchids are propagated by vegetative means as well through seeds. However, orchid seeds due to microscopic size i.e lack of endosperm and require a special fungal association (mycorrhiza) to germinate in nature. The rate of seed germination, therefore, is very poor, i.e., 0.2-0.3% in nature (Sungkumlong and Deb, 2008). The mycorrhizal association is believed to help in the
carbohydrate/auxin/vitamin transport. Knudson (1922) for the first time demonstrated the possibility of bypassing the fungal requirements during germination of Cattleya seeds in vitro with the supply of appropriate organic carbon in the medium, while Tsuchiya (1954) discussed the possibility of germinating orchid seeds from immature pods. The discovery of these two techniques led to the development of 'green pod culture' that enabled to rescue hybrid embryos from desired mating (Sagawa, 1963). However, it calls for devising protocols for rapid cloning for exploitation of elite hybrids. In vitro cloning of Phalaenopsis using uni-nodal floral stock cuttings was developed by Rotor (1949), whereas Thomale (1957) successfully cultured the shoot tips of Orchis maculata, but the possibility of using aerial roots for micropropagation was first suggested by Beechey (1970). Morel (1960) is credited for mass propagation of virus free Cymbidium clones from apical shoot meristem on Knudson 'C' medium. Shoot tips remain the most commonly used explants for micropropagating cymbidiums and other sympodial orchids but their utility is limited in monopodials as it involves the removal of the only growing apex, which endangers the survival of the mother stock. Endeavors should, therefore, be made toward exploring an alternative but equally effective technique whose excision will not be detrimental to the survival of the mother plant. Different workers have reported regeneration of plants in cultures using different explant sources like shoots, roots, seeds, axillary buds, pseudobulbs, leaf (Deb and Imchen, 2010; Deb and Temjensangba, 2005, 2007a, b; Deb and Sungkumlong, 2010; George and Ravishankar, 1997; Li and Xu, 2009; Nayak et al., 1997; Prasad et al., 2000; Sinha and Hegde, 1997; Temjensangba and Deb, 2005a, b, c, 2006; Vij and Pathak, 1999; Vij et al., 2000) and through callus induction and somatic embryogenesis (Ishii et al., 1998). Biotechnological tools like plant tissue culture techniques have thus opened new possibilities in conservation of threatened/endangered plants.
Many orchid species have been propagated successfully through this technique particularly the threatened orchid species and re-introduced into the wild ameliorating their status in nature. Different explants sources like seeds, leaf, rhizome, roots, inflorescence, etc have been used to propagate in vitro in various parts of the world for conservation programme. Following are some of the works done by various workers: *Aerides multiflora* Roxb. (seeds- Katiyar et al., 1987; foliar segment- Vij and Pathak, 1990; aerial roots- Vij- 1993); *Arachnis labrosa* (seeds- Temjensangba and Deb, 2005a; foliar segments- Deb and Temjensangba, 2007a; aerial roots- Deb and Temjensangba, 2006a); *Cleistostoma racemiferum* (seeds and leaf- Temjensangba and Deb, 2005b, c, 2006; aerial roots- Deb and Temjensangba, 2005); *Coelogyn porrecta* Lindl. (seeds- Abdul and Hairani, 1990); *C. suaveolens* Lindl (seeds- Sungkumlong and Deb, 2008, leaf – Deb and Sungkumlong, 2010); *Cymbidium elegans* Lindl. (seeds- Raghuvanshi et al., 1991); *Dendrobium chrysanthum* Wall. ex Lindl. (seeds- Raghuvanshi et al., 1986); *D. fimbriatum* var. oculatum Hk. f. (D.Don) (seeds- Devi et al., 1990); *D. nobile* Lindl. (seeds- Raghuvanshi et al., 1986); *D. primulinum* Lindl. (seed- Deb and Sungkumlong, 2009); *Eulophia alta* (L.) Fawcett & Rendle (seed- Johnson et al., 2007); *E. hormusjii* Duth. (rhizome segments- Vij et al., 1989); *Haemaria discolor* (Mandarin: Xue-ye-lan or Cai-ye-lan) (seeds- Shiau et al., 2005); *Luisia teretifolia* Gaud. (foliar segments- Vij and Pathak, 1990); *Malaxis khasiana* Soland ex. Swartz (seeds- Deb and Temjensangba, 2006b); *Rhynchostylis gigantea* (immature seeds- Li and Xu, 2009); *Rhynchostylis retusa* (L.) Bl. (seeds- Nath et al., 1991; aerial roots- Chaturvedi and Sharma, 1986; Sood and Vij, 1986; foliar segments- Vij and Pathak, 1990); *Taenia latifolia* Lindl. (seed- Deb and Sungkumlong, 2008; pseudobulb- Sungkumlong and Deb, 2009, leaf- Deb and Sungkumlong, 2010); *Vanda cristata* Lindl. (foliar segments- Vij and Pathak, 1990); *V.
**testaceae** (Lindl.) Reichb. f. (foliar segments- Vij and Pathak, 1990); *Vanda* Kasem’s Delight ‘Tom Boykin’ (aerial roots- Vij and Sharma, 1997).

A wide range of endangered plants including orchids have now been successfully propagated using *in vitro* techniques. There are many reports on *in vitro* multiplication of different types of orchids. Different workers have reported regeneration of plants in cultures using different explant sources like shoots, roots, seeds, axillary buds, pseudobulbs and leaves.

**Seed/Embryo Culture**

The technique is variously referred to as ovule/embryo/green pod/green fruit culture (Sagawa, 1963), which ensures better germination frequency and favours the production of virus free seedlings at a faster rate. Asymbiotic/non-symbiotic seed germination is the most common approach used in the propagation of tropical orchids, which tend to be easier to grow than their temperate relatives. The media used for asymbiotic germination are more complex than that for symbiotic germination, as all organic and inorganic nutrients and organic carbon source must be in a form readily available to the orchid without the intermediary fungus (Mc Kendrick, 2000). The technique involves an easy procedure for sterilization, ensures better frequency of germination, and reduces the time-lapse between pollination, sowing of seeds and production of virus free seedlings. Since all the seed/embryos are used in a single sowing in this technique, it is important to determine the harvest time of capsule or pod for getting optimal germination. The earliest stage at which the embryos can be cultured successfully varies with the orchid genotype and the local conditions. Very young ovules do not form suitable explants in orchids because the embryo sac development is a post pollination phenomenon and fertilization a prerequisite for obtaining seedlings. However as the ovules can be used for raising cultures immediately after fertilization, the
importance of information on time interval between pollination and fertilization has often been stressed (Valmayor and Sagawa, 1967). *Doritis* ovules from pollinated ovaries germinated readily after getting fertilized in vitro (Yasugi, 1984) suggesting that fertilization is a pre-requisite for germination. Yam and Weatherhead (1988) also noted that immature embryo germinates better than the mature ones due to their distended testa cells and metabolically awakened embryos; they also lack dormancy or inhibitory factors. *Arachnis labrosa* and *Cleisostoma racemiferum* embryos obtained between 16 and 18, and 16 weeks after pollination (WAP) respectively (Temjensangba and Deb, 2005a, c, 2006); readily germinate but their germination frequency declines sharply, when obtained from beyond this window period. Likewise, in *Satyrium nepalense*, *Nephalaphyllum cordifolium*, *Phaius tankervilliae* and *cymbidiums*, germination frequency shows sharp decline when the embryos are collected 3-4 weeks prior to fruit dehiscence. The fruit/capsule that develops prominent ridges along the valves and ceases to grow in diameter is considered a useful marker for selecting the right stage for embryo culture (Vij, 1995).

**Meristem Culture**

**Resident Meristem:** The embryo culture produces a great deal of heterozygosity in their progeny in orchids due to its out breeding characteristic. Because of this, it appears to be a disadvantageous proposition in cut-flower industry where pure lines of desired genotypes are preferred. The possibility of using excised shoot-meristem of *cymbidiums* for regenerating complete plant from in vitro was first demonstrated by Morel (1960), whereas Wimber (1963) formulated, described and published a procedure for the purpose. This technique of using resident meristem (shoot-tips, axillary bud) has opened new vistas in orchid micropropagation (Arditti and Ernst, 1993; Deb and Temjensangba, 2005, 2006a). Through this technique, upto 200,000 plants can be regenerated from a
single resident meristem within a year. However, it has limited utility in monopodial taxa as it involves the sacrifice of the growing tip thereby, endangering the survival of the mother plant.

**Adventive meristems:** The ability to use an adventive meristem is advantageous as it does not endanger the survival of mother plant. The regenerative competence or the proliferative potential has been positively tested in many orchid taxa, viz: leaf explants (Chaturvedi and Sharma, 1986; Deb and Temjensangba, 2007a; Deb and Sungkumlong, 2010; Mathews and Rao, 1985; Seeni, 1988; Seeni and Latha, 1992; Temjensangba and Deb, 2005b; Vij et al., 1984; Vij and Pathak, 1988, 1990), root (Chaturvedi and Sharma, 1986; Deb and Temjensangba, 2005, 2006a; Sood and Vij, 1986; Vij, 1993; Vij and Pathak, 1988); flower stalks (Kaur and Vij, 1995: Singh and Prakash, 1984; Vij et al., 1997). The source, genetic constitution and physiological age of the explants are however, some of the important factors for regeneration. The juvenile tissues from greenhouse grown plants respond better than the mature ones grown outdoors. Generally, the proliferative loci get activated in the sub-epidermal cells and soon develop into somatic embryos and or Protocorm-like-bodies (PLBs). Somatic embryogenesis is either direct or callus mediated development, and multiplication and differentiation of the PLBs is influenced by the chemical stimulus present in the nutrient pool (Seeni and Latha, 1992; Vij and Pathak, 1990).

The advantages of leaf and root segment culture are apparent for more than one reason: they are easy to obtain, easier to disinfect, and their excision does not endanger the mother plant. Furthermore, as the regeneration occurs in the dermal cells, which is cytologically more stable, mass production of genetically uniform plant from this is within the realm of reality (Vij, 2002).
Different species of orchids exhibit specific needs in respect to nutritional requirement and treatment with plant growth regulators (PGRs) for their growth and development. So, no standard media formulation can be prescribed for all the species. Most commonly employed basal media for orchid tissue culture are Knudson ‘C’ (1946), Mitra et al. (1976), Murashige and Skoog (MS) (1962), Nitsch and Nitsch (1969), Vacin and Went (1949). The use of α-Naphthalene acetic acid (NAA) and one of the cytokinins like Benzyladenine (BA) and kinetin (KN) yields a rich crop of PLBs in *Luisia trichorrhiza*, *Satyrium nepalense*, *Vanda cristata* and *Vanda testaceae* leaf segment culture (Vij, 1995). Similarly, in *Rhynchosytilis retusa*, a synergistic action of KN and indole 3-acetic acid (IAA) or NAA in peptone enriched medium favours enhanced production of PLBs; while yeast extract (YE) is obligatory for regeneration in *Aerides multiflorum*, *Papilionanthe teres* and *Satyrium nepalense* foliar cultures and peptone in those of Vanda (Vij, 2002).

**Plant Tissue Culture Media and Substratum**

Since its introduction as a gelling agent for microbial cultures more than 100 years ago, agar has been extensively used as gelling agent for microbial as well as plant tissue culture media (Babbar and Jain, 2006). Agar is useful for the purposes due to its stability, high clarity, non-toxic nature and resistance to its metabolism (Babbar and Jain, 2006; Henderson and Kinnersley, 1988; McLachlan, 1985). In the recent past several attempts have been made to look for some suitable substratum which can replace agar in the plant tissue culture media as well as microbial culture because of doubts about its inertness and non-toxic nature, fear of over-exploitation of its sources and above all the exorbitant price of tissue culture and bacteriological grade agar (Arnold and Ericksson, 1984; Babbar and Jain, 1998, 2006; Debergh, 1983; Jain and Babbar, 2002; Kohlenbach and Wernicke, 1978; Singha 1984; Zimmerman et al., 1995). During the last two
decades, a number of substances viz. agarose (Johansson, 1988), alginate (Scheurich et al., 1980), gelrite (Pasqualetto et al., 1988), isubgol (Babbar and Jain, 1998), starch (Nene et al., 1996; Zimmerman et al., 1995) etc. have been used with reasonable success as substitutes of agar. But these are not expected to find universal acceptance, for various reasons. Alginate gel only in the presence of specific ions and therefore are not suitable substitutes of agar, while agarose is cost prohibitive. Starch is not expected to find universal acceptance because of its inferior gelling ability, poor clarity and metabolizable nature, which leads to softening of the media. Isubgol, due to its polysaccharide nature, good gelling ability, resistance to enzymatic activity, and gel clarity it has a good potential to become a universal gelling agent for plant tissue culture media. But its high melting point (−70°C) necessitates adjusting of pH and fast dispensing (Babbar and Jain, 2006). But use of these gelling agents does not help in substantially reducing the production costs.

**Objectives of the Study**

The loss of primary forest in North-Eastern part of India is primarily due to practice of primitive form of agriculture commonly called “Jhum”, fragmentation of forest and unplanned developmental activities. As a result, many important plant species including orchids are under threat or on the verge of extinction before their commercial importance are being explored. Therefore, it calls for an immediate intervention and develop an alternate route to preserve the threatened and endangered plant species particularly orchids. A biotechnological tool like tissue culture technique comes in handy for successful achievement of the objectives. Plant tissue culture technique is in general a costly technique and faces difficulties in universal acceptance as a tool for commercial scale production of plants of horticultural/economic importance and *in vitro* conservation of rare and threatened taxa. To popularize the plant tissue culture technique
Figure 1: Selected orchids showing vegetative parts with flowers. a. *Cymbidium aloifolium* (L.); b. *Cymbidium iridioides* D. Don.
as a tool for commercial scale production of economically important plants and *in vitro* germplasm conservation, it is necessary to develop cost effective protocols.

With a view to mass multiply and conserve two rare/ and threatened orchids of North-East India and develop cost effective protocols, I had proposed to work on the following two economically important but over-exploited orchid species viz., *Cymbidium aloifolium* (L.) Sw. and *Cymbidium iridioides* D. Don of North-East India for my Ph. D. degree, with the following objectives:

1) *Initiation of cultures from various explants like immature embryos of various developmental stages, leaves, nodal explants and roots from in vitro raised plants.*

2) *Rapid mass multiplication of the selected species.*

3) *Screening of some low cost substratums as alternative to agar for initiation of cultures and mass multiplication of the selected orchids with an objective to reduce the production cost.*

4) *Reintroduction of in vitro raised plants in their natural habitats.*

**A Brief Account of the Two Selected Orchids**

1. *Cymbidium aloifolium* (L.) Sw. (*Orchidaceae*): It is an epiphytic herb which grows on the tree trunks in huge clumps. They are mostly found in the tropical forests. Pseudobulbs are ovoid, short, 6-9 x 3-4 cm, bilaterally flattened, sheathed. Leaves many, up to 60 cm long, linear, oblong, apex obliquely bilobed. Inflorescence raceme, arises from the base of the pseudobulbs, pendulous, many flowered. Flowers 3 cm across, yellowish with purple mid rib. Sepals oblong-lanceolate. Petals linear-oblong, obtuse. Lip whitish with maroon or purplish streaks; 3-lobed, side lobes oblong, obtuse; mid lobe ovate, acute, and reflexed; 2 yellowish lamellae; column purplish. It flowers in the months of April-August and last for about 25-30 days. *(Fig. 1 a).* The flowers have good
commercial value as cut flowers. Besides floricultural importance, the entire plant is used as purgative, emetic, tonic and in treating ear-ache. Due to its multipurpose use, the plants are ruthlessly collected from its natural habitat and the population is under threat.

2. Cymbidium iridioides D. Don (C. giganteum Wall. ex Lindl.) (Orchidaceae): This is an epiphytic or lithophytic, perennial herb. Pseudobulbs 6-7 cm long, broad, sheathed. Leaves up to 60 cm long, linear, lanceolate, base sheathing. Inflorescence many flowered raceme, arched. Flowers 7-8 cm across, brown with red streaks. Sepals and petals linear-oblong. Lip yellowish brown, side lobes reddish brown striped, ovate, acute; mid lobe yellowish with reddish brown transverse blotches, ovate-oblong, margins undulate; lamellae 2, terminating above the base of the mid lobe. Column yellowish brown. The species is predominantly found in North-east India. It flowers during the months of October-November. (Fig. 1 b). The flowers have high commercial value in cut flower industry due to its long lasting flowers. This species is under threat as a result of over-exploitation and indiscriminate destruction of forest cover to meet the demands of ever increasing populations and removal of ground vegetation and litters by forest fires for ‘Jhum/Slash and Burn cultivation’.