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Seed dormancy and germination of the subalpine geophyte *Crocus alatavicus* (Iridaceae)

Ziyan Fu[A], *Dunyan Tan*[A,D], *Jerry M. Baskin*[A,C] *and Carol C. Baskin*[A,B,C]

[A]Xinjiang Key Laboratory of Grassland Resources and Ecology and Ministry of Education Key Laboratory for Western Arid Region Grassland Resources and Ecology, College of Grassland and Environment Sciences, Xinjiang Agricultural University, Urümqi 830052, China.
[B]Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY 40546, USA.
[C]Department of Biology, University of Kentucky, Lexington, KY 40506, USA.
[D]Corresponding author. Email: tandunyan@yahoo.cn

Abstract. *Crocus alatavicus* Regel et Sem. is a cormous perennial primarily distributed in central Asia that may have potential in horticulture; however, relatively little is known about seed dormancy in the genus *Crocus*. The primary aim of the present study was to identify the dormancy breaking and germination requirements of seeds of *C. alatvicus* and to assign them to a dormancy category. In its natural habitat, the underdeveloped embryo in *C. alatavicus* seeds grows in early summer, and radicles emerge in early autumn. However, cotyledon emergence is delayed until the following spring. Radicle emergence was promoted by warm stratification and cotyledon emergence by cold stratification. GA_3 was ineffective in promoting either radicle or epicotyl emergence. We conclude that seeds of *C. alatavicus* have deep simple epicotyl morphophysiological dormancy of the type $C_{1b}B$(root) – C_3(shoot). To our knowledge, this is the first detailed study on the ecophysiology of seed dormancy and germination in the genus *Crocus*.

Additional keywords: epicotyl dormancy, morphophysiological dormancy, underdeveloped embryo.

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Introduction

Propagation of wild species is often inhibited by lack of information on how to germinate the seeds. Even if seeds of the desired species can be obtained, detailed information on dormancy-breaking and germination requirements is essential for development of efficient and cost-effective techniques of propagation. The first step in developing a protocol for plant propagation from seeds is to become familiar with the kind(s) of dormancy known to occur in the family. In the present study, we are concerned about how to break dormancy and germinate the seeds of *Crocus alatavicus* Regel et Sem. (Iridaceae), whose showy fragrant flowers suggest much potential for its utilisation in horticulture. This species also has medicinal value and is used in traditional Chinese herbal medicine to help reduce pain and swelling (Mao and Zhang 1994).

The most comprehensive classification system of seed dormancy was developed by Nikolaeva (1969, 1977), and it was organised by Baskin and Baskin (2004) into a hierarchical classification system consisting of the following five classes of dormancy: (1) physical dormancy (PY), with water-impermeable seed (or fruit) coat and a fully developed non-dormant embryo; (2) physiological dormancy (PD), with low growth potential of a fully developed embryo; (3) combinational dormancy (PY + PD), with water-impermeable seed (or fruit) coat and a fully developed embryo with PD; (4) morphological dormancy (MD), with an underdeveloped embryo that needs time to grow (the dormancy period) inside the seed before germination occurs; and (5) morphophysiological dormancy (MPD), with an underdeveloped embryo with PD. Some of these five classes of dormancy are further divided by Baskin and Baskin (2004) into levels and types.

Seeds of Iridaceae do not have water-impermeable seed coats, and thus they cannot have either PY or PY+PD (Baskin *et al.* 2000). According to Martin (1946), seeds of Iridaceae have linear embryos that may or may not be as long (or nearly so) as is the seed. Fresh seeds with a fully developed embryo and a water-permeable seed coat would be either non-dormant or have PD, whereas those with an underdeveloped embryo must undergo a period of embryo growth before radicle emergence, and, thus, would have either MD or MPD. Preliminary tests and observations of *C. alatavicus* seeds revealed that they were dormant and that the embryo was relatively small in relation to the size of the endosperm, which does not rule out PD, i.e. an embryo may be small and fully developed (Baskin and Baskin 2007).

If seeds have only MD, embryo growth would occur in a short period of time ($\leq$1 month) when the seeds are incubated under suitable temperature, moisture and light or dark conditions.

However, if seeds have MPD, they would require exposure to warm and/or cold (moist) temperatures to break PD and promote embryo growth and germination. Further, there are nine known levels of MPD (Baskin and Baskin 1998; Chien *et al*. 2011), and thus a full understanding of how to germinate seeds of *C. alatavicus* may require information about the level of MPD. Consequently, using a combination of field and laboratory studies, the objectives of our study were (1) to determine the class of dormancy in the seeds of *C. alatavicus*, and (2) if MPD was present, to determine the level of MPD and how to break it. To our knowledge, the present study is the first detailed study of seed dormancy and germination for a species of *Crocus*. Nikolaeva *et al*. (1985) listed *C. albiflorus*, *C. heuffelianus* and *C. iridiflorus* as having seeds with morphological dormancy but did not mention MPD as occurring in these species. Some seeds of *C. olivieri* subsp. *olivieri* began to germinate (percentage not given) in Petri dishes incubated at room temperature for 154–178 days (Vurdu *et al*. 2004).

Crocus alatavicus (Iridaceae) is a herbaceous perennial distributed primarily in the central Asian countries, including north-western China, Kazakhstan, Kyrgyzstan and Uzbekistan (Zhao *et al.* 2000). In China, the species occurs only in Xinjiang Uyghur Autonomous Region (north-western China) and is found in the Yili Valley in subalpine grasslands and forest edges, primarily at altitudes of 1200–2100 m (3000 m; Mao and Zhang 1994). It is an early spring-flowering geophyte that develops leaves and flowering shoots from buds on a perennial corm. Flowering begins in early–mid April, and the duration of individual flowers is 6–9 days. The inferior ovary is belowground at anthesis, whereas the other floral parts are aboveground. The peduncle elongates and raises the fruit above the soil level, and then the leaves die and the capsule dehisces. The flowers are fragrant and the tubular perianth is 2.5–6 cm in length, white with a yellow centre and striped or spotted grey or blue on the abaxial surface. Seeds have an elaiosome and are myrmecochorous.

Materials and methods

Seed collection

Seeds (capsules) were collected on 7 July 2011 and on 1 July 2012 from *C. alatavicus* plants growing in the subalpine zone of the western Tianshan Mountains (44°29′38″N, 81°10′35″E, 2080–2100 m asl), Xinjiang Uyghur Autonomous Region, China. This species inhabits grasslands and mountain slopes (Zhao *et al.* 2000). All experiments were started within 1 week after collecting the seeds; malformed seeds were not included in the studies. Mean annual temperature at the seed collection site is 0.5°C, and the lowest and the highest recorded temperature in January and July are –28.5°C and 21.0°C, respectively. Average annual rainfall is 300–600 mm, and relative humidity is ≥60% (Ma *et al*. 2003).

Imbibition test

The purpose of the present experiment was to verify (or not) that the seeds of *C. alatavicus* are water permeable. In July 2011, 20 fresh seeds of *C. alatavicus* were weighed with an analytical balance (0.0001 g) and then placed in a Petri dish on Whatman No. 1 filter paper (Shanghai Instruments, Shanghai, China) moistened with distilled water; the Petri dishes were wrapped with plastic film and kept for 24 h in the laboratory at room conditions (~25°C, 20–30% RH). Then, the seeds were removed from the dish, blotted dry with a paper towel and reweighed. Percentage increase in seed mass ($\%W_s$) was calculated as follows:

$$\%W_s = [(W_i - W_f)/W_f] \times 100,$$

where W_i = mass of seeds after 24 h of exposure to moist conditions and W_f = initial mass of fresh seeds.

Germination experiments

To determine whether fresh seeds would germinate over a range of temperatures, in July 2011, four replicates of 25 seeds each were placed in 9-cm-diameter plastic Petri dishes on two layers of Whatman No. 1 filter paper moistened with distilled water and incubated in light at 25/15°C, 20/10°C, 15/2°C and 5/2°C (day/night temperature). No seeds had germinated, as indicated by lack of radicle emergence, after 4 weeks. Thus, the dishes were placed back into the germinators for an additional 36 weeks and checked for germination at 7-day intervals. Seeds were watered as needed to keep them moist.

Although seeds of *C. alatavicus* are dispersed in summer, we do not know whether high summer temperatures are required for dormancy break (Baskin and Baskin 1998). Thus, a 'move-along' experiment (Baskin and Baskin 2003) was conducted to evaluate the role of summer and winter temperatures in breaking dormancy. Following the protocol of the move-along experiment, we simultaneously conducted the following two treatments, hereafter referred to as the warm (warm→cold→warm, . . .) and cold (cold→warm→cold, . . .) treatments. In the warm treatment, four replicates of 25 seeds each were incubated in Petri dishes on moistened Whatman No. 1 filter paper in the following sequence: 12 weeks at 25/15°C, 4 weeks at 20/10°C, 4 weeks at 15/2°C → 12 weeks at 5/2°C → 4 weeks at 15/2°C → 4 weeks at 20/10°C → 12 weeks at 25/15°C, and then reversing the sequence (i.e. 20/10°C → 15/2°C → 5/2°C, . . .) if any non-germinated seeds remained in the dishes. In the cold treatment, four replicates of 25 seeds each were incubated in the following sequence: 12 weeks at 5/2°C → 4 weeks at 15/2°C → 4 weeks at 20/10°C → 12 weeks at 25/15°C → 4 weeks at 20/10°C → 4 weeks at 15/2°C → 12 weeks at 5/2°C, and then reversing the sequence (i.e. 15/2°C → 20/10°C → 25/15°C, . . .) if any non-germinated seeds remained in the dishes. The cold treatment was used to determine whether seeds require cold stratification only, or cold followed by warm stratification, to germinate, whereas the warm treatment was used to determine whether seeds require a period of warm stratification only, or warm followed by cold stratification. Controls were kept continuously in light at 5/2°C, 15/2°C, 20/10°C and 25/15°C. Seeds were monitored for germination at 1-week intervals and watered as needed to keep them moist. Six extra dishes of seeds were included in each of the two sequences, so as to have seeds for measurement of the mean embryo length to seed length ratio (E : S ratio). Prior to transferring seeds to the next temperature in the sequence, the mean E : S ratio of 15 haphazardly selected seeds in the extra dishes was determined as described above.

Effect of dry storage on seed germination

To test whether an extended period of dry storage (after ripening) could alleviate dormancy, seeds collected on 7 July 2011 were stored dry for 0 (control), 1, 2, 3, 6 and 12 months under ambient room conditions (18–30°C, 20–30% RH). After each storage period, seeds were tested for germination in light and in darkness at 25/15°C, 20/10°C, 15/2°C and 5/2°C, using three replications of 25 seeds for each test condition. Seeds were checked for germination at weekly intervals for 2 months and the mean germination percentages were calculated.

Phenology of embryo growth and seedling emergence

Thirty seeds were placed in each of 10 fine-mesh polyester bags and buried at a soil depth of 5 cm in the natural habitat on 10 July 2011. Every 2 weeks, a bag was exhumed, and the mean (±s.e.) E : S ratio of 20 haphazardly selected seeds was determined. Seeds were cut in half and the embryo length and seed length were measured by using a dissecting microscope with an ocular micrometer. The final (critical) E : S ratio for germination was determined by measuring the embryo in seeds with a split seed coat, but with no radicle protrusion; this value was recorded for seeds that had germinated in the bags, i.e. they had obviously reached the critical length to germinate.

Phenology of seedling emergence was studied by burying seeds in the natural habitat of the species on 10 July 2011. Three replicates of 600 seeds each were placed into three mesh polyester bags and then the bags were buried at a depth of 5 cm in plastic pots filled with soil from the natural habitat. The pots were buried to soil level in an open place near the site of seed collection. Seedling emergence was monitored at 1-week intervals for 1 year, and each week emerged seedlings were counted and removed. Soil temperature at seed-burial depth was recorded at hourly intervals throughout the burial period, using Tiny Tag data loggers (Model MicroLite LITE5016, Fourier Technologies, Beijing, China).

Effect of gibberellic acid on dormancy break and germination

Information on the ability of gibberellic acid (GA_3) to overcome dormancy is required to help determine the level of MPD (Baskin and Baskin 1998). Thus, we tested the effect of GA_3 on the germination of *C. alatavicus* seeds. In July 2012, three replicates of 25 fresh seeds each were placed in Petri dishes on filter paper moistened with 0, 10, 100 or 1000 mg L^{-1} GA_3 and incubated for 12 weeks at 5/2°C, 15/2°C, 20/10°C and 25/15°C in light (12-h photoperiod, ~100 µmol m^{-2} s^{-1}, 400–700 nm, cool white fluorescent light). Germinated seeds (as indicated by radicle emergence) were counted and removed weekly.

A second experiment was set up to determine whether the seeds have epicotyl dormancy, i.e. a delay of epicotyl (shoot) growth for ≥30 days following radicle emergence, and if so, could it be broken by GA_3. Seeds with radicle emergence up to 2–5 mm in length were placed in Petri dishes with the same concentrations of GA_3 as in the experiment described above, and cotyledon emergence was monitored in light at 5/2°C, 15/2°C, 20/10°C and 25/15°C for 12 weeks.

Effect of warm temperature on radicle emergence

The purpose of this experiment was to determine whether seeds require a period of warm stratification for radicle emergence. In July 2012, three replicates of 20 fresh seeds each were placed in Petri dishes on Whatman No. 1 filter paper moistened with distilled water. Seeds were incubated in light (12-h photoperiod, hereafter light) at 25/15°C for 0, 2, 4, 6, 8, 10 or 12 weeks and then incubated in light at 20/10°C for six additional weeks. Controls were kept continuously at 5/2°C, 15/2°C, 20/10°C and 25/15°C for 18 weeks. Every 2 weeks, the percentage of seeds with emerged radicles was determined.

Effect of cold temperature on cotyledon emergence

The purpose of this experiment was to determine how much (if any) cold stratification is required to break shoot dormancy, which would be indicated by emergence of the cotyledon. In July 2012, 50 fresh seeds were placed in each of 100 Petri dishes (9 cm in diameter) on Whatman No. 1 filter paper moistened with distilled water in light at 20/10°C. Seeds with radicles emerged were removed from the dishes, and 10 each were placed on filter paper in 27 Petri dishes; three dishes were used for each treatment. The seeds were cold stratified in light at 5/2°C for 0, 2, 4, 6 and 8 weeks and then incubated in light at 20/10°C for 6 weeks. Controls were kept continuously in light at 5/2°C, 15/2°C, 20/10°C and 25/15°C for 14 weeks. Percentage of seedlings with an emerged cotyledon was determined every 2 weeks.

Data analysis

Mean and standard errors were calculated for percentages of radicle, seedling and cotyledon emergence and for embryo and seed lengths. The effects of GA_3 on final germination percentages were analysed by a one-way ANOVA. Two-way ANOVA was used to test for significance of main effects (light condition and storage time) and their interaction on germination in the 'Effect of dry storage on seed germination' experiment. Prior to analyses, data were checked for normality (Cochran test) and homoscedasticity (David test). If normal and homogeneous, data were subjected to further analysis. If data exhibited a non-normal distribution or if variances were not homogeneous, they were $\log_{10}$ or square-root transformed before analysis to ensure homogeneity of variance. In cases where the ANOVA assumptions continued to be violated following data transformation, treatment differences were assessed using the more conservative Kruskal–Wallis non-parametric test.

Results

Imbibition and germination experiments

The mass of fresh seeds had increased by 52% after 24 h on wet filter paper, demonstrating that seeds of this species have a water-permeable seed coat. No seeds germinated (as evidenced by a lack of radicle emergence) during the 4-week incubation period at 5/2°C, 15/2°C, 20/10°C or 25/15°C. However, after 8 weeks at 20/10°C, seeds began to germinate, and after 14 weeks, germination had reached the final percentage of 95.0 ± 2.5% (Fig. 1). Seeds incubated at 15/2°C began to germinate after 12 weeks, and the final germination percentage of 40.0 ± 2.8% was reached on Week 38. No seeds germinated during 40 weeks of incubation at 5/2°C or 25/15°C.

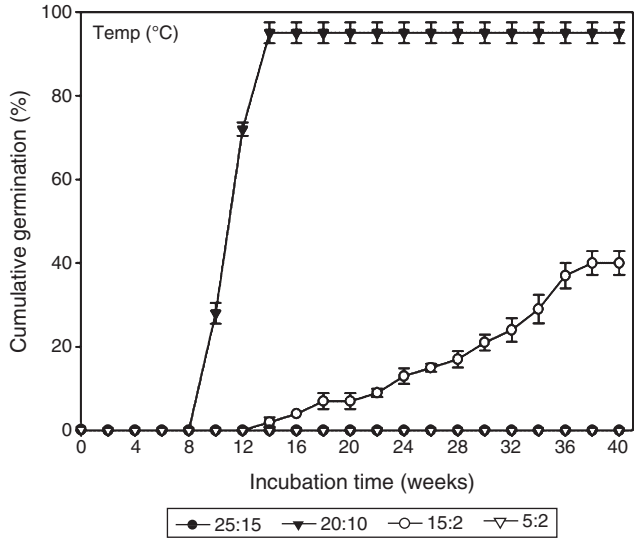

Fig. 1. Cumulative germination (radicle emergence) percentages (mean ± s.e.) for *Crocus alatavicus* seeds incubated at four temperature regimes for 40 weeks.

In the move-along experiment, seeds initially incubated in the warm treatment (12 weeks at 25/15°C) germinated (radicle emergence) after transfer to 20/10°C (Fig. 2*a*). Germination reached its peak during the 4-week period at 15/2°C, and the final germination percentage was 94.0 ± 2.6%. In contrast, seeds initially incubated in the cold treatment (12 weeks at 5/2°C) did not germinate until after they were transferred through the following sequence of temperatures: 12 weeks at 5/2°C → 4 weeks at 15/2°C → 4 weeks at 20/10°C → 12 weeks at 25/15°C → 4 weeks at 20/10°C → 4 weeks at 15/2°C, where 95.0 ± 2.5% of the seeds germinated, but only after they were transferred to 20/10°C the second time (Fig. 2*b*).

In the move-along sequence that began with a warm treatment, the mean E : S ratio was 0.44 ± 0.01 after the seeds were incubated at 25/15°C for 1 week (Fig. 2*a*). At the time when the seeds were transferred from 25/15°C to 20/10°C, the average E : S ratio was 0.54 ± 0.02, and no seeds had germinated. When the seeds were transferred to the first 12-week period at 15/2°C in the sequence beginning with a cold treatment, the E : S ratio was 0.46 ± 0.02, and it increased in the following 20 weeks to 0.59 ± 0.01, before seeds were transferred to the second 4-week sequence at 20/10°C, where they began to germinate (Fig. 2*b*).

Effect of different length of dry storage on seed germination

Regardless of the length of storage period, seeds germinated only when tested at 20/10°C (Fig. 3). Germination percentages peaked after 3 months of storage and then declined, and they were higher in seeds incubated in light than in those incubated in darkness at 20/10°C, although not significantly so ($P = 0.081$). Germination percentages were significantly affected by the length of dry storage ($P < 0.001$).

Phenology of embryo growth and seedling emergence

The E : S ratio of freshly matured seeds of *C. alatavicus* was 0.43 ± 0.01 (mean ± s.e.). From the time of burial on 10 July 2011

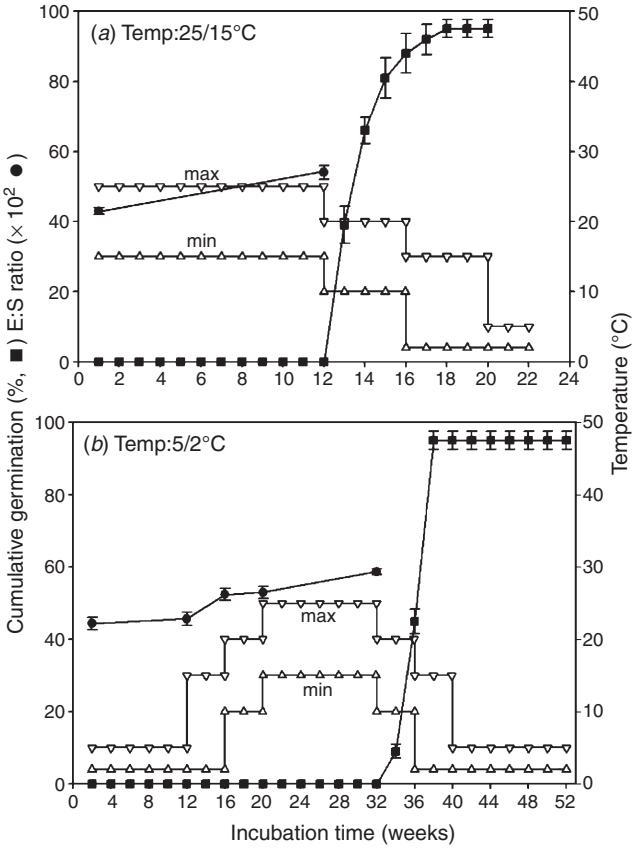

Fig. 2. Mean (±s.e.) embryo length to seed length (E : S) ratio (●) and mean (±s.e.) cumulative germination (radicle emergence) percentages (■) for *Crocus alatavicus* seeds in move-along experiment, beginning with (*a*) 25/15°C and (*b*) 5/2°C. Max, maximum temperature; min, minimum temperature.

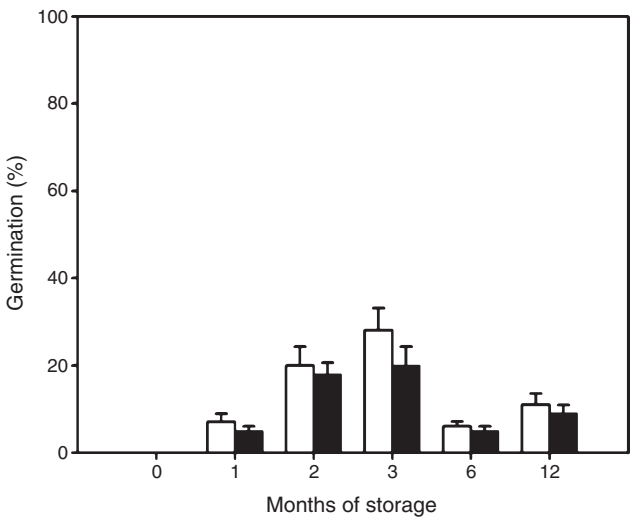

Fig. 3. Germination (radicle emergence) percentages (mean ± s.e.) of *Crocus alatavicus* seeds incubated in light (open bars) and darkness (solid bars) at 20/10°C for 2 months following 0, 1, 2, 3, 6 and 12 months of dry storage at ambient laboratory temperature. No seeds germinated after 0 months of storage.

until 7 August 2011, during which time mean weekly maximum and minimum soil temperatures were 21.5°C and 11.6°C, respectively, the E : S ratio increased to 0.46 ± 0.01, indicating that embryos had grown only slightly (Fig. 4). Significant growth of the embryo occurred between 7 and 21 August 2011, when mean weekly maximum and minimum soil temperatures were 20.4°C and 11.5°C, respectively. The embryo in 60% of the seeds exhumed on 21 August 2011 had reached the critical E : S ratio (0.62 ± 0.02) required for germination; no seeds had germinated. Final measurements of E : S ratio were made on seeds exhumed on 4 September 2011, at which time it was 0.62 ± 0.01, i.e. 100% of the length embryos must reach before the radicle emerges (critical length). About 93% of the seeds germinated (radicle emerged) in the bag between 21 August and 4 September 2011, when mean weekly maximum and minimum soil temperatures were 15.5°C and 9.4°C, respectively.

The period of seedling emergence of *C. alatavicus* in the natural habitat in 2012 was from 15 April to 10 June, with the peak between 15 and 22 April when daily maximum and minimum temperatures were 17.7°C and 1.2°C, respectively (Fig. 4). During this 1-week period, 53.6% of the seeds produced emergent seedlings. By 10 June 2012, 77.1% of the seeds had produced emerged seedlings; no seedlings emerged thereafter.

Effect of GA_3 on dormancy break and germination

No seeds germinated (root emergence) in the control or at any GA_3 concentration during incubation at 5/2°C, 15/2°C and 25/15°C for 12 weeks. However, after 12 weeks at 20/10°C, 68.0 ± 1.6% of the seeds had germinated in distilled-water control and 70.0 ± 3.3%, 72.0 ± 2.9% and 66.7 ± 3.2% in 10, 100 and 1000 mgL^{-1} GA_3, respectively ($P > 0.05$).

At 20/10°C, the highest cotyledon emergence was 86.7 ± 3.3% in the water control and 90.0 ± 3.3% in the 10 mg L^{-1} GA_3 solution ($P > 0.05$). At 20/10°C, cotyledons had emerged from 6.7 ± 1.7%, 10.0 ± 0.0% and 90.0 ± 3.3% of the seeds in 10 mg L^{-1} GA_3 solution after 4, 8 and 12 weeks, respectively, and from 6.7 ± 1.7%, 11.7 ± 1.7% and 86.7 ± 3.3% of the seeds in the water control, respectively. Cotyledons emerged from 10.0 ± 5.8%, 70.0 ± 5.8% and 26.7 ± 3.3% of control seeds (with the radicles emerged) kept continuously at 5/2°C, 15/2°C and 25/15°C, respectively. At 25/15°C, cotyledons emerged from 20.0 ± 2.9%, 15.0 ± 0.0% and 13.3 ± 1.7% of the seeds in the 10, 100 and 1000 mg L^{-1} GA_3 solutions, respectively.

Effect of warm temperature on radicle emergence

In general, percentage and rate of radicle emergence at 20/10°C increased with an increase in the length of the warm stratification period to which the seeds had been subjected (Fig. 5*a*). Radicle emergence from control seeds kept continuously at 20/10°C and 15/2°C for 18 weeks was 91.7 ± 3.3% and 10.0 ± 2.9%, respectively, but no radicles emerged from any seeds kept continuously at 5/2°C and 25/15°C.

Effect of cold temperature on cotyledon emergence

In general, percentage and the rate of cotyledon emergence at 20/10°C increased with an increasing length of the cold-stratification period to which the radicle-emerged seeds had been subjected (Fig. 5*b*). Cotyledons emerged from 13.3 ± 3.3%, 86.7 ± 3.3%, 93.3 ± 3.3% and 26.7 ± 3.3% of control seeds (with radicles emerged) kept continuously for 14 weeks at 5/2°C, 15/2°C, 20/10°C and 25/15°C, respectively. For seeds exposed to 5/2°C for 8 weeks and then transferred to 20/10°C, nearly 95% had an emerged cotyledon by 6 weeks. However, cotyledons required 14 weeks to emerge at 20/10°C from seeds with an emerged radicle that had not been cold treated.

Discussion

Seeds of *C. alatavicus* have a linear underdeveloped embryo at the time of dispersal in early summer, and the embryo increases ~44% in length inside the seed before the radicle emerges. In the natural subalpine habitat, embryos grow during summer, and radicles emerge when temperatures start decreasing in autumn. Therefore,

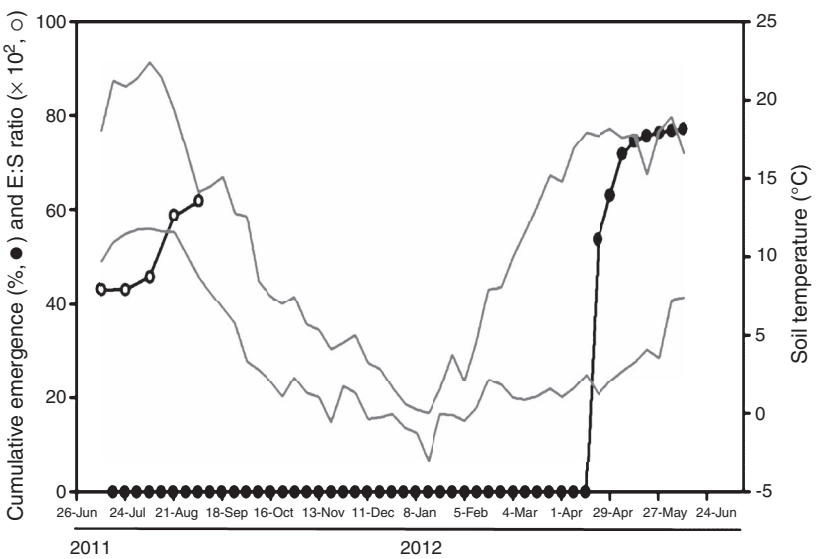

Fig. 4. Mean weekly maximum and minimum temperatures at a soil depth of 5 cm and phenology of embryo growth (○) and seedling emergence (●) in seeds of *Crocus alatavicus* buried in July 2011 in the natural habitat. All s.e. values were less than 2%.

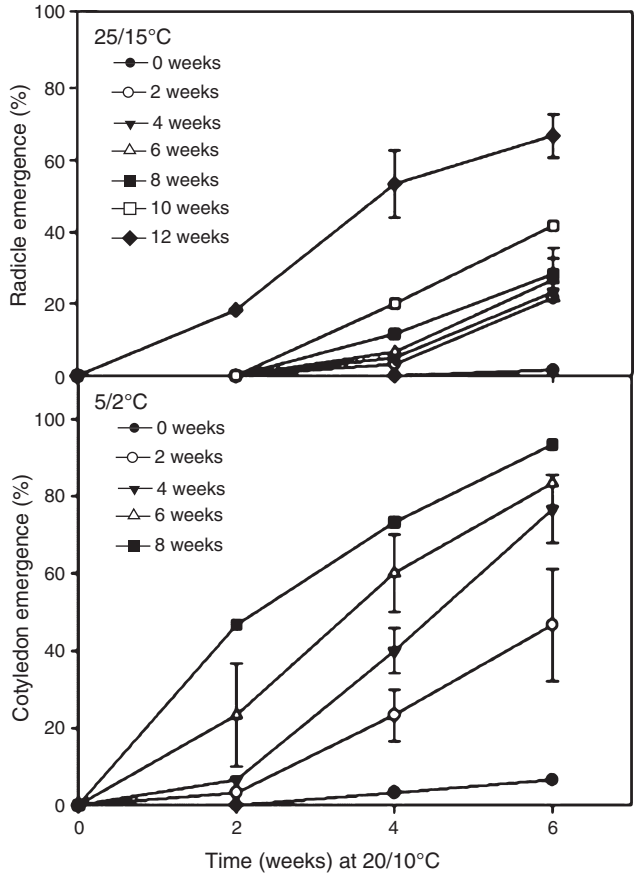

Fig. 5. (*a*) Cumulative radicle emergence (mean ± s.e., if ≥5%) of *Crocus alatavicus* seeds during 6 weeks of incubation in light at 20/10°C, following 0–12 weeks of warm stratification at 25/15°C. (*b*) Cumulative percentage cotyledon emergence (mean ± s.e., if ≥5%) of *C. alatavicus* seeds (with radicles emerged) during 6 weeks in light at 20/10°C, following 0–8 weeks of cold stratification at 5/2°C.

the embryo is underdeveloped and needs some time to grow to a critical length before germination occurs (Baskin and Baskin 1998).

No fresh seeds germinated within 4 weeks in any of the temperatures used in the move-along experiment, indicating that the underdeveloped embryos have PD at the time of seed maturity; thus, the seeds have MPD (Baskin and Baskin 1998). Results from the move-along experiment and the effect of warm temperature on radicle emergence showed that warm stratification promotes the breaking of PD. Also, some breaking of PD occurred during 3 months of dry storage. Thus, PD of the radicle is broken during summer.

The nine levels of MPD are subdivided into the following two categories, depending on the temperature requirement for embryo growth: (1) simple type, in which embryos grow during warm stratification; and (2) complex type, in which embryos grow during cold stratification (Baskin and Baskin 1998, 2004). Thus, seeds of *C. alatavicus* have some level of simple MPD (Baskin and Baskin 1998), because embryo growth occurred in the field in summer (Fig. 4) and during a long period of warm incubation in the laboratory (Fig. 5*a*). Although the radicle emerged from *C. alatavicus* seeds in the field in autumn when temperatures started to decrease, the cotyledons did not emerge until the following spring, after the seeds with an emerged radicle had been cold stratified (Fig. 5*b*). Further, a period of cold stratification (5/2°C) was required for cotyledon emergence in a laboratory experiment (Fig. 5*b*). Seedling emergence occurs in spring as soon as temperatures are high enough for shoot growth (Fig. 4). Thus, seeds of *C. alatavicus* have epicotyl MPD, and their response to GA_3 may help us determine the level of PD and, thus, the level of MPD. GA_3 stimulates the germination of seeds with non-deep and intermediate PD, but it does not break deep PD in intact dispersal units (Nikolaeva 1977; Baskin and Baskin 1998). GA_3 was not effective in breaking dormancy in roots or epicotyls of *C. alatavicus* seeds.

Thus, the level of PD in epicotyls of *C. alatavicus* is deep, and seeds have deep simple epicotyl MPD. The formula for this kind of dormancy is $C_{1b}B(root) - C_3(epicotyl)$. $C_{1b}B(root)$ indicates that the embryo is underdeveloped (B) and has physiological dormancy (C) that is non-deep (Subscript 1), requiring warm temperature (Subscript b) for the breaking of the PD. $C_3(epicotyl)$ indicates that the epicotyl has PD (C) that is deep, requiring a long cold period to break it (Subscript 3; Baskin and Baskin 2008).

The optimum temperature for maximum germination (radicle emergence) percentage in seeds of *C. alatavicus* was 20/10°C, and seeds began to germinate at this temperature regime after 8 weeks of incubation (Fig. 1). Further, a period of warm stratification was very effective in promoting radicle emergence at 20/10°C (Fig. 5*a*). Exposure of seeds to a sequence of temperatures that began with a warm treatment or with a cold treatment showed that the percentage of radicle emergence was highest at 20/10°C, after seeds had received 12 weeks of warm stratification at 25/15°C (Fig. 2).

Many seeds incubated continuously at 20/10°C and the controls for the GA_3 experiment kept at 20/10°C had a high percentage of emerged cotyledons. However, at this temperature regime, seeds received warm stratification during the day (20°C) and cold stratification (10°C) at night. Thus, whereas seeds with emergent radicles receiving 6 weeks of cold stratification had 70% cotyledon emergence, the controls kept at 20/10°C for 12 weeks (= 6 weeks of cold stratification at 10°C) had 90% cotyledon emergence. The reason that a high percentage of the GA_3-treated seeds with an emergent radicle produced an emergent cotyledon at 5/2°C, 15/2°C and 20/10°C is that these seeds received a daily cold-stratification treatment. At 25/15°C, where seeds with an emergent radicle did not receive a daily cold-stratification treatment, cotyledons emerged in only 13–20% of the seeds. Thus, GA_3 was not effective in promoting cotyledon emergence, as we would expect in the case of deep simple epicotyl MPD. However, this experiment did tell us that 10°C is a more effective cold stratification temperature than is 2°C.

Most seedlings (shoots) of *C. alatavicus* emerged between 15 and 22 April after epicotyl dormancy was broken during winter (Fig. 4). Thus, epicotyl dormancy may be adaptive in that the cotyledons are not exposed to predators and freezing temperatures in autumn and winter, and the seedlings have a well developed root system when the cotyledons expand (Baskin and Baskin 1983, 1985*b*; Masuda and Washitani 1990). Further, because the root system is already well established, seedlings can grow rapidly after temperatures increase in early spring. This

pattern of dormancy is an adaptation to the seasonal cycle in temperate regions (Baskin and Baskin 1983, 1985*a*, 1985*b*; Kondo *et al*. 2004). Regardless of what the adaptive advantages of deep simple epicotyl MPD might be for an individual species, this kind of dormancy is known in species of Amaryllidaceae, Aristolochiaceae, Boraginaceae, Caprifoliaceae, Melanthiaceae (*Trillium*), Paeoniaceae, Ranunculaceae and Liliaceae s.l., with limited occurrence in the Berberidaceae, Fumariaceae and Hyacinthaceae (Baskin and Baskin 1998). To our knowledge, the present results for *C. alatavicus* are the first report of this level of MPD in the Iridaceae.

Our study showed that *C. alatavicus* can be readily propagated from seeds by either subjecting seeds to the natural (or near-natural) sequence of warm summer, cool autumn, cold winter and cool spring temperatures found in temperate regions of the world. Also, both radicle and cotyledon dormancy can be broken if seeds are maintained at 20/10°C for 26 weeks, during which time 20°C breaks the PD of the radicle and 10°C breaks the PD of the shoot.

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