DORMANCY AND GERMINATION IN SHORT-LIVED *LEPIDIUM PERFOLIATUM* L. (BRASSICACEAE) SEEDS

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Abstract

To understand germination timing in an ecological context, the response to environmental events that effect seed dormancy is central and has to be combined with knowledge of germination responses to different ecological factors. In this study, seed dormancy, germination and seedling survival of annual short-lived clasping pepperweed *Lepidium perfoliatum* L. (Brassicaceae) were investigated. Three types of pre-treatments viz., various temperature dry storage, light and water stress were tested as possible dormancy- and survival-affecting environmental events. Fresh mature seeds were greatly dormant. Warm (30ºC) dry storage more facilitated breaking dormancy, they germinated well under apt conditions (e.g. 20ºC and 10/20ºC plus periodic light, 14 h/d). For those seeds which underwent after-ripening, they could germinate at a range of constant temperatures (4, 10, 15, 20, 25, and 30ºC) and one alternating temperature (10/20ºC). Under alternating temperature regimes, the final percent germination of *L. perfoliatum* seeds increased from 37ºC to 93% when temperature altered from 4/10ºC to 10/20ºC in light, then decreased with increasing temperature. The germination pattern under constant temperature conditions was similar to that under alternating temperature and significant differences in final percent germinations and rates of germination were observed among different temperatures. Under different light treatments, final germination of showed significant differences, only with 35% of germination percentage in dark, much lower than those in red and white light (i.e. 93% and 91%, respectively). GA_3 could promote the germination of non-dormant seeds in dark. When water potentials were reduced, final percent germination decreased dramatically, and few seeds germinated at -0.98 MPa (generated by PEG-8000). The changes of proline content in resultant seedlings were reverse to that of final percent germination with changing water potentials. The present findings show that the dormancy and germination patterns of *L. perfoliatum* are important mechanisms adaptive to the rigorous desert environmental conditions.

Introduction

Seed germination is a critical process in the life cycle of a plant. A number of models have shown that seed dormancy is a mechanism allowing plants to survive in spatio-temporally variable environments (Cohen, 1967; Venable and Brown, 1988; Rees, 1994; Tielbörger and Valleriani, 2005). Some seed traits including a mucilaginous coat have important attributes that favor dispersal and germination (Gutterman, 1997; Huang and Gutterman, 1998, 1999, 2000). For a species, at the gene level, genetic diversity can be secured either by sexual reproduction or by persistence of established individuals. Many short-lived species are more dependent than long-lived species on a sexual reproductive system that ensures regeneration every year, such as *Atriplex aucheri* (Wei *et al*., 2003), *Malcolmia scorpioides*, *Tetracme recurvata*, *T. quadricornis* and *Lappula semiglabra* (Pan and Wang, 1995). Once their sexual regenerations fail for one year or more, the annual short-lived species have to tend to extinction. However, many species including desert short-lived plants are still growing in the deserts for thousands of years for their specific mechanisms to adapt to desert environments.

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Recently, the ephemeral flora has attracted attention of ecologists and seed biologists because of particular morphologic characteristics and physiological processes to survive in highly unfavorable environments (Gutterman, 1993, 1997; Wesche *et al*., 2006). Moreover, desert annuals have been used as model organisms for empirical and theoretical investigations on the adaptive value of seed dormancy (Tiebörger and Valleriani, 2005). However, seed responses of many desert ephemerals to ecological factors have not been well understood. Thus, detailed studies of the germination eco-physiology are needed.

In the Junggar Basin, Xinjiang, northwest China, more than two hundred desert ephemerals belong to twenty seven plant families, such as Brassicaceae, Compositae, Gramineae and Liliaceae (Mao and Zhuang, 1994). Clasping pepperweed (*Lepidium perfoliatum* L) is a short-lived plant from the plant family of Brassicaceae, only depending on sexual reproduction, with a life cycle of approximately 60 days. To our knowledge, information that has been documented and published on this species focuses on its taxonomy (Mao and Tian, 1994), anatomical and morphological acclimation (Zhuang and Tian, 1990a, b), reproductive allocation (Wei et al., 2003), and habitat and the range of its geographical distribution (Mao and Zhang, 1994). However, there has been little experimental research regarding seed germination responses to ecological factors for clasping peppwerweed.

In the present study, we mainly determined the seed dormancy pattern and germination preferences of annual short-lived *L. perfoliatum* that is used for dune rehabilitation, particularly with regard to water availability in sand. We examined the effects of temperature, light and polyethylene glycol (PEG)-8000 on seed germination and the changes of proline in resultant seedlings.

2. Materials and methods

Seed collection: Fresh-mature seeds were collected randomly from one natural population in 29 May 2006 in the Jiangjun Mountain of the Jungger Basin in north Xinjiang, China (44°11.77′N, 86°5.16′E, Alt. 620~640 m), where the annual precipitation is about 200 mm, which falls mainly in summer (Table 1). Mean annual temperature is 5.8°C, with mean daily maximum and minimum temperatures of 39.4°C and -36.8°C, respectively (Gan *et al*., 1995). Ripe fruits were collected directly from 200 adult plants, from which seeds were obtained. Immediately after collection, seeds were mixed thoroughly and divided into batches of equal size. Seeds were immediately taken to the Xishuangbanna Tropical Garden, Chinese Academy of Sciences, China, and stored in paper bags at room temperature (20±2°C), 4°C and 30°C, respectively, until used.

Germination experiments: For fresh-matures seeds, germination tests were carried out at a alternating temperature (10/20°C) and a constant temperature of 4, 10, 15, 20, 25, and 30°C, respectively under 14 hr d^{-1} warm fluorescent light (HPG-280B Illuminating Incubator; Har'erbin Electronic Apparatus Manufactory, Har'erbin, China, providing a Photosynthetic photo flux density (PPFD) of approximately 40μmol s^{-1} m^{-2} (LI-COR, Inc., Nebraska, USA). Four replicates of 50 seeds each were incubated in Petri dishes of 9 cm diameter with a sheet of round filter paper moistened by distilled water. Seeds were inspected daily for germination for 40 days, and protrusion of the radicle was the criterion for germination. The respective experiment was described in detail as following:

Table 1. Effects of temperature regimes in light on the germination of *Lepidium perfoliatum* seeds stored for four months at 4ºC and 30ºC, respectively.

Temperature (ºC)	Germination (%) ± SD of seeds stored at 4ºC		Germination (%) ± SD of seeds stored at 30ºC		Germination (%) ± SD of seeds stored at RT[*] in light	
	Raw	SD	Raw	SD	Raw	SD
4	9h	1.9	11h	2.8		
10	37f	2.2	58f	2.7		
15	59c	2.2	71c	1.9	59c	3.8
20	67b	2.7	83b	3.2	67b	2.2
25	42e	3.8	62e	2.8		
30	7h	1.7	13h	4.0	3.0d	1.5
4/10	27g	4.1	37g	3.0		
4/15	39f	3.0	61e	2.2		
4/20	44e	3.8	66d	1.5		
10/20	76a	1.5	93a	0.7	73a	4.2
10/25	51d	2.2	64e	1.5	66b	1.5

*RT, room temperature; for each column the mean values with the same superscript letters among temperatures are not significantly different at 5% level of probability (Duncan's multiple comparisons test).

Effects of temperature on germination: For fresh-matured or dry-stored seeds, germination tested were carried out at the following constant temperatures (i.e., 4, 10, 15, 20, 25, and 30ºC) and altering temperatures (i.e., 4/10, 4/15, 4/20, 10/20 and 10/25ºC), respectively, with 14 h daily photoperiod.

Effects of dry storage on germination: Before sowing, some seeds were stored for 4 months at room temperature (20 ± 2ºC) (used as control), 4ºC and 30ºC, respectively, at 45% relative humidity (RH). After four-month dry storage, germination was tested in incubators as described above.

Effects of light on germination: For incubation in darkness, Petri dishes with seeds were wrapped in a 4-layer black cloth. For red light (650±20 nm) treatment, Petri dishes with dormancy-broken seeds were put in the incubator equipped with red lamp-houses (LED-R, Tokyo Rikakikai CO., LTD. Japan). For white light treatment, the incubators as described above were used. A green safe light (Baskin and Baskin, 1998) was used to examine the dark-incubated seeds. All the germination tests were conducted at the altering temperature of 10/20°C.

GA₃ responses: For seeds stored for 4 months at 30ºC, a simple description on sensitivity to applied GA_3 was made. To determine GA_3 response germination was tested at a range of GA_3 concentrations prepared according to Derkx and Karssen (1994). Before measuring the final percentage germination in an incubator, batches of seeds were imbibed for 24 hours in a certain GA_3 solution, and then were placed on a filter paper moistened by 10 ml solutions of 0.05, 0.1, 0.3, 0.5 and 1.0 m mol/L GA_3 solution, respectively, at 10/20ºC in darkness. Germination was observed every two days and certain GA_3 solution was added into corresponding Petri dishes in time.

Water stress during germination: Different solutions of Polyethylene Glycol 8000 (PEG-8000) were prepared to simulate five different water potential conditions (-0.15, -0.34, -0.49, -0.98, -1.27 and -2.04 MPa) at 20ºC (Michel, 1983). In these treatments, we

poured 10 ml of the PEG-8000 solution into each Petri dish and placed a piece of gauze on the surface of the liquid. Freshly-matured or four-month dry-stored seeds at 30ºC were sown on the gauze to prevent them from sinking in the PEG solution. Each Petri dish was wrapped with transparent plastic foil to maintain constant humidity. The control of this experiment was added 10 ml distilled water. Petri dishes were placed in incubators with same parameters as described above.

Proline extraction and assay of seedlings: Free proline was extracted and determined according to Bates *et al*., (1973). The seedlings produced by the seeds treated by PEG solutions were pulverized using a liquid nitrogen-chilled mortar and pestle and homogenized in 5 ml of 3% (w/v) sulfosalicylic acid. The extract was centrifuged for 10 min at 8000*g*. The pooled supernatant was used for proline determination by UV/Visible-800 spectrophotometer (Beckman Coulter, Inc., USA).

Statistical analyses: Percentage values were arcsine transformed prior to statistical analysis. However, values in Table 1, Fig. 1, Fig. 2 and Fig. 3 are untransformed data. The results were expressed as mean values of four replications with standard deviation. Means were compared by the least significant difference (LSD) intervals method at $p = 0.05$, and one-way ANOVA and Duncan multiple comparison tests were carried out using SPSS 12.0 software package.

Results

Effects of temperature: Fresh seeds did not germinate well under given conditions and all the germination percentages were very low (20%) (Fig.1). After four-month dry storage at 4ºC and 30ºC, respectively, germination was significantly affected by temperature; and 10/20ºC was found to be the optimum temperature for germination (Table 1), followed by 20ºC. The favorable range of constant temperatures appeared between 15ºC and 20ºC. With an increase or decrease of the temperature outside this range, the final germination decreased significantly. When the temperature was at or over 30ºC, the seeds hardly germinated. Compared with several constant temperatures, daily alternating temperatures of 4/10, 4/15, 4/20, 10/20 and 10/25ºC did not have a positive effect on final germination percentages. The alternating temperature of 10/20ºC best favored the germination of stored clasping pepperweed seeds among a few daily fluctuating temperatures.

Influence of light on germination: Apparently, light had a strong effect on the germination of clasping pepperweed seeds. The percent germination in red light was a little higher (93%) than that (91%) in white light. Only a small set of seeds (35%) germinated in dark (Fig.1). Thus, seeds showed significant differences in light responses ($F=100.6$, $p<0.05$).

Sensitivity to GA₃: Germination of dormancy-broken seeds was significantly promoted by GA$_3$ solution. For all the GA$_3$ treatments, the germination percentage (i.e. from 54% to 92%) was much higher than 43% of the control. However, with further increase of GA$_3$ concentration (from 0.5 to 1.0 m mol/L), the final germination percentage declined sharply to 62% (Fig. 2).
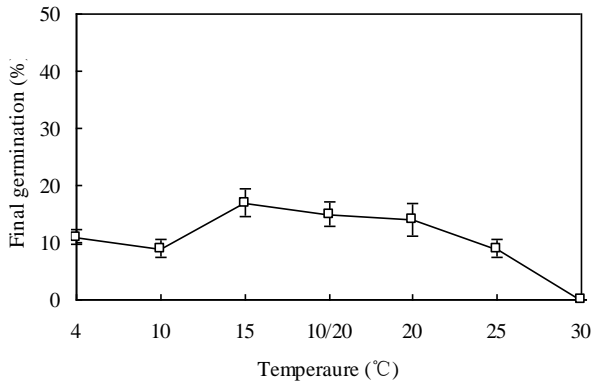
Fig. 1. Percent germination of fresh seeds of *Lepidium perfoliatum* under different constant temperatures. Error bars indicate ± 1 SD.
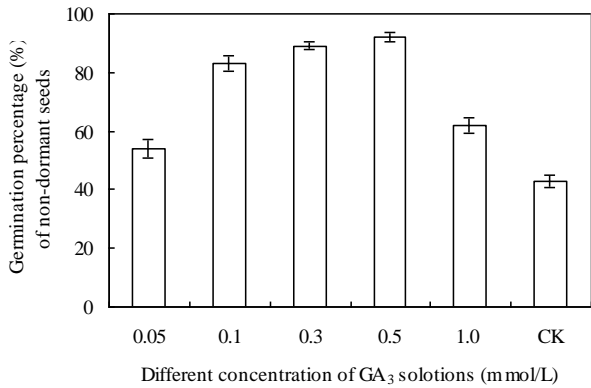

Fig. 2. Seed germination of *Lepidium perfoliatum* over a range of GA$_3$ concentration at 10/20°C in darkness of seeds stored for four months at 30°C and freshly-matured seeds, respectively. Error bars indicate ± 1 SD.
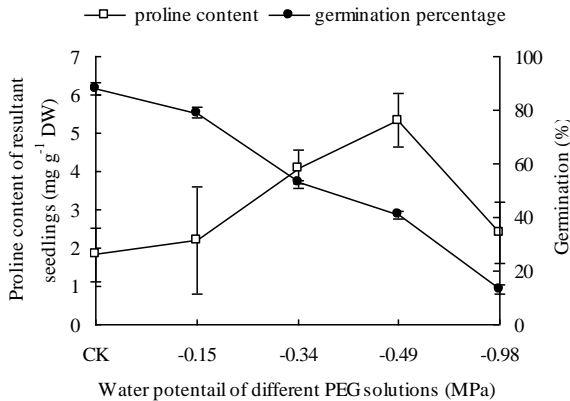

Fig. 3. The effects of different PEG solutions on the germination and proline content of seedlings of *Lepidium perfoliatum* at 20°C. Error bars indicate ± 1 SD.

Water potential: When water potentials were reduced, final percent germinations decreased dramatically. The final percent germinations declined from 88% to 13% along decreasing water potentials of CK (0), -0.15, -0.34, -0.49 and -0.98 MPa. The germination did not occur at all at -1.27 and -2.04 MPa (Fig. 3).

Proline content of resultant seedlings significantly increased from 1.8 to 5.3 mg g^{-1} dry weight (DW) under decreasing Ψs (i.e. 0 to -0.49 MPa), and then sharply fell to 2.4 mg g^{-1} DW at -0.98 MPa, slightly higher than that of CK (Fig. 3).

Discussion

Lepidium Perfoliatum which grows in the Junggar Basin, Xinjiang, northwest China, is an annual desert short-lived species with moderate xeromorphic characteristics in its vegetative organs (Zhuang & Tian, 1990). For this species, its reproductive age is younger, and its reproductive period is longer, which takes three fourths of life cycle (Wei *et al*., 2003a). *Lepidium perfoliatum* produces large numbers of small, light and mucilaginous seeds (data not shown). It is inferred that mucilaginous coat of clasping pepperweed seeds is ecologically significant, as has been found in other plants with mucilaginous seeds (Gutterman & Shem-Tov, 1997). Absorbing water rapidly, mucilaginous seed coat has key ecological influence to assure the germinating for its adhesion to the soil crust for some desert plants (Gutterman & Shem-Tov, 1997; Huang and Gutterman, 2000).

Non-dormancy for some short-lived species would be a mechanism to ensure regeneration (Wang *et al*., 1998; Liu *et al*., 2003; Weshce *et al*., 2006), but dormancy is also a common adaptive characteristic for many temperate plants (Baskin & Baskin, 1988; Gutterman, 1993). A dormancy pattern in *Arabidopsis thaliana* has been described by Baskin and Baskin (1983), reporting that at the time of seed dispersal, which occurs around May in Kentucky, USA, *Arabidopsis thaliana* seeds were dormant. Also, *Rhexia mariana* var. interior (Baskin *et al*., 1999) and *Papaver aculeatum* (Karlsson & Milberg, 2007) show a dormancy cycle in different environmental conditions. Wesche *et al*., (2006) also reported that fresh seeds of seven short-lived species and two herbaceous perennials among 26 species tested had low germination below 30%, while seed viability was equally high, suggesting that these species produce dormant seeds in dry Central Asian steppes.

In the present study, freshly harvested mature seeds of *L. perfoliatum* were characterized by low germination percentages under the given conditions (Table 1; Fig. 2). Thus, we concluded that freshly-matured seeds of *L. perfoliatum* were dormant. Interestingly, after four months storage either at 30°C or 4°C, even at room temperature, the final percent germination was enhanced markedly (Fig. 1; Table 1). Although our methods did not allow differentiation between conditional dormancy and non-dormancy, but requirements of after-ripening was apparently expected for this short-lived species (Fig. 1; Table 1). Moreover, during dry storage, the rate of undergoing after-ripening at 30°C seemed faster than that at 4°C. In comparison, we thought that some seeds stored at 4°C for four months were still in the state of dormancy. Thus, we inferred that fresh seeds of *L. perfoliatum* were non-deep physiologically dormant and the rate of dry after-ripening depends on storage temperature. In contrast, In *Eremurus inderiensis* seeds, the freezing treatment (-18°C) was better than cold storage (0~4°C) for after-ripening. Apparently, temperature requirements for after-ripening or breaking dormancy are associated with species.

Temperature not only regulates the dormancy-breaking processes, but also influences subsequent germination (Baskin & Baskin, 1988; Brändel & Schütz, 2005). Indeed, suitable temperature plays a central role in seed germination. Tobe *et al*., (2001) reported five shrubs needed at least 10ºC for germination. Seeds of *Eremurus inderiensis*, a typically desert short-lived plant, germinated best at 10ºC in light (Wu *et al*., 2005). In *Artemisia ordosica* (Huang & Gutterman, 2000) and *Artemisia sphaerocephala* (Huang & Gutterman, 1999), optimum germination occurred at temperatures of 15-25ºC. *Hedysarum fruticosum* seeds can germinate to high percent germination (i.e. from 87.2% to 99.2%) when alternating temperature increased from 5/15 (night: day) to 10/20ºC (Zheng *et a*l., 2005). In addition, Weshce *et al*. (2006) found several plant species also showed low germination preferences (4ºC darkness / 8ºC light). In the current study, for undergone after-ripening seeds stored at 30ºC, it was found that under alternating temperature regimes, final percent germination increased from 37% to 93% when temperature increased from 4/10ºC (night/day) to 10:20ºC, then decreased with increasing temperature. Besides, the germination pattern under constant temperature conditions was similar to that under alternating temperature and there were significant differences in final percent germination and germination rate between alternating and constant temperature (Table 1). Considering temperature in the habitat, discrepancy in day-and-night temperature is much, favoring the germination of *L. perfoliatum* seeds. Seed dispersal of clasping pepperweed starts from late-May to early June when daily temperature is above 25ºC, even over 30ºC. After shedding, seeds have to experience natural hot treatment. After the special hot treatment, although most of seeds are released from dormancy, temperature and water availability (discussed subsequently) become limiting factors for germination. From late September on, daily low temperature (15ºC, even 0ºC) is not helpful to seed germination of clasping pepperweed (Table 1). In fact, we observed clasping pepperweed seedlings only in next late March and early April in the field. This germination pattern is an extremely important adaptive mechanism for this ephemeral species studied. Interestingly, contrary to these species above, *Atriplex aucheri* produces black seeds which were nondormant and germinated very well at the low temperature of 5~8ºC (Wei *et al*., 2003b). The phenomenon suggests that temperature preference for germination also depends on the genotype of species as well as environmental conditions.

Light is also one of the most important environmental regulatory signals that regulate seed dormancy and germination (Pons, 2000). In the absence of light, hydrated Grand Rapids lettuce seeds maintained in darkness at 25ºC fail to germinate (Bewley & Black, 1972). In *Eremurus inderiensis*, the germination is better in light than in dark at optical temperature of 10ºC (Wu *et al*., 2005). A seasonal dormancy pattern in *Arabidopsis thaliana* is regulated by changes in seed sensitivity to light (Derkx & Karssen 2004). Also, Rojas-Aréchiga *et al*., (1997) reported that seeds of *Echinocactus platyacanthus* and *Ferocactus flavovirens* showed significant differences in response to different light treatments. Flores *et al*., (2006) reported that almost 28 species (Cactaceae) require light for seed germination. Of course, light can also inhibit seed germination (Koller 1956; Thanos *et al*., 1994) and induced dormancy (Malik & Vanden, 1987). In the present study, the spring ephemeral plant of *L. perfoliatum* germinated to low value (35%) in darkness after breaking-dormancy. The phenomenon suggested that once clasping pepperweed seeds locating underground deeper or under snow cannot germinate well for the absence of light. The light-requiring characteristic suggests that even though dormancy-broken seeds can form a soil seed bank, to an extent, associated with low temperature inhibition in winter, to favor lasting its populations in adverse environments.

As is reported by some authors, during the process of breaking dormancy, endogenous phytohormone level, such as GA, ABA and kinetin, is related to dormant state (Malik and Vaden, 1987; Toyomasu et al., 1993; Finch-Savage, 2006). Of course, the addition of GAs overcomes dormancy or promotes germination for many species. For example, GA_3 promoted the germination of the wild type of *Aradidopsis thaliana* seeds (Li *et al*., 2000) and Eriobotrya japonica seeds (El-Dengawy, 2005). In *Galium spurium*, light-induced dormancy was overcome by the application of nitrates and GA_3, respectively, and by the combination of kinetin and GA_3 (Malik and Vanden, 1987). In the present study, GA_3 promoted the germination of non-dormant clasping pepperweed seeds whose dormancy was broken during dry storage at 30ºC (Fig. 3) although GA_3 solutions had not effective influence on the germination of the control. Thus, sensitivity to GA_3 seemed concerned with the dormant state of clasping pepperweed seeds and the application of GA_3 can enhance the germination of *L. perfoliatum* seeds after after-ripening in practice.

In deserts, drought is a common stress factor, and plants must be adapted to this stress to survive. Species with seeds that can germinate at low water potentials have the advantage of becoming established in some areas where species with drought-sensitive seeds can not do so. Consequently, selection favors environmental cueing mechanisms that ensure the germination and future success. Drought escape, avoidance and tolerance are the three main types of plant behavior developed in order to cope with water stress in natural conditions (Jones *et al*., 1981). In desert environments, where rainfall is rare and uncertain, glycophytes and halophytes respond to osmotic stress in a similar way during the germination stage, i.e., the germination process is delayed under such conditions (Khan & Ungar, 1997) and the seedling growth are significantly inhibited by scarce rainfall water in the harsh sand-dune condition (Villagra & Cavagnaro, 2006). The present study showed that both germination percentage and rate (data not shown) decreased gradually with decreasing water potential (Fig. 3), suggesting that the water potential is a limiting factor for the germination of clasping pepperweed seeds, even if temperature is favorable. Water content of top sandy soil (0~5 cm) is about 5.7% of in the habitat during autumn (Tang, 2008). Moreover, the wind is so strong that top sandy soil becomes dry quickly by strong evaporation. Therefore water stress becomes a lethal factor for the germination of clasping pepperweed seeds. Similarly, most studies report optimum germination occurs at osmotic potentials between 0 and -1.0 MPa (Wesche *et al*., 2006). Although these data are from different plant species, general patterns are quite clear, namely, these ephemeral plant species display similar drought-adaptive strategies when subjected to drought stress.

When plants are subjected to water deficit during plant establishment, levels of some metabolic products in plants, such as sugar, lycine and amino acid, change significantly. Thus, metabolites, as the terminal products of cellular metabolism, are valuable indicators of how a biological system responds to environmental changes (Fiehn, 2002). Among these, free proline acts as a protection against dry-induced damage to cell membranes, which is a common mechanism used by all desiccation-tolerant organisms, that is to say, Proline potentially plays roles in gene expression and antioxidative defense systems (Hare *et al*., 1999; Hong *et al*., 2000) and in osmoregulation under water deficit (Liu *et al*., 2007). In our study, with water potential decreased, changes of proline content in resultant seedlings was the reverse of the germination percentages of clasping pepperweed seeds (Fig. 3). For clasping pepperweed, elevated levels of proline content might assist in osmotic adjustment during water stress. Interestingly, with further

decreasing the water potential to -0.98 MPa, the proline content of clasping pepperweed seedlings abruptly declined, which could be for disruption of protein synthesis by serious osmotic stress, suggesting that clasping pepperweed seedlings were being subjected to a lethal injury. This result further indicated that the germination of clasping pepperweed seeds cannot occur under injury-induced water potential condition and sowing non-dormant seeds should be in spring.

Acknowledgements

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