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Ex situ conservation of rare and threatened orchid: *Diplomeris hirsuta* (Lindl.)

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Abstract

The *Diplomeris hirsuta* Lindl. is a rare and endangered orchid species indigenous to India. This delicate species faces numerous threats, including landslides and road expansion, which have decreased its population and placed the species at risk of extinction. However, there is hope for this species, as the paper has reported the successful *ex situ* conservation of *D. hirsuta* plants. The researchers achieved this feat by creating a simulated natural habitat that closely mimicked the conditions of the plant's native environment. This simulated habitat provided the ideal environmental conditions necessary to sustain the plant's growth and development, including temperature, humidity, light, and soil composition. As a result, the live plants of *D. hirsuta* were successfully conserved. This study's *ex situ* conservation method is essential in preserving species' survival, especially when their natural habitats are at risk or destroyed. The success of this conservation effort provides a potential model for future conservation efforts of other threatened plant species, giving hope for the protection and preservation of rare and endangered plant species worldwide.

Key words: Conservation, *Diplomeris hirsuta*, *ex situ*, habitat, snow orchid, threatened, watering

Introduction

The Snow Orchid (*Diplomeris hirsuta* Lindl.) is a lithophytic orchid species found in India, Northern Myanmar, Southern China, Northern Vietnam, North-East Thailand, Bhutan, and Nepal (Iamwiriyakul and Kaewphung, 2008). "Diplomeris" is derived from the Greek word for "divided stigma," whereas "hirsuta" is derived from the Latin word for "hairy" (Singh, 2012). It is found in India (Fig. 1) in Sikkim, Arunachal Pradesh, West Bengal, and the Western Himalaya (Rao, 2010).

This orchid species has large ovate-oblong hairy leaves and produces single or occasionally two white flowers. It grows in cool and shady places on moist rocks near running water streams, along with other vegetation such as liverworts, ferns, and wild begonia. Unfortunately, due to anthropogenic activities such as the clearing of land for road widening, landslides, and scraping of retaining walls for beautification of roads, populations of this orchid are vulnerable to extinction. There are no records of conservation or successful rehabilitation of this species in *ex situ* environments (Behera *et al.,* 2012).

Ex situ conservation, the practice of conserving a species outside of its natural habitat, is a valuable strategy for orchid conservation. This strategy can be implemented by cultivating orchids in specialized facilities like botanical gardens, greenhouses, and other controlled environments.

In the case of *D. hirsuta*, there is a lack of information regarding its growth cycle, watering, and substrate requirements, which can be crucial for successful *ex situ* conservation and rehabilitation. In this study, we aimed to investigate the role of watering on *D. hirsuta* using live plants.

Understanding the watering requirements of endangered orchid species is crucial for their successful conservation and rehabilitation. To achieve this, *D. hirsuta* plants should be grown in a controlled environment and subjected to different watering regimes. Such an approach will help understand the significance of providing optimal watering conditions for the species' *ex situ* conservation and rehabilitation efforts. By ensuring optimal

Fig.1. Distribution of *Diplomeris hirsuta* (Lindl.) in Sikkim and West Bengal.

watering conditions, the survival and growth rates of this species can be improved, which will increase the chances of its successful conservation and reintroduction to its natural habitat. Therefore, understanding the watering requirements of endangered plant species, such as *D. hirsuta*, is essential to conserve and protect them for future generations.

The study involved growing live *D. hirsuta* plants in a controlled environment and subjecting them to different watering regimes. The growth, survival, and physiological parameters of the plants, such as leaf water potential, chlorophyll content, and photosynthetic rate, were monitored for uderstanding the optimal watering requirements for successful *ex situ* conservation and rehabilitation of *D. hirsuta*, and how do different watering regimes affect the growth, survival, and physiological parameters of this species

Materials and methods

Live plants of Diplomeris hirsuta were collected from its natural habitat in Assam Linzey, East Sikkim, during the year 2017-18. To create simulated natural habitat conditions, the collected plants were placed on bricks along with its associated vegetation, replicating the conditions found in nature (Fig. 2).

The experiment was conducted in the semi-automated Glasshouse at ICAR-National Research Centre for Orchids, Pakyong, Sikkim. The experiment followed a randomized block design with three treatments and seven replications. The treatments consisted of watering the live plants at different intervals, including T_1: watering in February, T_2: watering in March, and T_3: watering in April. The aim was to study the effect of watering on the growth and flowering of *D. hirsuta*.

Fig. 2. *Diplomeris hirsuta* in controlled condition (*ex situ* conservation)

Observations were recorded at different stages of plant growth, including days to plant emergence, leaf length, leaf width, number of leaves per plant, days to bud initiation, days taken for flowering from bud initiation, and days to flower withering.

Statistical analysis: This experiment was carried out in a completely randomised design (CRD) with ?? replicates per treatment. Statistical significance between mean values was evaluated using one-way ANOVA (Gomez and Gomez, 1984).

Results and discussion

Diplomeris is considered as an endangered and threatened orchid (Ayensu, 1986; Jalonen *et al.,* 2009). It is found growing in the rocks along the roadside. Habitat destruction, landslides and the widening of roads lead to this orchid extinction's vulnerability (Burman and Devdas, 2013). These orchids need artificial assistance to migrate or translocate from such hostile environments to new places where they can be conserved (Swarts and Dixon, 2009).

To successfully conserve Diplomers hirsuta in *ex situ* conditions, live plants were subjected to watering treatments to create natural habitat. The results indicated that during the first year, significant differences were observed for leaf length and leaf width among the treatments. Maximum leaf length and leaf width was recorded in T_1 (10.97 and 3.22cm) followed by T_2 (8.27cm and 2.53cm) and T_3 (7.78cm and 2.38cm). However, no significant difference was observed for number of leaves. Days to bud initiation and days to flower emergence were earlier in T_3 i.e April watering (35 days) followed by T_1 and T_2 (50 and 50.85 days, respectively). However, the plant emerged earlier in T_1 between 12-26th, May as compared to the other two treatments between 30th May-6th June and 17th June to 24th June, respectively. Significant differences were observed for days to flower emergence in all the treatments. However, T_1 and T_2 were at par. Minimum days for flowering were recorded by T_3 (67.28 days) followed by T_1 (80.28 days) and T_2 (83 days). For days to flower withering, all the treatments showed insignificant differences.

During the second year, similar observation was recorded for leaf length, width, and number of leaves. The leaf length and width were smaller than the previous year for all the treatments. Maximum leaf length and leaf width were recorded by T_1 (6.52cm and 1.85cm) followed by T_2 (5.13cm and 1.47cm) and T_3 (4.62cm and 1.38cm), respectively. Plant emergence was earlier in all the treatments as compared to the previous year, *i.e.,* in T_1 (5-15th May), T_2 (14-21st May) and T_3 (17-29th May). Though the plant emergence was earlier in T_1, the minimum days taken for bud initiation was recorded by T_3 (41.86) followed by T_2 (48.57) and T_1 (49.43). For days to flower opening, all treatments showed significant differences. T_2 significantly differed from T_1 and T_3 and recorded minimum days for flower opening (60.85). However, flower withering was observed earlier in T_3 (14.62 days) from flower opening (Table 1).

It was observed that the plant emergence was earlier during 2019

Table 1. Effect of watering treatment on growth and flowering of *Diplomeris hirsuta*

Treatment	First Year (2018)						Second Year (2019)					
	Leaf length (cm)	Leaf width (cm)	Number of leaves/ plant	Days to bud initiation	Days to flower emergence	Days to flower withering	Leaf length (cm)	Leaf width (cm)	Number of leaves/ plant	Days to bud emergence	Days to flower emergence	Days to flower withering
T1	10.97[a]	3.22[a]	2.14	50.00[a]	80.28 [a]	20.14	6.52[a]	1.85[a]	1.86	49.43[a]	74.43[a]	15.57[b]
T2	8.27[b]	2.53[b]	2.00	50.85[a]	83.00 [a]	21.14	5.13[b]	1.47[b]	2.00	48.57[a]	60.85[b]	28.72[a]
T3	7.78[b]	2.38[b]	2.00	35.00[b]	67.28 [b]	19.00	4.62[b]	1.38[b]	2.00	41.86[b]	72.43[a]	14.57[b]

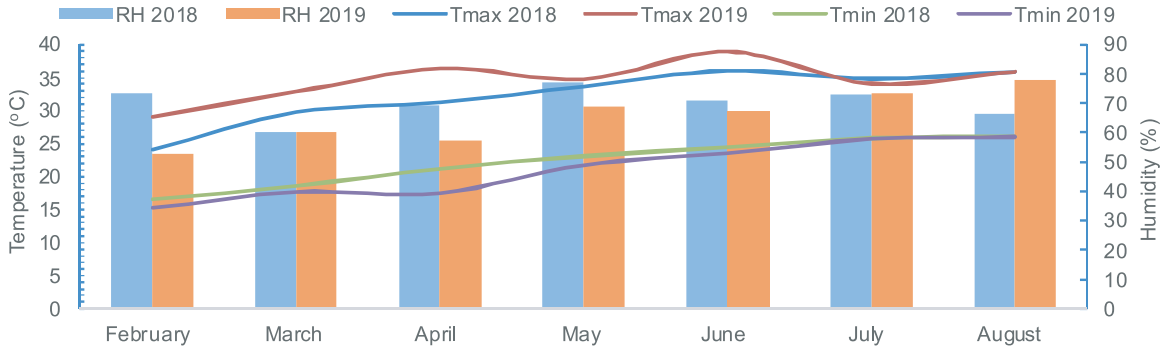

Fig. 3. Average maximum, minimum temperature (°C) and relative humidity (%) during peak growing season of *Diplomeris hirsuta* (Lindl.)

compared to 2018 which extended till last week of June, whereas all the plants of 2019 in all treatments emerged within May. It may be because the plant requires a warm and humid climate during its active growing stage and the temperature recorded during 2019 was higher than the previous year (Fig 3).

It was observed that all the plants emerged between May to June in both the years in all the treatments. Vegetative parameters such as leaf length and width were highest in T_1 during both the years followed by T_2 and T_3. This is because the plants remain dormant after flowering (Aug-Sept) and survives by a tuber and reappears in May-June with water availability and favorable climate. So, a continuous water supply from February led to good vegetative growth of the plants in T_1. However, T_3 plants were exposed to watering treatment for a short duration till its germination; hence, their leaf length and width were smaller than other treatments. Also, the flower size was smaller in T_3 than in T_1 and T_2. Pradhan (1974a); Pradhan (1974b) also reported that *D. hirsuta* could be grown by keeping the plants in a warmer area, protected from direct sunlight with good moisture during the growing season and keeping the media dry during winter. The peak flowering was during August for all treatments in both the years irrespective of treatments though there was some variation in number of days in different treatments. Water played an important role in the overall growth and survival of the plants.

Ex situ conservation efforts were undertaken to preserve this species, and the study found that watering treatments played a crucial role in maintaining natural habitat conditions and promoting growth and survival of the plants. The results showed that continuous water supply during the initial and active growing stage led to good vegetative growth and increased chances of survival. The study provides useful insights into the conservation of rare and endangered plant species and highlights the importance of *ex situ* conservation efforts in ensuring the survival of such species.

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