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Seed dormancy release and germination characteristics of *Corispermum lehmannianum* Bunge, an endemic species in the Gurbantunggut desert of China

Características de la ruptura de la dormancia y la germinación de *Corispermum lehmannianum* Bunge, una especie endémica del desierto de Gurbantunggut en China

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Abstract. Seed dormancy release and germination of *Corispermum lehmannianum* Bunge were tested using various treatments: temperature, cold stratification, gibberelins (GA3), dry storage and sand burial. Results showed that temperature and light did not affect the germination of fresh seeds, cold stratification and GA3 could improve seed germination, whereas dry storage and sand burial did not. The germination percentage was highest at 35/20 ˚C after the cold stratification and GA3 treatments. *Corispermum lehmannianum* seeds were classified as non-deep, Type-2, physiological dormancy (PD), whose seed dormancy could be released by cold stratification and GA3.

Keywords: Seed germination; Seed dormancy; Gurbantunggut desert.

Resumen. Se estudiaron la ruptura de la dormancia y la germinación de *Corispermum lehmannianum* Bunge utilizando varios tratamientos: temperatura, estratificación (frío), ácido giberélico, almacenaje en seco y entierro en arena. La temperatura y la luz no afectaron la germinación de las semillas frescas, la estratificación y el ácido giberélico podrían mejorar la germinación, mientras que el almacenaje en seco y entierro en arena no pudieron hacerlo. El porcentaje de germinación más alto se obtuvo a 35/20 ˚C después de aplicar los tratamientos de estratificación y ácido giberélico. Las semillas de *Corispermum lehmannianum* se clasificaron como no profundas, Tipo-2, dormancia fisiológica (PD). Sus semillas podrían salir de la dormancia usando estratificación y ácido giberélico.

Palabras clave: Germinación de semillas; Dormancia de las semillas; Desierto de Gurbantunggut.

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INTRODUCTION

Primary dormancy occurs in a freshly matured seed (or other germination units) (Hilhorst, 1995, 1998; Bewley, 1997) when germination is blocked physiologically or physically. Dormant seeds do not germinate under any favorable conditions (strict dormancy) (Baskin & Baskin, 2004). In many species, when freshly matured seeds are dispersed, germination is delayed due to primary dormancy, and seeds enter into the soil to form a transient or persistent seed bank (Murdoch & Ellis, 2000). Dormancy is therefore considered an important species-specific characteristic that responds to environmental changes, and determines the germination periods and environments (Finch-Savage & Leubner-Metzger, 2006). Consequently, the maintenance or release of dormancy depends on various environmental signals. These signals might stimulate many apparently different physiological characteristics. Elucidating the mechanism responsible for the maintenance or release of dormancy is important for understanding the ecological adaptation and regeneration of plants in natural environments.

Cold stratification, exogenous application of gibberellins (GA_3), dry storage (after ripening) and sand burial can overcome physiological dormancy and promote germination of seeds of angiosperms and gymnosperms (Singh & Banerji, 1983; Bewley & Black, 1994; Bungard et al., 1997; Baskin & Baskin, 1998). They could shorten the time from sowing to germination of dormant seeds and thus the time required for seedling production, depending on the species (Bewley & Black, 1994; Baskin & Baskin, 1998). Information on seed germination and dormancy-release characteristics of desert plants is crucial to understand the adaptive strategies and seedling reestablishment in harsh habitats.

Corispermum lehmannianum Bunge occurs in Central Asia (including northwest China), Iran, Turkey and Caucasia (Zhang & Chen, 2002). In China, the species only occurs in the cold, desert region of the Gurbantunggut Desert in Xinjiang. It is a very common annual/spring ephemeral and can stabilize sand dunes. Little information is known regarding the seed germination and dormancy release of this species. This study examines the effect of a sequence of temperatures that could occur in the natural habitat at the timing of seed germination. The effects of cold stratification, gibberellic acid (GA_3), dry storage and sand burial pretreatments on dormancy-break and germination were also tested to determine dormancy types. This information may be useful for the conservation and restoration of this plant species, and pertinent to its survival in the most extreme environments.

MATERIALS AND METHODS

Study area. The Gurbantunggut Desert, the second largest desert in China with an area of 48800 km^2, is located in the center of the Junggar Basin of Xinjiang, China. Blocked by the Tien Shan and Altay Mountains, and far away from any oceans, this desert is a large expanse of drought-exposed terrain. The mean annual rainfall is approximately 80 mm, and it occurs mostly during spring and winter. Number of snowfall days in winter total from 100 to 160. The snow melts in late March and becomes an important water source for seed germination and seedling establishment. The annual mean potential evaporation is 2606 mm. The average annual temperature in this area is 7.26 ˚C; January (-17 ˚C) and July (25.6 ˚C) are the coldest and hottest months, respectively (Zhang et al., 2006).

Seed collection. Freshly matured seeds (brown colour) of *C. lehmannianum* were collected from the Gurbantunggut Desert in late September of 2010. Seeds were harvested from more than 20 randomly selected individuals. Unhealthy seeds with pathogenic bacteria or insect damage were discarded.

Seed mass and imbibition. Seed mass was determined by weighing three replicates of 100 seeds each using an electronic balance (0.0001 g).

An imbibition test was conducted at room temperature (21-25 ˚C, 45% relative humidity) using three replicates of 100 dry seeds each. The dry mass of each replicate was determined (time 0), and the seeds were then placed on Whatman No. 1 filter paper in 5 cm diameter Petri dishes, moistened with distilled water. Seed mass was measured again after 0.5, 1, 2, 4, 6 and 8 h from water imbibition. The relative increase in fresh mass (Wr) was calculated as Wr = [(Wf -Wi) /Wi]*100, where Wi is the initial seed mass and Wf the mass after a certain time (Baskin et al., 2004). The test was repeated three times, and the standard error was calculated.

Effect of light and temperature on germination of the seed. To investigate the germination behavior, four replicates of 25 fresh seeds each were incubated on two layers of Whatman No.1 filter paper moistened with 2.5 mL of distilled water in 5 cm diameter plastic Petri dishes. The dishes were sealed with parafilm and incubated at daily (12/12 h) temperature regimes of 5/15, 5/20, 10/25, 15/30 and 20/35 ˚C in dark/light (12 h daily photoperiod) and in continuous darkness (seeds in black bags) for 14 d. These thermoperiods represent the mean daily minimum and maximum monthly temperatures in the natural habitat during the growing season: 5/15 ˚C in early April and October, 5/20 ˚C in late April, 10/25 ˚C in May and September, 15/30 ˚C in June and August and 20/35 ˚C in July. The seed was considered germinated when the radicle had emerged. Germination was examined every day under light conditions during 14 d, and germinated seeds were removed after each inspection. Seeds incubated in darkness were checked only after a 14-day period. The final percentage of germination (FPG) was estimated as follows: FPG=GN/SN, where GN is the total number of germinated seeds and SN the total number of viable seeds tested.

Effect of gibberellic acid (GA_3) on germination. GA_3 is a plant growth regulator that can break non-deep physiological dormancy (non-deep PD) on seeds. To test the effects of GA_3 on the dormancy break, four replicates of 25 seeds each were incubated in 0 (distilled water, control) and 1 mmol/L GA_3 solutions at a daily temperature regime of 5/15, 5/20, 10/25, 15/30 and 20/35 ˚C in dark/light for 14 d. Seeds were checked every day for emergence of the radicle over the 14 d period.

To examine whether excised embryos produced normal seedlings, we carefully used the blade to strip embryo from seeds, and then put them to the flowerpot in the incubator.

Effect of cold stratification treatment on germination. Quartz sand washed and moistened with distilled water (sand moisture content 11-13%) was placed beneath two layers of filter paper in 10 cm deep, 20 cm diameter metal boxes. Fresh seeds used within two weeks of collection were placed on the filter paper, and the metal boxes were closed and placed in a refrigerator for 2, 4, 8 and 12 weeks at a constant 5 ˚C. After retrieval, the seeds were transferred to Petri dishes with filter papers moistened with distilled water. Germination conditions were the same as those aforementioned on the effects of light and temperature on seed germination.

Effect of storage treatment on germination. The fresh seeds were stored dry in paper bags for about seven months at ambient room temperature (20 ˚C; 20-30% RH). The seeds were also buried at a depth of 2-5 cm in selected, marked locations in the desert and left to overwinter (-20 to 10 ˚C; v/v 40-50%). In the following spring (late April), the seeds were excavated from the soil.

After retrieval, the seeds were transferred to Petri dishes with filter papers moistened with distilled water. Germination conditions were the same as those aforementioned on the effects of light and temperature on seed germination. Untreated seeds collected at the same time were used as the control.

Data analysis. The means ± SEs were calculated, and the data were analyzed using SPSS 15.0 software (SPSS, Chicago, IL). The figures were created with Origin 8.0. All data on seed germination were transformed (arcsine) before statistical analysis to ensure homogeneity of variance. A two-way analysis of variance was used to test for significance among different temperatures, light and cold stratification period at the 95% level.

RESULTS

Seed weight and imbibition. The seed mass of *C. lehmannianum* was 0.073 ± 0.01mg. Seeds imbibed water readily, and followed a typical pattern of rapid initial water uptake, with seed mass increasing by 48.7 ± 1.3 % after 1 h, 62.5 ± 0.8% after 2 h and 64.3 ± 1.4% after 8 h (Fig. 1).

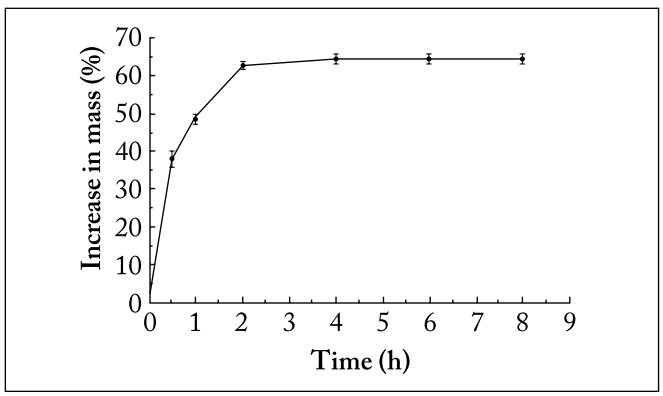

Fig. 1. Mass increase of untreated dry *Corispermum lehmannianum* seeds during imbibition at various time periods at ambient laboratory temperature (21-25 °C). Error bars are ± SE.
Fig. 1. Incremento de peso de semillas secas no tratadas de *Corispermum lehmannianum* durante la imbibición a varios períodos de tiempo a temperature ambiente de laboratorio (21-25 °C). Las barras verticals reprsentan 1 ± E.E.

Effect of light and temperature on germination. Fresh seed germination was not significantly affected by temperature (F=0.076, p=0.989; Table 1), light (F=1.058, p=0.312; Table 1) or their interaction (F=0.14, p=0.966; Table 1). Less than 5% of the fresh seeds germinated in light and dark at all temperatures (Fig. 2).

Table 1. Results of two-way ANOVA procedure on germination of fresh *Corispermum lehmannianum* seeds at different temperature and light regimes.
Tabla 1. Resultados del ANOVA doble sobre la germinación de semillas cosechadas en el año en que fueron producidas de *Corispermum lehmannianum* a diferentes regímenes de temperatura y luz.

Source	SS	df	MS	F-value	p-value
Temperature (T)	5.600	4	1.400	0.076	0.989
Light (L)	19.600	1	19.600	1.058	0.312
T * L	10.400	4	2.600	0.140	0.966

Effect of gibberellic acid (GA3) on germination. Compared to non-treated seeds, GA_3 treatments significantly increased germination percentage to 20% in light, but not in dark at all tested temperature periods (Fig. 2). In addition, excised embryos produced normal seedlings.

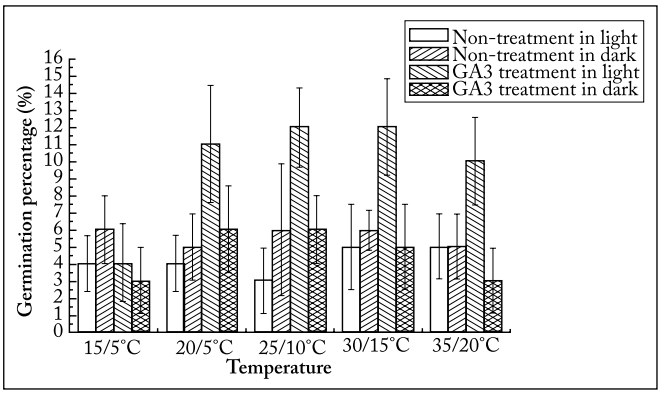

Fig. 2. Effect of temperature and light on germination of non-treated fresh and GA3 treated seeds of *Corispermum lehmannianum*. Error bars are ± SE.
Fig. 2. Efecto de la temperature y la luz en la germinación de semillas frescas no tratadas y semillas tratadas con ácido giberélico de *Corispermum lehmannianum*. Las barras verticals representan 1 ± E.E.

Effect of cold stratification on germination. Seed germination has significantly increased after cold stratification compared to the fresh seeds (Fig. 3). As stratification time increased from 0 to 12 weeks, the germination percentage also increased. Seed germination at 20/35 °C increased from 5.0±1.91% at 0-week-stratification (control) to 39.0 ± 2.5% at 12-week-stratification (Fig. 3). There was also a significant increase in germination at the 5/15 °C from 4.0±1.63% at 0-week-stratification (control) to 12.0±1.63% at 12-week-stratification. Effect of incubation temperature ($F=18.298$, $p<0.001$; Table 2), length of cold stratification period ($F=56.771$, $p<0.001$; Table 2), and the interaction between the length of cold stratification period and incubation temperature ($F=3.309$, $p<0.001$; Table 2) on germination were statistically significant.

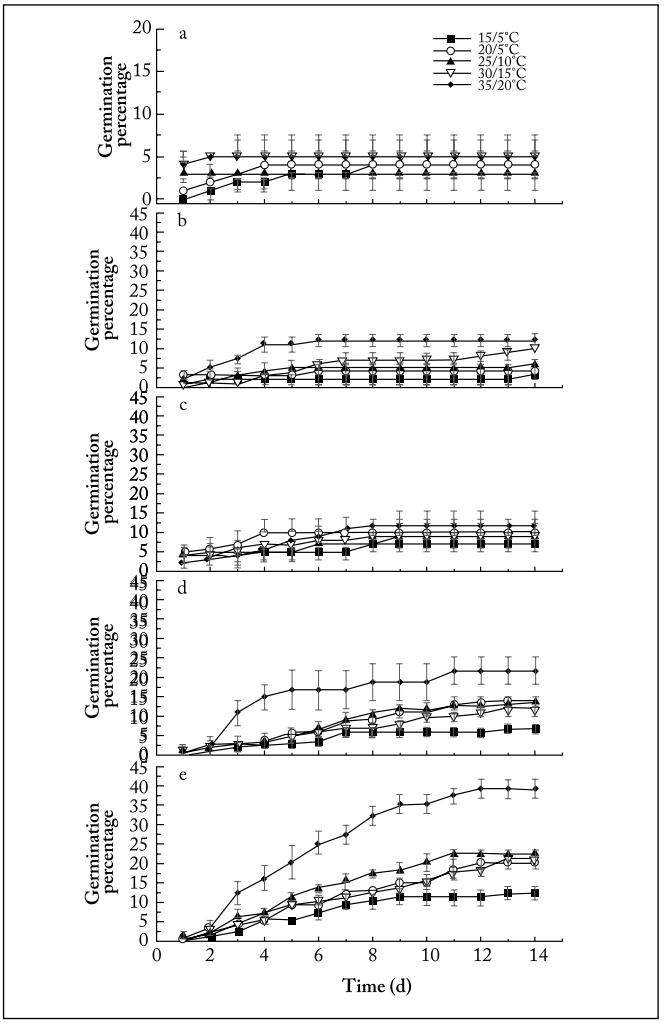

Fig. 3. Cumulative germination of (a) fresh, (b) 2-week cold-stratified, (c) 4-week cold-stratified, (d) 8-week cold-stratified, and (e) 12-week cold-stratified seeds of *Corispermum lehmannianum* incubated at different temperature regimes in light for 14 d. Error bars are ± S.E. Note the change of scale in panel (a).
Fig. 3. Germinación acumulativa de semillas de *Corispermum lehmannianum* (a) frescas, (b) estratificadas en frío por 2 semanas, (c) estratificadas en frío por 4 semanas, (d) estratificadas en frío por 8 semanas, y (e) estratificadas en frío por 12 semanas. Las semillas se incubaron a varios regímenes de temperatura bajo condiciones de luz por 14 días. Las barras verticales son ± 1 E.E.
Note el cambio de escala en el panel (a).

Table 2. Results of two-way ANOVA procedure on germination of *Corispermum lehmannianum* seeds after different stratification treatments, at different temperature regimes.
Tabla 2. Resultados del ANOVA doble sobre la germinación de semillas de *Corispermum lehmannianum* después de diferentes tratamientos de escarificación a diferentes regimenes de temperatura.

Source	SS	df	MS	F-value	p-value
Temperature (T)	1354.560	4	338.640	18.298	<0.001
Cold stratification (CS)	4202.560	4	1050.640	56.771	<0.001
T * CS	979.840	16	61.240	3.309	<0.001

Effect of dry storage and sand burial on germination. Seeds slightly germinated after dry storage and sand burial treatments (Fig. 4). The treatments, however, neither significantly increased seed germination percentage (Fig. 4) nor broke seed dormancy.

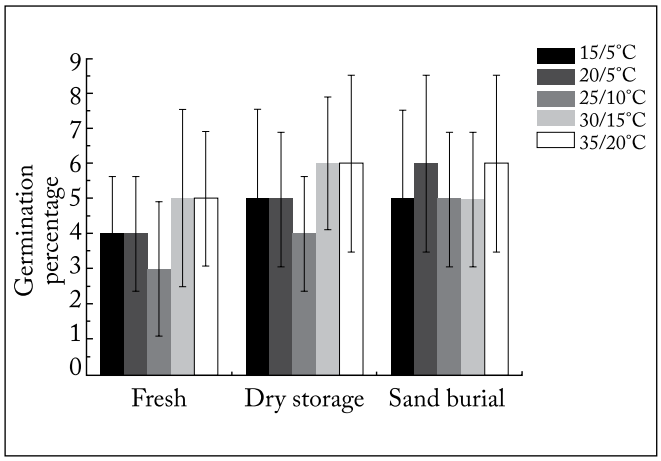

Fig. 4. Effect of temperature and storage treatment on germination of seeds of *Corispermum lehmannianum*. Error bars are ± S.E.
Fig. 4. Efecto de la temperature y tratamiento de almacenaje en la germinación de semillas de *Corispermum lehmannianum*. Las barras vertcales son ± 1 E.E.

DISCUSSION

In the dormancy classification system designed by Baskin and Baskin (1998, 2004), seeds with physiological dormancy (PD), morphological dormancy (MD) and morphophysiological dormancy (MPD) have a seed coat permeable to water. According to our observation, *C. lehmannianum* seeds had fully developed embryos and a water-permeable coat. However, percentage germination of fresh seeds was significantly low suggesting that the seeds had PD. According to Baskin and Baskin's classification regarding dormancy (1998, 2004), PD is divided into three levels: deep, intermediate and non-deep. Our experiments showed that 12 weeks of cold stratification could break dormancy of *C. lehmannianum* seeds. Further, GA_3 promoted germination in light, and excised embryos produced normal seedlings. Based on these characteristics, it can be concluded that *C. lehmannianum* seeds have non-deep PD. Most species with non-deep PD have either Type 1 or Type 2 seeds (Baskin & Baskin, 2004). Results of this study showed that after different duration of cold stratification, germination of seeds increased from high to low temperature regimes, inferring that seed dormancy of *C. lehmannianum* can be classified as Type 2, non-deep PD.

Annuals species in Gurbantunggut desert, such as *Ceratocarpus arenarius, Bassia dasyphylla* and *Cousinia affinis* (Liu et al., 2013) adapt to a seasonal arid habitat through the seeds entering physiological dormancy. These authors stated that seeds may require a particular temperature after ripening. This phenomenon can regulate the annual germination of seeds of these species in the field (Probert, 2000). Dry storage had limited effect on the germination of *C. lehmannianum* seeds, demonstrating that the seeds of this species do not ripen further during dry storage. The observation also shows cases where species with physiological dormancy have mechanisms that ensure seeds from individual plants to germinate at different time periods, often over several years (Tieu et al., 2001; Schütz et al., 2002). Also, the dry, summer season is likely to be a predominant period for releasing seed dormancy in these species (Commander et al., 2009). This bet-hedging strategy is an adaptation to the unpredictable environmental conditions in the desert (Venable & Lawlor, 1980; Venable, 1985; Philippi & Seger, 1989).

Seeds buried in sand controlled the dynamics of dormant seed states, which occur in a continuous fashion (i.e., dormancy continuum/dormancy cycle) (Baskin & Baskin, 1998). Germination of *C. lehmannianum* seeds was not affected by the burial treatment, so perhaps these seeds do not have the capacity to respond to such treatment. This finding showed that burial treatments were unsuitable for seedling establishment and survival. The possible explanation might be that seeds absent of germination after treatments may require a longer period of cold and moist conditions for breaking dormancy. This might occur under natural conditions in the spring, and it may be mimicked in the lab by cold stratification under moist conditions and low temperature (Baskin & Baskin, 1998). However, most seeds were dormant and formed a persistent seed bank which plays a major role in the regeneration of this mature vegetation.

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