Brief communication

Epicotyl morphophysiological dormancy in seeds of *Lilium polyphyllum* (Liliaceae)

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Dormancy-breaking and seed germination studies in genus *Lilium* reveal that the majority of *Lilium* spp. studied have an underdeveloped embryo at maturity, which grows inside the seed before the radicle emerges. Additionally, the embryo, radicle or cotyledon has a physiological component of dormancy; thus, *Lilium* seeds have morphophysiological dormancy (MPD). A previous study suggested that seeds of *Lilium polyphyllum* have MPD but the study did not investigate the development of the embryo, which is one of the main criteria to determine MPD in seeds. To test this hypothesis, we investigated embryo growth and emergence of radicles and epicotyls in seeds over a range of temperatures. At maturity, seeds had underdeveloped embryos which developed fully at warm temperature within 6 weeks. Immediately after embryo growth, radicles also emerged at warm temperatures. However, epicotyls failed to emerge soon after radicle emergence. Epicotyls emerged from >90% seeds with an emerged radicle only after they were subjected to 2 weeks of cold moist stratification. The overall temperature requirements for dormancy-breaking and seed germination indicate a non-deep simple epicotyl MPD in *L. polyphyllum*.

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1. Introduction

The genus *Lilium* contains approximately 110 species of herbaceous perennials (Mabberley 2008), which have been classified into 5 to 10 sections or subgenera, geographically distributed in the northern hemisphere (North America, Europe and Temperate Asia) (Siljak-Yakovlev *et al.* 2003). *Lilium polyphyllum* D. Don ex Royle is a medicinal woodland perennial herb native to the Himalayan region of temperate Asia (Afghanistan) and tropical Asia (India, Nepal and Pakistan) (USDA-ARS 2011). In India *L. polyphyllum* falls into a critically endangered category and is found in Jammu and Kashmir, Himachal Pradesh and Uttarakhand in the western Himalayas (Ved *et al.* 2003). The species inhabits cool and moist sites and has been exploited for its medicinal bulbs to meet the demand of the pharmaceutical industries (Dhyani *et al.* 2010a; Rana and Samant 2011). However, overexploitation from its natural habitats has caused its population depletion to a great extent (Rana and Samant 2011).

Medicinal plant management programs encourage the planting of seed and/or root material to either augment pre-existing forest populations, or establish new populations in forests where they are locally absent (Albrecht and McCarthy 2006). To achieve this task a good knowledge of seed germination ecophysiology and environmental conditions to break seed dormancy of an individual species is required. However, detailed reliable information on class and level of seed dormancy (*sensu* Baskin and Baskin 2004) and germination requirements of many medicinal

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plants of the Indian subcontinent is currently unavailable except in a recent study on seed dormancy and germination in the Giant Himalayan Lily (Phartyal *et al.* 2012). To fill gaps in reliable information on seed dormancy and germination ecophysiology in medicinal plants of the temperate Himalayas, we selected *L. polyphyllum*, which grows naturally in the outer range of the Western Himalayas in India. Seeds of *L. polyphyllum* matured and dispersed in October–November have at maturity an underdeveloped embryo (Dhyani *et al.* 2009), but nothing is known about the class and level of seed dormancy and germination requirements of this species.

There are many plant species, including those of Liliaceae, which have underdeveloped embryos (Martin 1946) and embryos must grow prior to radicle emergence (Baskin and Baskin 1998; 2004). In morphological dormant (MD) seeds, the embryo grows without any special dormancy-breaking treatment and the seed begins to germinate within about 30 days (Baskin and Baskin 1998, 2004). Seeds with MD, which have a physiological component of dormancy and require warm and/or cold stratification to break physiological dormancy (PD) and to encourage the growth of the underdeveloped embryo, are classified as morphophysiological dormant (MPD) seeds (Baskin and Baskin 1998; 2004). Currently, nine different levels of MPD have been identified based on required temperatures at the time of embryo growth, radicles and cotyledons emergence, and the ability of GA_3 to substitute for a cold and/or a warm temperature requirement to overcome dormancy (Baskin & Baskin 1998; 2004; Baskin *et al.* 2008). Out of the nine levels of MPD, two levels fall under epicotyl dormancy, i.e. deep simple epicotyl MPD and non-deep simple epicotyl MPD (Baskin *et al.* 2009).

Epicotyl MPD has been reported in 10 families and 6 orders that also include family Liliaceae in order Liliales (Baskin and Baskin 2010). Four genera of Liliaceae, i.e. *Erythronium, Fritillaria*, *Gagea* and *Lilium*, have been reported to have seeds with epicotyl MPD (see Kondo *et al.* 2006). So far four different levels of MPD have been reported in seeds of genus *Lilium* ranging from deep simple epicotyl MPD to deep complex MPD (Barton 1936; Crocker and Barton 1957; Nikolaeva *et al.* 1985). The majority of these studies did not experimentally define the specific temperature requirements for embryo growth in *Lilium* species. However, in these studies, it was speculated that seeds require a warm temperature for radicle emergence and further low temperatures to initiate growth of the dormant epicotyl.

Taking all these facts together, we hypothesize that seeds of *L. polyphyllum* could have MPD as its seed has an underdeveloped embryo at maturity. An earlier study on the phenology of *L. polyphyllum* reported epicotyl dormancy (Dhyani *et al.* 2010b) without considering any specific temperature requirements for dormancy-breaking and seed germination. Thus, the specific objectives of this investigation were to determine whether seeds of *L. polyphyllum* show MPD, and if so, what level of MPD and when embryo growth occurs.

2. Methods

Ripe seeds of *L. polyphyllum* were collected on 20 October 2007 from plants growing under a canopy of coniferous or mixed forest woodlands at 2200 m above sea level (latitude 30°25′ N, 78°15′ E) in the Tehri district of Uttarakhand state of India. Mature capsules were brought to the laboratory and then dried for 2 days at ambient room temperature, and seeds were removed from the capsule by hand, winnowed and later spread onto trays to dry for 5 days.

2.1 *Temperature requirements for embryo growth*

On 28 October 2007, 15 fresh seeds (average seed length 7.25 mm) that had been allowed to imbibe water overnight at room temperature were cut into thin sections manually with a razor blade. Embryo length (initial embryo length) of each seed was measured using a microscope equipped with a micrometer. On the same day, five petri dishes containing 50 seeds each were placed on moist filter paper and incubated at constant temperature of 0°C and 25°C. Three seeds were randomly chosen and removed from each of the five dishes in each temperature treatment at weekly intervals, and the length of the 15 embryos was measured as previously described.

2.2 *Temperature requirements for radicle emergence*

The temperature requirements for the emergence of radicles were determined by incubating seeds in 12/12 h light/dark conditions at three constant temperature regimes (15, 20 and 25°C) and three daily alternative temperature regimes (15/6, 20/10 and 25/15°C). In each temperature treatment, three replicates of 50 fresh seeds were placed in petri dishes on moist filter paper and regularly monitored for radicle emergence for 8 weeks. The criterion for radicle emergence was adopted from Kondo *et al.* (2006), i.e. the percentage of seeds with radicle tip emerged 1 mm or more from the seed coat.

2.3 *Temperature requirements for epicotyl emergence*

In another set of experiments, seeds with emerged radicles took a prolonged period of 90 days to produce 35% epicotyl in a continuous warm temperature of 20°C (personal

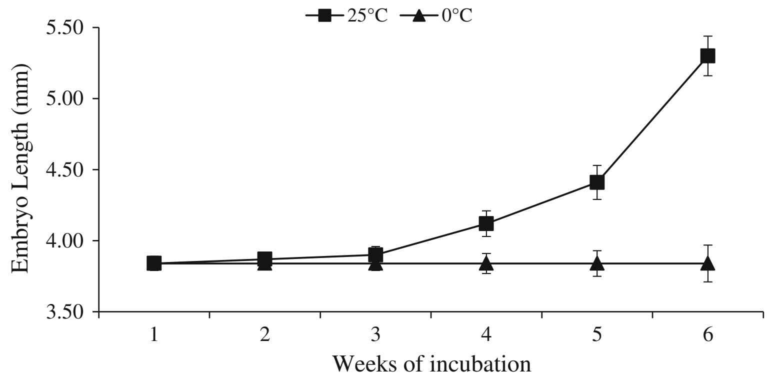

Figure 1. Effect of temperatures on embryo growth in seeds of *Lilium polyphyllum*. Error bars ± SD.

observation). In nature, seeds of *L. polyphyllum* disperse in autumn and radicles emerge during June (summer). After radicle emergence, bulblets develop in July and epicotyls emerge next year during March (spring). This sequence indicates that after radicle emergence the seed faces cold winter temperature that might be needed for epicotyl emergence (Dhyani *et al.* 2010b). Thus, we tested the effect of low temperature followed by warm temperature on epicotyl emergence. Seeds with emerged radicles and bulblets from previous experiments were cold moist stratified at 0–4°C for a period of 0, 7, 15, 30 and 45 days, and then three replicates of 50 seeds with emerged radicles and bulblets were buried about 5 mm deep in soil in three small plastic containers and incubated in 12/12 h light/dark conditions at 15, 20, 25, 15/6, 20/10 and 25/15°C. Epicotyl emergence was monitored daily for 3 weeks. The criterion for epicotyl emergence was adopted from Kondo *et al.* (2006), i.e. when the cotyledon tip emerged completely above soil surface, and was recorded as a percentage of seeds that showed epicotyl emergence.

3. Results

3.1 *Temperature requirements for embryo growth*

Mean embryo length in fresh mature seeds was 3.84± 0.73 mm. These grew up to 5.3±0.16 mm when the radicle began to protrude. Thus, embryo length increased by 1.46 mm within 6 weeks of incubation at a warm (25°C) temperature before radicle emergence. In a cold temperature regime of 0°C, embryos did not grow at all (figure 1).

3.2 *Temperature requirements for radicle emergence*

Immediately after embryo growth, radicles began to emerge in warm temperatures. Radicles emerged from >90% of the

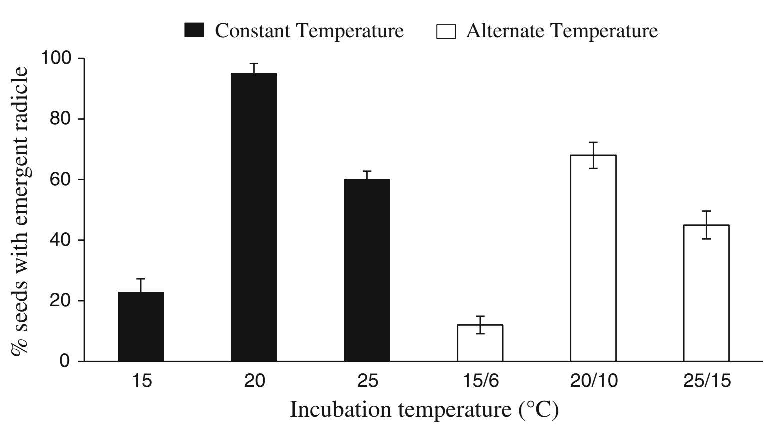

Figure 2. Effect of constant and daily alternating temperatures on radicle emergence in *Lilium polyphyllum* seeds. Bar graphs indicating results up to 8 weeks after seed sowing. Error bars ± SD.

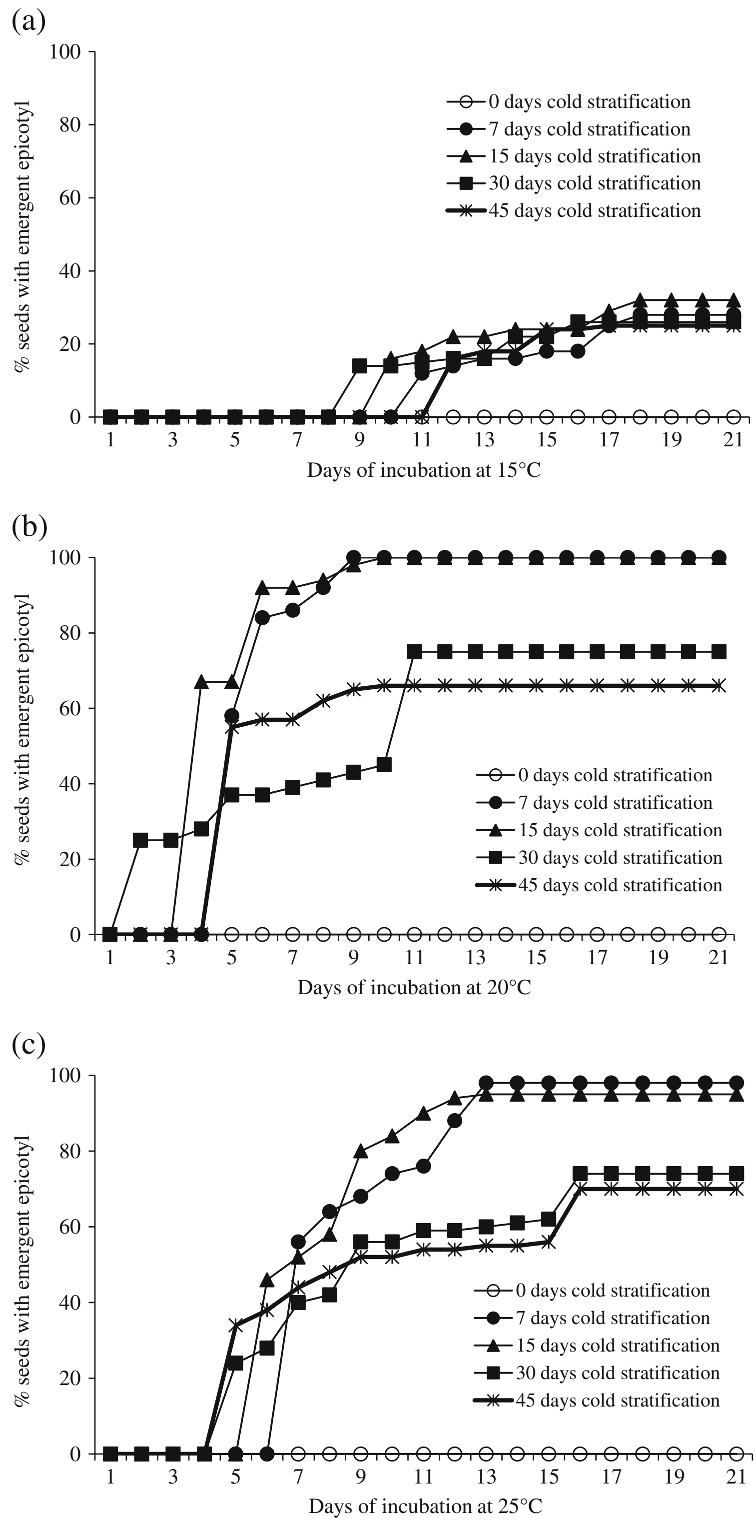

Figure 3. Effect of constant temperatures and period of cold stratification on epicotyl emergence in seeds of *Lilium polyphyllum*.

seeds (figure 2) by 8 weeks of incubation at constant 20°C. The percentage of radicle emergence was low at other temperatures. For example, radicles emerged from <25% seeds at a constant 15°C and alternating 15/6°C (figure 2).

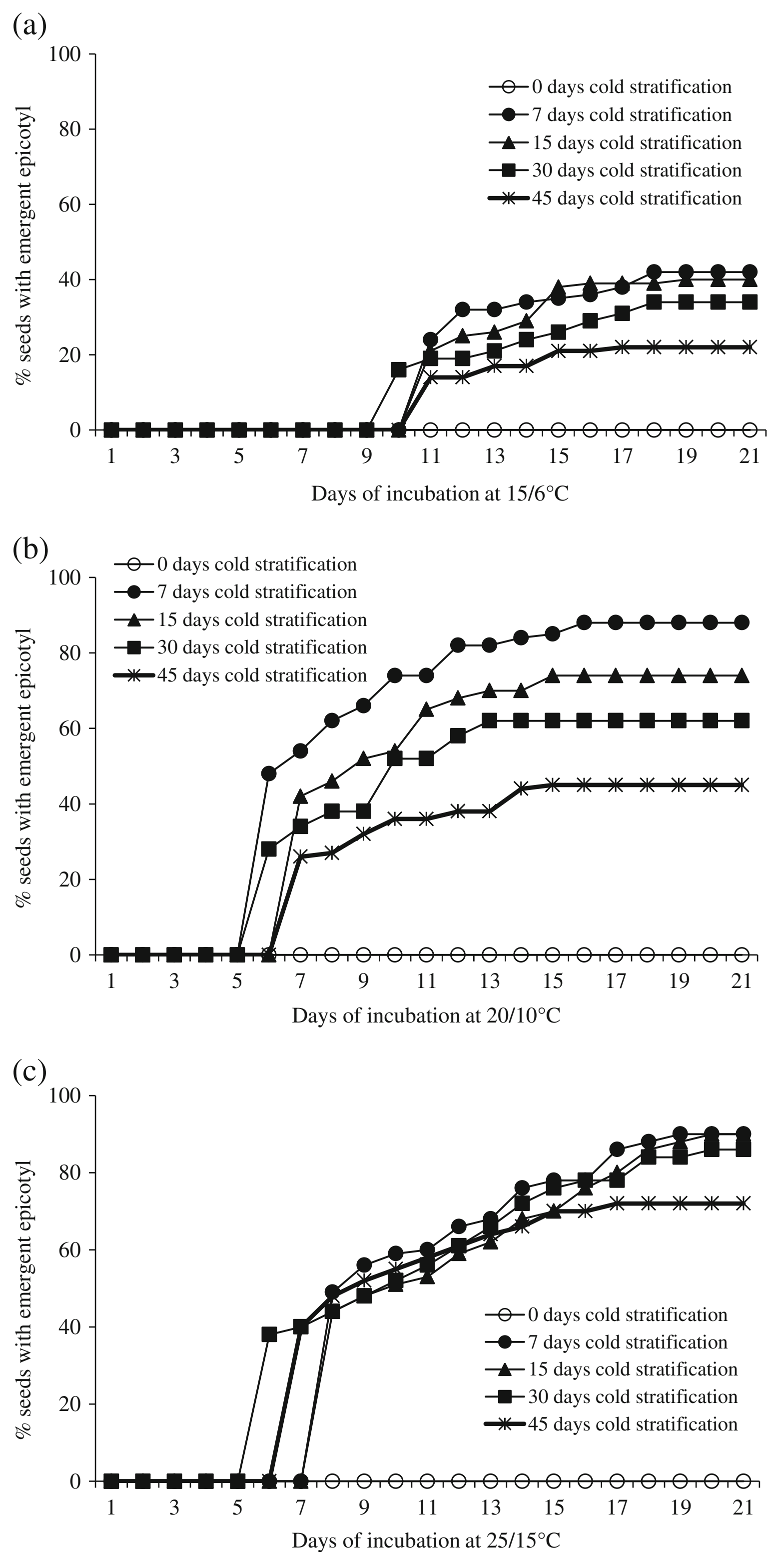

Figure 4. Effect of daily alternating temperatures and period of cold stratification on epicotyl emergence in seeds of *Lilium polyphyllum*.

3.3 *Temperature requirements for epicotyl emergence*

During a 3 week incubation period, epicotyls failed to emerge in seeds which were not stratified (0 days cold stratification). However, in 7 and 15 day cold moist stratified seeds, epicotyls emerged from >80% seeds at constant 20° and 25°C. Cold stratification of seeds with emerged radicles for 30 and 45 days resulted in low epicotyl emergence at all incubation temperature regimes (figures 3 and 4). At a constant 15° and alternate 15/6°, 20/10° and 25/15°C, epicotyl emergence was low irrespective of the cold stratification period (figures 3 and 4).

4. Discussion

This study revealed that at maturity in autumn, seeds of *L. polyphyllum* had underdeveloped embryos, which elongated fully at warm temperatures before radicle emergence; thus these seeds have MD. Radicles emerged at moderate/warm temperatures immediately after embryo development. No epicotyl emerged when seeds with emergent radicle were incubated at warm temperatures for 3 weeks. However, when seeds with emergent radicle were exposed to <4°C for 3 weeks and then moved to warmer temperatures, the epicotyl emerged from >80% seeds within 3 weeks, indicating PD in epicotyls.

Radicle emergence in a natural habitat, in autumn dispersed seeds of *L. polphyllum*, occurs in the warm temperatures of the following summer (June). Immediately after radicle emergence, bulblets begin to develop in the monsoon; epicotyls begin to emerge in the second spring after seed dispersal (Dhyani *et al.* 2010b). Although autumn and spring temperatures are almost similar (average 12–13°C) in the natural habitat of *L. polyphyllum*, the epicotyl does not emerge at autumn temperatures immediately after radicle emergence. This indicates that seeds with emerged radicles have a component of PD in their epicotyl, which needs a cold stratification treatment before emergence. Results of laboratory experiments on temperature requirement for embryo growth and for radicle and epicotyl emergence were in agreement with the phenology of seed germination (emergence of radicle and cotyledon) in natural habitats.

Based on the overall pattern of seed germination in *L. polyphyllum*, we conclude that this species possesses MPD like other species of *Lilium* genus. Furthermore, epicotyl emergence requires cold stratification. Thus, we categorize epicotyl MPD in *L. polyphyllum* seeds, similar to its congeners. To date, four different levels of MPD have been reported in the *Lilium* genus, viz. deep simple epicotyl MPD in *L. auratum*, *L. canadense*, *L. japonicum*, *L. rubellum*, *L. superbum* and *L. szovitsianum* (Barton 1936; Crocker and Barton 1957); non-deep complex epicotyl MPD in *L. pensylvanicum*; deep complex MPD in *L. martagon* and non-deep simple MPD in *L. speciosum* (Nikolaeva *et al.* 1985). Recently Baskin *et al.* (2008) reported a new level of MPD in *Viburnum odoratissimum*, i.e. non-deep, simple, epicotyl MPD, based on PD in epicotyl. There are three kind of PD in epicotyls: Non-deep PD can be broken by exposing seeds to relatively short periods of warm or cold stratification (sometimes by dry after ripening), depending on the species, and dormancy may be broken by GA_3. Intermediate PD is broken by relatively long periods of cold stratification, and GA_3 may promote germination. Deep PD usually is broken only by long periods of cold stratification, and GA_3 does not promote germination (Nikolaeva 1977; Baskin and Baskin 1998; Baskin *et al.* 2008). In *L. polyphyllum*, when seeds with emerged radical are incubated continuously at warm (20°C) temperature for prolonged periods of 90 days, epicotyl emergence occurred in only 35% seeds (personal observation). However, a short period of cold stratification for 2 weeks increased both percentage and rate of epicotyl emergence. This confirms that *L. polyphyllum* requires a short period of cold stratification to break PD in the epicotyl. In this study, the role of GA_3 in replacing the requirement for warm and/or cold stratification was not monitored. Thus, we are not able to conclude the exact level of MPD in *L. polyphyllum* but we predict a non-deep simple epicotyl MPD in *L. polyphyllum*, which would be a first report in the genus *Lilium*. This information about the kind and level of dormancy in *L. polyphyllum* will be useful to persons trying to germinate seeds of this species to establish new populations in forests where they are locally absent or to augment pre-existing forest populations.

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