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Short communication

Population biology of *Lilium polyphyllum* D. Don ex Royle—A critically endangered medicinal plant in a protected area of Northwestern Himalaya

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ABSTRACT

Lilium polyphyllum D. Don ex Royle, a Critically Endangered medicinal plant of the Himalayan Region is exploited for its roots to meet the demand of pharmaceutical industries. Over exploitation from the natural habitats has caused population depletion to a great extent. Therefore, population status of *Lilium polyphyllum*, effect of soil factors and associated species, and methods to improve seed germination were investigated. Species were sampled in habitats, seeds were collected and subjected to various treatments viz., soaking, chilling, plant growth regulators (indol acetic acid, indol butyric acid and gibbrelic acid) and chemical compounds (potassium nitrate and sodium hypochlorite). *Lilium polyphyllum* was recorded in only two habitats between 2338 and 2574 m with density range of 1–6.7 plants m^{-2}. Density had significant correlations with soil moisture content ($r = 0.463$, $P < 0.05$) and nitrogen ($r = -0.96$, $P < 0.01$). In control conditions seed germination (35%) and Mean Germination Time (91 days) was poor. All the treatments applied to the seeds improved germination and MGT significantly ($P < 0.01$) over control. Sodium hypochlorite (5 min) improved the germination percentage to 92% and indol acetic acid (100 μM) reduced mean germination time to 20 days. Species had high sensitivity to soil factors (i.e., nitrogen and moisture), non-native species and habitat destruction in natural habitats. Therefore, regular monitoring of its habitats, mass multiplication using germination protocol and transplantation are suggested to conserve the species.

Introduction

Lilium polyphyllum D. Don ex Royle is locally and commercially known as Kakoli or Ksheerkakoli and belongs to the family Liliaceae. It is a Critically Endangered (IUCN Red List) medicinal plant (Ved et al. 2003). It grows commonly in humus-rich forest floors between 2100 and 3000 m amsl. Plants are bulbous herbs and grow up to 1 m height. Leaves are narrow, alternate, lanceolate, 8–12 cm × 0.5–1 cm, sessile, flashy. Flowers are white with purple dots and capsules three-angled with winged seeds (Dhaliwal & Sharma 1999). Flowering occurs during June–July (rainy season) and fruiting during September–October (autumn season).

Lilium polyphyllum is restricted to its native habitats in the Himalayan Region. It is found between Afghanistan and Uttarakhand state in India. In the Indian Himalayan Region (IHR), the species has a very small population, and reported only from a few places namely Dhauladhar and Shimla in Himachal Pradesh, Chatru and Doda in Jammu and Kashmir, Chakrata and Raath in Uttarakhand (Ved et al. 2003), Pulga-Kullu, (Dhaliwal & Sharma 1999), Chakisain, Garhwal (Gaur 1999) & Gargia-Pithoragarh (Samant 1987) (Fig. 1). Bulbs have soothing, astringent and anti-inflammatory properties. They are used in traditional and modern medicines and used as refrigerant, galactogogue, expectorant, aphrodisiac, diuretic, antipyretic and tonic in cough, bronchitis, vitiated conditions, seminal weakness, strangury, burning sensation, hyperdipsia, intermittent fever, hematemesis, rheumatalgia and general disability (Jain 1991). Bulbs are also used in revitalising night cream and *Chywanaprasha* (an ancient ayurvedic herbal preparation) (Anonymous 2007). The bulbs are traded under the trade name Kakoli/Ksheerkakoli in the local and national markets. In nature, plants regenerate both by vegetative means (through bulbs) and by seeds. Over-exploitation for trade and habitat degradation due to heavy grazing (Rana & Samant 2010) coupled with climate change and proliferation of invasive species has led to considerable depletion of its natural populations. This has resulted in listing this species as Threatened in the Red Data Book of Indian Plants (Nayar & Sastry 1990) and subsequently as Critically Endangered by the International Union for Conservation of Nature and Natural Resources (IUCN) (Ved et al. 2003) and necessitated a study of the population biology to suggest in-situ and ex-situ conservation measures.

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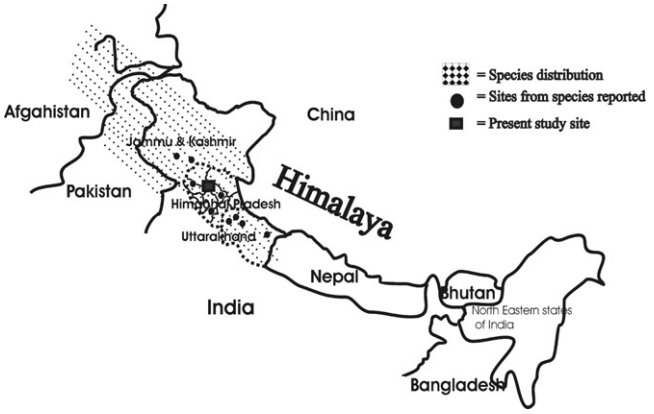

Fig. 1. Distribution of *Lilium polyphyllum*.

Review of literature showed that to date very little was known about the population ecology and seed biology of this critically endangered species. Therefore, in view of conservation and socio-economic values of the species, the present study was conducted to: (i) examine the population status of *Lilium polyphyllum* in natural habitats; (ii) understand its associated species; (ii) identify the optimum conditions that could enhance seed germination and reduce germination time; and (iii) give recommend conservation and management of the species.

Materials and methods

Study site

The study was conducted in Manali Wildlife Sanctuary (77°03′–77°10′ E latitudes and 032°13′–32°17′ N longitudes; area: 29.03 km^2) of Himachal Pradesh, India. The climate of the study area is temperate, subalpine and alpine types and consists of mainly three distinct seasons: summer season (mid April–mid June); rainy season (mid June–September); and winter season (November–March). However, short autumn (mid September–October) and spring (mid March–mid April) seasons also prevail in the area. Average maximum and minimum temperature ranges between 14.0 to 30.0 °C and −7.0 to 17.0 °C, respectively and mean annual rainfall is 1080 mm in the study area.

Sampling methods

Surveys were conducted during 2005–2006 throughout the sanctuary in accessible areas and total 93 sites representing 13 habitats and all aspects between 2050 and 4800 m were randomly selected and sampled. Habitats were identified based on physical features. For example, sites having closed canopy with high percent of humus and moisture were considered as shady moist habitats whereas the sites having >50% area under rocks as rocky habitat. The geo-references for each site were obtained with hand held Global Positioning System (GPS). At sites/habitats in which target species was recorded, a plot of 20 m × 20 m was laid and 20 quadrats of 1 m × 1 m were placed randomly within the plot. The individuals of all the species were recorded in each quadrat. Samples of each species were collected and identified with the help of local floras (Dhaliwal & Sharma 1999; Khullar 1994; Khullar 2000; Singh & Rawat 2000).

Soil sampling and analysis

Soil samples up to 20 cm depth were collected from the centre and four corners of each site. A composite sample approximately weighing 200 g from each site was brought to the laboratory in an air-tight polythene bag. Fresh soil sample (10 g) was kept in oven for 5 h at 105 °C to determine moisture content immediately after reaching the laboratory. Remaining sample was dried at room temperature for 4–7 days, sieved over a 2 mm sieve and stored in an air-tight polythene bag. The pH was determined in a 1:4 (w:v) suspension of soil in water using a pH meter. Carbon and organic matter were determined by Walkely and Black method. Total nitrogen (%) was ascertained by the Kjeldhal procedure after digestion with concentrated H_2SO_4 (Singh et al. 2005).

Seed collection

Mature fruits (i.e., capsules) were collected during the 2nd week of October 2006 from all sampled sites and pooled. Seeds separated from fruits and dried for a month at room temperature and stored in a brown bag until the start of germination experiment at room temperature. Aborted and predated/damaged seeds were discarded.

Viability test

Seed viability test was determined using the tetrazolium test at the start of experiments. Thirty seeds were immersed in 1% solution of 2,3,5-triphenyl tetrazolium chloride in a Petri dish. After 24 h, seeds were longitudinally sectioned so that it could display embryo. Seeds with a red stained embryo and white endosperm were classified as viable (ISTA 1996). Seed germination was monitored up to 15 weeks for untreated seeds kept at room temperature whereas maximum seeds under treatments were germinated in 8–9 weeks. All the ungerminated seeds were rotten. Therefore, viability of ungerminated seeds was not determined.

Treatments and experimental conditions

Seeds selected for treatments were surface sterilised with $NaHClO_3$ (4%) for 1 min and washed thoroughly with double distilled water (DDW). Details of various treatments tried for the purpose of improving seed germination of *Lilium ployphyllum* are presented in Table 1. Selection of the treatments and concentrations were based on experimental trails conducted earlier and published results of the responses to various treatments in other

Table 1
Details of treatments tried in present study.

S.N.	Treatments/Conditions	Concentration/Duration	Experimental condition
1	Incubator	Untreated	Kept at 25 °C
2	Soaking in dark	24 h	Kept in dark at 25 °C
3	Chilling	24 h	Kept at 4 °C
4	Indol acetic acid (IAA)	50, 100, 150 μM	Seed soaked for 24 h
5	Indol butyric acid (IBA)	50, 100, 150 μM	Seed soaked for 24 h
6	Gibbrelic acid (GA_3)	50, 100, 150 μM	Seed soaked for 24 h
7	Potassium nitrate (KNO_3)	50, 100, 150 μM	Seed soaked for 24 h
8	Sodium hypochlorite ($NaHClO_3$)	5, 10, 15 min	Kept in solution

Fig. 2. Flower (a) and mature fruits (b) of *Lilium polyphyllum* in Manali wildlife sanctuary.

related species (Butola & Badola 2004; Chauhan & Nautiyal 2007). Treated seeds were washed with DDW and three replicates of each treatment containing 20 seeds were placed in Petri dishes lined with Qualigens (615 A°) filter paper. Petri dishes were kept in an incubator at 25 °C, since 20–30 °C is optimum temperature for seed germination of many species (Baskin & Baskin 1998). Untreated seeds kept at room temperature were considered as control. Filter papers were moistened daily using DDW and observations were taken on seed germination every day after first germination. Seeds were considered germinated on the emergence of cotyledon.

Data analysis

Field data were analysed for density, frequency, abundance and species diversity (Shannon & Weaver 1963; Simpson 1949). Major associated species were recognised based on relative density of the identified species. Species having their origin or reported first from the Himalayan region were considered as natives (Anonymous 1883–1970; Samant et al. 1998) and the remainder as non-natives.

Mean Germination Time (MGT) was calculated as $MGT = \sum(fx)/\sum x$, where, x is number of newly germinated seeds on each day and f is the number of days after seeds were set to germinate (Nichols & Heydecker 1968) and final percentage of seed germination was also calculated. Differences in germination and MGT among treatments were tested with one-way analysis of variance (ANOVA). Data for germination percentage and MGT did not show normal distribution on applying normality test. Therefore, both germination percentage and MGT were transformed using Johnson's transformation (Transformation function: $1.148 + 1.63 \times A\sinh(X - 90.8)/15.13$) before carrying out ANOVA. Pairwise difference was calculated and treatment means were compared with control by Dunnett's comparison test. Hsu's Multiple Comparisons with the Best was used to identify best treatment. Statistical software Minitab 15 (Minitab Inc. 2007) was used to transform data and analyse these tests.

Viability adjusted germination (VAG) was calculated following Roche et al. (1997).

$$VAG = \frac{\text{Seed germinated}}{\text{Total number of seeds} \times \text{viability}/100} \times 100$$

Results

Site characteristics

Of the total 93 sites selected and sampled, *Lilium polyphyllum* (Fig. 2) was recorded in five sites i.e., Panjali (2515 m), Madogh Thatch (2505 m), Dhungri Gor (2338 m), Goya Dugh (2415 m), and Orkhad (2574 m) representing two habitats for the first time from the Manali Wildlife Sanctuary. The species was found in shady moist and rocky habitats, and north and northwest aspects only between 2338 m and 2574 m amsl. All the sites were located between 32°14.701′–32°14.940′ N and 77°09.117′–77°09.798′ E. Soil analysis of the sampled sites revealed pH range from 6.14 to 7.37; nitrogen, 0.25–0.55%, moisture content, 23–33%, organic matter, 5.78–12.77%, carbon, 3.53–6.81% and carbon:nitrogen ratio, 7.20–22.13% (Fig. 3).

Ecological evaluation

The density ranged between 1 and 6.7 individual/m^2 (Table 2). It was significantly higher in the shady moist habitat than rocky habitat. Distribution was clustered (aggregated) in all sites and species diversity (H') among habitats ranged from 3.00 to 3.52. The density had a significant positive correlation with moisture content ($r = 0.463$, $P < 0.05$, $n = 5$) and carbon/nitrogen ratio ($r = 0.791$,

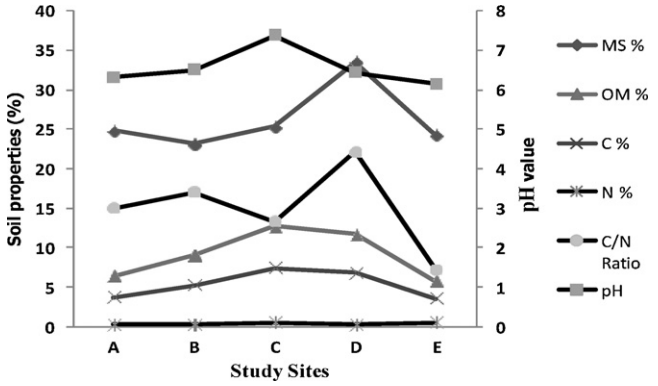

Fig. 3. Soil composition at different sites of the study area. Abbreviations used: MS = Moisture (%); OM = Organic Matter (%); C = Carbon (%); N = Nitrogen (%), A = Panjali; B = Madogh Thatch; C = Dhungri Gor; D = Goya Dugh and E = Orkhad.

Table 2
Habitat wise distribution of *Lilium polyphyllum* in MWLS.

Habitat	SR	Density (Ind/m^2)	RD (%)	Abundance	Species diversity	Nativity of associate species (%)
Shady moist Forest	4	6.7	12.2	7.0	3.52	52.3
Rocky	1	1.0	1.1	3.0	3.00	65.6

Abbreviations used: SR = Sites represented; Ind = Individual; m = Metre and RD = Relative density.

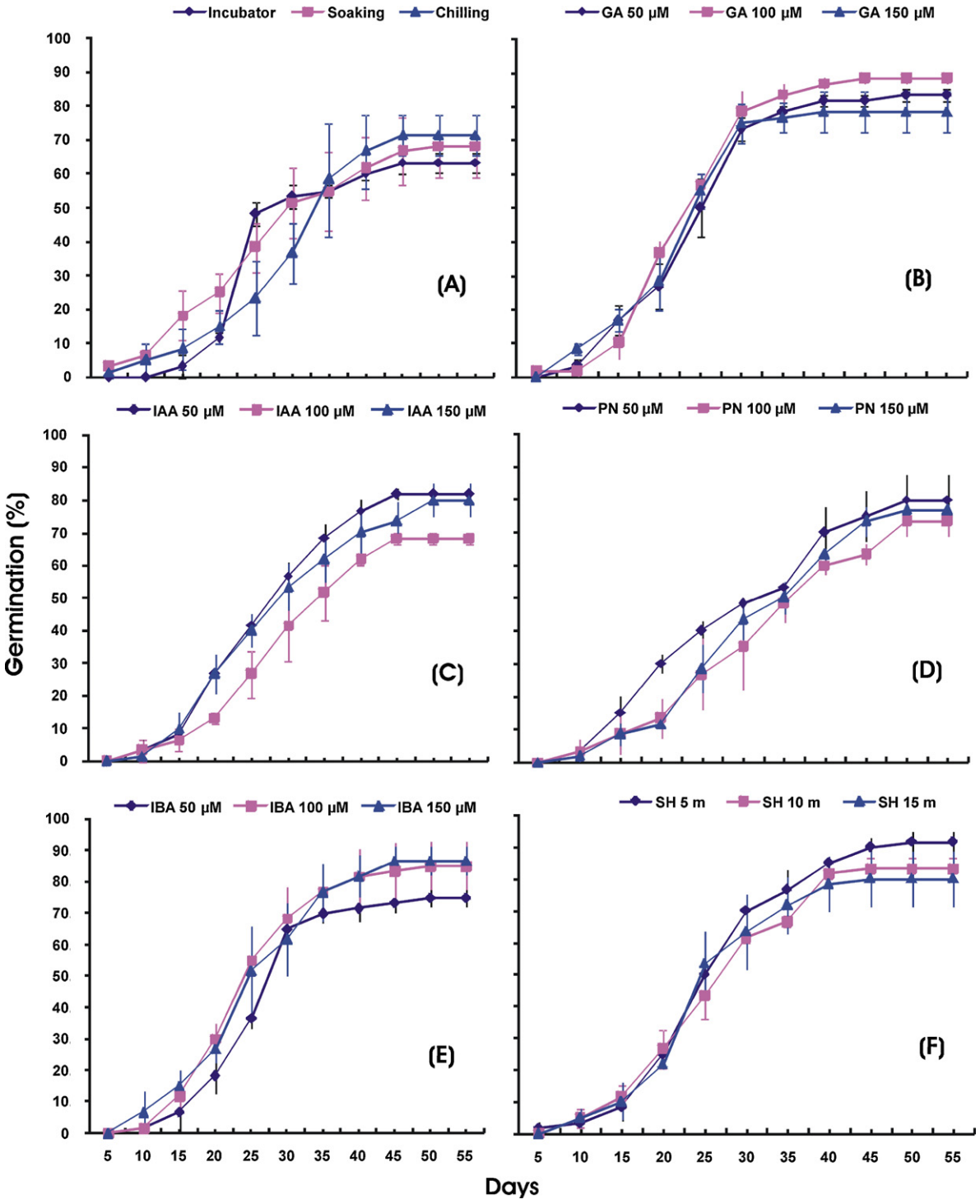

Fig. 4. (A–F) Cumulative seed germination of *Lilium polyphyllum* in different treatments. Abbreviations used: IAA = Indol acetic acid; IBA = Indol butyric acid; GA = Gibberelic acid; PN = Potassium nitrate (KNO_3); and SH = Sodium hypochlorite ($NaHClO_3$).

$P < 0.05$, $n = 5$), but significant negative correlation with nitrogen ($r = -0.963$, $P < 0.01$, $n = 5$).

Strobilanthes atropurpureus (10.3%), *Fragaria vesca* (6.2%), *Adiantum capillus-veneris* (5.9%), *Viola canescens* (4.9%) and *Strobilanthes wallichii* (4.08%) were the major associate species in shady moist habitat whereas *Carex filicina* (19.70%), *Fragaria vesca* (11.5%) and *Strobilanthes atropurpureus* (10.2%) in rocky habitat. Of the total 74 species identified, 41 were native to the Himalayan region. Shady moist habitat had only 52.3% of native species, whereas rocky habitat had 65.6% native species (Table 2).

Seed germination

Viability of the seeds as determined by tetrazolium was 90%. First germination was observed between 7 and 15 days in treatments at 25 °C but 80 days in control condition (room temperature: 5–15 °C). Cumulative germination increased at rates up to 45 days in all treatments (Fig. 4). In control condition, germination was 35% and MGT was 91 days but in incubator at 25 °C it improved to 63% and 35 days, respectively. One-way analysis of variance (ANOVA) showed differences in germination ($F = 3.76$, $P < 0.001$) and MGT ($F = 3.33$, $P < 0.001$) between treatments, were significant. Soaking and chilling for 24 h increased germination to 68% and 72% and reduced MGT to 29 and 24 days, respectively (Table 3). Among the plant growth regulators used, GA (50, 100 μM), IAA (50, 150 μM) and IBA (100, 150 μM) improved the germination percentage and MGT significantly ($P < 0.05$) but difference among their concentrations was non-significant. Among the concentrations of growth regulators, highest germination percentage (88.3%) was achieved with GA 100 μM and minimum MGT (20 days) in IAA 100 μM (Fig. 4 & Table 3). Increase in concentration of IBA improved the germination percentage.

Seeds treated with the chemical compounds (KNO_3 and $NaHClO_3$) also improved germination percentage and MGT. The highest percentage germination (91.7%) was achieved with $NaHClO_3$ (5 min) and minimum MGT (24 days) with $NaHClO_3$ (15 min), further increase in treatment duration decreased germination percentage and MGT (Table 3).

The confidence interval for Dunnet's comparison test (Critical value: 3.67) inferred that difference in germination percentage between control and treatments i.e., GA (50, 100 μM), IAA (50, 150 μM), IBA (100, 150 μM), PN and SH (5, 10, 15 min) was statistically significant. Whereas, difference in MGT between control and treatments i.e., Chilling, GA (50, 100, 150 μM), IAA (50, 100 μM), IBA (50, 100, 150 μM) and SH (5, 10, 15 min) were significant. However, Hsu's method identified $NaHClO_3$ (5 min) with 91.7% as best treatment for germination percentage whereas IAA (100 μM) with 20 days for MGT.

Discussion

Quantitative assessment of a species is a prerequisite for setting its conservation priorities; for this reason, the floristic diversity of *Lilium polyphyllum* which is identified by IUCN as a Critically Endangered species globally, and found in only few patches in the study area, was investigated. Habitat evaluation of the species indicated poor status in habitats/sites which also clearly confirmed the current conservation status of species in the study area. Restriction of *L. polyphyllum* into only two habitat types might be due to habitat degradation, over exploitation and habitat specificity. Evaluation of species like *L. polyphyllum* becomes more important in view of the fact that wild populations of critically endangered species are decreasing continuously in the area (Bhatt et al. 2005; Rana 2007). Occurrence of more than 35% non-native species in the habitats clearly indicates its habitat degradation. In addition to grazing and over-exploitation, these invasive species can add further pressure on species like *L. polyphyllum* for survival. The narrow altitudinal range, habitat restriction and relationship with edaphic factors i.e., moisture content, C:N ratio and nitrogen revealed the high sensitivity of the species to environmental factors.

Seeds of *L. polyphyllum* possess high viability. Most of the treatments used in the present study improved germination and MGT. Increase in seed germination in soaking and suitable temperature conditions in the incubator indicates that seeds were sensitive to water and temperature stress. Most of the treatments applied had no significant effect on germination responses over soaking and incubator conditions, this suggest that treatments have no special advantage over simple facilitated imbibitions in soaking and suitable temperature condition. On the contrary, treatments GA

Table 3
Germination responses of seeds in different treatments.

Treatments	Seed germination (%) ±SE	Viability adjusted germination (%) ±SE	First germination time ±SE (days)	Mean germination time ±SE (days)
Room temperature (Control)	35.0 ± 5.8	38.9 ± 6.4	80.3 ± 0.7	91.0 ± 1.8
Incubator (25 °C)	63.3 ± 3.3	88.9 ± 3.2	15.3 ± 1.2	34.2 ± 7.9
Soaking (24 h)	68.3 ± 9.3	87.0 ± 10.3	9.0 ± 1.5	29.0 ± 1.6
Chilling (4 °C, 24 h)	71.7 ± 6.0	79.6 ± 6.7	11.7 ± 3.8	23.8 ± 3.5
IAA				
50 μM	81.7 ± 1.7	90.7 ± 1.9	11.7 ± 2.3	25.4 ± 1.0
100 μM	68.3 ± 1.7	75.9 ± 1.9	12.3 ± 2.0	20.0 ± 7.1
150 μM	80.0 ± 5.0	88.9 ± 5.6	12.7 ± 2.3	26.8 ± 0.7
IBA				
50 μM	75.0 ± 2.9	83.3 ± 3.2	13.7 ± 2.8	24.5 ± 0.9
100 μM	85.0 ± 7.6	94.4 ± 8.5	11.3 ± 0.9	24.6 ± 1.4
150 μM	86.7 ± 4.4	96.3 ± 4.9	10.0 ± 2.1	25.2 ± 1.5
GA				
50 μM	83.3 ± 1.7	92.6 ± 1.9	10.3 ± 0.7	23.1 ± 0.7
100 μM	88.3 ± 1.7	98.1 ± 1.9	14.3 ± 0.7	22.1 ± 0.4
150 μM	78.3 ± 6.0	87.0 ± 6.7	7.3 ± 1.3	22.8 ± 1.7
KNO_3				
50 μM	80.0 ± 7.6	88.9 ± 8.5	9.3 ± 2.3	27.1 ± 1.0
100 μM	73.3 ± 4.4	81.5 ± 4.9	15.0 ± 1.0	30.4 ± 2.7
150 μM	76.7 ± 1.7	85.2 ± 1.9	12.0 ± 1.2	30.3 ± 0.9
$NaHClO_3$				
5 min	91.7 ± 3.3	101.9 ± 3.7	10.0 ± 3.5	25.1 ± 1.4
10 min	83.3 ± 3.3	92.6 ± 3.7	9.7 ± 0.9	25.1 ± 1.6
15 min	80.0 ± 8.7	88.9 ± 9.6	10.3 ± 2.8	23.9 ± 0.9

(100 μM), IBA (100, 150 μM) and SH (5 min) only, improved the germination and GA (100 μM) and IAA (100 μM) reduced MGT significantly ($P < 0.05$). This suggests that these treatments were quite effective to overcome the physiological dormancy. Physiological dormancy is common in temperate climatic species and it has been found in species of many different families (Baskin & Baskin 1998). Applications of IAA and $NaHClO_3$ are known to break seed dormancy in many plant species (Chauhan & Nautiyal 2007). GA is also reported to play important role in improving germination in several plant species (Baskin & Baskin 1990). The study has provided a useful understanding of the best treatments for better germination percentage as well as lower MGT in *L. polyphyllum*. Though, the IAA (100 μM) gave the best response for MGT but SH (5 min) can be recommended as the most suitable treatment for propagation of species (91.7% with 25 days MGT).

In natural populations, intraspecific variability in germination response of different species is widely known (Fox et al. 1994). Therefore, the study of germination behaviour of different populations is needed to suggest suitable treatments for mass propagation of *L. polyphyllum*. In spite of high viability and simple dispersal mechanism of seeds through wind, the species is unable to proliferate in the natural habitats. This may be due to heavy grazing, trampling and competition among the associate species. In addition, failure of seedling establishment might be due to its criticality of optimum temperature, moisture, altitudinal range and nitrogen concentration.

For in-situ conservation of the species, regular monitoring of the sites/habitats and complete protection of the habitats is suggested. Strict implementations of the rules and regulations in the protected areas are also required. In addition, seed germination protocols developed may be used for mass multiplication of the species and seedlings should be transplanted in comparable habitats so that viable population of the species can be maintained. Species may also be introduced in cultivation in nearby villages to conserve this critically endangered species, and provide socio-economic benefit to the inhabitants.

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