

Effects of chemical treatments and environmental factors on seed dormancy and germination of shepherd's purse (*Capsella bursa-pastoris* (L.) Medic.)

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ABSTRACT

Shepherd's purse (*Capsella bursa-pastoris*) is a problematic weed in citrus orchards and crop fields in northern Iran. In a series of laboratory and greenhouse experiments, we evaluated the effects that treatment with gibberellic acid (GA₃) and potassium nitrate (KNO₃), as well as environmental factors, including temperature, the duration of pre-chilling (wet and dry), drought stress, salt stress, pH, and sowing depth, have on seed dormancy breaking and germination in *C. bursa-pastoris*. Treatment with GA₃ strongly stimulated germination of *C. bursa-pastoris* in conditions of light/dark and continuous darkness. The germination rate was highest (40.08%) for seeds treated with 400 ppm of GA₃ in the light/dark condition. Treatment with KNO₃ did not significantly influence seed germination. Longer wet pre-chilling promoted germination and was more successful in seed dormancy breaking than was dry pre-chilling. Seed germination occurred at 10-30°C and within a range of pH of 3-11. Drought and salt stress both strongly inhibited germination. Seedling emergence decreased in proportion to sowing depth. The rates of *C. bursa-pastoris* germination and seedling emergence were highest for seeds on the soil surface.

Key words: Drought and salt stress, gibberellic acid, light, temperature, sowing depth

Introduction

Shepherd's purse (*Capsella bursa-pastoris* (L.) Medic.), a member of the family Brassicaceae, is an annual winter weed that occurs in Asia, Africa, Australia, Europe, North America, and South America (Holm *et al.* 1979). In Iran, *C. bursa-pastoris* is often found in citrus orchards, as well as fields of wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.). It is a prolific seed producer, a single *C. bursa-pastoris* plant being able to produce several thousand seeds (Karimi 2001).

Seed germination is strongly affected by seed dormancy. Seed dormancy is a complex mechanism that has evolved to ensure future seed germination and plant establishment. The dormancy of seeds is controlled by environmental factors that ensure synchronization with the optimal growing season (Baskin & Baskin 1989). Breaking dormancy or inducing germination is influenced by several factors,

including alternating temperatures, light, and nitrate (Bouwmeester & Karssen 1992; 1993). In addition, growth regulators such as gibberellic acid (GA₃) can induce seed germination without after-ripening in plants (Bewley & Black 1994).

Various environmental factors, such as light, temperature, soil moisture (Chauhan & Johnson 2010), and pH, interact to influence seed germination (Koger *et al.* 2004). Temperature and light play critical roles in regulating seed germination (Chauhan *et al.* 2006a). A non-optimal temperature could result in the inhibition of germination and ultimately in the induction of dormancy (Toorop *et al.* 2011). Light can induce dormancy in positively photoblastic seeds when those seeds are sown in the soil (Toorop *et al.* 2008). Sowing depth also influences germination and seedling establishment (Koger *et al.* 2004).

The principal *Capsella bursa-pastoris* strategy for survival and dispersal in agroecosystems is prolific seed

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production. Therefore, an understanding of the dormancy breaking methods and germination requirements may help us in predicting emergence and its potential behavior in field crops, which could be critical in devising effective control methods. The objectives of this study of *Capsella bursa-pastoris* were to determine the effects of gibberellic acid (GA_3) and potassium nitrate (KNO_3) on seed dormancy breaking; to investigate the effects that environmental factors, including temperature, light, pre-chilling duration, salt stress, drought stress, and pH, have on seed germination; and to assess the effect of sowing depth on seedling emergence.

Material and methods

Seed collection

Capsella bursa-pastoris seeds were collected from naturally ripened pods of intact plants growing in a wheat field at Qarakheyl Crop Research Station (36°27'N; 52°46'E), within the province of Mazandaran, Iran, in the summer of 2010. Seeds were removed from pods and stored in paper bags at room temperature ($20\pm 5^\circ C$) until use. The weight of 1000 *C. bursa-pastoris* seeds was 0.08 g. Before the experiments, seed viability was tested by immersion of the seeds in 1% tetrazolium chloride solution (Peters 2000). The tetrazolium chloride test showed that approximately 98% of the seeds were viable.

General germination

Fifty seeds were placed in a 9-cm plastic petri dish containing one filter paper disk moistened with 5 ml of distilled water or test solution including GA_3 , KNO_3 , sodium chloride (NaCl), polyethylene glycol, and pH buffer solutions. Dishes were sealed with a plastic film and placed in a germinator (X630; Fater Electronic, Tehran, Iran) set at 25/15°C (day/night). Light was provided by fluorescent lamps to produce a light intensity of $300 \mu mol m^{-2} s^{-1}$. The photoperiod was set at 12 h (day/night) for all germination tests except continuous darkness. For the germination test in continuous darkness, the petri dishes were wrapped in two layers of aluminum foil and seed germination was quantified only after 4 weeks. Germinated seeds were counted at 4 weeks after the start of tests. Seeds were classified as germinated when the cotyledons and radicle emerged from the seed coat.

Seed dormancy breaking

Effects of light and GA_3

Germination of *Capsella bursa-pastoris* seeds in response to 0, 25, 50, 100, 200, 400, 600, and 800 ppm of GA_3 was evaluated in the 12/12-h light/dark and continuous darkness conditions.

Effect of KNO_3

The effectiveness of KNO_3 in breaking the dormancy of *Capsella bursa-pastoris* seeds was evaluated at concentrations of 0, 0.01, 0.5, 2, 5, and 10 mmol of KNO_3 in the 12/12-h light/dark condition.

Effect of the duration of pre-chilling (wet and dry)

The effect of pre-chilling duration on the promotion of seed germination of *Capsella bursa-pastoris* was studied by placing seeds in wet or dry conditions for 15, 30, and 45 days at $5\pm 1^\circ C$. For wet pre-chilling treatments, seeds were wrapped in moistened towels. After pre-chilling, seeds were set to germinate immediately.

Environmental factors

Effects of constant temperature and light

The effect of five constant temperatures (10, 15, 20, 25, and $30^\circ C$) was evaluated in the 12/12-h light/dark and continuous darkness conditions to determine the response of seed germination to variations in temperature and light.

Effect of drought stress

To evaluate the effect of drought stress, aqueous solutions with osmotic potentials of 0, -0.1, -0.25, -0.5, -1 and -1.5 MPa were prepared by dissolving 0, 99.4, 157.1, 222.2, 314.2, and 384.8 g, respectively, of polyethylene glycol 8000 (Merck, Darmstadt, Germany) in 1 L of distilled water (Michel 1983).

Effect of salt stress

The effect of salt stress on *Capsella bursa-pastoris* seed germination was tested by treating seeds in solutions of 0, 10, 20, 40, 80, and 160 mM sodium chloride (Merck).

Effect of pH

The effect of pH on seed germination was determined with the use of buffer solutions of pH 3, 5, 7, 9, and 11, prepared according to Chachalis & Reddy (2000).

Effect of seed sowing depth

Fifty *Capsella bursa-pastoris* seeds were planted in soil in 15-cm-diameter plastic pots at depths of 0, 0.5, 1, 2, and 4 cm. Control pots were assumed to have no background seed bank of *C. bursa-pastoris* in the soil. No *C. bursa-pastoris* seedlings emerged in the control pots by 45 days after sowing, suggesting there was no background *C. bursa-pastoris* seed bank in their soil.

In this experiment, we used a silty soil containing 29.4% clay, 51.4% silt, and 19.2% sand, and 0.41% organic carbon,

with a pH of 7.8. Pots were irrigated as needed to maintain soil moisture at field capacity. The greenhouse temperature was set at 25/15°C (day/night) with a natural photoperiod. Seedling emergence was defined as the appearance of the two cotyledons, and emerged seedlings were counted at 45 days after planting.

Statistical analyses

Seed germination and seed planting depth experiments were conducted in a complete randomized manner with three replicates and were performed in duplicate. We performed ANOVA of the arcsine transformed data obtained as percent germination. Means were separated by Fisher's least significant difference test or standard error bars at the 0.01 probability level.

For the salt stress experiment, we used regression analysis to determine the response of the data. To that end, we employed a functional three-parameter logistic model (Kleemann *et al.* 2007), plotted to the seed germination rates (%) at different osmotic potentials:

$$G(\%) = G_{max} / [1 + (x/x_{50})^{c_{rate}}] \quad [1]$$

where G indicates the total seed germination (%) at osmotic potential x , G_{max} is the maximum seed germination (%), x_{50} is the osmotic potential for 50% inhibition of the maximum seed germination, and G_{rate} is the slope of the curve.

Results and discussion

Seed dormancy breaking treatments

GA₃

Treatment with GA₃ had a significant influence on *Capsella bursa-pastoris* seed germination (Tab. 1), strongly stimulating germination in the 12/12-h light/dark and continuous darkness conditions. The proportion of seeds germinated was highest (40.08%) among those treated with 400 ppm of GA₃ in the 12/12-h light/dark condition. That proportion was markedly lower at 600 ppm and 800 ppm than at 400 ppm (Fig. 1). In the continuous darkness condition, the germination rate was highest at concentrations of 100-600 ppm and was significantly lower at 800 ppm (Fig. 1). Dormancy is a potential problem for most species of the Brassicaceae family. Considerable research on gibberellins as seed germination promoters has shown that application of GA₃ to dormant seeds can eliminate their natural chilling requirement (Gashi *et al.* 2012). The physiological role of GA₃ as a promoter of dormant seed germination, through the induction of hydrolytic enzymes, has been documented in a wide range of plant species (Rogis *et al.* 2004; Zhang *et al.* 2006; Baskin & Baskin 2004).

KNO₃

Application of KNO₃ did not have a significant effect on seed germination (Tab. 1). Among the seeds treated with KNO₃, proportional germination was highest (17.57%, significantly higher than that obtained for the control) for those treated at a concentration of 2 mmol (Fig. 2). Proportional germination did not differ significantly among the various concentrations of KNO₃ (Fig. 2). Chauhan *et al.* (2006b) found that the germination rate of Oriental mustard (*Sisymbrium orientale*) seeds increased in direct proportion to increasing concentrations of KNO₃, although only up to 0.02 M, after which it decreased. Promotion and inhibition of germination at low and high concentrations of KNO₃ have previously been reported by Wei *et al.* (2010) and Foley & Chao (2008), in different plants.

Pre-chilling duration

The effect of wet pre-chilling on the germination of *Capsella bursa-pastoris* seeds was significant (Tab. 1). The germination rate increased markedly as the duration of wet pre-chilling increased. After 45 days of wet pre-chilling, 48.51% of the seeds germinated (Fig. 3). Dry pre-chilling had no significant effect on seed germination (Tab. 1) and did not promote the germination of dormant seeds (Fig. 3). Pre-chilling of many annual plant seeds could be effective in breaking dormancy and stimulating germination (Huang *et al.* 2004). However, in many plants, wet pre-chilling has been shown to be more successful than is dry pre-chilling in promoting dormant seed germination and is considered the standard method of inducing germination (Ren & Guan 2008). Feghahati & Reese (1994) demonstrated that dry pre-chilling of *Echinacea angustifolia* was not effective in dormancy breaking. Wet pre-chilling can accelerate seed germination by providing enough moisture to activate the hydraulic enzymes (Nkomo & Kambizi 2009). Our results show that wet pre-chilling, especially that of longer duration, was considerably more successful than was dry pre-chilling in breaking the dormancy of *C. bursa-pastoris* seeds.

Table 1. ANOVA of the effects of treatments on *Capsella bursa-pastoris* seed germination.

Source of variation	df	MS
Gibberlic acid	5	**
Potassium nitrate	5	ns
Wet pre-chilling duration	3	**
Dry pre-chilling duration	3	ns
Temperature	4	**
pH	4	ns
Sowing depth	4	**

**Significant at $P \leq 0.01$; ns: Not significant.

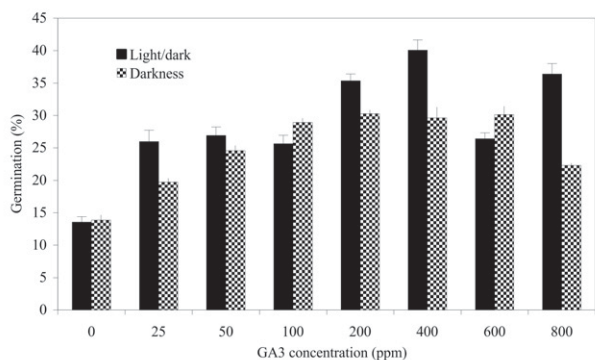


Figure 1. Effects of treatment with gibberellic acid (GA_3) and light conditions (12/12-h light/dark cycle and continuous darkness) on the germination of dormant *Capsella bursa-pastoris* seeds incubated for 4 weeks. Vertical bars represent standard errors of the mean.

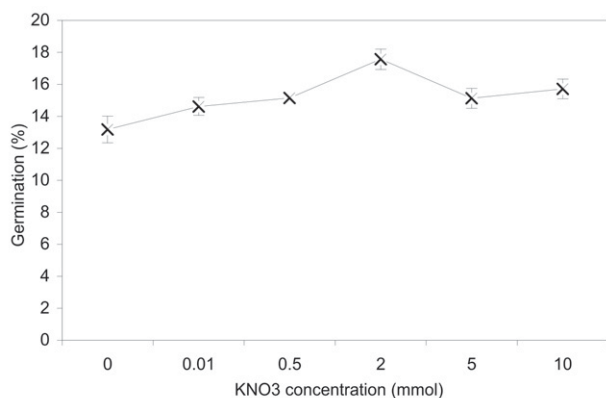


Figure 2. Effect of potassium nitrate (KNO_3) on the germination of dormant *Capsella bursa-pastoris* seeds incubated at 25/15°C (day/night), on a 12/12-h light/dark cycle, for 4 weeks. Vertical bars represent standard errors of the mean.

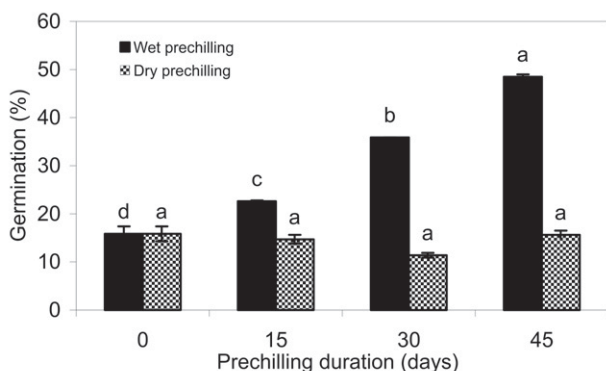


Figure 3. Effect of pre-chilling, by duration, on the germination of dormant *Capsella bursa-pastoris* seeds incubated at 25/15°C (day/night), on a 12/12-h light/dark cycle, for 4 weeks. Vertical bars represent standard errors of the mean.

Seed germination treatments

Temperature and light

The impact of temperature on seed germination was significant, in 12/12-h light/dark and continuous darkness conditions alike (Tab. 1). With increasing temperature, the germination of *Capsella bursa-pastoris* seed increased markedly in both conditions (Fig. 4). In the 12/12-h light/dark condition, the germination rate was highest at 30°C, compared with a range of 20-30°C for the continuous darkness condition (Fig. 4). In general, *C. bursa-pastoris* seeds germinated over a range of 10-30°C. Within the 10-25°C range, seed germination did not differ significantly between the 12/12-h light/dark and continuous darkness conditions. However, at in temperature range from 10°C until 25°C, but in temperature of 30°C the germination rate was significantly higher in the 12/12-h light/dark condition (Fig. 4). Temperature and light conditions are important factors in seed germination. The optimum conditions of temperature and light required for the germination of seeds is strongly dependent on the plant species (Egley & Duke 1985). In the literature we reviewed (e.g., Mulligan & Bailey 1975; Cousens *et al.* 1994; Chauhan *et al.* 2006b), there is evidence that the Brassicaceae seed germination response to temperature and light could be variable. Chauhan *et al.* (2006c) showed that seed germination of turnipweed (*Rapistrum rugosum*) was unaffected by variations in temperature but was influenced by the light conditions. However, Cousens *et al.* (1994) concluded that light conditions have only a slight influence on the germination of *R. rugosum* seeds.

Drought stress

A nonlinear regression model $\{G(\%) = 35.01/[1 + (x/0.24)^{1.97}]\}$, $R^2 = 0.971$ was fitted to seed germination (%) values obtained at different osmotic potentials (Fig. 5). Drought stress greatly reduced seed germination of *Capsella bursa-pastoris*. As can be seen in Fig. 5, the osmotic potential required for 50% inhibition of maximum germination was calculated by the model at -0.24 MPa. Germination at -1 MPa reached zero, indicating that seeds of *C. bursa-pastoris* are intolerant to high drought stress conditions (Fig. 5). According to our review of the literature, germination tolerance to drought stress varies among Brassicaceae species. Ray *et al.* (2005) reported that seeds of London rocket (*Sisymbrium irio* L.) germinated up to an osmotic potential of -1.2 MPa.

Salt stress

Germination of *Capsella bursa-pastoris* seeds was decreased by increasing NaCl concentrations ($y=34.462e^{-0.0146x}$, $R^2=0.90$). There was no significant change in seed germination at NaCl concentrations from 10 mM to 80 mM. At high

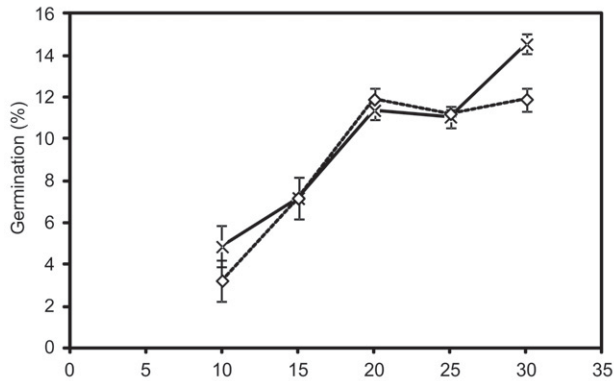


Figure 4. Effects of constant temperature and light conditions (12/12-h light/dark cycle and continuous darkness) on the germination of dormant *Capsella bursa-pastoris* seeds incubated for 4 weeks. Vertical bars represent standard errors of the mean.

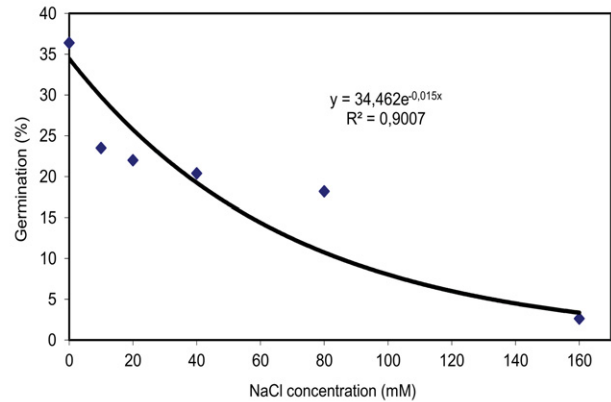


Figure 6. Effect of salt stress on the germination of dormant *Capsella bursa-pastoris* seeds incubated at 25/15°C (day/night), on a 12/12-h light/dark cycle, for 4 weeks.

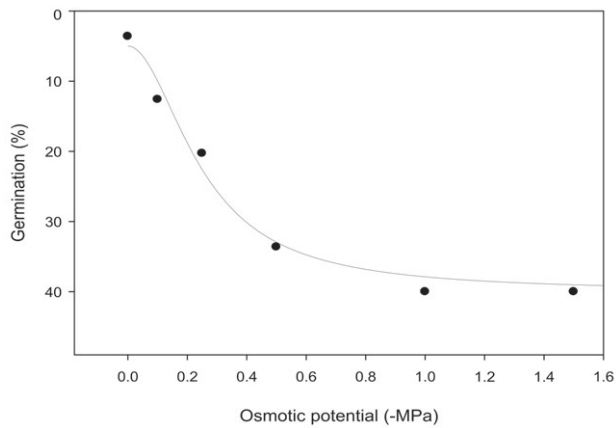


Figure 5. Effect of drought stress on the germination of dormant *Capsella bursa-pastoris* seeds. The line represents the functional three-parameter logistic model— $G(\%) = G_{max}/[1 + (x/x_{50})^{c_{rate}}]$ —fitted to the data.

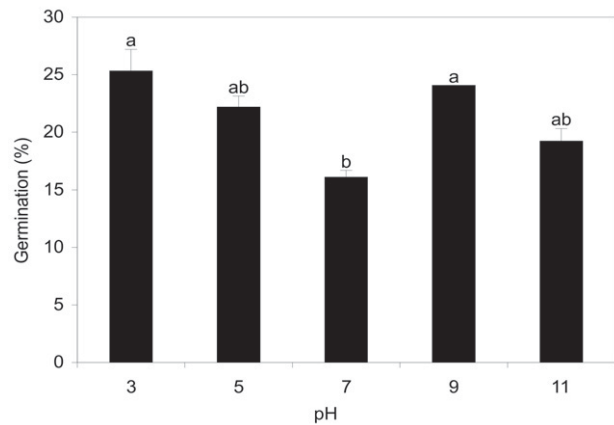


Figure 7. Effect of pH on the germination of dormant *Capsella bursa-pastoris* seeds incubated at 25/15°C (day/night), on a 12/12-h light/dark cycle, for 4 weeks. Vertical bars represent standard errors of the mean.

salinity (160 mM), 2.63% of seeds germinated that showing the potential of *C. bursa-pastoris* seeds to germinate in conditions of high salinity (Fig. 6). Other authors have also reported a salt stress-related reduction in the germination of seeds of different plant species (Ray *et al.* 2005; Mulligan & Bailey 1975; Chauhan *et al.* 2006b; Gordin *et al.* 2012). In northern Iran, which is a main habitat of *C. bursa-pastoris*, conditions of high salinity can occur in fields and orchards near the seashore because of high depletion of ground water.

pH

The effect of pH on the germination of *Capsella bursa-pastoris* seed was not significant (Tab. 1). Figure 7 shows that germination of *C. bursa-pastoris* seeds occurred in a wide range of pH values (from 3 to 11). The germination rate was significantly higher at pH 3 and pH 9 than at pH 7. According to our results, pH is not a critical factor ger-

mination of *C. bursa-pastoris*. Similarly, Ray *et al.* (2005), Chauhan *et al.* (2006b) and Chauhan *et al.* (2006c) have been reported Brassicaceae seeds germinated in a broad range of pH. These data suggest that pH is not a limiting factor for Brassicaceae family seed germination. In addition, other authors (e.g., Zhou *et al.* 2005; Fani Yazdi *et al.* 2013; Rezvani & Fani Yazdi 2013) have suggested that pH is not a limitation for germination of seed in different plant families.

Sowing depth

The effect of sowing depth on *Capsella bursa-pastoris* seedling emergence was significant (Tab. 1). Seedling emergence decreased markedly in parallel with increasing seed sowing depth (Fig. 8). The highest rates of seed germination and seedling emergence were observed for seed scattered on the soil surface. When the sowing depth was 4 cm, no seedlings emerged (Fig. 8). Our results suggest that light

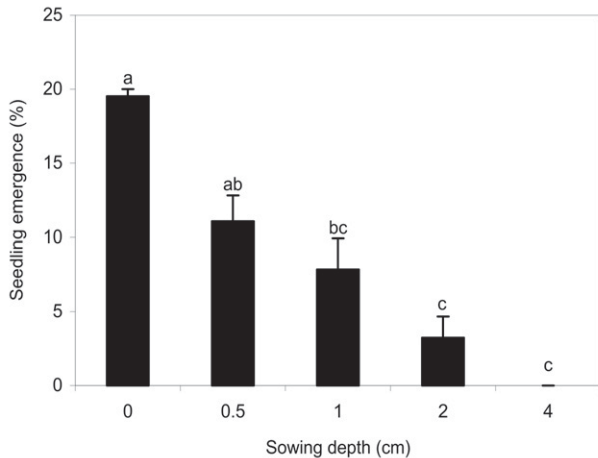


Figure 8. Effect of sowing depth on seedling emergence in *Capsella bursa-pastoris* seeds incubated at 25/15°C (day/night), on a 12/12-h light/dark cycle, for 4 weeks. Vertical bars represent standard errors of the mean.

is a critical factor for the germination of *C. bursa-pastoris* seed. Similarly, Chauhan *et al.* (2006a; 2006b) showed that, in small-seeded Brassicaceae, the rate of seedling emergence decreases in proportion to sowing depth. Our results indicate that sowing *C. bursa-pastoris* seeds to a depth ≥ 4 cm can result in a significant reduction in the density of this species in next crop.

Conclusions

We found that treatment with GA₃ at a concentration of 400 ppm strongly promoted germination of *Capsella bursa-pastoris* seeds in the 12/12-h light/dark condition. Among the seeds treated with KNO₃, proportional germination was highest (and significantly higher than that observed for the control) for those treated at a concentration of 2 mmol. In addition, wet pre-chilling for 45 days was more successful in breaking the dormancy of *C. bursa-pastoris* seeds than was dry pre-chilling, treatment with GA₃, and treatment with KNO₃ (17.57%). We found that *C. bursa-pastoris* seeds germinated at a range of 10–30°C and at pH values between 3 and 11, indicating that the latter is not a critical factor. We also found that *C. bursa-pastoris* seed germination rates decreased in parallel with increases in the levels of salt and drought stress. However, our data suggest that *C. bursa-pastoris* seeds can germinate at high salinity. Moreover, we found that sowing depth (i.e., light) is a critical factor for the germination of *C. bursa-pastoris* seeds.

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