The biodiversity in a given area is markedly influenced by climatic changes and pressures from various anthropogenic activities. While some of the species tend to succumb to the anthropogenic forces, others occasionally change their distribution pattern by extending the range to their occurrence to some adjacent regions; some may even evolve neotypes as an adaptive strategy in response to the changing climatic conditions. According to Lokesha and Vasudeva (1993), certain specific syndromes with respect to habit, distribution pattern, phenology, and modes of pollination and seed dispersal enhance the possibilities of species extinction, whereas the actual rate of extinction of the species in wild is hastened by human induced perturbations. A periodic evaluation is therefore highly sought-after to keep eye on changing trends of nature and to keep abreast of the current status of biodiversity in a given area.

Present study was planned to explore and document orchid diversity in various climatic zones (subtropical, warm temperate, cold temperate, subalpine and alpine) of Himachal Pradesh. Information on the distribution pattern of different species, range of habitats, flowering and fruiting periods, and possible threats was gathered along with quantitative analysis (density, frequency, abundance) of their populations and soil characteristics of natural habitats. The taxonomic, phylogenetic and phytogeographical importance of seed characters was assessed. Additionally, biochemical studies were made in three polymorphic species with an aim to investigate genetic relationship among their morphotypes. The results have already been presented in the preceding pages and in what follows these are discussed in the light of information available in literature.

5.1. Orchid species studied presently

A total of 39 orchid species under 23 genera were presently collected from 78 localities (643-3979 m) of Himachal Pradesh. Two of them (*Habenaria aitchisonii, H. pubescens*) were recorded as new additions to the state flora. Subfamily Cypripedioideae was represented by a single species, Orchidoideae by 24 and Epidendroideae by 14 species. Such a representation of different
subfamilies bespeaks of the predominance of temperate climate in the study area and is in accord to the general distribution pattern of family Orchidaceae. According to Zotz (2013), nearly 70% of total orchids are epiphytic (belonging to advanced Epidendroideae) and are distributed mainly in tropical and subtropical climates. The remaining 30% (belonging to Cypripedioideae, Orchidoideae, Vanilloideae and Epidendroideae), on the other hand, usually dwell in temperate regions. Emergence of Habenaria as the largest genus (nine species) suggests successful adaptation of its species throughout varied climatic regimes from subtropical (Habenaria commelinifolia, H. digitata, H. marginata, H. plantaginea, H. stenopetala) to cold temperate (Habenaria aitchisonii, H. intermedia, H. pectinata, H. stenopetala). In majority of these species, the seed set was rather high and increment in the natural populations was frequently observed in some of these. On the other hand, the genera represented by a single species each, generally showed habitat specificity and narrower distribution range. According to Hegde (1982), orchids are not only habitat specific but within a habitat also, they require unique micro-climatic conditions to survive and perpetuate.

5.2. Species habits, distribution pattern and habitats

Majority of the presently studied species were ground growing in habit and only two (Gastrochilus calceolaris, Rhynchostylis retusa) grow in tree canopies as epiphytes. Two of the terrestrials (Cymbidium macrorhizon, Gastrodia falconeri) exhibited leafless mycoheterotrophic life mode. The epiphytic orchids were restricted in distribution subtropical to warm temperate climates within an altitudinal range of 640-1450 m. Total absence of epiphytic orchids in other regions of the study area seems to be related to factors such as comparatively low humidity and temperature, and severe winters. The epiphytes possessed well developed velamen tissue in their roots and have fleshy leaves as contrivances for absorption and storage of atmospheric water. Five broad leaved trees (Bauhinia variegata, Dalbergia sissoo, Ficus benghalensis, F. glomerata, Mangifera indica) were observed to host orchids in present study area. Vij (2006) suggested the importance of substratum dynamics and adaptive flexibility of orchids in promoting diversification of phorophyte based species. Distribution of epiphytic species is limited by the availability of seeds and their dispersal to suitable hosts.
(Male and Roberts, 2005), and according to Verma et al. (2009), one of the most important factors that determines whether an epiphyte can grow on a particular host is the ability of its seeds to germinate upon it. Earlier, Allen (1959) also demonstrated that seed germination is the most critical phase while preferring a particular host; mature seedlings can, however, grow well on plants other than the actual host. Both of the present species were observed to prefer rough barked phorophytes. The rough bark provides crevices for orchid seeds to lodge whereas at smooth bark there is a little chance for the seeds/seedlings to get even established. A soft, spongy bark with roughened surface is also better for water retention. The epiphytic orchids normally grow in close association with lichens and mosses, both of which are known for their water retention capacity. At many places in study area, there is predominance of coniferous trees but they were never observed to host any epiphytic orchid. According to Vij et al. (1983), the resinous bark of conifers is not well suited for orchid seed germination despite of its rough nature.

Depending upon their climatic requirements, the terrestrial orchids showed different distribution pattern in the study area. Majority of the species, including both mycoheterotrophs were distributed in subtropical to temperate climates (up to 3000 m). Only one species (Herminium monorchis) was recorded exclusively from alpine climate (>3000 m). Since the ground orchids are able to draw nutrition from soil, their growth and development is directly influenced by edaphic characteristics in the habitats. Moreover, as climatic requirements of the species are different from each other, different orchid species inhabit different ecological niches even within a given geographical area. According to Case (1962) orchid habitats vary on the basis of biotic and abiotic factors and these differences are well reflected in the species they support. Environmental factors like available space, exposure to light, temperature, moisture and mycorrhiza, act together in determining the character of orchid habitat rather than any one of the factors, however favourable that may be (Misra, 1995). The terrestrial orchids come out of soil during rainy season immediately after summers and go dormant as the entire leafy shoots dry up and disappear on the onset of winters; new vegetative shoots appear again after next summers. They have specialized organs like
rhizomes (Cymbidium, Cypripedium, Goodyera), tubers (Androcorys, Brachycorythis, Dactylorhiza, Eulophia, Gastrodia, Habenaria, Herminium, Pecteilis, Peristylus, Platanthera, Satyrium), fleshy roots (Spiranthes) and pseudobulbs (Crepidium, Dienia, Liparis, Oreorchis) to store water and food and to perennate through unfavourable growth periods. Majority of the present species were well embodied in shaded forests and shrubby grasslands due to good humidity and favourable temperature with less variation. Vij et al. (1998) demonstrated that the species which dwell on exposed areas usually require less humidity, more light, quick water drain off, and can possibly withstand strong winds and high fluctuations in day and night temperatures prevailed in such niches. The species inhabiting shady places, on the other hand, require less exposure to direct sunlight and less fluctuation between day and night temperatures. A few species (Goodyera repens, Dithrix griffithii, Habenaria commelinifolia, H. intermedia, H. marginata, H. pectinata, Herminium lanceum, H. monorchis, Satyrium nepalense) were also found to occur in disturbed habitats such as road embankments and forest borders. Such habitats are ecologically marginal and apparently more xeric with respect to availability of water and moisture. According to Bartholomew et al. (1973), such a variable and fluctuating climate either cause periodic reduction in population size leading to much local extinction, or a few individuals, if left growing, may upon the return of favourable conditions, give rise to local populations which may be more adapted to local conditions than the core populations. The species occupying a variety of habitats (Crepidium acuminatum, Epipactis helleborine, H. commelinifolia, H. intermedia, H. marginata, H. pectinata, Herminium lanceum, Platanthera clavigera, P. edgeworthii, Satyrium nepalense) bespeaks of their wider ecological adaptability. Due to smaller geographical size of the present study area, no apparent relationship of species distribution was observed with respect to latitudinal and longitudinal coordinates.

5.3. Flowering and fruiting

Majority of the presently studied orchids flowered during July (28 species) and August (26 species). None of the species was observed in flowering during November-March. Wells et al. (1998), Kindlmann and Balounova (1999) and Pfeifer et al. (2006) suggested that orchids vary in flowering time depending on
their population characteristics, and sensitivity of growth and flower induction to environmental factors. According to Lokesha and Vasudeva (1993), majority of Indian orchids flower during the month of August; the high altitude ones follow a seasonal pattern of blooming, while those at lower elevations may flower during different months of the year. Occurrence of early summer blooming species at lower elevations and late summer flowering species at higher elevations seems to be correlated with prevalence of lower temperature, more rainfall and high humidity during summer months at higher altitudes (Vij et al., 1982). Present species also conform to such a generalization but none of them was free flowering in nature. *Gastrochilus calceolaris* and *Rhynchostylis retusa* were observed to flower first of all (April) among presently studied orchids; both of these are distributed in low hills. Literature studies reveal that besides the maturity level of the tissues, the blooming season in orchids depend upon difference in day and night temperature, and availability of pollinators (Abraham and Vatsala, 1981). According to Kochmer and Handel (1986), the seasonal availability of pollinators can be a powerful selective force shaping flower phonologies in animal pollinated plant species such as orchids.

A wider and successful distribution of orchids, like other flowering plants, depends upon success of seed (fruit) production and their effective dispersal thereafter. Moreover, the production and germination of seeds in any species at a given area directly depends upon its population structure (more individuals, chances of more fruit and seed production) and the habitat characteristics (that decide seed germination). Presently, the fruit set ranged between 60-90% in majority of cases; it was lowest (26.45%) in terrestrial *Crepidium acuminatum* and highest (96.26%) in epiphytic *Rhynchostylis retusa*. It has been observed that the species having higher (>90%) fruit set (*Rhynchostylis retusa, Gastrochilus calceolaris, Platanthera edgeworthii, P. latilabris*) generally produced small sized seeds with smaller air space (11.122-34.052%) inside them (Table 4.6). On the other hand, orchids with low percentage (25-50%) of seed set (*Crepidium acuminatum, Liparis odorata, L. rostrata, Cypripedium cordigerum*) produced comparatively larger seeds with larger air space (82.127-95.650%). The epiphytes produced more fruits (per inflorescence) due to easy access (less competition) to pollinators as well as lesser damage to their plants from herbivores. Such an observation suggests that despite of lesser fruit set
in ground growing taxa, the seeds are well adapted (high air space) for effective dispersal to longer distances and therefore newer localities. Inflorescences of *Crepidium acuminatum* possessed many (10-25) flowers but fruit production was very low; only 1-2 fruit(s) were produced in population comprising of 15-20 individuals. According to Calvo (1990) the larger inflorescence size in orchids did not necessarily result in increased fruit set percentage but tend to increase the chances to produce at least one fruit. This appears true in case of closely allied *Crepidium acuminatum, Liparis odorata* and *L. rostrata*.

### 5.4. Geographical affinities of the species

The Himalaya is a meeting ground for floristic components of various neighboring countries and geographical regions. None of the presently studied orchids was found to be endemic to the state of Himachal Pradesh and they show greater affinities with those of adjacent areas (Table 4.2). Only three species (*Androcorys monophylla, Goodyera biflora, Oreorchis indica*) are restricted in distribution to northwest Himalaya. Hajra and De (2010) presented a phytogeographic analysis of Indian orchids and suggested four major orchid zones (Eastern Himalaya and northeastern India, northwestern Himalaya, Peninsular India, Andaman and Nicobar Islands) in the country. Indian Himalayan Region (IHR) is an important orchid habitat for about 850 species (Singh, 2001). In addition to the prevalence of congenial ecological conditions for large-scale mutations (frequent seismic activity), wide matings (proximity to several immigrant taxa from adjoining areas of Bhutan, China, Nepal, Pakistan, Tibet, etc.) and better growth and development environment (high humidity and thick vegetation) has attributed immensely for such a great orchid diversity in this region. The western Himalayan region differs from its eastern counterpart in having larger size, cooler and drier climate and wider mountain mass. The orchid flora in this part is considered to be the extension of that of the eastern regions and orchids of Himachal Pradesh are no exception. The orchids especially epiphytes are less in number here, due to comparatively lower rainfall, reduced humidity, heavy snowfall and predominance of coniferous forests (Verma *et al.*, 2009). Where many terrestrial orchids are better represented in Himachal Pradesh, the state represents the western limit for the distribution for epiphytic orchids; only
one epiphytic species (*Rhynchostylis retusa*) has further extended its distribution westwards to the state of Jammu and Kashmir.

According to Vij et al. (1983) the long and/or short range of seed dispersal and the availability of suitable substratum are two very important deciding factors for distribution potential in orchids. The importance of seed buoyancy in their dispersal has also been stressed by Arditti et al. (1979). The surface sculpturing of orchid seeds makes them very resistant to wetting which helps them to disseminate to longer distances (Verma et al., 2012). Orchid seeds can adapt to a variety of conditions (Misra, 2004) and are known to traverse seas (Bose et al., 1999). Though a large number of biotic and abiotic factors are responsible for successful seed germination and seedling establishment in orchids, but still a possible correlation between the aerodynamics of orchid seeds and species distribution has been suggested (Vij et al., 1992).

### 5.5. Population analysis and associated vegetation

Biodiversity study is fundamental to our understanding of life on earth and we increasingly count on species diversity for a wide variety of ecosystem functions and services. According to Coates et al. (2006) investigation of demographical data is vital for obtaining a real picture of population size in any area. The quantitative information collected for various species in any community can be used for formulating conservation strategies in a better way (Uniyal et al., 2002). Orchids in present study area occupied a variety of habitats across the climates ranging from tropical to alpine, and their species were not distributed uniformly in various landscapes. Differences were observed in population structure (density, abundance, frequency) of various species in their natural habitats. The quantitative analysis of various terrestrial orchids revealed minimum species density [individual(s)/ m²] in *Habenaria commelinifolia* (0.77±0.29), which dwell in shady forests and shrubby grasslands. This species was found to have narrow preferences toward soil pH, EC, N, P and K. *Androcorys monophylla*, which was found occurring in a wider range of habitats (shady forests, shrubby grasslands, grassy slopes, road embankments), was observed to have maximum density (6.54±1.60). Such an adaptability of this species in various habitats ranging from subtropical to temperate seems to be responsible for its highest density in the study area. It was also found to inhabit soils with wider range of moisture content (20.20-33.60%) and pH (7.10-8.09). Earlier, Jalal and Rawat (2009) also recorded the
highest density in case of orchid species that occupy shady herbaceous habitats. Kull and Hutchings (2006) demonstrated that terrestrial orchids generally prefer shady and moisture rich forest floors and their populations show a gradual decrease towards relatively open drier habitats. In Epidendroideae, *Crepidium acuminatum* was observed to have maximum species density (3.37±1.27). This species, with wider range of habitats, generally grows as large clumps of underground pseudobulbs. The species was found to exhibit lesser habitat specificity and can successfully grow on sandy loam and loamy soils. Vij et al. (2013) reported a lithophytic mode of existence for this species. During present surveys, no lithophytic individual was found in the study area but this information bespeaks of better adaptability of *Crepidium acuminatum* as different life forms. Both mycoheterotrophs were found to have moderate species density ranging between 1.37±0.40 and 1.40±0.49. Such taxa, because of their total dependence on mycorrhizal endophytes throughout their life, are highly habitat specific (Vij et al., 1998) when compared with their terrestrial or epiphytic counterparts. Like density, species abundance was also recorded high in case of widely distributed species preferring wider range of habitats. The species growing in mid hill zone (subtropical-temperate) are more abundant than those growing in low and high hills. Bulafu et al. (2007) also observed same pattern of species richness in terrestrial orchids of Uganda. Though majority of orchids grow in tropical and subtropical regions of the world but they exhibit epiphytic mode of existence. The predominance of temperate climates in Himachal Pradesh bespeaks of terrestrial orchid abundance in the state. Presently, *Cypripedium cordigerum* was observed to have minimum (20%) and *Satyrium nepalense* (90%) maximum frequency. Such a difference can be correlated with the habitat characteristics and fruit set percentage. *Satyrium nepalense* has been found to inhabit in all five identified orchid habitats whereas *Cypripedium cordigerum* only in two of these. The lady slipper orchids are reported to be habitat specific, with much pronounced microclimatic requirements (Huang et al., 2008). They generally dwell in shady and moist locales and follow a patchy distribution across larger geographic ranges. Fruit set was also comparatively low in *Cypripedium cordigerum*. These factors might be responsible for its lower frequency in Himachal Pradesh. In subfamily Orchidoideae, *Habenaria aitchisonii* was found to be least frequent. This species has recently been recorded from the study area during present surveys and found to be highly localized (Verma et al., 2010); it is still in the process of expanding its populations in the state. Majority of
orchids were seen to grow in small patches of 2-6 plants. Such a distributional pattern seems to be correlated to the prevalence of favourable microclimates (abiotic, biotic) in different niches which help in initial establishment of orchid seeds by helping them to germinate. According to Focho et al. (2010) the initial orchid establishment is mostly dependent upon successful root establishment and presence of lower plants that aid to trap their seeds; the species can, however, thrive well with a minimum amount of nutrients in soil.

Associated vegetation showed quite variations in different localities of the study area. Where Cedrus deodara, Pinus roxburghii, P. wallichiana, Quercus leucotrichophora and Rhododendron arboreum represented the higher trees in majority of the localities, Rubus niveus, Rosa webbiana, Berberis aristata, Prinsepia utilis, Strobilanthes artopurpureus, Woodfordia fruticosa, Hypericum lanceolatus, etc. were the dominant shrubby elements. The herbaceous vegetation though varies in different localities (Table 4.3), ferns, mosses, and grasses were found to be the most common elements noticed invariably in all of the habitats. The ferns are believed to either compete with orchids or aid their germination, whereas the mosses and liverworts are often beneficial to orchids due to their better water absorption capacity which ensure an uninterrupted water availability enabling orchids to withstand excessive evapo-transpiration (Lecoufle, 1964; Sanford, 1974). Tall grasses, on the other hand provide protective cover to orchids and save them from strong wind currents. The orchids invariably shared their ecological niches with other vegetation due probably to lack of interspecific competition between them (Vij et al., 1998).

5.6. Possible threats to orchid species and conservation

The Himalayan range is geodynamically young, and the top soil layer here, that actually sustains the flora and fauna, is thin and fragile. Majority of natural habitats across the Himalaya are under a variety of anthropogenic pressures. All of the presently studied orchids are facing threat to their natural populations because of loss or shrinkage of habitats due to varied reasons (constructing houses, hydro-electric projects, roads, expanding agricultural fields). Intact and healthy (least disturbed) habitats are necessary not only for the germination, growth and reproduction of orchids, but also for those of other two
specific biotic elements (mycorrhiza, pollinators) that are obligatory for undergoing all processes from seed germination to seed formation.

Another major activity seen in orchid habitats was overgrazing. Some tribes in Himachal Pradesh used to wander from one place to other along with their buffalo and sheep to provide them better food. This is in fact their livelihood and they get authorized licenses from local government for this. The grazing animals eat away young inflorescences and/or fruits (that affect fruit/seed production) and also uproot the underground perennating plant parts (roots, rhizomes, pseudobulbs, tubers). This therefore, affects the natural increment in orchid populations both through sexual and asexual means. The overgrazed areas are also more prone to soil erosions. As the rate of young plants mortality is high in areas of frequent grazing, it should be regulated (Kant et al., 2012). Same area should not be used for this purpose year after year so that roots can get enough time to establish themselves better.

Some of the Himachal orchids possess high therapeutic properties. Their parts are extracted from wild and this practice leads to gradual decrease in population size. Illegal collection of the plant germplasm should be checked. There is also an urgent need to link the indigenous knowledge of medicinal plants including orchids to modern research activities so as to accelerate the rate of drug discovery. Studies are required to search alternative synthetic molecules as done in Vanillin (4-hydroxy-3-methoxybenzaldehyde) that can successfully substitute the growing demand of herbal orchids so as to aid in their conservation.

Majority of villages in Himachal Pradesh depend on forests for collecting fuel wood. As orchids have a long vegetative phase in their life cycle, during fodder collection activities each year, many seedlings were cut immature enough before reaching their flowering and fruiting stage. According to Woodrich (2007), the species of terrestrial orchids that occur in grassland vegetations are the worst affected by fodder collection and overgrazing activities.

Himachal Pradesh is an important tourist destination. Though the state government has put a complete ban on plastic bags, tourists usually carry these with them. Littering with polythene bags, biscuit/chips wrappers, plastic glasses/bottles has been observed only in those habitats that fall in and around some popular tourist destinations (Kasauli, Kothi, Kufri, Khajiar, Taradevi, etc.). This non-biodegradable
matter goes on accumulating year after year in the forest/ grassland/ riverbed and ultimately gets buried under soil forming layers. Such soils become plastic contaminated, and are not suitable for the growth and development of plant species. Light and MacConaill (2007) studied the effects of trampling on a terrestrial orchid (*Cypripedium parviflorum* var. *pubescens*) environment and suggested that tourism related activities have a negative impact on its natural populations.

Another important reason for decline in populations of native species (including orchids) is invasive species. According to Jalal (2012) these weeds are widely spreading in northwestern Himalaya and resulting in gradual replacement of local taxa. Lastly, the frequency of orchid populations might face shrinkage due to forest fires and landslides. Local people usually set fire in forests/ grasslands in want of better grass for fodder. Such fires destroy whole vegetation in grasslands and under vegetation in forests. Rising flames also prove dangerous for epiphytic vegetation. Landslides, on the other hand, leads to habitat fragmentation that affects seed set by altering pollinator population and behavior in addition to overall loss in species populations.

Conservation is the maintenance of essential ecological processes and life-support systems, the preservation of genetic diversity and the sustainable utilization of species and ecosystems (Talbot, 1980). According to Salazar (1996), destruction, modification and fragmentation of natural forests, as well as illegal extraction of orchids, hasten their local extinction. So, to conserve the species, initiatives must be taken at local level where it actually grows (*in situ*). Species can be better conserved in Protected Areas (National Parks, Wild Life Sanctuaries, Biosphere Reserves), where anthropogenic interference is very low. Moreover, at such places, the conservation is done on community basis rather than targeting a single specific species, and therefore the ecological equilibrium is well maintained. Baade (1960) stressed upon the importance of protecting orchids in their wild habitats, besides selecting areas suitable for their plantation and propagation. For *ex situ* conservation cost effective and highly reproducible protocols need to be developed for the mass multiplication by means of *in vitro* micropropagation where only very small amount of plant material is required. It will help reducing the collection pressure on the wild populations and to fulfill their increasing demand in the pharmaceutical units. The micropropagated seedlings (*ex situ* raised) after better
acclimatization, could successfully be rehabilitated back to their natural or natural-like artificial abodes with desired substratum characteristics.

5.7. Soil analysis

The nature of substratum (soil where a species grows) decides the floristic composition of a given area. Characterization of physical and chemical soil properties of orchid habitats is important to understand the effect of various abiotic factors on their growth and development (Ors et al., 2011). A good knowledge of genetic diversity and substratum preferences has also been stressed for developing conservation strategies for the species (de Lange and Norton, 2004; Pillon et al., 2007). Presently, 12 species were found to grow on loamy and 11 on sandy loam soils. Fourteen species, however, dwelled invariably on both types of soils, and more or less uniformity towards soil texture was observed at genus level. The species producing underground tubers or pseudobulbs (Dactylorhiza hatagirea, Gastrodia falconeri, Habenaria spp., Herminium spp., Platanthera spp., Satyrium nepalense, Liparis spp., etc.) preferred sandy loam soils. Such soils have lower water holding capacity and better aeration (Buckman and Brady, 1971), and the species occurring on them require proper aeration and less moisture around their roots. Where the roots and/or perennating underground parts of the species preferring loamy soils can withstand poor aeration, they usually require an optimal moisture level in their microclimate. According to Vij et al. (1998), the capacity of some species to grow on different types of soils reflects their better adaptability in different habitats. The moisture content (%) in presently analyzed soils ranged between 20.040±0.971 (Crepidium acuminatum) and 33.921±0.001 (Cymbidium macrorhizon). Both of these species belong to advanced subfamily Epidendroideae but differ with respect to their habits. Such an ability to grow across a wider range of moisture regimes bespeaks of the wider ecological preferences of Epidendroid taxa that might have been responsible for greater diversification of species and habits (terrestrial, lithophytic, mycoheterotrophic) in this subfamily. According to Tsiftsis et al. (2012), soil texture and moisture are important for deciding the germination and growth of orchid as well as its mycorrhizal associate.

Majority of the present species preferred to grow on slightly acidic to slightly alkaline soils (pH 6.20-7.80). The preferences towards soil pH reflected
significant differences among species of same genus. Literature study reveals that orchids usually prefer to occupy soils with pH ranging between 5.9 and 6.9 (Vij et al., 1998; Bowles et al., 2005; Tsiftsis et al., 2008; Ors et al., 2011); some species, however, can thrive well on soils having pH value as low as 4.40 (Focho et al., 2010) or as high as 8.4 (Davies et al., 1984; Sieg and Ring, 1995). Our results are in line with these reports; the soil pH has been observed to be lowest in case of Goodyera biflora (5.076±0.062) and highest in Herminium monorchis (8.062±0.024). Knudson (1951) suggested that soil pH is critical only during the initiation of seed germination in orchids and the seedlings are less sensitive to its fluctuations. Total availability of salts in ground growing species is directly related to the soil Electric Conductivity (dSm⁻¹); it was observed to be lowest (0.114±0.001) in case of Goodyera biflora and highest (2.338±0.407) in Herminium monorchis. Excessive soil salts are often inhibitory to moisture and mineral uptake by the plants (Khanna and Yadav, 1979; Ors et al., 2011). Vij et al. (1998) demonstrated that the size and frequency of orchid populations are usually inversely proportional to the soil conductivity and majority of the present species were no exception to this.

Present study reveals that there is a sort of habitat differentiation with respect to the organic C, N, P and K preferences of orchids as their amount show significant differences in many cases. Since fungal endophyte has been reported to augment the carbohydrate transport in orchids, carbon content in soil is an important aspect of orchid nutrition (Withner, 1974). The mycorrhizal partner also facilitates orchid seed germination by supplying necessary vitamins and auxins (Hijner and Arditti, 1973). In many genera, significant differences were observed towards the organic carbon (%) content of soils; its value ranged between 0.462±0.013 (Spiranthes sinensis) and 6.422±0.010 (Oreorchis indica). In a study conducted by Tsiftsis et al. (2008) in 56 orchid species, the carbon content in soil has been found to range from 0.58 to 21.03%. Such a range of C in orchid supporting soils reflects the qualitative rather than quantitative importance of carbon in orchid nutrition (Vij et al., 1998). Low carbon content in soil also lowers its moisture holding capacity and therefore affects mycorrhizal colonization in natural habitats (Bowles et al., 2005).
The amount of nitrogen (Kg/ha) was observed to range between 311.626±1.135 in *Habenaria stenopetala* (Orchidoideae) and 941.252±0.947 in *Oreorchis indica* (Epidendroideae). Nitrogen rich soils are often considered to be conducive for orchid growth (Sheehan, 1961). A variable preference towards N nutrition of orchids seems to be related to their ecological plasticity as also reported earlier in many studies (Vij *et al*., 1982, 1998). According to Bichsel *et al.* (2008), inadequate levels of N in orchid soils affect the vegetative growth of plants and nitrogen deficient plants are more prone to leaf loss; however, very high amount of soil nitrogen may lead to their poor flowering. The effect of N concentration on flowering has not been investigated presently, but significant differences in amount of N in soils supporting different species bespeaks of their wider ecological amplitude. *In vitro* studies suggest that the requirement for either ammonium or nitrate as a source of nitrogen varies with the orchid species (Sembi *et al*., 2011).

Like nitrogen, species showed significant differences towards the soil phosphorous; it was lowest in *Dithrix griffithii* (8.762±0.433) and highest in *Oreorchis micrantha* (64.638±0.449). Orchids generally prefer soils with low phosphorous content (Penningsfeld and Fast, 1973; Dijk and Grootjans, 1998). It has not been considered as a limiting factor for orchid growth as mycorrhizal associate is thought to fulfill its requirement in these plants (Vij *et al*., 1998; Tsiftsis *et al*., 2008). According to Rodrigues *et al.* (2010), the initial P requirement in young orchids is greater than that of the adult plants. Naik *et al.* (2006) demonstrated that the epiphytic orchids generally have more P requirement as compared to their ground growing counterparts.

Most significant differences were observed in case of the available potassium contents (Kg/ha) in soils. It was found to range between 161.552±3.588 (*Habenaria stenopetala*) and 656.532±1.073 (*Platanthera edgeworthii*). Potassium is required in comparatively higher amounts in orchids; where its varied levels have been shown to influence vegetative plant characteristics (number and size of pseudobulbs) in these plants, it is reported to have little effect on flowering (Bichsel *et al*., 2008). Potassium level in plant is highly mobile and it can be easily redistributed in various plant parts (Mengel and Kirby, 1982). According to Davidson (1960), the exogenous supply of K is not a limiting factor for orchid growth as it can be translocated from older
tissues for reutilization in development of new organs. Pooley and Seeley (1978), Vij et al. (1998) and Kusum et al. (2013) also observed the satisfactory occurrence of orchids at soils with low K concentrations.

5.8. Seed characteristics

Orchid seeds are the smallest in plant kingdom and they are produced in very large numbers. Generally, such a huge number of seeds are produced by those plants that have some very specific requirement(s) for their germination (Rauh et al., 1975; Rasmussen, 1995) and Orchidaceae are no exception to this. Despite of the large numbers in which they are produced, the seed germination is exceptionally low (less than 1%) in orchids. Their embryos lack access to nutrient reserves, and the successful germination of seeds, in vivo, is obligatory to a fungal stimulus (mycotrophy). Present investigation on seed characteristics of 31 Himachal orchids yielded interesting results that have taxonomic, phylogenetic and phytogeographic implications. As many as 50% of the presently studied species spread over three different subfamilies produced fusiform seeds, and there is no uniformity in seed shape even at the tribe level. Seed shape was found uniform at generic level in case of Epipactis, Liparis and Platanthera. Chase and Pippen (1988) observed a remarkably consistent seed shape over a large and variable set of Oncidiod genus, and Tsutsumi et al. (2007) reported uniform occurrence of fusiform seeds in some Liparis species irrespective of their life mode (terrestrial/ epiphytic). Aybeke (2007) studied seed morphometry of eight Ophrys species and found uniformly fusiform seeds in all of them. The differences in seed shapes of presently studied species of Habenaria (fusiform, spathulate) and Eulophia (fusiform, ovoid), however, reflect that this character could not be employed for delimitation of different genera with certainly. Seed characteristics support the transfer of three Habenarias (H. clavigera, H. edgeworthii, H. latilabris) to genus Platanthera (Govaerts et al., 2013). Their seeds were uniformly spathulate and smaller (Table 4.6) as compared to presently studied species of Habenaria. Only H. aitchisonii possessed spathulate seeds, which depicts closer affinities between these two genera (Habenaria, Platanthera). Shape of the seeds is thought to have a phylogenetic significance also. Fusiform seeds that are met in all of the subfamilies (Arditti et al., 1979, 1980; Healey et al., 1980; Rasmussen, 1995; Verma et al., 2012; present study) appear to be basic in orchids from which all other seed shapes might have evolved. Arditti et al. (1979) and Vij et al. (1992) demonstrated that seeds show least shape variability and are usually
fusiform in primitive orchids (Cypripediodeae) but exhibit great variations (fusiform, ovoid, elliptical, filamentous, cylindrical) in advanced Epidendroid orchids. Present results are in line with these findings. Such observations also suggest a possible correlation between the number of seed shapes and the extent of species diversification in different orchid subfamilies.

Seeds show a significant variation in their length (0.05-6.0 mm) as well as width (0.01-0.9 mm), and such differences are of good taxonomic importance at genus and/or species level (Arditti and Ghani, 2000). Presently, quite variable seed sizes were observed in Epidendroideae. Vij et al. (1992) also observed such variations and demonstrated that the seeds are generally long and intermediate sized in primitive Cypripedoid orchids, short and intermediate in Orchidoid orchids, and short, intermediate and long in advanced Epidendroid orchids. Seed length is very important in calculating the degree of seed truncation. Presently, 27 species were found to possess truncated seeds where L/W<6.0. Elongated (L/W>6.0) seeds were observed only in four species irrespective of their taxonomic position. According to Arditti et al. (1979), the relative degree of truncation of orchid seeds is directly correlated with an increase in their length rather than their width. Chase and Pippen (1988) demonstrated that during seed drying process there is no significant change in seed length but the width may decrease by 25-40%. Such findings suggest that L/W ratio is not of much conservative value. Arditti et al. (1979) suggested that instead of length and width, the volume should be considered as a better reflection of seed size in orchids. On an average, seed volume was recorded high in presently studied Epidendroid orchids. When compared with ground growing species, the seed volume (mm$^3$×10$^{-3}$) was found comparatively lower in the epiphytic ones [0.105±0.020 in Rhynchostylis retusa, 0.146±0.029 in Gastrochilus calceolaris]. Kiyohara et al. (2012) in a study on 68 Japanese orchids also observed the lowest seed volume in an epiphytic species (Eria reptas). According to Clifford and Smith (1969), Vij et al. (1992), Rasmussen (1995), Swamy et al. (2004) and Verma et al. (2012), the seed size show a direct correlation with plant habit; epiphytic orchids generally possess smaller seeds as compared to the terrestrials. However, the data presented by Arditti and Ghani (2000) on numerical and physical characteristics of orchid seeds suggest that this is not a rule.

Seed coat in orchids is generally represented by a single layer of elongated and transparent testa cells derived from the outer integument (Vij, 2006). The testa cells
were quadrilateral in shape in majority of the presently studied species. Earlier, Clifford and Smith (1969), Vij et al. (1992) and Verma et al. (2012) suggested that quadrilateral testa cells are found commonly in ground growing species, and they are generally fusiform in epiphytes. But such differences were not seen presently. Some orchids possess uneven deposition of thickening materials on their periclinal or anticlinal walls, and such ornamentation(s) is of taxonomic importance. Kurzweil (1993) suggested that the seed coat in orchids may be made of cells that differ in shape and ornamentation; cells are generally concave and elongate with straight or slightly undulate anticlinal walls. The surface walls were bereft of any cross band thickenings in most of the presently studied taxa. However, such structures were present in six species (Dactylorhiza hatagirea, Dithrix griffithii, Spiranthes sinensis, Oreorchis micrantha, Eulophia herbacea, E. hormusjii). Such wall thickenings were earlier reported in Spiranthes sinensis (Clifford & Smith, 1969; Vij et al., 1992), Dendrobium longicornu, Orchis habenaroides (= Gymnadenia orchidis) and Goodyeya repens (Vij et al., 1992), S. romanzoffiana (Healey et al., 1980), Eulophia guineensis (Barthlott, 1976) and species of Calypso (Arditti et al., 1980). The cell walls in all epiphytic species possessed very thick depositions. Such a profuse development of wall thickenings is of common occurrence in epiphytes and is thought to have an adaptive significance. They provide rigidity to seed coat and thus protect embryo (Swamy et al., 2004). Shimizu (2012), on the other hand, demonstrated that seed coat patterns are independent of plant habit and these only make seeds to ride upon the winds to help in wider species distribution. The number of testa cells and their size showed variations in different species (Table 4.6). They were generally short sized both at apical and chalazal ends. Ortunez et al. (2006) and Gamarra et al. (2007) while investigating seed micromorphology of Cypripedium calceolus and genus Neotinea respectively, also demonstrated the same pattern of testa cell lengths (short apical and chalazal cells, elongated medial cells) in these taxa. According to Arditti et al. (1979), for any given orchid genus one might expect either testa cell number or the cell length to be somewhat consistent. Present observations agree with this generalization. The number of testa cells in different Habenaria spp. and Platanthera spp. was found almost similar. On the other hand, two species of Eulophia, though, showed significant difference in number of testa cells (11.200±0.918 in E. herbacea, 2.600±0.516 in E. hormusjii), but exhibit uniformity in their size.
Like seeds, the orchid embryos are also minute and present species were no exception. A wide range of embryo volume was seen in advanced Epidendroideae (0.018±0.005-1.646±0.217 mm$^3$×10$^{-3}$). Embryo volume directly affects the percentage of air inside seed, and therefore has an important role in seed dispersal and species distribution. Fahn and Werker (1972) distinguished the wind dispersed (anomochores) seeds into flyers (meteoranomochores) and rollers (chamaechores), and the orchid seeds, because of their small size and light weight, belong to the former category (flyers). The tiny nature of embryos makes them exceedingly air-filled therefore helping them to float (fly) across longer distances in air for a wider dispersal (Arditti, 1992; Yam et al., 2002). Burgeff (1936) experimentally demonstrated the relationship between seed to embryo (s/e) volume ratio and seed buoyancy; seeds with high value of s/e ratio are found more buoyant as they possess more air space. In presently investigated species, the highest s/e volume ratio (25.854±8.457) was obtained in Liparis odorata and lowest (1.182±0.265) in Rhynchostylis retusa, both of which are members of subfamily Epidendroideae. The seeds of epiphytic species were found to possess comparatively low s/e volume ratio and less percentage of air space than the terrestrials. According to Tsutsumi et al. (2007) the comparative s/e volume ratio has evolutionary implication; seeds of epiphytic (advanced) orchids are generally shorter but possess large sized embryos. These authors suggested that the larger sized embryos seem likely to be developmentally advanced and have potential to germinate earlier than the smaller ones. Therefore large embryos are thought to play an important role in early and better establishment of seedlings in epiphytic life style. Moreover, in epiphytes, as the seeds are released from more height, they remained in air for more time and consequently could disperse to longer distances despite of being less buoyant. Earlier, Garg et al. (1992) also mentioned that the seeds of terrestrial orchids are voluminous with a larger air space (smaller embryos) than those of epiphytes. All of the presently studied Epidendroid orchids are widely distributed except Gastrodia falconeri (Table 4.1). This species produce highly floating (>90% air space) seeds but exhibits a restricted distribution in the Himalayan range. Similar is the case with presently studied lady slipper (Cypripedium cordigerum) which despite of its much buoyant seeds (air space>85%), is a Himalayan endemic and exhibits patchy distribution along mountain ranges. Both of these orchids seem to be highly habitat specific. According to Vij et al. (1998) some orchid species exhibit higher habitat specificity (narrow preferences towards exposure and shade, moisture,
soil pH, mineral elements in soil, etc.) and this phenomenon is much pronounced in the mycoheteroprophs. The buoyant seeds that can successfully disperse to long distances in such species might not be able to germinate and/or establish themselves in absence of suitable biotic (mycorrhizal) and abiotic (desired habitat and substrates) factors. According to Benzing (1981, 1987) both the large number and the physical characteristics (size, air space) of orchid seeds help in wider distribution of Orchidaceae by effective dispersion and successful colonization of at least a few of them at new substrates in newer localities. Such findings explain the discontinuous (patchy) distribution of many orchid species, including presently studied *Cypripedium cordigerum*, across larger ecosystems like the Himalaya.

### 5.9. Polymorphic species and biochemical studies

Conservation planning begins with a comprehensive evaluation of regional biodiversity. Knowledge of organisms’ life histories, species richness, endemism, rarity, range of morphological and genetic variability, and evolutionary histories are all part of a biodiversity assessment, but the necessary first step is identification of the organisms themselves (Steele and Pires, 2011). Genetic diversity between different morphotypes in each of the three polymorphic orchid species (*Crepidium acuminatum*, *Herminium lanceum*, *Satyrium nepalense*) was observed low with \( P = 15.1\% , 13.2\% \) and \( 11.4\% \) respectively. The mean number of allele per locus \( (A) \) was \( 1.15, 1.32 \) and \( 1.14 \) respectively. These parameters have been studied in several orchid species and a low genetic variability has been observed (Schlegel *et al*., 1989; Scacchi *et al*., 1991; Rossi *et al*., 1992; Kull and Paaver, 1997; Ackerman and Ward, 1999; Wallace and Case, 2000; Brzosko *et al*., 2002a, 2002b, 2004, 2006, 2011, 2013). Very low values for these parameters have been observed in *Listera ovata* \( (P = 9.4\%, A = 1.09; \) Brzosko and Wroblewska 2003). Similarly low value of \( P \) and \( A \) has been found for *Encyclia cochleata* \( (P = 11\%; \) Ortiz-Barney and Ackerman, 1999), *Cypripedium acaule* \( (P = 5.3-15.4\%, A = 1.05-1.15; \) Bornbush *et al*., 1994) and *Goodyra procera* populations \( (P = 13-33\%, A = 1.1-1.3; \) Wong and Sun, 1999). Our results in case of all of the species are in line with these results. Where in *Cypripedium arietinum* and *Cephalanthera damasonium* no genetic diversity \( (P = 0\%) \) was documented (Bornbush *et al*., 1994; Case, 1994), in *Goodyra repens* its value was as high as 50\% (Brzosko *et al*., 2013). Present estimates of genetic variations in three polymorphic
orchid species revealed the occurrence of low intra-specific variation in them. According to Wong and Sun (1999) and Chung and Chung (2010) various populations of *G. repens* have been found to be more genetically variable than those of other species from this genus (*G. procera, G. rosulacea*).

The low value of mean observed heterozygosity was observed for *Satyrium nepalense, Herminium lanceum* and *Crepidium acuminatum* ($H_o = 0.15, 0.32$ and $0.13$ respectively). This value was observed very low ($H_o = 0.058$; Brzosko and Wroblewska, 2003) for *Listera ovata* and higher for *Goodyra repens* ($H_o = 0.21$; Brzosko *et al.*, 2013). The low value of observed heterozygosity ($H_o$) is therefore related to the low genetic diversity among different morphotypes of the species. FIS value (a parameter of F- statistics) ranged between -1 to 1 for presently studied species. The -1 value represents the over abundance of homozygotes, while +1 represents the predominance of heterozygotes.

The factors that underlie the genetic structure of the plant population are very much varied (Karaca, 2013). Propagation behaviour and breeding system are the main factors that directly influence the amount of genetic variation found in plant species (Hamrick and Godt, 1989). The species where self pollination is predominant exhibit low values for several genetic parameters than the cross pollinated ones. From the theoretical predictions and a number of experimental studies, smaller population size has been expected to show reduced level of polymorphism (Young *et al.*, 1996). In present studies, the various orchid species were observed to have very small population size. The low genetic variation in their morphotypes may be due to overabundance of homozygotes, or it can also be the resultant of small population size that was initially larger, but the gradual reduction in population size caused a detectable loss in genetic variation. The frequent occurrence of intraspecific variations strongly indicates that different variants (morphotypes) do not suffer much in selection and may be responsible for the wide distribution of this successful family.