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Conservation of a medicinal orchid through ex-situ cultivation: an experimental trial on Saalampanja (*Dactylorhiza hatagirea* D.Don) Soó RET species in North Western Himalaya

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ABSTRACT

Dactylorhiza hatagirea (D.Don) Soó (Orchidaceae), popularly known as Saalmpanja, is a high value perennial herb. It inhabits sub-alpine to alpine regions, ranging at elevations between 2500 - 5000 m.a.s.l., Due to over harvesting, it has now been declared as 'Critically endangered' medicinal orchid in Himalayan region. *Ex-situ* production and sustainable harvesting from wild habitats are viable options for its conservation. An experimental trial following Randomized Block Design was carried out to analyze plant growth, phenology and productivity (per ha) under cultivation as compared with the plants of the same age group growing naturally in nearby forest area. Seed germination was found nil however all the tubers sprouted without any treatment. Comparatively, cultivated plants were found to be superior in every aspect of growth, biomass, and productivity. The study concludes that there are high possibilities of *ex-situ* production of this species in high altitude zones resembling to its wild habitats. The plants can be easily raised through tubers; however, intensive studies are suggested here to under seed germination mechanism in this species. Due to its high demand in Ayurvedic formulations, ex-situ production of the species will open new avenues for consistent supply of raw materials and thus, good returns to the high-altitude farmers. The study recommends an extensive scientific effort to develop GAP (Good Agricultural Practices) and sustainable GFCP (Good Forest Collection Practices) in such Himalayan RET (Rare Endangered Threatened) plant species.

Key words : *Conservation, Dactylorhiza hatagirea, Growth, Medicinal orchid, Phenology, Productivity, RET, Saalampanja*

Introduction

Dactylorhiza hatagirea (D.Don) Soó (Family-Orchidaceae), inhabiting sub-alpine to alpine regions, occurs at elevations between 2500 and 5000 m.a.s.l. (Pant and Rinchen, 2012). Of about 1,229 species (184 genera) of orchids, distributed in tropical to alpine regions of India (Chowdhery, 1999), 960

species (158 genera) are reported from Indian Himalayan Region (Samant and Pant, 2006). Saalampanja is one of the most valuable and widely known species. It has palmately lobed tubers and lanceolate leaves having a sheathing leaf base, it bears pink flowers with purple-colored notches and a curved spur (Wani *et al*., 2020). A perennial, beautiful herb, 30-50 cm tall, it is native and near endemic to Himalayan region (Samant *et al*., 1998; Figure 1); This species is distributed in India, Pakistan, Afghanistan, Nepal, Tibet and Bhutan. In India, it is distributed in Jammu and Kashmir, Uttarakhand and Himachal Pradesh (Chauhan *et al*., 2014). In India, it is reported from Jammu and Kashmir (J&K, Dhar and Kachroo, 1983), Uttaranchal (U.A., Hajra and Balodi, 1995) and Himachal Pradesh (H.P., Aswal and Mehrotra, 1994). In H.P., the plant grows along open grassy slopes and alpine meadows between 2500-4000m. Being palmate lobed, like a hand with fingers open, the plant is locally called as panja, salampanja, hath-panja or hatajari in Uttarakhand; in Kashmir known as salem panja and in various parts of Ladakh known as wanglak or angulagpa (Pant and Rinchen, 2012). In Nepal 'panchaule' (Magar *et al*., 2020). The plant is used in various Indian System of Medicine including Ayurveda, Siddha and Unani medicine and also practiced traditionally in curing dysentery, diarrhoea, chronic fever, cough, stomachache, wounds, cuts, burns, and bone fracture, general weakness particularly in debilitated women after delivery and for increasing regenerative fluid (CSIR, 1966; Lal *et al*., 2004; Pant and Rinchen, 2012; Magar *et al*., 2020; Singh *et al*., 2021). The fleshy tuber contains starch, mucilage, sugar, phosphate, chloride and glucoside-loroglossin (CSIR, 1966). Tuber yields 'Salep', which is used as farinaceous food, expectorant, aphrodisiac and as nervine tonic (Singh *et al*., 2021). Due to high medicinal and edible values, the species has great demand in national and international markets (Olsen and Helles, 1997).

Cultivation of orchids in India is mostly done for cut flowers. Some of native genera like *Arachnis, Cymbidium, Dendrobium, Paphiopedilum* and *Vanda* are cultivated on a large scale for cut flower production. Among these genera, *Dendrobium* is most popular tropical orchid getting fame as cut flowers in India as well as in the world. The *Cymbidium* is mainly grown in NEH region, Sikkim, Darjeeling hills, Arunachal Pradesh and Assam. Tropical orchids are cultivated in Kerala and Tamil Nadu. So far, there is no report available on systematic cultivation of Saalampanja that exists in India. Extraction of raw material from its wild populations is the only source to meet the market demand. Excessive extraction has resulted in further depletion of its rare, patchy and limited populations in Kumaun (Uniyal *et al*., 2002). Severe extraction threats are noticed in other parts too (Bhatt *et al*., 2005). Owing to unsustainable harvesting and illegal trade of its natural populations, the species is categorized as critically endangered for J&K, U.A. and H.P. (CAMP, 2003), and placed under negative list of exports of the Export and Import Policy, 1997-2002 (InfodriveIndia, 1999-2000) and in Appendix-II of CITES. Conservation and sustainable utilization of this species through *ex-situ* cultivation is suggested in India (Nautiyal and Nautiyal, 2004) and Nepal (Kunwar, 2006).

Seeds of *D. hatagirea* are very minute in size and exhibit very low viability (Nautiyal and Nautiyal, 2004). A couple of reports suggest the association of mycorrhiza is essential for its seed germination (Chauhan, 1999; Nautiyal and Nautiyal, 2004). Based on the available reports, Dhar *et al.* (2002) listed this species very difficult for cultivation. The current study is the first scientific field trial initiative in this direction, which aimed determining future potential of *D. hatagirea* as cash crop, by comparing with wild plants, for substituting raw material harvesting from natural habitats, and highlighting associated marketing. The objectives of the present study were (i) comparison assessment of plant growth, phenology, and productivity in terms of both biomass and monitory per unit area, with the plants grown in wild habitats, and (ii) to explore the local market value and demand of the selected species.

Materials and Methods

Mature tubers and seeds of Saalampanja were collected from the plants growing in forest gaps, open grassy slopes and shady moist areas (3100 m-3040 m- amsl) in Bhandag area of Parvati valley, H.P. Sustainable harvesting practices were followed during the collection. The tubers were stored beneath sand and the seeds after drying at room temperature (12-20 ^{o}C) for 10 days were stored in a hermetic plastic container and placed them under refrigerator (4 ^{o}C) until used experimentally in October last week. Density of the plants in wild was noted and 9 mature individuals (one year old originated from tuber) were additionally sampled to record their growth and biomass.

Two north-facing, mild sloppy plots (15 to 20°) of 10 m x 10 m each were selected for the cultivation trials. These plots, with brownish red clay soil, were located at the fringe of an apple orchard (2500 m amsl) near the village Silha, Parvati valley; district Kullu, H.P. The district lies between 31^0 25′ and 32^0 35′ north latitudes and 76^0 9′ and 77^0 9′ east longitudes, covering an area of 5,503 sq. Km (9.9 percent of the state) along altitudinal variations between 900 m to over 6000 m amsl. During The study period, the climatic data were recorded at 2200 m station along the same altitudinal transect [mean daily minimum temperature: 5.42±0.18 ^{0}C (January) to 12.5±2.03 °C (July); mean maximum temperature: 7.11±0.34 °C (January) to 17.3±2.26 ^{0}C (July); mean minimum relative humidity: 22.9±0.78 % (February) to 45.0±8.92 % (July) and the maximum from 29.3±0.88 % (January) to 67.5±20.7 % (August)]. The monthly rainfall data obtained from nearby station of forest department were: 11.2±7.0 mm for mean minimum (November) and 194.4±14 mm for mean maximum (July).

For the preparation of plots, digging and ploughing were done and the clay lumps were crushed and stones removed. For soil amendment, farm yard manure and forest humus at the rate of 2 Kg m^{-2} each (estimated 20 tone h^{-1}) were mixed thoroughly. The amended soil pH ranged from 6.6 to 6.9, electrical conductivity from 0.122 to 0.149 mhos/cm, and the soil had 2.5 % carbon on dry weight basis. Randomized Block Design (RBD) was followed for the experimentation.

For seed propagation, trenches were made in the plot and a bulk of seeds (approximate 1 gram; very minute dark brown powdery) directly sown, mixed with sand, with the help of plastic spoon at a spacing of 15 cm × 15 cm (between points) and 0.5 cm depth. The plot was irrigated just after seed sowing and monitored daily for the seedling emergence.

For vegetative propagation, 300 young tubers were planted at a distance of 30 cm × 30 cm between plants (16 plants/m^2) and 5 cm depth in the plot. Proper tilling, watering and weeding were done from time-to-time. Weeding was practiced twice monthly during rainy season. The survival and pheno-phases of the plants were recorded. However, from December, 2001 to March, 2002, the experimental plot remained covered with the snow.

During first week of September, the seeds from cultivated plants were collected and tested immediately for germination in laboratory. Approximately 1gm seed per replicate (in triplicate) were placed in a petridish lined with moistened filter paper (Qualigens, 615 A), using double distilled water. The petridishes were placed in BOD (25 °C) equipped with alternate light regimes (16 hrs. light and 8 hrs. dark).

In September end, about half of the plants were harvested and among them, 9 plants randomly selected for recording their growth and biomass. Remaining plants were planted in nurseries and herbal garden of the Institute. Fresh tubers were sun dried for three weeks to estimate their biomass. The final survival data were used to estimate dry matter productivity on per hectare basis and local market rates were used to calculate the monetary value of the belowground products (dry tubers). Productivity and monetary value were projected for the plants under domestication, per unit area basis, and compared with wild harvesting. The monetary value was calculated using average minimum prices of dry tubers, *i.e.*, Rs. 1000/kg.

The data were statistically analyzed using MS-Excel 2000. Analysis of variance (ANOVA). Mean and standard deviation were used for processing the data in various ways. Least Significant Difference (LSD) was estimated (Snedecor and Cochran 1967) to calculate variation in parameters.

Results and Discussion

In The present study, seed germination was found nil in field as well as laboratory condition although no any seed treatment was given. Nautiyal and Nautiyal (2004) have reported 20-30 % seed germination in this species while sowing them in their natural habitat or in the soil thereof. It can be assumed that the seeds of this species, being very minute in size, can easily disperse by wind and owing to their low regeneration they further become least helpful in population expansion. This may be one of the reasons of patchy or restricted distribution of this species (Uniyal *et al*., 2002). However, its propagation is limited in nature due to its non-endospermous seeds requiring mycorhizal fungal association for germination (Magar *et al*., 2020). *D. hatagirea* can be considered inherently slow growing and poorly regenerating species, because of pollinator specificity and requirement for mycorrhizal association (Bhatt *et al*., 2005). Singh *et al.,* (2021) concluded that short life cycle, poor seed germination rate, specific microhabitat requirement, low popula-

tion density and high commercial value restrict the widespread distribution of the species. The vegetative propagation through splits and divisions of the tuber having stem portion with bud, is relatively found successful in this species. Badoni (1997) found that the application of 50 mM of STIK or 1000 mM of GA_3 on cut parts of dormant shoot buds was effective in increasing sprouting, length and gross weight of roots in this species. Giri and Tamta, (2012) revealed that to improve vegetative multiplication, tubers were treated with α-naphthalene acetic acid (NAA), indole-3- butyric acid (IBA) and indole acetic acid (IAA) before planting. Rooting was observed in only apical segments. Maximum rooting (38.88%) was induced with 50.0 µM IBA. In current case, full young tubers were propagated to minimize the probability of failure in the first-hand experience of field trials. Inevitably, 90 % tubers were sprouted and matured into plants, in which 70 % were survived. The maximum mortality was observed during July, which could be related to heavy rainfall during this month, washing away the upper layer of soil exposing roots of some plants. During harvesting domesticated plants, it was noted that the finger tips of 30 % old (mother) tubers were partially rotten and 5 % tubers got detached and decomposed in soil. This proposes harvesting after a long period may be harmful for the tubers and yearly period is sufficient for the same, however, the time may vary with the environmental conditions. In traditional domestication of the species, harvesting was reported after three years of planting (Maikhuri *et al*., 1998).

Studies suggest high utility of phenological observations in developing management and conservation plans for valuable plant species (Martinez-Calvo *et al*., 1999; Baumgartner and Hartmann, 2000, etc.). In the present study of *D. hatagirea*, the comparison of pheno-phases between plants under field trial and wild state showed a notable variation in timing, while commencement of all phenological stages were recorded very early under field trial (Table 1). Altitudinal difference between trial site and wild habitats may be an important factor for such variations in timings. Along altitudinal difference, the temperature is a major factor, determining phenology (Sparks, 2002). In present case, tubers propagated in the last week of October, started sprouting in March 3rd week after complete snow melting, which continued up to 1st week of April. By May 2nd week, the plants showed young leaf stage and leaves matured by June 4th week. Floral buds appeared during June 3rd week and subsequently it followed the flowering. July 2nd week witnessed fruiting initiation, mature seeds produced by the August 1st week and shedding begun from August 3rd week. By the September end, a few stems were in senescence stage, however, 100 % plants dried before October last. In general, collection of tuber is done during September to November after seed ripening and fall.

Table 2 presents data on the growth and biomass of plants, both collected from wild and those harvested after one growing season under cultivation. Plant height, collar diameter and tuber length in domesticated plants were significantly ($P<0.05$) superior than those from wild. The either source yielded only one new tuber per plant but the num-

Table 1. Comparison of various phenological phases between the plants grown in field and those recorded in wild habitats of Saalampanja

Pheno-phases	Under cultivation (2500 m amsl)	Wild habitats* (>3000 m amsl)
Vegetative bud sprouting initiation	3rd week of March to 1st week of April	April-May (after snow melting)
New leaf initiation	Between 1st to 3rd weeks of April	April-May (as temperature rises)
Young leaf stage	2nd week of May	May-June
Leaf maturation	Between 1st to 4th weeks of June	June-July
Floral bud stage	3rd week of June	June-July
Flowering	3rd to 4th weeks of June	June-July
Fruiting	2nd to 4th weeks of July	August-September
Seed maturation	1st week of August	August-September
Seed shedding	3rd week of August to 1st weeks of September	September-October
Leaf senescence/drying	3rd weeks of August to 4th weeks of September	September-October
Stem senescence/drying	4th week of September to 1st week of October	October end

*Based on various field visits of the authors in high altitudes of Parvati valley in district Kullu, H.P.

ber of fingers per tuber in domesticated plants (Fig. **1**; 4.22±0.97; range 3-5) were significantly higher than that observed for wild (2.56±0.73; range: 2-4). In general, the tubers with higher number of fingers

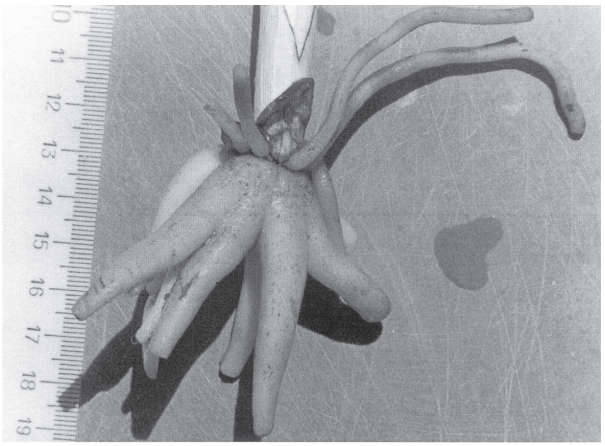

Fig. 1. Mother tuber of Saalampanja attached with newly produced tuber

are said to be superior to those with fewer fingers (Lama *et al*., 2001).

Fresh weight of tubers (10.81±2.97 g) from cultivation was significantly (P<0.05) higher than that from wild (3.09±0.59 g). The moisture content was also higher in domesticated tubers (368.0 %) than wild tubers (286.3 %). Smaller size as well as high moisture content of the tubers is the main reason of extracting large number of individuals of this species from wild to get maximum dry weight (Uniyal *et al*., 2002). The productivity (3.70 quintal/ha) and monetary value (Rs. 3,69,600/ha) projected for 100 % survival of the plants under cultivation came 5 folds higher over wild harvesting (Fig. 2). It is also higher than the report of Shrestha and Shrestha (2004) which estimated a total yield of dried tubers of 2.5-3.0 quintal/ha in natural habitats. Higher growth and biomass under cultivation could be related to proper management, less environmental hazards and no anthropogenic pressure (grazing, trampling, harvesting, etc.) as compare to that in wild. Additionally, nutrients' supplement to the plants using organic matters has contributed maximum to good results. For soil enrichment in the current trial, only organic matters were used. Incidentally, the use of chemical fertilizer (Urea) in cultivation attempts of this species, by a farmer in Pattan valley of Lahaul and Spiti area of H.P. It had re-

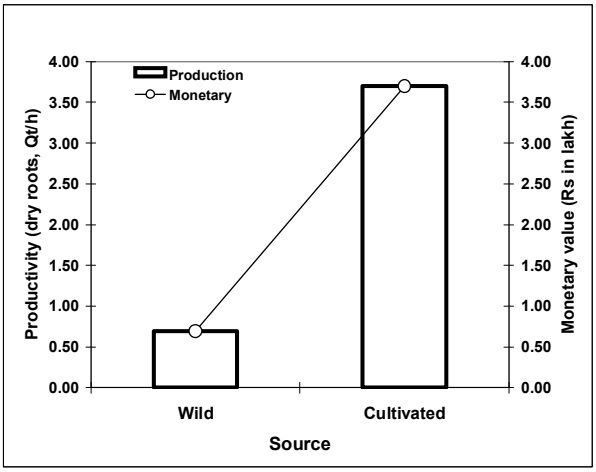

Fig. 2. Productivity and Monetary value of Saalampanja from wild harvesting and under cultivation (based on 100 % survival of plants)

Table 2. Growth and biomass of Saalampanja from wild population and after one growing season under cultivation

Parameters	Wild (3100 m amsl)	Cultivated (2500 m amsl)	LSD (P<0.05)	F
Plant height (cm)	30.76±1.41	38.08±4.46	3.31	22.02*
Stem diameter (mm)	8.21±0.86	7.89±1.51	1.23	0.313^{ns}
Collar diameter (mm)	6.11±0.91	8.28±1.93	1.51	9.359*
Number of new tubers	1.00±0.00	1.00±0.00	-	-
Number of fingers (range)	2.56±0.73 (2-4)	4.22±0.97 (3-5)	0.86	16.98*
Tuber length (mm)	24.58±5.79	99.73±17.19	12.82	154.5*
Tuber width (mm)	19.05±6.18	20.35±3.94	5.18	0.283^{ns}
Above ground fresh weight (g)	12.90±1.42	11.99±4.29	3.19	0.366^{ns}
Tuber fresh weight (g)	3.09±0.59	10.81±2.97	2.14	58.37*
Tuber dry weight (g)	0.80±0.13	2.31±0.56	0.41	61.73*
Moisture content (%)	286.3	368.0	-	-
Density (no. h^{-1})	30000-40000	160000	-	-

*=significant at P<0.05 level; ns=non significant; ±=standard deviation

sulted in high mortality of plants (Authors' personal communication). Small scale domestication of the species following traditional farming system is reported in Nanda Devi Biosphere Reserve in Uttarakhand (Maikhuri *et al*., 1998).

Based on actual survival (70 %) of the plants, the productivity and monetary value were estimated to be 2.59 quintal/ha and Rs. 2,58,720/ha, respectively. These outputs may offer encouraging possibilities of economically viable cultivation of this species in North Western Himalaya. Production of orchids as cut flower has already found beneficial while cultivated in Sikkim. From an area of 500m^2 containing 3000 plants of *Dendrobium* growers can earn upto 5 lakhs in 3 years period after selling of 3000 number of cut spikes and 3000 mother plants (De and Medhi, 2015).

Author's market (Kullu, Dehradun and Delhi) survey showed increasing trend in market price over the years. The rates of dry tubers/kg were recorded many folds higher in year 2021 (maximum Rs. 9000) than that in the year 1999 (Rs 500). However, the rates varied amongst and within the markets. It may be due to largely grey marketing for threatened medicinal plants, which provides the harvesters poor chances of negotiations. However, the traders in interviews revealed that the demand of species has been increasing continuously. To their opinion, commercial cultivation of the species is the only option to meet ever increasing demand of pharmacies.

The study concludes that

(i) There are high possibilities of *ex-situ* production of replace with Saalampanja in high altitude zones resembling to its wild habitats;

(ii) Considering poor understanding on seed germination of Saalampanja, the plants can be easily raised through tubers; however, intensive studies on seed germination mechanism is required;

(iii) For sustainable harvesting, collection of mother plant takes place by leaving one immature tuber in the soil. Wild collection of plant is done by applying rotation system.

(iv) Market demand and price of the species are continuously increasing and therefore, commercial production will provide good returns to the farmers.

(v) Conservation and management need a proper understanding of reproductive biology and strategic action to protect its rare habitats.

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