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DEVELOPMENT OF AGRO-TECHNIQUES FOR *EX SITU* CONSERVATION OF *DACTYLORHIZA* NECK. EX NEVSKI (ORCHIDACEAE) SPECIES GROWING IN KASHMIR HIMALAYA, INDIA

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Abstract

Development of agro-technique is one of the basic conservation strategies for sustainable utilization of plant wealth. The present study was carried out to develop agro-techniques for an important orchid genus *Dactylorhiza*, so as to conserve this highly prized plant species under *ex situ* conditions. Present study revealed that mixture of sand, soil, charcoal, rice straw, brick chips, *Sphagnum* moss, *Betula* bark was suitable for the successful growth of this plant species. Timing of transplantation and proper irrigation proved crucial for proper growth upon transplantation. It has also been observed that treatment of tubers with different concentrations of gibberellic acid prior to their transplantation resulted in an increase in shoot length, number of leaves, and flowers. The present communication may thus prove helpful for mass cultivation of this plant species under *ex situ* conditions and for their sustainable utilization.

Introduction

ORCHIDS CONSTITUTE an important component of natural vegetation. These species are in great demand for medicinal value, as well as for commercial purposes as ornamentals (Pathak *et al.,* 2010; Prakash and Pathak, 2019; Shapoo *et al*., 2013a). Orchid trade, a million dollar industry in several countries has contributed substantially to the economy of many ASEAN (Association of the SouthEast Asian Nations) countries (Hew, 1994; Laws, 1995). Orchids are currently the second most valuable potted crop in the United States with a total wholesale value of US$ 288 million of potted orchids for the year 2014-2015 (U.S. Department of Agriculture, 2016). Thailand, which is the world's sixth largest exporter of cut flowers, earns US$ 30 million a year from orchid exports, and Singapore earns US$ 16 million a year (Reddy, 2008). India's annual flower production stands at around 1000 tonnes and its floriculture industry have a miniscule 0.01% share in the international market (Chug *et al.,* 2009; De and Pathak, 2018; Janakiram and Baskaran, 2018; Patnaik *et al.,* 2017; Singh *et al.,* 2008). Although orchid cultivation and commerce in India is still at a nascent stage, yet orchids have made their presence felt in the Indian cut-flower trade (Chug *et al.,* 2009; Singh *et al.,* 2008). In addition to ornamentals, some orchids are employed as herbal medicines and are in high demand. Orchids have been used since Vedic period as medicine; *Ashtavarga* is a group of 8 drugs in Ayurvedic system which is used for preparation of tonics, such as *Chyavanprash*. Around 40 other orchid species are being used in indigenous medicinal systems (Barua *et al.,* 2019; Bhandari *et al.,* 2018; Devi *et al.,* 2018; Kaushik, 2019; Kumar *et al.,* 2017, 2019; Kumar *et al.,* 2018; Ninawe and Sapna, 2017; Pathak *et al*., 2010; Prakash and Pathak; 2019; Prakash *et al.,* 2018; Shapoo *et al.,* 2013b; Sharma *et al.,* 2017; Singh and Tiwari, 2007; Singh *et al.,* 2019). Many orchid species play a significant role in traditional systems of medicine because they are rich in alkaloids, flavonoids, glycosides, and photochemical contents (Rosa, 2010). This high demand in the ornamental and herbal industry has put enormous pressure on the wild populations leading to destruction in natural populations. The species of the genus *Dactylorhiza* constitute both medicinal and ornamental species growing in Kashmir Himalaya which has been rendered threatened because of overexploitation, as this plant species has not been cultivated for commercial purposes. Moreover, most of the species of the genus has been categorized as Critically Endangered (IUCN, 2017). Thus, conservation efforts are of immediate need to save these species, without which the species may be wiped out from the earth (Dulloo *et al.,* 2010; Somashekhar, 2001).

Conservation of species diversity is one of the main goals of the 2010 biodiversity target. *Ex situ* conservation is a basic part of conservational strategies and is becoming more important as a backup technology (Dulloo *et al.,* 2010; Kaur *et al.,* 2017; Lozoya, 1994; Nautiyal and Nautiyal, 2004). Information on the propagation of medicinal plants is available (Anuprabha and Pathak, 2019; Anuprabha *et al.,* 2017; Bhatti *et al.,* 2017; Decruse and Gangaprasad, 2018; Gurudeva, 2019; Lekshmi and Decruse, 2018; Madhavi and

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Shankar, 2019; Mohanty and Salam, 2017; Pathak *et al.,* 2016, 2017; Vasundhra *et al.,* 2019) for less than 10% species and agro-technology is available only for 1% of the total known plants globally. This trend shows that developing agro-technology should be one of the thrust areas for research (Kuniyal *et al.,* 2005). Furthermore, in order to meet the escalating demand of these plants, farming of these plant species is imperative. Apart from meeting the present demand, farming may help in conserving the wild genetic diversity of this valuable flora. Farming permits the production of uniform material, from which standardized products can be consistently obtained. Cultivation also permits better species identification, improved quality control, and increased prospects for genetic improvements (Dulloo *et al.,* 2010). Selection of planting material for large-scale farming is also an important task. The planting material therefore, should be of good quality, rich in active ingredients, pest- and disease-resistant, and environmental tolerant (Kala *et al.,* 2006). Keeping in view the rising demands of orchids and increasing threats in their natural habitats, the present study was carried out to develop the *ex situ* conservation strategies for the sustainable development of four species of the genus *Dactylorhiza* growing in Kashmir Himalaya.

Material and Methods

During the present investigation, extensive surveys were conducted in Kashmir Himalayas for the collection of different species of *Dactylorhiza* from their natural habitats (Figs. 1-2; Table 1). Tubers measuring 7±2 cm were collected and transplanted at Kashmir University Botanical Garden (KUBG). The tubers were transplanted in the clay (21 × 10 × 21 cm) and plastic (21 × 15 × 21 cm) pots. The tubers were collected from April to August and consequently were transplanted at KUBG, 1-2 days after collection. Five tubers were placed in each pot in 15 replicates; however, keeping in view the small populations of *D. viridis,* its 5 replicates were used. All the pots were kept in partial shade conditions. The different agro-techniques employed are depicted in Table 2.

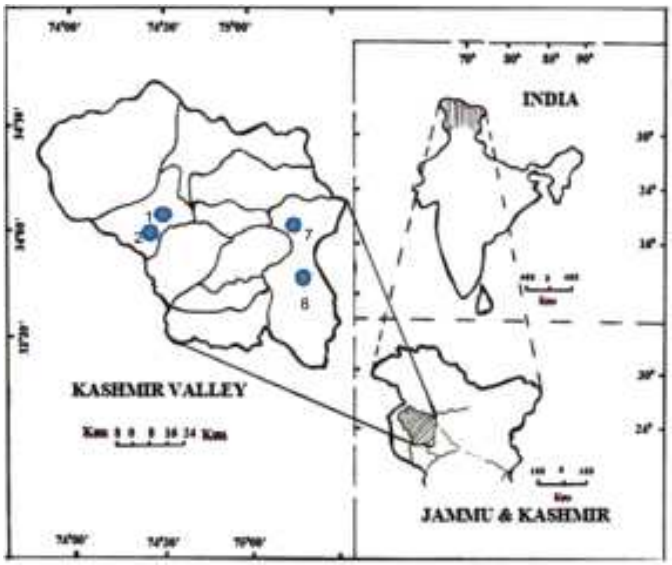

Fig. 1. Map of Jammu and Kashmir showing collection sites of different species of genus *Dactylorhiza*: 1, Ferozpura; 2, Gulmarg; 3, Mamar; 4, Tulail; 5, Vishensar; 6, Sonamarg; 7, Pahalgam; 8, Daksum.

Tuber cuttings with and without shoot apex were treated with double distilled water and various concentrations of GA_3 under controlled conditions in petriplates at 25±2^{0}C and photoperiod of 16 hr light so as to record the sprouting.

Fig. 2. A-D. Four species of genus *Dactylorhiza* selected during present study: A, *Dactylorhiza hatagirea* (D.Don) Soo; B, *Dactylorhiza kafiriana* Renz; C, *Dactylorhiza viridis* (L.) R.M.Bateman, A.M.Pridgeon & M.W.Chase; D, *Dactylorhiza umbrosa* (Kar. & Kir.) Nevski.

Traits Analyzed

The different traits analyzed include sprouting of tubers, average plant height, number of leaves produced, and number of plants entering flowering phase.

Statistical Analysis

The data was analyzed using one way ANOVA. Tukey's test was performed to determine post hoc differences. All the statistical analysis was performed using SPSS (16.0) software.

Results and Discussion

During the present study, different agro-techniques such as mixture of sand, soil, charcoal, rice straw, *Sphagnum* moss, *Betula* bark was used to standardize protocols for successful establishment of the plant species under *ex situ* conditions (Table 3, Fig. 3). The addition of rice straw and peat moss in the substratum enhanced the water-holding capacity. The peat and coir based substrates had greater water-holding capacities therefore increased the root and shoot fresh weight of some bedding plants and these plants thrived well when coir or peat based substrates were used (Evans *et al.*, 1996). The charcoal and moss provide enough support to the plants and also act as carbon source; it holds nutrients and on subsequent watering releases nutrient slowly (Devadas *et al.*, 2008). The timing of tuber sowing is important for successful sprouting of tubers and for the growth of plants under *ex situ* conditions. It was observed that month of April is suitable for the transplantation of tubers. The vegetative propagules *i.e.* tubers were transplanted in different pots supplemented with various ingredients. The results of transplantation in pots with different substrates *i.e.* soil, sand compositions, and addition of different ingredients and the treatment with gibberellic acid prior to transplantation is depicted in Table 3. It was observed

Fig. 3. A-L. Development of *ex situ* conservation strategies (Agro-techniques) for *Dactylorhiza* species growing in Kashmir Himalaya: A, Tubers; B, GA_3 treatment; C, Sprouting of tubers; D, Regeneration in loam soil; E-H, Sprouting in *Sphagnum* moss and charcoal; I-L, Flowering in different potting mixtures.

Table 1. Geo-coordinates of various collection sites of 4 different species of *Dactylorhiza*.

Species	Collection site	Latitude (N)	Longitude (E)	Altitude (m amsl)
Dactylorhiza hatagirea (D.Don) Soo	Ferozpura-Baramulla	34°02.983´	74°25.497´	2230
	Gulmarg-Baramulla	34°02.930´	74°23.616´	2684
	Pahalgam- Anantnag	34°05.543´	75°15.598´	2502
	Daksum-Anantnag	34°22.321´	74°03.321´	3549
D. kafiriana Renz	Sonanarg-Ganderbal	34°19.179´	75°01.223´	3165
	Mamar-Ganderbal	34°13.554´	74°59.777´	2828
	Tulail-Bandipora	34°31.334´	75°10.757´	3212
D. umbrosa (Kar. & Kir.) Nevski	Vishensar-Ganderbal	34°24.109´	75°08.122´	3645
D. viridis (L.) R.M.Bateman, A.M. Pridgeon & M.W.Chase	Vishensar-Ganderbal	34°24.210´	75°08.101´	3704

that addition of different ingredients to soil and sand in 1:1 proportion with prior treatment of gibberellic acid resulted in an increase in shoot elongation, number of leaves, and flowers produced in case of *D. hatagirea*. Giri and Tamata (2012) also observed that Plant Growth Regulators (PGRs) improved vegetative propagation in *D. hatagierea* when tubers were treated with PGRs before planting. In case of *D. kafiriana,* loamy soil and sand in 1:1 ratio showed maximum, sprouting of tubers, height of plants, and number of leaves. The maximum sprouting, plant height, and number of leaves was observed in Loamy soil : Sand : Moss : Charcoal : Rice straw + Brick chips + Gibberellic acid (100 ppm) in

Table 2. Different agro-techniques employed.

Ingredients used	Ratio
Loamy soil	-
Loamy soil : Sand	1:1
Loamy soil : Sand : Moss : Charcoal : Rice straw	1:1:1:1:1
Loamy soil : Sand : Moss : Charcoal : Rice straw + Brick chips + *Betula* bark + Gibberellic acid*	1:1:1:1:1:1:1 + GA_3 (100-1000 ppm)

*Different concentrations of Gibberellic acid treatment were given to tubers prior to transplantation.

case of *D. viridis* and *D. umbrosa*. The transplanted tubers needs to be irrigated immediately after transplantation. Irrigation should be done twice a wk for a month; afterwards frequency of irrigation is to be reduced to once a wk. Frequent watering is useful for survival of *ex situ* cultivation of orchids; the amount of water used depends on prevailing environmental conditions and the condition of plant (Hew, 2001; Tali *et al*., 2015). Thus, present investigation revealed that mixture of sand, soil, charcoal, rice straw, brick chips, *Sphagnum* moss, and *Betula* bark was suitable for successful growth of these plant species with proper irrigation, under *ex situ* conditions.

It has been reported that more than 50% of the orchid species are threatened because of their indiscriminate utilization (IUCN, 2017). Therefore, present study may prove useful in conservation and sustainable utilization of these prized plant species. Most of the orchid species have both medicinal and ornamental value. Cultivation of medicinal and ornamental orchid species can be practiced by farmers/companies/entrepreneurs along with traditional healers for the profit making. There are three prominent methods of conservation of genetic resources of orchid species namely, legislative measures, *in situ* conservation in Sanctuaries/Reserves, and *ex situ* conservation in Orchidaria/Botanic gardens by cultivation (Hegde, 2012), and *ex situ* conservation is an effective method for sustainable utilization of these economically important plant species. The cultivation practices of such economically important plant species can prove effective in employment generation and improving economic conditions of huge chunk of population particularly in far flung areas. The present study may prove useful in large-scale cultivation of present species and other related commercially

Table 3. Response of tubers of four species of *Dactylorhiza* growing in Kashmir Himalaya, upon transplantation to different potting mixtures.

Species	Ingredients used in potting mixture	Percentage sprouting	Height of plants (cm)	Number of leaves produced	Number of plants entering flowering phase
D. hatagirea	Loamy soil	99.00±0.21[a]	12.40±0.34[a]	2.00±0.00[a]	Only up to bud formation
	Loamy soil : Sand	99.60±0.16[a]	12.93±0.24[b]	2.00±0.00[a]	Only up to bud formation
	Loamy soil : Sand : Moss : Charcoal : Rice straw	99.73±0.08	18.2±0.52[c]	4.00±0.00b[a]	20% plants produced flowers
	Loamy soil : Sand : Moss : Charcoal : Rice straw + Brick chips + Gibberellic acid (100 ppm)	99.86±0.09[a]	22.01±0.02[d]	6.00±0.00[c]	74% plants produced flowers
	Loamy soil : Sand : Moss : Charcoal : Rice straw + Brick chips + *Betula* bark + Gibberellic acid (500 ppm)	100.00±0.00[a]	26.03±0.40[e]	8.00±0.00[d]	84% plants produced flowers and pods
	Loamy soil : Sand : Moss : Charcoal : Rice straw + Brick chips + *Betula* bark + Gibberellic acid (1000 ppm)	100.00±0.00[a]	30.80±0.34[f]	8.00±0.00[d]	95% plants produced flowers and pods
D. kafiriana	Loamy soil	80.80±0.38[d]	8.26±0.10[b]	2.00±0.00[a]	Only up to bud formation
	Loamy soil : Sand	99.46±0.15[e]	10.03±0.09[a]	6.00±0.00[c]	95% plants produced flowers and pods
	Loamy soil : Sand : Moss : Charcoal : Rice straw	74.86±0.03[c]	7.14±0.08[b]	4.00±0.00b[b]	60% plants produced flowers
	Loamy soil : Sand : Moss : Charcoal : Rice straw + Brick chips + Gibberellic acid (100 ppm)	70.40±0.16[b]	7.31±0.07[b]	4.00±0.00[b]	55% plants produced flowers
	Loamy soil : Sand : Moss: Charcoal : Rice straw + Brick chips + *Betula* bark + Gibberellic acid (500 ppm)	64.66±0.18[a]	6.04±0.02[b]	2.00±0.00[a]	Only up to bud formation
	Loamy soil : Sand : Moss : Charcoal : Rice straw + Brick chips + *Betula* bark + Gibberellic acid (1000 ppm)	65.53±0.25[a]	5.68±0.10[b]	2.00±0.00[a]	Only up to bud formation
D. viridis	Loamy soil	20.60±0.40[a]	5.36±0.15[b]	2.00±0.00[a]	Only up to bud formation
	Loamy soil : Sand	35.00±0.31[b]	5.80±0.10[c]	2.00±0.00[a]	Only up to bud formation
	Loamy soil : Sand : Moss : Charcoal : Rice straw	40.00±0.31[c]	5.92±0.48[c]	2.00±0.00b[a]	40% plants produced flowers
	Loamy soil : Sand : Moss : Charcoal : Rice straw + Brick chips + Gibberellic acid (100 ppm)	79.40±0.40[d]	6.68±0.09[d]	4.00±0.00[b]	65% plants produced flowers and pods
	Loamy soil : Sand : Moss: Charcoal : Rice straw + Brick chips + *Betula* bark + Gibberellic acid (500 ppm)	74.40±0.50[b]	6.22±0.02[c]	2.00±0.00[a]	25% plants produced flowers
	Loamy soil : Sand : Moss : Charcoal : Rice straw + Brick chips + *Betula* bark + Gibberellic acid (1000 ppm)	59.40±0.40[c]	4.16±0.08[a]	2.00±0.00[a]	Only up to bud formation
D. umbrosa	Loamy soil	98.86±0.25[b]	14.53±0.25[a]	6.00±0.00[a]	75% plants produced flowers and pods
	Loamy soil : Sand	98.73±0.44[b]	17.73±0.70[a]	6.00±0.00[a]	85% plants produced flowers and pods

Table 3. Response of tubers of four species of *Dactylorhiza* growing in Kashmir Himalaya, upon transplantation to different potting mixtures (contd.).

Species	Ingredients used in potting mixture	Percentage sprouting	Height of plants (cm)	Number of leaves produced	Number of plants entering flowering phase
D. umbrosa	Loamy soil : Sand : Moss : Charcoal : Rice straw	98.00±0.96^{b}	22.00±0.69^{b}	8.00±0.00b^{a}	75% plants produced flowers
	Loamy soil : Sand : Moss : Charcoal : Rice straw + Brick chips + Gibberellic acid (100 ppm)	99.01±0.91^{b}	22.93±1.60^{b}	8.00±0.00^{a}	95% plants produced flowers and pods
	Loamy soil : Sand : Moss: Charcoal : Rice straw + Brick chips + *Betula* bark + Gibberellic acid (500 ppm)	92.93±2.47^{ab}	21.06±0.37^{b}	6.00±0.00^{a}	65% plants produced flowers
	Loamy soil : Sand : Moss : Charcoal : Rice straw + Brick chips + *Betula* bark + Gibberellic acid (1000 ppm)	86.93±2.97^{a}	21.46±0.27^{b}	4.00±0.00^{a}	45% plants produced flowers

Different letters (a, b, c, d, e, and f) indicate means that are significantly different-Tukey test; $P \leq 0.05$, N=15 (5 in case of *D. viridis*); It was also observed that concentration 50 and 100 ppm of GA_3 induced maximum sprouting in tubers with shoot apex and the tubers without shoot apex do not sprout at all.

important species for cut flower industry so as to improve the economy of the concerned region.

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