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Domestication and *ex situ* conservation of three species of *Dendrobium* Swartz (Orchidaceae) under greenhouse conditions

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Abstract

The family Orchidaceae is the most diverse group of plants with an estimate of over 28,000 species. The numbers of orchids are declining at an alarming rate even in regions known earlier for species diversity and richness. This rapid decline in number is attributed mainly to anthropogenic activities. Orchids are thus suffering from an uncertain future through over exploitation, habitat loss due to human activities and impact of climate change. The present paper focuses on the domestication of three different species of commonly found orchid *Dendrobium* like *D. fimbriatum* Hooker, *D. jenkinsii* Wallich ex Lindley, and *D. polyanthum* Wallich *ex* Lindley which have now been recognised as vulnerable, under green house conditions and highlights the various aspects of sustainable means of their cultivation and conservation.

Key words**:** *Dendrobium fimbriatum*, *Dendrobium jenkinsii*, *Dendrobium polyanthum*, Domestication, Conservation, Greenhouse

INTRODUCTION

The orchids (members of Orchidaceae) are undoubtedly the royal but highly mysterious members of plant kingdom. The beautiful and long lasting blooms, unparalleled range of peculiarities in floral structure, unique pollination mechanisms, vegetative morphology and rarity of many species have captured the attention of not only flower enthusiasts but researchers as well for over the last two centuries. Orchids are cosmopolitan plants which occur in all habitats except glaciers (Evans *et al.* 2013). Majority of orchids are perennial epiphytes or lithophytes which grow anchored on trees, shrubs or on rocks. They are very sensitive to environmental change and are a subject of great concern at present for their conservation (Medhi & Chakrabarti 2009; De *et al*. 2012).

Population declines of different orchid species have been obviously due to over-collection, habitat degradation and climate change (Maschinski *et al.* 2006; Soto Arenas *et al.* 2007). Consequently, orchids are frequent, if not prominent, occupants of endangered species lists and all the 28,000-plus species of the family (Govaerts *et al.* 2010) have been placed on either appendix I or II of the Convention on International Trade in Endangered Species (CITES). Moreover, several books have focused on orchid conservation (e.g., OSG 1996; Koopowitz 2001; Dixon *et al*. 2003).

Northeast India is a hotspot of biodiversity and occupies 7.7% of India's total geographical area supporting 50 % of the flora (ca. 8000 species), of which 31.58 %

(ca. 2526 species) is endemic (Hedge 2005). Of about 1331 species of orchids belonging to 186 genera reported from India, Northeast India sustains the highest number of about 850 species (De & Medhi 2014). As many as 34 species of orchids from Northeast India are listed among the threatened plants of India (Nayar & Sastry 1990; Ahmedullah *et al*. 1999) and 85 species are endemic to this region (Das & Deori 1983). In India orchids are now treated as protected plants and a large number of initiatives have been taken to conserve the orchids both *in situ* and *ex situ* conditions. Some states have taken special initiatives for the protection of orchids in their natural habitats (*in situ* conservation) like Appangala in Karnataka, Loleyangaon in Darjeeling District, West Bengal have been declared as Orchid Reserves by their State Governments (Behera *et al*. 2013). Orchid Sanctuaries have been set up in Deorali and Singtam in Sikkim and Sessa in Arunachal Pradesh (Hegde 1983, 1984). Living germplasm collection is maintained by Regional Plant Resource Centre (RPRC), Odisha, accompanied by mass propagation through *in vitro* (*ex situ*) culture (Behera *et al.* 2013).

Maintaining a wide diversity of species in each ecosystem is necessary to preserve the web of life that sustains all living things (De & Medhi 2014; De & Singh 2015). Biological diversity is also essential for preserving ecological processes, such as fixing and recycling of nutrients, soil formation, circulation and cleansing of air and water, global life support, maintaining the water balance within ecosystems, watershed protection, maintaining stream and river flows throughout the year, erosion control and local flood reduction (Chatterjee *et al*. 2006). Hence there is an urgent need to preserve the orchid diversity too and to look for various methods of conservation which may be *in vitro* (Hegde 2012), *in vivo* or biotechnology assisted measures for conservation (Kumaria & Tandon 2007).

The Orchidaceae have had a history of ecological resiliency and evolutionary flexibility, which provides us some degree of assurance and relief **(**Ackerman 2014). But this should not be an excuse for complacency since without some form of intervention the pace of change underway may be more than what orchid populations can overcome. Therefore, there is an urgent need for human intervention especially for conservation of threatened species. The present paper highlights some of the most commonly used methods of domestication, cultivation and conservation which can easily be practiced by both amateur growers and professionals for orchid germplasm conservation before these precious plant species completely disappear from this planet. For the present work three wild species of *Dendrobium* Swartz, namely *D. fimbriatum* Hooker, *D. jenkinsii* Wallich ex Lindley, and *D. polyanthum* Wallich *ex* Lindley were selected as these are all potential ornamentals which have originated from north eastern region (Kumaria & Tandon 2007).

Dendrobium Swartz is the most interesting, extremely large and diverse genus of Orchidaceae.. The genus has as many as 1500 species (Shashidhar 2012) and is distributed in Assam, Khasi, Darjeeling hills, Sikkim, Manipur, Nagaland, Meghalaya in the Eastern Himalaya as well as in Orissa, Sunderbans, Andaman and Nicobar Islands, Deccan peninsula, Nilgiri and Anamalai Hills in India (Gogoi *et al*. 2014). In India there are 103 species of which 84 are known to grow in Eastern Himalaya and NE India (Gogoi *et al*. 2014; Shashidhar 2012). Growing some of the species in gardens or in other *ex situ* conservatories is a challenge to even some of the most seasoned growers because of their specific habitat and nutritional requirements. Diversities in plant forms, attractive flowers, and distribution has lured the collectors, growers and breeders towards this genus. This popularity has almost led to immense commercial exploitation of some of its species resulting in over collection which is continuing till date.

MATERIALS AND METHODS

The plant materials required for the present investigation were the three different species of *Dendrobium* viz. *D. fimbriatum* Hooker, *D. jenkinsii* Wallich ex Lindley, and *D. polyanthum* Wallich *ex* Lindley collected by professional collectors from the forests of Manipur in Northeast India which is considered to be very rich in orchid diversity (Chowdhery 2001, 2009). The mature plants procured from the collectors of Manipur were then acclimatized under greenhouse conditions (Elliott 1994) and successfully cultivated and propagated by vegetative means (Shashidhar 2012) for *ex situ* germplasm conservation. All three species were found to prefer tropical environment for their normal growth.

Cultural requirements and Cultivation Regimes

All the three species under study were found to grow robustly during spring and summer and remain stunted in late summer or autumn and drop their leaves during the winter and remain dormant.

Temperature: Since these orchid species were found to experience mild to warm daytime temperatures in spring and summer, and moderately cooler temperatures in winter in their natural habitats, extreme care was taken to maintain the temperature in the greenhouses using exhaust fans, khus khus screen, etc. The night temperature was maintained during extremely cold winters by using high voltage tungsten bulbs.

Light: The plants were grown under green agronets having different levels (50 – 70 %) of light penetration depending upon the size and age of plants. Initially, the plants were strictly grown under filtered light in green houses and were later shifted to open conditions under shade.

Water: The plants were watered regularly when they were actively growing but watering was withdrawn gradually as the plants entered into their resting phases during winter.

Fertilizer: The plants were regularly fed with both organic and inorganic water soluble fertilizer, N : P : K :: 19 : 19 : 19 when they were growing actively in the frequency of once a week. The feeding was reduced during autumn, completely stopped during winter and resumed after flowers were finished. The feeding dose was regulated according to the size and maturity of plants and dilution was made strictly according to the manufacturer's instruction.

Potting: The pendent stems of *D. polyanthum* and *D. fimbriatum* made it difficult to grow them in pots especially when the plants were large, so they were mounted on fern blocks, wood pieces and coconut husk or in hanging baskets or were planted in pots with brick pieces and stone-pebbles (Plate I).

Potting and Bedding Materials: The aim of potting is to provide a confined space for the roots in conditions that favour healthy growth. But not all pots and potting materials are suitable for the growth of epiphytic orchids especially in tropical conditions. Therefore, the authors used the most conventional potting materials consisting of charcoal, broken brick pieces and durable materials like tree-fern roots. All the potting materials used here had a certain degree of porosity that allowed water retention, proper aeration and supported the healthy growth of orchids.

Problems: Pests like snails and slugs were controlled by mechanical removal, saline water treatment, use of vegetable and fruit peels for snails and mild fungicides were used to avoid any fungal infection.

Natural Distribution of the species:

Dendrobium fimbriatum Hooker is a native to India and also found in Southern China, Malayan Peninsula, Thailand, Vietnam, etc. and blooms between March to May (Sashidhar 2012; Lucksom 2008).

Dendrobium jenkinsii Wallich ex Lindley is a miniature which is distributed from Eastern Himalaya to China (South Yunnan) and North Indo-China. They flower at different times depending on the place of cultivation. However, flowering usually takes place during February to June.

Dendrobium polyanthum Wallich *ex* Lindley mostly occur in North Eastern Himalayas, Western Himalaya, Indo-China, Laos, Myanmar, Thailand and Vietnam at elevations ranging from 500 – 1000 m. Flowers of this species bloom from winter to summer usually between February and May.

The plant selected for the present study showed distinct variations in their sizes and forms. *D. jenkinsii* appeared like a miniature orchid producing bright yellow flowers while *D. polyanthum* and *D. fimbriatum* were found to possess either upright or long pendant canes. *D. polyanthum* produce fragrant flowers. Considering their diverse forms, extreme care was taken to meet their cultural and nutritional requirements under green house conditions.

Propagation

Successful propagation of different orchid species by any means and rapid multiplication followed by cultivation under greenhouses are the key steps regulating its conservation. Gurung & Gurung (2014) reported that knowing exactly the right time of propagation of the orchid of interest is very important behind the success. Studies carried out earlier have revealed that propagation by division of clusters is best done at the re-potting time, when the plant has completely outgrown its pot.

***Propagation from old pseudobulbs*:** Old but healthy pseudobulbs at dormant stage were taken, pulled off the papery covering and dead leaves and placed those in moist Sphagnum-peat and coir fibres with the eyes facing upwards and kept in a warm place. When new growths were noticed, the plants were brought in the greenhouse environment and allowed to grow as usual in well-ventilated areas. Watering was done approximately twice a week, when the surface of the potting mixture was found to be dry. The plants were then moved into bright, indirect light and provided up to 12 – 14 hours of artificial light per day. This method of propagation was tried with all three test species with varying degree of success rates (Table 1.)

***Propagation form Keikis*:** Different species of *Dendrobium* produce small plantlets called "keikis" (Plate 1, Fig. F) which grow as offshoots from nodes on the mother stem, under filtered light of greenhouses. Propagation of different species of *Dendrobium* by keikis in the present study was done by severing the stem 2.5 to 5 cm below and above the node. Keikis were then planted in a porous medium or in a bed of jute/ coconut fibres after the keiki developed three to four roots.

Propagation by division of rhizome: The rhizome was severed between sympodials with a sterilized knife or razor. In plants with no pseudobulb, rhizome was cut into pieces with at least one live eye. The pseudobulbs or severed pieces of rhizomes were then placed on moist Sphagnum-peat in a low-light environment with good ventilation. The new plants produced were potted using the conventional potting mixture of pebbles, brick pieces and cocoanut chips.

RESULT AND DISCUSSION

The observations and results of the present study clearly indicate that even indigenous orchid species growing in wild forests can be successfully domesticated and cultivated under greenhouse conditions simply by mimicking their natural habitats under controlled conditions. The successful cultivation under green houses in turn can compensate for some of the numbers lost from their natural habitats which in turn can also act as a key step in the conservation of different orchid species.

Table 1. Table showing various methods of propagation and their success rates for three species of *Dendrobium* Swartz

Name of the plant	Method of propagation using	Success (%)	Vigour of new plants	Remarks
D. *fimbriatum*	Pseudobulb	85	++	
	Keikis	90	+++	
	Rhizome	95	+++	
D. jenkinsii	Pseudobulb	70	++	
	Keikis*	nt	nt	*Keikis were neither strong nor prominent but very weak and fibrous
	Rhizome	85	+++	
D. polyanthum	Pseudobulb	75	++	
	Keikis*	nt	nt	
	Rhizome	80	+++	

Very Good: +++; Good: ++; Average: +; nt: not tested

It was observed that the crucial factors for successful domestication and conservation under green houses were the temperature regulation and close monitoring of watering schedule (Table 2). Observing the tips of the canes or pseudobulbs in the autumn when they stop producing new leaves was found to be very crucial as this was the signal that the plant had finished growing for the season. The watering was then gradually reduced allowing the plants to dry somewhat between successive waterings. Growth was found to start again in the late winter or early spring at about the same time that the flower buds began to appear; once the buds were formed and new growth appeared watering was increased. But, none of the species tested here preferred complete dryness even during their resting period. Prolonged period of dryness was found to either delay the flowering or inhibit flowering completely (Table 2).

All three test species of *Dendrobium* in the present study were found to prefer bright but filtered light. The requirement for bright intensity of light was found to be more especially during cold winter months. This need may be correlated to slight degree of warmth which the sunlight or any artificial lights provided as these species were found to prefer mild to warm days in winter, but much cooler nights (Table 2).

Earlier reports and studies (Awasthi *et al*. 1995; Yoder *et al*. 2000) did not highlight the need to feed epiphytic orchids as they are known to absorb all nutrients through the slow breakdown of tree bark and accumulated debris from air and also benefit from their mycorrizal association of roots with beneficial microoganisms. But the observations recorded in the present study showed that feeding was very essential for orchids in contrary to earlier belief. Though the authors tested the use of both organic and inorganic fertilizers commercially available, the inorganic fertilizers were found to be much more convenient to use as they were odourless. It was found that the ratio of N:P:K was another crucial

TABLE – I. Culturing wild species of *Dendrobium*: A & B. *D. jenkinsii* tied to coconut fibres; **C.** *D. jenkinsii* growing in a greenhouse; **D.** *D. fimbriatum* tied to fern block; **E.** Blooms of *D. fimbriatum;* **F:** *D. fimbriatum* showing formation of Keikis on old sticks; **G & H:** *D. polyanthum* in vegetative stages; **I.** *D. polyanthum* in flowering stage

Table 2. Variations in performance of three different species of *Dendrobium* cultivated at a temperature of 25 – 28º C, humidity of 70 – 80 % under filtered light during summer and 12 – 14 hours of artificial light during winter in a greenhouse

Name of the plant	Watering Schedule		Fertilizer N:P:K (Half Strength)*		Flower initiation	No. of flowers per spike/ per plant	Quality of blooms
	Summer	Winter	19:19:19	P & K rich (Tracil) 1gm in 2 litres of water			
D. fimbriatum	Daily	Daily	Once a week	Once a week	Delayed	14	++
	Alternate	Daily			Delayed	12	++
	Daily	Alternate			In time	20	+++
	Alternate	Alternate			In time	18	+++
	Daily	No			No flowering	00	-
D. jenkinsii	Daily	Daily	Once a week	Once a week	Delayed	02	+
	Alternate	Daily			Delayed	02	+
	Daily	Alternate			Timely	05	+++
	Alternate	Alternate			Timely	04	+++
	Daily	No			No flowering	00	-
D. polyanthum	Daily	Daily	Once a week	Once a week	Delayed	10	++
	Alternate	Daily			Delayed	12	++
	Daily	Alternate			Timely	18	+++
	Alternate	Alternate			Timely	16	+++
	Daily	No			No flowering	00	-

Very Good: +++; Good: ++; Average: +

Feeding the plants regularly with only N:P:K and no Tracil resulted in only vegetative growth and no initiation of flowering

factor in promoting growth or inducing flowering. Fertilizer high in nitrogen content only led to vegetative growth while the one with higher proportion of P and K were found to promote flowering (Table 2). Hence mere application of general purpose fertilizer was not found to be enough in the successful domestication and cultivation under green house conditions. Further, it was observed that all three species required extra care and attention only in their initial period of acclimatization, but once they were established, they could be gradually taken outdoors and cultivated in garden on tree trunks (Plate 1, Fig. E). Introduction outdoors also promoted profuse development of aerial roots and considerably reduced their dependency upon artificial feed.

CONCLUSIONS

Orchids are known to offer a lot in the study of interaction of plants, fungi and animals especially pollinators. Hence, it is natural for the structure and composition of orchid floras to change as populations respond evolutionarily through adaptation, extinctions and migrations. But any drastic changes leading to an alarming decline in numbers and extinction of any species is not desirable under any circumstances. Yumnam (2008) and Medhi *et al.* (2012)

have earlier reported that the conservation of orchids is now a matter of universal concern. There is an urgent need to protect and conserve the valuable orchids in green houses if not in their natural habitats as they are highly sensitive to ecological disturbances (Medhi & Chakrabarti 2009). Conservation measures must be strengthened and all forms of conservation including domestication and cultivation under green houses must be encouraged to preserve these precious gifts of nature before they become extinct from this planet.

If propagation in such much simplified conditions become successful, as in the present case, then those species can be multiplied in large number and can be used for the regeneration of their population in their wild habitat.

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