2. REVIEW OF LITERATURE

2.1. Orchids

Orchids have a long history traceable back to the ancient Greeks. Their earliest mention appeared in book ‘Enquiry into Plants’ written by Theophrastus (370-285 B.C.) who referred to a group of plants having paired testiculate tubers as *Orchis* (Singh, 2001). Swartz (1800) was the pioneer to propose a classification system for these plants; he also reported the occurrence of monandrous and diandrous conditions in orchids. Lindley did extensive work on documentation of orchids in different continents and in 1826 proposed a classification scheme by dividing orchid family into 8 tribes; he is therefore known as ‘Father of Orchidology’. A number of classification systems were purposed thereafter but the major accepted ones were of those by Dressler (1981, 1993), Szlachetko (1995) and Cameron *et al.* (1999). Most of the above listed systems agree with one another in broader outline, but differ with respect to the systematic position of several taxa mainly due to reticulate pattern of continuous morphological variability met in them.

Presently, more than 26,500 species of orchids are on record (Govaerts *et al.*, 2013) from across the globe. However, as interspecific and/or intergeneric crosses can be made with ease in orchids, this has resulted in development of thousands of artificial hybrids in this group. The successful development of the first man made orchid hybrid of *Calanthe* (*Calanthe masuca* × *Calanthe furcata*) by John Dominy, which dates back to 1852 (flowered in 1856) proved a turning point in the history of orchid commercialization. Today with more than one million registered hybrids, the orchid floriculture is a multimillion dollar industry in the world (Chowdhery and Agrawala, 2013). The hybrids outclass the parental species in their floral excellence and are the mainstay of the orchid based floriculture.

Orchids grow in a wider range of habitats where their growth is influenced by the physical, chemical and biotic factors. Nearly 70% species are epiphytic (Zotz, 2013) and are distributed mainly in tropical and subtropical climates. The epiphytes have well developed velamenous roots and fleshy leaves as contrivances for
absorption and storage of atmospheric water. Presence of thick cuticle, water storage cells and sunken stomata are additional features associated with water conservation and storage in epiphytes (Kaushik, 1983). The terrestrials, on the other hand, possess specialized organs like rhizomes, tubers, fleshy roots, and pseudobulbs to store a plenty of water and food, and to perennate through unfavourable periods.

Orchids were first discovered for their therapeutic values and many species are still used as folklore remedies against a variety of human ailments. Several orchids are used in ayurvedic formulations for their rejuvenating and restorative properties (Hossain, 2011). It is, however, the exquisite quality of blooms of their long-lasting flowers for which they are appreciated and revered the world over. Though the cultivation of orchids has evolved from a hobby to a highly lucrative vocation in many countries, their commercial farming is still in its infancy in India due mainly to lack of proper planting material and cultivation procedures (Vij and Pathak, 2012).

### 2.2. Historical account of Indian orchids with special reference to study area

Though ‘Charaka Samhita’- a classic Indian medicinal treatise (around 100 A.D.), has been found to contain notes on medicinal importance of some orchids (Singh, 2001), the first scientific account of Indian orchids comes through Van Rheede’s 12-volume classic, ‘Hortus Malabaricus’ published during 1678-1693. The subsequent taxonomic works on Indian orchids (Roxburgh, 1832; Griffith, 1851; Lindley, 1857, 1858; Drury, 1869; Aitchison, 1869; Atkinson, 1882) culminated into the monumental work of Sir J. D. Hooker (1890, 1894), which included descriptions of some 1200 species belonging to 113 genera from the erstwhile British India. King and Pantling (1898) presented well illustrated descriptions on Sikkim Himalayan orchids (448 species in 91 genera). Duthie (1906) made a detailed study on the orchids of northwestern Himalaya and recorded 173 orchid species from here. Many regional floras like that of Shimla (Collett, 1902), Bengal (Prain, 1903), Presidency of Bombay (Cooke, 1906), Nilgiri and Pulney Hilltops
(Fyson, 1914), Travancore (Rao, 1914), Presidency of Madras (Fischer, 1928), Lahore district (Kashyap, 1936), Bombay (Santapau and Kapadia, 1966), Punjab Plains (Nair, 1978), Kashmir Himalaya (Dhar and Kachroo, 1983), Himachal Pradesh (Chowdhery and Wadhwa, 1984), Haryana (Jain et al., 2000; Kumar, 2001), and Cold Deserts of western Himalaya (Murti, 2001), contributed significantly towards the knowledge of orchids in these regions. Bose and Bhattacherjee (1980) described 834 orchid species from the country and outlined the cultural notes for many of these. Jain and Mehrotra (1984) presented a preliminary inventory of Indian orchids, listing about 925 species under 144 genera. Sathish Kumar and Manilal (1994) catalogued 1141 species in 166 genera. Comprehensive accounts of orchids of northwest Himalaya (Deva and Naithani, 1986; Chowdhery and Agrawala, 2013), Arunachal Pradesh (Chowdhery, 1998), Kerala (Sathish Kumar and Manilal, 2004), Nagaland (Hynnieeta et al., 2000), Manipur (Sathish Kumar and Suresh Kumar, 2005), Meghalaya (Kataki, 1986), Nilgiris (Joseph, 1987), Sikkim (Lucksom, 2007) Karnataka (Swamy et al., 2004) and Orissa (Misra, 2004), have brought to the fore the orchid wealth of the respective regions. Singh (2001) presented an overview of orchid diversity (1229 species) in India, highlighting their phytogeographical affinities, endemism, economic importance, and conservation status. Misra (2007) has authenticated the occurrence of nearly 1331 orchid species in the country.

The earliest collection of orchids from northwestern Himalaya was probably made by Major General Thomas Hardwick in 1796. Later, Royle (1839) made extensive collection of plants including orchids from Kashmir to Garhwal and Himachal Pradesh. Hooker (1890, 1894) in his ‘Flora of British India’ listed 33 species in 19 genera from areas comprising Himachal Pradesh. Collett (1902) described 38 orchid species from Shimla and adjacent hills and provided drawings for six of these. Duthie (1906) reported the occurrence of 46 orchid species from the state. Nair (1977) described 43 orchid species from Bashahr province. Vij et al. (1982) listed as many as 54 species from Shimla and adjacent hills. Chowdhery and Wadhwa (1984) described 53 species under 23 orchid genera in their ‘Flora of Himachal Pradesh’. Later, Deva and Naithani (1986) published Orchid Flora of northwest Himalaya
wherein out of a total of 239 species, 64 were reported to occur in Himachal Pradesh. Dhaliwal and Sharma (1999) included 16 orchid species from Kullu district, and Singh and Rawat (2000) provided notes on 16 orchids found in The Great Himalayan National Park, Kullu. Kaur and Sharma (2004) recorded six species from Sirmaur. Subsequent additions were made to orchids of Himachal Pradesh by Vij and Verma (2005, 2007a, b), Lal and Rawat (2008), Lal et al. (2008, 2010a, b), Vij et al. (2008), and Agarwala and Lal (2012). All of these reports finally culminated into the recently published comprehensive account of Himachal Pradesh orchids (Vij et al., 2013); it reported the occurrence of 85 orchid species in the state under 44 genera. More recently, another orchid species (Neottia pinetorum) has been added by Singh et al. (2013) from Kullu district.

2.3. Population analysis and associated vegetation

The importance of demographical data has been strongly advocated for obtaining an accurate picture of population size and conservation status of a plant group in any geographical area (Coates et al., 2006). Quantitative information also plays a vital role to help formulating effective conservation plan for a particular area (Uniyal et al., 2002). Orchid species are not distributed uniformly in various habitats due to their higher specificity towards varied microclimates (Samant, 2002). Hence it is desirable to study various population parameters (density, abundance, frequency) of the species across different habitats. Many studies have been carried out in different regions of the world to document orchid species diversity and their associated vegetation (Wells, 1967; Catling et al., 1986; Gentry and Dodson, 1987; Bowles et al., 1992; Zimmerman and Olmsted, 1992; Gillman and Dodd, 1998; Ackerman and Ackerman, 2001; Wolf and Flemenco, 2003; Montero et al., 2005; Brzosko et al., 2006; Hietz et al., 2006; Bulafu et al., 2007; Huang et al., 2008; Islam and Huda, 2008; Landi et al., 2009; Mageto et al., 2013).

Literature on population studies of Indian orchids is, however, scanty. Annaselvam and Parthasarathy (2001) made observations on diversity and distribution of some herbaceous epiphytes in Western Ghats. Sinu et al. (2011) assessed the epiphytic orchid diversity in Western Ghats. There are a few reports of population and habitat studies on western Himalayan orchids. Dhar and Samant (1997) made observations
on structural diversity of forest vegetation in Kumaun Himalaya. Kumar et al. (2004) studied the community structure of forests in Garhwal Himalaya. The conservation status of Dactylorhiza hatagirea was studied by quadrat based data by Sharma et al. (2005) and Giri et al. (2008). Kharkwal et al. (2005) investigated species richness in some oak forests of Nainital region. Bhatt et al. (2005) also studied the population details of Dactylorhiza hatagirea in some protected and non protected areas of northwest Himalaya. Species richness of Habenaria intermedia was investigated by Chauhan et al. (2007) in Uttarakhand. The abundance and distributional details of orchids in Gori Valley was assessed by Jalal et al. (2008). Jalal and Rawat (2009) also studied habitat types and population structure of medicinal orchids of Uttarakhand. Species diversity and conservation status of orchids of Askot wildlife sanctuary were documented by Samant (2009). Rai et al. (2012) investigated the community structure along a timberline ecotone in western Himalaya and correlated their findings with micro-topography and habitat disturbances. Density and frequency of Satyrium nepalense was assessed by Mishra and Saklani (2012). Dad and Khan (2013) studied the conservation status of five threatened medicinal plants in high altitude grasslands of Kashmir and found Dactylorhiza hatagirea to show lowest frequency. Population assessment and habitat categorization of Malaxis acuminata was investigated by Lohani et al. (2013) in northwestern Himalaya.

In Himachal Pradesh, several reports are available on orchid species diversity (Vij et al., 2013). But studies related to their population structure are rather limited; there has been only a little work done for evaluation of orchid species richness (abundance, frequency, density) in the state. Vij et al. (1998) provided information on substratum analysis and distribution pattern for orchids of Shimla and adjacent regions. Rana et al. (2008) made an ecological evaluation of 24 orchid species in Kullu district by using quadrat method; population density was found minimum in Calanthe tricarinata and maximum in Malaxis muscifera. Orchid diversity at Bandli wildlife sanctuary (Mandi district) was studied by Verma et al. (2011), and the highest density was recorded in case of Habenaria marginata. More recently, Marpa and Samant (2012) made observations on diversity and conservation status of
orchids in and around Prashar sacred shrine documenting as many as 16 species but without any population analysis.

2.4. Threats and conservation

Orchids are facing higher levels of threats to their survival due to various anthropogenic forces. They are at the front-line of extinction with more species under threat globally than any other plant family (Kull et al., 2006). Habitat destruction has played havoc with orchid populations around the world (Swarts and Dixon, 2009). Change in land use patterns which cause transformation of natural habitats for various purposes is one of the key factors responsible for loss or change in biodiversity in various ecosystems (Gonzalez et al., 2011). The widespread deforestation has lead to shrinkage of orchid populations, and according to Pradhan (1975), if it goes on continuing with same pace, many of the species may vanish even before their existence and biological importance will be established. Salazar (1996) also demonstrated that destruction, modification and fragmentation of natural forests hasten the local extinction process in orchids along with their heavy illegal extraction from wild. According to Srivastava (2010), loss of habitat, grazing, collection of fuel wood and herbs, and land and snow slides are some of the main threats to natural plant populations in western Himalayan region. Requirement of unique habitats and specific pollinators are the main drivers of rarity in majority of the orchid species.

Speaking internationally, the whole Orchidaceae have been placed in Appendix II of CITES where the international trade is controlled to ensure their conservation. In India, a number of orchids have become threatened due to their over exploitation and habitat destruction. Though several studies have been carried out on exploring orchid flora in various regions of the country, the data on extant of species distribution and conservation is still inadequate. The information available on conservation of Indian orchids is quite fragmentary and can be seen often appended to some of the books/ research papers dealing with other aspects (cytology, distribution, morphogenesis, taxonomy, embryology, etc.) of orchid biology. Katak (1976) listed 35
species of Indian orchids, which were found to be scarce or near extinct in their natural localities at that time. Ten Indian species (9 species of *Paphiopedilum, Renanthera imschootiana*) find mention in Appendix I of CITES and their export has been completely banned. As many as 43 species of Indian orchids (*Anoectochilus nicobaricus, A. rotundifolius, A. tetrapertus, Aphyllorchis gollani, Archineottia microglossis, Bulbophyllum acutiflorum, B. alboidum, B. Aureum, B. elegantulum, Coelogyne mossiae, C. treutleri, ChrysoGLOSSum halbergei, Corymboskis veratrifolia, Cymbidium eburneum, C. hookerianum, C. whiteae, Cypripedium elegans, C. himalaiacum, Didiciea cunninghamii, Diplomeris hirsuta, D. pulchella, Eria occidentalis, Eulophia mackinnonii, Flickingeria hesperis, Habenaria barnesii, Ipsea malabarica, Liparis biloba, Malleola andamanica, Paphiopedilum druryi, P. fairrieanum, P. insigni, P. specerianum, P. venustum, P. villosum, P. wardii, Phalaenopsis speciosa, Pholidota wattii, Pleione lagenaria, Renanthera imschootiana, Taeniophyllum andamanicum, Vanda wightii, Vanilla wightiana, Zeuxine pulchra*) have been listed in first volume of Indian Red Data Book (Nayar and Sastry, 1987). Subsequent publications by these authors included 17 species (*Aphyllorchis parviflora, Bulbophyllum kaitiens, Bullerjia yunnanensis, Calanthe alpina, C. anthropophora, C. mannii, C. pachystalix, Coelogyne rossiana, Corybas purpurens, Cymbidium tigrinum, Cypripedium cordigerum, Dendrobium arachites, D. aurantiacum, Eria albiflora, Eulophia nicobarica, Neottia inayattii, Oberonia brachyphylla*) in volume 2 (Nayar and Sastry, 1988) and five species (*Dendrobium tenuicaule, Habenaria andamanica, H. pandhganianensis, Paphiopedilum hirsutissimum, Vanda coerulea*) in its volume 3 (Nayar and Sastry, 1990). Out of these species, about 13 are on record from western Himalaya (Jalal, 2012); two of these (*Cypripedium cordigerum, C. himalaiacum*) also occur in present study area. Jain and Hajra (1976) highlighted the possible role of national parks and sanctuaries in orchid conservation. As many of the orchid species are habitat-specific, the preservation of their genetic stock necessitates the preservation of their habitats (Arora and Mukherjee, 1983). According to Cribb *et al.* (2003), where ecological specialization has contributed to the great species diversity in Orchidaceae, it is also been responsible for triggering the high level of threat in this family. The conservation of natural homes by establishment of protected areas and
rehabilitation of the endangered species in germplasm centres are important approaches to conserve orchids (Hore and Sharma, 1990). The Government of India has now imposed a complete ban on collection and sale of orchids from their natural abodes.

Arditti (1992) highlighted the role of local Orchid Societies in conservation and suggested that in addition to imposing ban on advertisements for the sale of wild collected orchid plants, they must exclude such orchids from exhibits/judging. The Orchid Society of India, Chandigarh is playing an important role in generating awareness on sustainable utilization and conservation of orchids in the country.

2.5. Soil analysis

Orchids are sensitive to changes occurring in and around their external surroundings. According to Sylvain and Wall (2011), many factors including the physical and chemical properties of soil, climate, the composition and type of vegetation govern the biogeography of soil biota. Environmental factors like available space, exposure to light, temperature, moisture and micorrhiza, act together in determining the type of orchid habitat rather than any one of the factors, however favourable that may be (Misra, 1995). The occurrence and distribution of orchids has been found to be influenced by a multitude of factors such as latitude, altitude, soil types, climatic conditions, atmospheric humidity and temperature (Pieris, 1968). While the epiphytes do not directly depend upon available ground resources, the soil conditions (aeration, pH, minerals, water content, texture and structure, etc.) directly influence the germination, growth and development of terrestrial orchids (Rasmussen, 1995). Focho et al. (2010) demonstrated that the initial orchid establishment is the most crucial step that depends upon altitude, root establishment and presence of lower plants that aid to trap their seeds; the species can later thrive well with a minimum amount of nutrients in soil. Vij et al., (1998) conducted studies on substratum analysis of Shimla hill orchids, and suggested that the soil requirements of the species are different from each other.
Because of their highly attractive flowers, the diversity, morphology, phenology, etc. of orchids have been studied more intensively than those of other plants (Schlegel et al., 1989). However, many of characteristics where the orchids prefer to grow still await proper documentation. Abiotic site conditions, such as the soil water relations and nutrients affect plant growth directly or may determine the strength of competitors (Chesson and Huntly, 1989). According to Sieg and Bjugstad (1994), different species of Poa and Euphorbia were found to be of common occurrence in soils supporting orchid populations at Sheyenne National Grassland. Studies to document soil characteristics and associated plant species in orchids have been conducted by many authors (Sheviak and Bowles, 1986; Bruckner, 1997; Caron and Nkongolo, 1999; Jorg, 2000; Stancato et al., 2002; Naik et al., 2006; Wang, 2007; Bichsel et al., 2008; Maciejewska-Rutkowska et al., 2008; Duffy et al., 2009; Rodrigues et al. 2010; Mroz and Kosiba, 2011; Ors et al., 2011; Tsiftsis et al., 2008, 2012). Soil characteristics are of utmost importance to rehabilitate orchids from degrading habitats to some newly created ones (Stewart, 1987; Kusum et al., 2013).

The terrestrial orchids have much pronounced associations with mycorrhizal fungi for seed germination and growth (Curtis, 1936; Warcup, 1973; Clements and Ellyard, 1979; Clements et al., 1986; Rasmussen, 1995). During adult phase, majority of orchids become photosynthetic but the leafless taxa (mycoheterotrophs) have to depend on their mycorrhizal partner for carbon nutrition throughout their life (McKendrick et al., 2000). Several soil characteristics have been reported to play important role in colonization of mycorrhizal fungi in orchid habitats (Sheviak, 1974; Ramsay et al., 1986; Masuhara and Katsuya, 1994; Taylor and Bruns, 1999). Physical and chemical soil properties (especially pH, base concentration and available moisture) strongly influence the survival of different plant species including orchids (Larcher, 1975; Orozco et al., 1997). These soil characteristics may vary in response to the regional and the vegetation difference, and therefore affect the establishment and distribution of various orchid species (Stuckey, 1967). Where majority of orchids thrive in soils with pH ranging between 6.0 and 7.0 (Knudson, 1945; Ito, 1955; Raguvanshi et al., 1985), some are found to display a even bimodal distribution with respect to pH and nutrient concentrations (Sheviak, 1974; Neimann, 1975; Sheviak, 1983; Case, 1987). According to Knudson (1951), the pH value is critical only during
early stages of orchid germination; the seedlings, however, are less sensitive to its fluctuations. Moisture holding capacity of soil influences the occurrence of orchids in various ways; *Platanthera leucophaea* was found to be highly sensitive to drought (Bowles *et al*., 1992). Bowles *et al*. (2005) further investigated the relationship between soil characteristics, distribution and restoration of *Platanthera leucophea* in its natural habitats and found pH and various other soil parameters to affect its survival. Adhikari *et al*. (2012) studied the impact of micro-cite conditions on epiphytic orchids of Nepal and found bark rugosity and pH as important factors for their growth and distribution. According to Davidson (1960), the mineral content in soil is more critical for terrestrial orchid growth as compared to pH.

Soil texture is an important physical parameter of soil which is based on its particle type and size (silt, clay, sand). In a study conducted by Sieg and Ring (1995) in orchid habitats, average percentage of sand particles was found to range from 74.7 to 81.1, silt from 7.5 to 9.8 and clay from 11.4 to 16.1. The organic matter percentage ranged between 4.1 and 4.6 in normal and 1.7 in case of grazed areas. Sandy and sandy loam soils are better aerated and have poor water holding capacity when compared with loamy soils (Buckman and Brady, 1971), and such soils have been found to be better suited for growth of orchids having tubers or pseudobulbs as water storage strategy (Vij *et al*., 1998). Soil moisture is another important driver of many above and belowground ecological processes (Stephenson, 1998; Churkina *et al*., 1999; Weltzin and McPherson, 2000; Urban *et al*., 2000; McKenzie *et al*., 2003; Xiao and Moody, 2004). Precipitation patterns and available moisture are likely to change under various climate change scenarios (Harte and Shaw, 1995; Gordon and Famiglietti, 2004). In addition to high rains, soil moisture and forest fires have been reported to influence the dormancy and flowering initiation in orchids (Sheviak, 1974; Bowles, 1983; Mehrhoff, 1989; Calvo, 1990; Falb and Leopold, 1993; Sieg and Ring, 1995). Presence of leaf litter maintains higher moisture levels in soil; it also adds good amount of organic matter on which soil fungi (including orchid mycorrhiza) depend (Garrett, 1956; Hutchings, 1989). The low organic matter content, on the other hand, reflects low soil moisture content (Sieg and Ring, 1995) and depicts the interdependency of both the parameter with each other.
Orchids require less fertilizer because of their slow growth (Scully, 1951). According to Stoutamire (1974), orchids have an ability to adapt in varied nutritional regimes and sometimes even capable of surviving under nutritionally marginal conditions. Poole and Seeley (1978) investigated the mineral (N, K, Mg) requirements in three orchids genera (*Cymbidium, Phalaenopsis, Cattlea*) and found nitrogen concentration to be the most important factor determining growth in all of these. Poole and Sheehan (1982) further suggested that nutrient requirement of orchids generally vary from genera to genera. At higher nitrogen concentration, an increase has been recorded in leaf number as well as number, length and width of pseudobulbs (Miwa and Ozaki, 1975). Though nitrogen rich substrates are often conducive for orchid growth (Sheehan, 1961), its very high amount in soil has been reported to cause root injury in them (Wang, 1998). Penningsfeld and Fast (1970, 1973) hinted at the low requirement of orchids for phosphorous. Potassium at varied levels was shown to have a little effect on growth and development of some orchids (Miwa and Ozaki, 1975). The study conducted on requirement of N, P, and K for *Dendrobium* (Bichsel *et al*., 2008) revealed that the requirement of these elements vary from species to species. Potassium and organic carbon were related to soil structure and texture properties, and thus are indirectly rather than directly related to seed germination in orchids (Garrett, 1956); the former often relates to the presence of more clay in soil that affects soil water holding capacity (Batty *et al*., 2001). An exogenous application of potassium is not a limiting factor for orchid growth as it can be mobilized from older tissues and reutilized to meet the growth requirements of new organs (Davidson, 1960). According to Poole and Seeley (1978), the vegetative growth of orchids is quite satisfactory at low potassium concentration and is not affected much by its different levels. Total availability of soluble salts to the ground growing plants is directly correlated with the soil electric conductivity. The size and frequency of orchid populations has been found to be inversely proportional to the soil EC (Khanna and Yadav, 1979; Vij *et al*., 1998) suggesting thereby that high concentration of salts is not conducive for their growth and development.

**2.6. Seed characteristics**

Orchid seeds are the smallest in plant kingdom. They are produced in such a large number that if all of them manage to germinate, the jungles on earth will be
converted into thick orchid mats with beautiful colour combinations. Because of their minute size, the seeds are extremely light weighted; the heaviest seeds weigh just 14-17 µg in species of *Galeola*, while they were the lightest (0.3-0.4 µg only) in *Anguloa* spp. (Arditti and Ghani, 2000). Cellular organization of seeds is also very simple; they are just an undifferentiated mass of embryonal cells that is enclosed within a more or less transparent seed coat (Arditti *et al.*, 1979). Despite of their dust-like nature and so simple organization, a great deal of diversity is met in size, shape and structure of orchid seeds. Barthlott and Ziegler (1981) recognized 20 different seed types in orchids based primarily upon their shape, relative elongation of seed coat (testa) cells, cell wall sculpturing and presence of intercellular gaps and beadings. Seed characteristics are quite conservative than vegetative and floral ones (Chase and Pippen, 1988), and therefore are of good taxonomic, phylogenetic and phytogeographic importance in orchids (Clifford and Smith, 1969; Barthlott, 1976; Healey *et al.*, 1980; Molvray and Kores, 1995; Arditti and Ghani, 2000; Aybeke, 2007; Chemisquy *et al.*, 2009; Akcin *et al.*, 2010; Gamarra *et al.*, 2008, 2010, 2012). Also, as seeds are directly responsible for regeneration and distribution of species, they play an important role in orchid conservation.

Depending on the dispersal capacity of seeds and nutritional and microclimatic requirements for their germination, orchids experience different distributional patterns across the Himalayan range. The Western Himalaya supports more of ground growing species whereas the epiphytes dominate in its Eastern part. Despite of their rich diversity in the Himalaya, only a few orchids have so far been studied for their detailed seed morphometric characteristics (Garg *et al.*, 1992; Vij *et al.*, 1992; Rani *et al.*, 1993; Sharma *et al.*, 2004; Verma *et al.*, 2012).

2.7. Polymorphic species and biochemical studies

Systematic position of several orchid taxa stays quite controversial due to morphological variability resulting through free flow of genes across the specific limits. Such a morphological variability, though a boon for commercial growers and hobbyists, poses a challenging situation for orchid taxonomists. Assessment of genetic variation and its partitioning within and
between populations is necessary for formulating effective conservation strategies. Many orchid species exhibiting varied levels of polymorphism are available in Himachal Pradesh, and it is highly desirable to solve these taxonomic hurdles by employing some taxonomic marker(s) other than gross morphology. Polymorphism in any taxon occurs due to certain genetic or environmental differences or both. However, when different morphotypes are sympatrically distributed, it hints towards the possible genetic variations among them.

Electrophoretic analysis of isoenzymes has been used successfully to provide rapid and quantitative estimation of the extent of genetic variation between and within species of animals (Powell, 1975), fungi (Micales et al., 1988; Sariah, 1988; Bonde et al., 1993; Aly et al., 1996, 2003; Sharma et al., 2004; Adhikary et al., 2006; Siddiquee et al., 2010; Abdel- Fatah et al., 2013) and angiosperms (Kuhns and Fretz, 1978; Loveless and Hamrick, 1984; Mowrey et al., 1990; Aoki and Hattori, 1992; Zoro et al., 1999; Paisooksantivatana et al., 2001; Philomina and Surendran, 2003; Guma et al., 2006; Patial et al., 2007; Colich et al., 2009; Hammad, 2009; Kubiak, 2009; Fernandez et al., 2011; Padmanaban et al., 2013). The term isoenzyme was proposed by Markert and Moller (1959). Isoenzymes (also called isozymes) are defined as the multiple molecular forms of a protein that usually have similar enzymatic properties, but differ in their physical characteristics such as molecular mass, electrical charge, shape, structures, etc. (Buth, 1984). They can be identified from different tissues and they offer one of the most reliable single gene markers (Bryne, 1989; Weeden and Wendel, 1989). The polymorphism at enzyme loci has been shown to be stable under varied environmental conditions (Arulsekar and Parfitt, 1986). The utility of isozymes as genetic marker (Cheniany et al., 2007) has generally been attributed to their polymorphism, codominance, simple inheritance, simple assay and obliquity in plant tissues or organs (Simpson and Withers, 1986). Therefore, isozymes of a particular molecular weight can be considered as a direct manifestation of the blue print of the specific gene loci (Abiden and Vijayakumar, 2002).

Tanksley and Orton (1984) stressed upon use of isozymes in genetics and breeding of higher plants. They have also been successfully used to investigate genetic relationships among various orchid taxa such as Cypripedium spp. (Case, 1994),