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SOIL PROFILING OF SOME ENDANDERED TERRESTRIAL ORCHIDS OF SHIMLA HILLS (HIMACHAL PRADESH), NORTHWESTERN HIMALAYAS

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Abstract

Shimla hills are part of NorthWestern Himalayas and are well known for their diverse habitats, climatic conditions, and rich biodiversity; this area has mainly evergreen forests including dry sub-tropical forests and moist temperate evergreen forests. However, only terrestrial orchids have so far been recorded from here. As nature of substratum (soil) is an important factor affecting vegetation in a given area, soil profiles were presently analysed so as to determine the actual ecological preferences of different selected orchid species. It was observed that orchids were distributed from shady, semi-shaded and moisture rich area to dry and open grassland fields along roads or embankments with slightly acidic to alkaline soil pH. Substratum was found to be rich with Carbon, Nitrogen, Potassium, and Calcium and poor with Sodium, Sulphur, and Phosphate contents. After soil profiling a standard nutrient media can also be devised for their successful *in vitro* species specific asymbiotic seed culture, mass propagation, and conservation.

Introduction

THE FAMILY Orchidaceae is a diverse and widespread family of flowering plants and most advanced amongst the monocots (Willis, 2017). This family comprises of about 28,484 species distributed in 850 genera in the world (Govaerts *et al*., 2017). Orchids are easily distinguished from other plants, as they have characteristic features namely, bilateral symmetry of the flower, highly modified petal (labellum), fused stamens, highly complex pollination mechanism, and extremely small non-endospermic seeds, dependent on mycorrhizal fungus for their germination in nature (cf. Prakash and Pathak, 2020). In India, orchids are represented by about 1,256 species in 155 genera and in NorthWestern Himalayas (Jammu and Kashmir, Himachal Pradesh, and Uttarakhand), 135 genera and 368 orchid species have been recorded (Singh *et al*., 2019). While the orchid diversity is relatively very less in Himachal Pradesh, it supports natural, unique, and socio-economically important orchids (Chauhan, 1999; Deva and Naithani, 1986); the state is represented by 43 genera and 84 species (Singh *et al*., 2019). Geographically, the capital town, Shimla (31°11'16''N latitude and 77°20'30''E longitude) and adjacent hills are located at an altitude 1600-2800 m amsl. The study area has mainly evergreen forests including dry sub-tropical evergreen forests and moist temperate evergreen forests. However, only terrestrial orchids are recorded from Shimla hills, so far. Orchids are basically terrestrial in habit but they have effectively utilized epiphytic mode during species diversification (Vij, 1995). Due to the lack of endosperm, most orchids are thought to begin their life cycles aided by mycorrhizal fungi that provide the seeds with the nutrients necessary for germination (Manoharachary, 2019). Thus, orchid species are myco-heterotrophic during germination and germinate very poorly in nature (less than 1%) (Anuprabha and Pathak, 2020; Arditti, 1992; Merckx, 2013). As compared to biological interaction such as competitors and antagonists, abiotic factors attract less attention, but still significantly affect terrestrial orchid distribution especially light/shade, moisture, substrate chemistry, and soil texture (Bowles *et al*., 2005; Djordjevic *et al*., 2016). According to Stoutamire (1974), seed germination is the most critical phase of orchid life cycle; after germination, orchids possess an ability to adapt to comparatively wider nutritional regimes, and at times, even capable of surviving under nutritionally marginal conditions. Soil profiling refers the measurement of nutrients present in the soil which may be estimated by using standard methods for respective nutrients; this analysis will provide an appropriate information for understanding nutrient requirements of a particular species. Nitrogen, Potassium, and Phosphorus are very essential for plant growth and development. Nitrogen is an essential constituent of protein, nucleic acids, chlorophyll, and growth hormones (Barker *et al*., 1974). The total level of native Phosphorus in soil is low compared to other plant nutrients and is present in strongly adsorbed or insoluble inorganic forms with the remainder present in organic forms (White, 1995). Potassium is present in elemental form, exchangeable form or as a part of mineral lattices and used for flowering purpose; it is also required for building of proteins, photosynthesis, fruit quality, and

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reduction of diseases. Calcium and Magnesium interfere in soil activity as well as activate a number of plant enzyme systems. The deficiency of any of these elements has retarding effect on the growth of plants.

Literature studies reveals that success of *in vitro* asymbiotic seed germination depends on seed conditions, physical conditions, and the constituents in the growth media (Decruse and Gangaprasad, 2018; Gurudeva, 2019; Kaur *et al.,* 2017; Lekshmi and Decruse, 2018; Mohanty and Salam, 2017; Thakur and Pathak, 2020). Work on orchid habitats, distribution, soil profiling, and nutritional analysis has been reported by a few workers including Bowles *et al*. (2005); Devi *et al*. (2018); Djordjevic *et al*. (2016); Janeckova *et al*. (2006); Kull (1998); Kull and Hutchings (2006); Kusum *et al*. (2013); Lal and Pathak (2020); Naik *et al*. (2009); Poole and Seeley (1978); Tsiftsis *et al.* (2008); Verma *et al*. (2014); Vij *et al*. (1998); Wang *et al*. (2015); Wright *et al*. (2007) *etc*.

The present investigation, substratum profiling of some therapeutically significant and/or endangered orchids from Shimla hills was undertaken with a view and to know their nutritional preferences.

Material and Methods

Soil profile is an important aspect for the terrestrial orchids and during the present investigation, substratum analysis with different aspects such as habit, habitat, associated vegetation, and different physico-chemical parameters including soil pH, electrical conductivity, and nutrient contents were studied in six orchid species namely, *Cephalanthera longifolia* (L.) Fritsch., *Cypripedium cordigerum* D.Don, *Goodyera biflora* (Lindl.) Hook.f., *Habenaria intermedia* D.Don, *Liparis odorata* (Willd.) Lindl., and *Satyrium nepalense* D.Don (Fig. 1).

Study Area

The study area *i.e.,* Shimla and adjacent hills (Himachal Pradesh) with an altitude ranging between 1600-2800 m amsl. The vegetation mainly comprises of sub-tropical, temperate, and sub-alpine forest types. The area receives heavy rainfall in rainy season and snowfall during winter. Soil samples were collected, clearly labelled, and sealed in clean plastic bags from three different localities (Kufri, Taradevi, and Kaithalighat), after digging (10 cm) substratum with the help of trowel at three nearby zones and prepared the final sample for further analysis.

Preparation of Soil Extract (S.E.) and Soil Analysis

The soil samples were oven dried at 40-45°C, followed by grinding with the help of mortar and pestle and subsequently filtered through a sieve. Soil extract (S.E.) was prepared with 20 g soil dissolved in 100 ml distilled water, kept on shaker overnight followed by filtration through Whatman filter paper; soil analysis was done for pH, electrical conductivity, and nutrients (Sodium, Potassium, Calcium, Magnesium, Nitrate, and Phosphate).

The analysis of different physico-chemical soil properties was performed according to the standard methods (Chand *et al*., 2011; Trivedy *et al*., 1987). Soil pH was measured by digital electrical pH meter and electrical conductivity with electrical conductivity meter. CHNS elemental analyser was used for the detection of Carbon (C), Hydrogen (H), Nitrogen (N), and Sulphur (S) by using dry and powdery soil samples with dynamic flash combustion method. Flame-photometer was used to detect Sodium (Na) and Potassium (K) with soil extract while, Calcium (Ca) and Magnesium (Mg) were measured through volumetric titration (titrimetrically). Later, soil extract was analyzed for Nitrates (NO3-), and Phosphates (PO43-) through spectrophotometer.

Results and Discussion

During the present investigation, it was observed that orchid species had variety of habitats such as shady and moist (*Cephalanthera longifolia* and *Cypripedium cordigerum*), shaded forest floor (*Goodyera biflora*), and shaded to semi-shaded forests areas (*Satyrium nepalense*) (Table 1). *Goodyera biflora*, *Habenaria intermedia,* and *Liparis odorata* were observed to need more aeration and cannot tolerate high moisture in soil so generally prefered clayey and clayey-loam soil while *C. longifolia* and *C. cordigerum* occupied loamy soil as they can grow in poor aeration. *Satyrium nepalense* was observed as having greater plasticity and inhabited both kind of habitats. Kull and Hutchings (2006) demonstrated that terrestrial orchids generally grow in shady and moisture rich locales and their populations showed a gradual decrease towards relatively open drier habitats. Presently, soil pH (6.64-7.12) ranged from slightly acidic to alkaline for orchid species. pH is a measure of the acidity of the soil and it is important because it affects the availability of nutrients through both chemical and biological processes. *Liparis odorata* and *S. nepalense* were found in soil with slightly alkaline pH and all other species were found in slightly acidic soil. According to Knudson (1951), pH is of critical importance only during the early stages of orchid seed germination; their seedlings, however, are less susceptible to its fluctuations. Mineral contents of soil are more critical in orchid growth than its pH (Davidson, 1960). According to Arditti (2008), the pH varying from 4.5 to 8 has also been reported to increase the availability of macronutrients such as Nitrate, Phosphate, Calcium, and micronutrients such as Iron, Boron, Copper, Zinc *etc.* Besides soil pH, electrical conductivity was low for all species which showed that high concentration of salts is not conducive for their growth (Table 2). The size and frequency of orchid populations is generally inversely proportional to the soil conductivity (Vij *et al*., 1998).

Initial germination of seeds *in vivo* is obligatory to presence of a suitable fungus that is believed to augment the carbohydrate transport (Withner, 1974). The nutrient requirement of orchid seeds in terms of quantity as well as form may vary at different stages of development (Arditti and Ernst, 1984). Availability of different elements is quite important for growth and development of orchids (Ors *et al*., 2011; Tsiftsis *et al*., 2008; Vij *et al.,* 1998). Presently, Carbon and Hydrogen rich substratum was occupied by *C. longifolia* and *C. cordigerum* and same elements were observed as minimum for *H. intermedia.* Earlier, population density of *C. cordigerum* was found to be directly related to quantity of Carbon (Verma *et al*., 2014). Nitrogen was found to be growth conducive for *C. longifolia, H. intermedia,* and *S. nepalense* as substratum was Nitrogen rich. High Nitrogen content in complex additives has reported to stimulate initial stages of seed growth and differentiation in many orchid species

Table 1. Orchid species with their localities, habit and habitat, altitude range, vegetation associated, and therapeutic uses.

Species	Locality	Habit and habitat	Altitude range (m amsl)	Vegetation associated	Therapeutic uses	References
Cephalanthera longifolia (L.) Fritsch.	Kufri	Terrestrial; occupies shaded to semi-shaded, litter rich, high moisture forest floor	2450-2700	Ferns, grasses, orchids (*Epipactis helleborine, Goodyera repens*, *Oreorchis indica,* and *Malaxis muscifera*), and mixed forests	Used as an aphrodisiac, tonic and for curing cough, paralysis	Prakash and Pathak (2019); Sharma *et al*. (2017)
Cypripedium cordigerum D.Don	Kufri	Terrestrial; occupies shaded, low moisture, forest boarders	2400-2650	Mosses, arisaemas, composits, labiates, orchids (*Epipactis helleborine*, *Goodyera repens*), and mixed forests	Used as tonic and for curing mental disorder and as a vegetable	Kumari and Pathak (2020); Prakash *et al*. (2018)
Goodyera biflora (Lindl.) Hook.f.	Taradevi	Terrestrial; occupies shaded, litter and humus rich, moist forest floor	1800-1950	Mosses, ferns, liverworts, scrophs, orchid (*Neottia listeroides*), and mixed forests	Used as an aphrodisiac, blood purifier, and for curing cold	Sharma *et al*. (2017)
Habenaria intermedia D.Don	Taradevi	Terrestrial; occupies semi-shaded, meadows or grassy slopes, road embankments, and forest borders	1900-2100	Mosses, ferns composits, labiates, orchids (*Habenaria edgeworthii*, *H. pectinata*, *Herminium lanceum*, and *Satyrium nepalense*), and mixed forests	One of the ingredients of Ashtavarga of Ayurveda; used as tonic, and paste is used to cure various diseases such as hyperpiesia, fever, cough, asthma, leprosy, and skin diseases	Kumar *et al*. (2019); Pant (2013)
Liparis odorata (Willd.) Lindl.	Kaithalighat	Terrestrial; occupies exposed or humus rich open grassy slopes	1650-1800	Mosses, composits, potentillas, grasses, orchids (*Eulophia dabia*, *Herminium lanceum*, and *Satyrium nepalense*)	Used to treat cancerous ulcers, gangrene, fever, and dropsy	Singh and Dey (2005)
Satyrium nepalense D.Don	Taradevi	Terrestrial; occupies semi-shaded, humus rich, grassy slopes, road embankments, and forest borders	1900-2100	Mosses, ferns, labiates, composits, asterals, grasses, Orchids (*Epipactis helleborine*, *Eulophia dabia*, *Habenaria edgeworthii*, *H. intermedia*, *H. pectinata*, and *Herminum lanceum*), and mixed forests	Used to cure diarrhoea, dysentery and malaria. Anti-mycobacterial activity has also been revealed	Balkrishna *et al*. (2020); Linthoingambi *et al*. (2013)

Table 2. Different physico-chemical parameters of soil samples of presently studied orchid species.

Species	pH	Electrical conductivity (mS)	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Sulphur (%)
Cephalanthera longifolia (L.) Fritsch.	6.69	0.24	3.913	1.273	1.218	BDL
Cypripedium cordigerum D.Don	6.64	0.30	4.269	1.188	0.047	BDL
Goodyera biflora (Lindl.) Hook.f.	6.93	0.15	2.966	1.126	0.014	BDL
Habenaria intermedia D.Don	6.83	0.32	1.682	0.679	0.050	BDL
Liparis odorata (Willd.) Lindl.	7.12	0.27	1.869	0.855	0.024	BDL
Satyrium nepalense D.Don	7.05	0.28	1.695	0.935	0.097	BDL

BDL, Below detection limit.

including *Dendrobium hookerianum* (Paul *et al*., 2012), *D. parishii* (Buyun *et al*., 2004), *Vanda teres* (Sinha and Roy, 2004), and *V. tessellata* (Roy and Banerjee, 2002). Nitrogen has been reported not to have a significant role on seed germination of *Cymbidium eburneum* but its stimulatory effect on protocorm development and growth was observed by Gogoi *et al.* (2012). Similarly, the soil which was supporting growth of *Eulophia herbacea* was found to possess high percentage of Carbon as well as Nitrogen (Kusum *et al.,* 2013). According to Yates and Curtis (1949), the shoot to root ratio in orchids is markedly affected by a variation in the sucrose content of the external medium and each species exhibits distinct optima for shoot and root growth, the root optimum being consistently higher than the shoot optimum.

During the current study, substratum inhabited by *L. odorata* and *S. nepalense* was Sodium rich, but was low for *G. biflora* and *H. intermedia* (Table 3). Potassium was high for all the species and was recorded as maximum for *S. nepalense*. An exogenous supply of Potassium is not a limiting factor in orchid growth; it can be mobilised (translocated) from old tissues and reutilized to meet most of the growth requirements of new organs (Davidson, 1960). Calcium and Magnesium were, however, observed in good quantity. High Calcium was recorded for *H. intermedia* and *L. odorata* while Magnesium rich substratum was occupied by *G. biflora* and *L. odorata*. Mineral soil habitats supporting *Platanthera leucophaea* had higher Potassium, Calcium, and Magnesium concentrations (Bowles *et al*., 2005). Nitrate concentration was invariably found low in the substratum of all the species studied; it was found minimum in case of *L. odorata*. These observations are in line with the earlier observations of Ponert *et al*. (2013) which state that Nitrate strongly inhibites both germination and protocorm development, even at surprisingly low concentrations. Presently, Phosphate was also found very low and even below detection limits in the substratum of *C. longifolia*, *H. intermedia*, and *S. nepalense*. Similarly, soil profile of *Eulophia herbacea* was also observed earlier, with low Phosphorus content (Kusum *et al*., 2013). According to Tsiftsis *et al*. (2008), Phosphorus does not seem to affect orchid distribution.

In vitro mass propagation of plants is currently used world-wide in numerous species ranging from ferns to trees. *Cymbidium* orchid was the first plant to be

Table 3. Analysis of mineral contents of soil samples, in presently studied orchid species.

Species	Sodium ($mgkg^{-1}$)	Potassium ($mgkg^{-1}$)	Calcium ($mgkg^{-1}$)	Magnesium ($mgkg^{-1}$)	Nitrate ($mgkg^{-1}$)	Phosphate ($mgkg^{-1}$)
Cephalanthera longifolia (L.) Fritsch.	13.85	68.50	133.33	48.50	1.83	BDL
Cypripedium cordigerum D.Don	13.45	64.68	133.33	81.00	3.88	0.41
Goodyera biflora (Lindl.) Hook.f.	8.26	68.81	93.33	210.83	2.13	0.18
Habenaria intermedia D.Don	10.06	65.25	240.16	32.16	6.18	BDL
Liparis odorata (Willd.) Lindl.	20.05	66.45	226.83	186.50	1.30	1.15
Satyrium nepalense D.Don	15.06	69.95	146.66	89.16	1.63	BDL

BDL, Below detection limit.

Table 4. Composition of devised M medium for presently studied orchid species.

Species	$MgSO_4.7H_2O$ (gml^{-1})	KNO_3 (gml^{-1})	$(NH_4)SO_4$ (gml^{-1})	NaH_2PO_4 (gml^{-1})	$Ca(NO_3)_2.4H_2O$ (gml^{-1})	Minor (mlL^{-1})	Vitamins (mlL^{-1})	Sucrose (gml^{-1})	EDTA (mlL^{-1})
Cephalanthera longifolia (L.) Fritsch.	0.010	0.014	12.18	0.003	0.027	1.0	1.0	39.13	3.0
Cypripedium cordigerum D.Don	0.016	0.013	0.47	0.003	0.027	1.0	1.0	42.69	3.0
Goodyera biflora (Lindl.) Hook.f.	0.042	0.014	0.14	0.002	0.019	1.0	1.0	29.66	3.0
Habenaria intermedia D.Don	0.006	0.013	0.50	0.002	0.048	1.0	1.0	16.82	3.0
Liparis odorata (Willd.) Lindl.	0.037	0.013	0.24	0.004	0.045	1.0	1.0	18.69	3.0
Satyrium nepalense D.Don	0.018	0.014	0.97	0.003	0.029	1.0	1.0	16.95	3.0

$MgSO_4.7H_2O$ (Mg source); KNO_3 (K source); $(NH_4)SO_4$ (N source); NaH_2PO_4 (Na source); $Ca(NO_3)_2.4H_2O$ (Ca source); and sucrose (Carbon source).

propagated commercially using this method (Morel, 1960; Wimber, 1963). Knudson (1922) formulated method for asymbiotic seed germination of orchids. In plant tissue culture, different workers have modified the nutrient media (Knudson, 1950; Lugo, 1955; Vacin and Went, 1949; Wynd, 1933; Yates and Curtis, 1949). The most common used nutrient media during *in vitro* culture include VW (Vacin and Went, 1949), modified KC (Knudson, 1946), M (Mitra *et al*., 1976), and MS media (Murashige and Skoog, 1962). Amongst these, M and MS media are considered as complete nutrient media providing all the nutrients or components required for growth and development. Terrestrial orchids as compared to the epiphytic orchids are considered very hard to germinate (Arditti *et al*., 1982) because, these plants have hardened seed coats

Table 5. Composition of devised MS medium for presently studied orchid species.

Species	$MgSO_4.7H_2O$ (gml^{-1})	KNO_3 (gml^{-1})	NH_4NO_3 (gml^{-1})	KH_2PO_4 (mgl^{-1})	$CaCl_2.2H_2O$ (gml^{-1})	Minor (mlL^{-1})	Vitamins (mlL^{-1})	Sucrose (gml^{-1})	EDTA (mlL^{-1})
Cephalanthera longifolia (L.) Fritsch.	0.010	0.014	12.18	-	0.027	1.0	1.0	39.13	10
Cypripedium cordigerum D.Don	0.016	0.013	0.47	0.08	0.027	1.0	1.0	42.69	10
Goodyera biflora (Lindl.) Hook.f.	0.042	0.014	0.14	0.03	0.019	1.0	1.0	29.66	10
Habenaria intermedia D.Don	0.006	0.013	0.50	-	0.048	1.0	1.0	16.82	10
Liparis odorata (Willd.) Lindl.	0.037	0.013	0.24	0.23	0.045	1.0	1.0	18.69	10
Satyrium nepalense D.Don	0.018	0.014	0.97	-	0.029	1.0	1.0	16.95	10

$MgSO_4.7H_2O$ (Mg source); KNO_3 (K source); NH_4NO_3 (N source); KH_2PO_4 (P source); $CaCl_2.2H_2O$ (Ca source); and sucrose (Carbon source).

Fig. 1. A-F. Some of the selected orchids of Shimla hills: A, *Cephalanthera longifolia;* B, *Cypripedium cordigerum*; C, *Goodyera biflora*; D, *Habenaria intermedia*; E, *Liparis odorata*; F, *Satyrium nepalense*.

and more stringent requirements for germination *in vitro.* Keeping these in view, the standard nutrient M and MS media may be modified based on the observations of the substratum analysis for the currently studied orchid species; in these media, two major salts namely $MgSO_4.7H_2O$ (Mg source) and KNO_3 (K source) and sucrose (Carbon source) are common (Table 4-5). There are different Nitrogen sources in inorganic form; $(NH_4)SO_4$ in M medium and NH_4NO_3 in MS medium were, however, considered as Nitrogen source, while devising these media. Amongst the medium components, Nitrogen often has been regarded as one of the key ingredients. The source of Nitrogen in the culture medium has been shown to affect the germination of different orchid species such as *Bletia purpurea* (Dutra *et al*., 2008), *Calopogon tuberosus* (Kauth *et al*., 2006), *Habenaria macroceratitis* (Stewart and Kane, 2006), and *Platanthera ciliaris* (Anderson, 1996).

In devised MS medium, the concentration of KH_2PO_4 (P source) is negligible for *C. longifolia, H. intermedia,* and *S. nepalense* because Phosphorus was below detection limit for these species. However, in modified M medium, NaH_2PO_4 was taken as Sodium source; and if it has to be taken as Phosphorus source, concentration of NaH_2PO_4 will be similar to KH_2PO_4 in modified MS medium. Both modified nutrient media have no change in the concentration of minor, vitamins, EDTA, and Agar. pH is very critical and a medium with high acidic pH does not solidify while, medium with more alkalinity becomes very hard, so pH can be adjusted in a range 6.64-7.12 as observed for the species (Table 2). Different kinds of growth additives such as plant hormones (auxins/cytokinins) and organic supplements can be further used for good plant growth and development, during asymbiotic seed germination.

The present studies conducted on soil profiling and nutritional assessment in some endangered terrestrial orchids with a view to understanding the nutritional status of these plants proved useful for devising modified species specific nutrient media for their mass propagation and conservation, using *in vitro* asymbiotic seed culture technique and such studies may be extended to other related orchid taxa.

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