

# Environmental factors on seed germination and seedling emergence of *Phleum paniculatum* Huds.

Han Wu<sup>1</sup>, Pei Zhang<sup>1</sup>, Guoqi Chen<sup>1</sup>, Lang Pan<sup>1</sup>, Jun Li<sup>1</sup>, and Liyao Dong<sup>1\*</sup>

<sup>1</sup>Nanjing Agricultural University, College of Plant Protection, Nanjing 210095, China. \*Corresponding author (dly@njau.edu.cn).

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## ABSTRACT

*Phleum paniculatum* Huds., commonly known as British timothy, is an increasingly serious weed in wheat field of China. However, the biology of its seed germination and seedling emergence remain unclear. In the present study, the effects of environmental factors on seed germination and seedling emergence of *P. paniculatum* were explored. *Phleum paniculatum* seeds had a shallow dormancy (20-30 d) when stored at room temperature ( $25 \pm 5$  °C). Seeds could germinate at constant temperatures between 10 and 25 °C, except for 5 or 30 °C. Light was not essential for seed germination, and pH values from 4 to 10 did not inhibit germination. Seeds were moderately adaptable to water potential and NaCl concentration, and germination rates would be decreased 50% when water potential was -0.4 MPa or NaCl concentration was 130 mM. Increased soil burial depth decreased the seedling emergence, and no seeds emerged when the burial depth was more than 4 cm. Taken together, our results provide useful information of the germination and emergence of *P. paniculatum* seed, and strategies such as proper drainage systems managed and deep plowing are recommended to limit its detrimental effects on agricultural production.

**Key words:** Burial depth, dormancy, light, osmotic potential, salt, temperature.

## INTRODUCTION

*Phleum paniculatum* Huds., known as British timothy, widely distributed along the Yangtze River, and throughout several provinces including Shanxi, Shaanxi, and Gansu in China as well as in other regions of temperate Eurasia ([www.efloras.org](http://www.efloras.org)). As a short winter annual grass, the life cycle of *P. paniculatum* coincides with that of winter wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) in China (Flora of China; FOC, 2006). *Phleum paniculatum* frequently thrive on riversides, field margins, and roadsides. Nevertheless, in the recent years, some researchers reported that *P. paniculatum* occurred in wheat fields and even became one of the dominant malignant weeds in Shanxi Province (Gao et al., 2011). Jin et al. (2009) reported that in Zhouzhi county of China, difenzoquat and fenoxaprop could not effectively control *P. paniculatum*. According to our field observations, *P. paniculatum* developed into secondary malignant weeds in wheat fields at the Yangtze River downstream areas. *Phleum paniculatum* seeds are tawny, obovate, and about 1 mm long and 0.2 mm wide (Flora of China; FOC, 2006). *Phleum paniculatum* commonly produce 1500 to 2900 seeds per plant and seed is quite small, with a thousand-seed weight of only 0.17 to 0.23 g, which can be dispersed by water, wind or farm operation in the fields because of its light weight (Wu personal observation). Moreover, this grass weed could serve as a host for Northern cereal mosaic virus (Ruan et al., 1982) and cereal cyst nematode (*Heterodera avenae* Wollenweber) (Zhao et al., 2013). Therefore, as a new problematical weed and host of pests in wheat fields, a better understanding of *P. paniculatum* is essential for developing effective management strategies against it.

Seed germination and seedling emergence are the initial steps for weed population establishment, which are influenced by numerous environmental factors, such as temperature, water, light, and seed burial depth (Singh et al., 2012; Javaid and

Tanveer, 2014). To date, biology on seed germination and seedling emergence of *P. paniculatum* has not been reported. Here, we conducted a serial of experiments on the dormancy, germination and emergence of this weed species. The objective of this research was to increase our understanding of the effects of temperature, light, pH, salt stress, osmotic stress, and burial depth on seed germination and emergence of *P. paniculatum* and find recommend effective strategies for its control in winter wheat.

## MATERIALS AND METHODS

*Phleum paniculatum* seed of each individual sample were collected and combined from several fallow fields in Suining county (34°01'0.86" N, 117°53'13.95" E), China, in May 2017. The climate in Suining County is temperate monsoonal, with a mean annual temperature of 14 °C and annual precipitation of 991 mm (National Meteorological Information Center, <http://www.nmic.cn/en/>). All seeds were cleaned and stored in paper bags at room temperature (25 ± 5 °C) until used, except a portion of the seeds that were stored at 4 °C for a seed dormancy experiment.

Seed germination experiments were conducted at Nanjing Agricultural University (32°02'3" N, 118°50'6" E). In each germination trial described below, 25 seeds of *P. paniculatum* were placed in 9-cm glass petri dishes containing two pieces of filter paper moistened with 5 mL distilled water (pH 6.8) or with a treatment solution. Dishes were sealed with Parafilm and placed in an incubator with 12 h of white fluorescent light (140 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetic photon flux density) and a photoperiod of 12:12 h with 20 °C/10 °C light/darkness temperatures (unless stated otherwise). Seeds were considered to have germinated when the coleoptile was visible (about 2 mm in length). Percent germination was determined 15 d after sowing (Rao et al., 2008; Wu et al., 2016; Du et al., 2017). All experiments were conducted in a completely randomized design with four replicates. Each experiment was conducted twice.

To test for seed dormancy of *P. paniculatum*, germination experiments were conducted at 1, 10, 20, and 30 d after seed were collected (Wu et al., 2015). Portions of the seeds used for the dormancy trial were stored at both 4 °C and 25 ± 5 °C to evaluate the effect of low temperature on dormancy. All other environmental conditions were the same as described in the general germination protocol.

To evaluate the effect of temperature, dishes containing non-dormant *P. paniculatum* seeds were placed in incubators at constant temperatures ranging from 5 °C to 30 °C in increments of 5 °C or with alternating light/darkness temperatures of 30 °C/20 °C, 25 °C/15 °C, 25 °C/10 °C, 20 °C/15 °C, 20 °C/10 °C, 15 °C/5 °C, 10 °C/5 °C. Germination was determined daily beginning 15 d after seeds were placed in the incubators.

Seed exposed to different periods of light: no light (24/0 h dark/light), constant light (0/24 h dark/light), or alternating light and dark conditions (12/12 h dark/light), were evaluated to determine the effect of light on seed germination. To completely exclude light, petri dishes were wrapped with two layers of aluminum foil immediately after being placed in petri dishes.

Non-dormant seeds were incubated in buffered solutions at a pH of 4, 5, 6, 7, 8, 9, or 10 to determine if pH affects seed germination of *P. paniculatum* (Chachalis and Reddy, 2000; Wu et al., 2016). Seed incubated using distilled water (pH 6.8) was used as a control for this experiment.

To evaluate the effect of osmotic stress, seeds were incubated in aqueous solutions with osmotic potentials of 0, -0.1, -0.2, -0.3, -0.35, -0.4, -0.45 -0.5, -0.6, and -0.7 MPa, prepared by dissolving polyethylene glycol (PEG) 6000 (Solarbio, Beijing, China) in distilled water (Michel and Kaufmann, 1973; Javaid and Tanveer, 2014; Wu et al., 2016). Seeds were placed in incubators at a constant temperature of 15 °C and a 12:12 h light:dark cycle.

Effect of salt stress was evaluated in seeds exposed to seven levels of increasing salinity using 5 mL of NaCl solutions prepared at concentrations of 0, 10, 20, 40, 80, 120, 160, or 320 mM NaCl. The solutions were prepared by dissolving 0, 0.58, 1.17, 2.34, 4.68, 7.02, 9.35, or 18.70 g NaCl L<sup>-1</sup> distilled water (Wu et al., 2016).

Effect of burial depth was evaluated in 50 seeds sown at depths of 0 (soil surface), 0.5, 1, 2, 3, 4, 5, or 6 cm in plastic pots (15 cm in diameter and 13 cm in height) with drain holes at the bottom (Wu et al., 2016). The soil media consisted of a 1:1 mixture, by weight, of medium loam soil (pH 8.06, with 0.67% organic matter) and organic matter (pH 8.23, with 40.4% organic matter). Seedling emergence was recorded when a coleoptile emerged through the soil surface and was determined 21 d after sowing.

### Statistical analysis

Germination and emergence rates were reported as the percentage  $\pm$  95% confidence interval. A completely randomized design with four replicates was used for all experiments and each experiment was repeated twice. Differences among treatments were examined with ANOVA analysis. There was nonsignificant ( $p > 0.05$ ) trial-by-treatment interaction for each experiment; therefore, data were pooled for the final analysis. Germination and seedling emergence data were analyzed with SPSS software (version 22.0, SPSS Inc., Chicago, Illinois, USA) using Tukey's test ( $p \leq 0.05$ ).

The germination percentages of *P. paniculatum* at different salt concentrations, or at different osmotic potentials were fitted to a functional three-parameter logistic model using SigmaPlot (version 10.0, Systat Software Inc., San Jose, California, USA) with the following equation (Wu et al., 2015):

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

where  $G (\%)$  represents the total germination (%) for the NaCl concentration, or osmotic potential  $x$ ;  $G_{max}$  is the maximum germination (%);  $x_{50}$  is NaCl concentration or osmotic potential for 50% of the maximum germination; and  $g$  indicates the slope.

The seedling emergence (%) obtained at different burial depths was fitted to a sigmoidal decay curve (Eslami, 2011) using SigmaPlot with the following equation:

$$E (\%) = E_{max} \{1 + \exp[-E_{rate}(x - x_{50})]\} \quad [2]$$

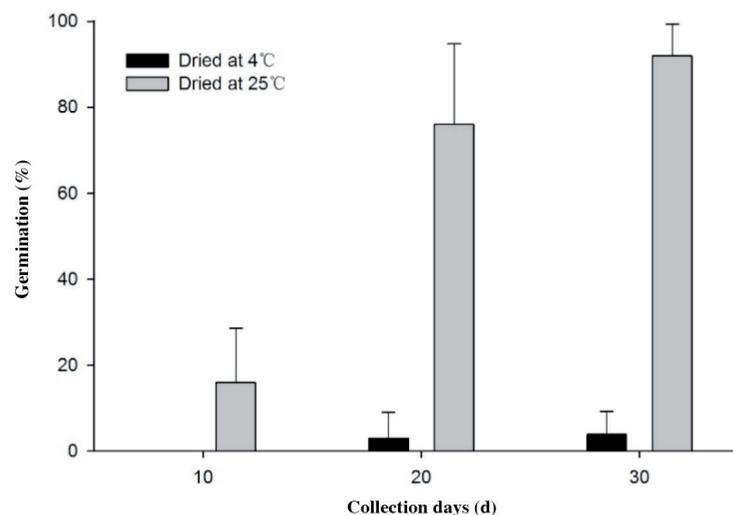
where  $E$  denotes the total seedling emergence (%) at burial depth  $x$ ,  $E_{max}$  is the maximum seedling emergence (%),  $x_{50}$  is the depth with 50% of maximum seedling emergence, and  $E_{rate}$  indicates the slope.

## RESULTS

### Seed dormancy

Whether stored at 4 °C nor at room temperature ( $25 \pm 5$  °C), did not germinate when tested immediately after being harvested (Figure 1). The percent germination for seed stored at room temperature increased rapidly when tested 10, 20, and 30 d after collection. A remarkable increase in germination was observed at 20 d after collection ( $76 \pm 18.7\%$ ), and germination rate exceeded 90% at 30 d after collection. In contrast, germination was almost completely inhibited when seed were stored at 4 °C, reaching only  $12.2 \pm 3\%$  at 30 d after collection. These results indicate that *P. paniculatum* seeds has a shallow dormancy (20-30 d) when stored at room temperature ( $25 \pm 5$  °C), while a low storage temperature, such as 4 °C, maintained seed dormancy.

**Figure 1. Germination of *Phleum paniculatum* sown at 1, 10, 20, 30 d after storage. Seeds were incubated at 20 °C/10 °C with a 12:12 h photoperiod for 30 d. Error bars represent 95% confidence interval of the means.**

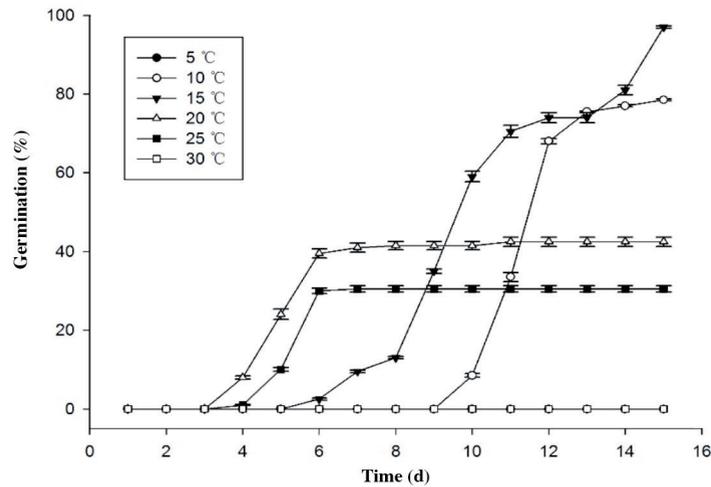


### Effect of temperature and light on germination

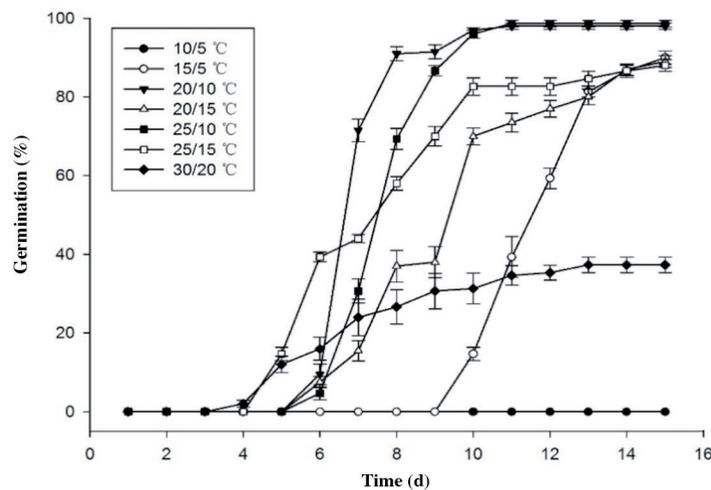
*Phleum paniculatum* seed germinated at constant temperatures between 10 and 25 °C, with 15 °C as the optimum temperature for germination. No seed germinated at 5 or 30 °C constant temperatures (Figure 2). Seed germination of *P. paniculatum* was 87% or more under all fluctuating temperature regimes except the 30/20 °C where 38% of seeds germinated and the 10/5 °C treatment that had 0% germination (Figure 3). Our results indicate that *P. paniculatum* germination is adapted to the temperatures of autumn and spring where temperature typically range for 10 to 25 °C. Hot temperature > 25 °C typically experience during the summer months and cold temperatures < 10 °C typically experience during the winter months could inhibit seed germination.

Seed germination rate of *P. paniculatum* was  $99 \pm 3.7\%$  without light, compared with  $99 \pm 3.2\%$  with constant light, and  $98 \pm 3.7\%$  with alternative light and dark at 15 d after sowing, respectively (Table 1). These results indicate that germination of *P. paniculatum* is not inhibited nor promoted by darkness or light.

**Figure 2. Effect of constant temperature on germination of *Phleum paniculatum* after incubation with a 12:12 h photoperiod for 15 d. Error bars represent 95% confidence interval of the means.**



**Figure 3. Effect of fluctuating temperature on germination of *Phleum paniculatum* after incubation with a 12:12 h photoperiod for 15 d. Error bars represent 95% confidence interval of the means.**



**Table 1. Effect of different photoperiods of light on germination of *Phleum paniculatum* incubated at 20 °C/10 °C for 15 d. Error bars represent 95% confidence interval of the means.**

Photoperiod (h)	Germination (%)
0	99 ± 3.7a
12	98 ± 3.7a
24	99 ± 3.2a

Same letter represents nonsignificant difference among all treatment according to Tukey's test ( $p \leq 0.05$ ).

### Effect of pH on germination

*Phleum paniculatum* seeds germination percentages were  $97.5 \pm 1.1\%$ ,  $97.0 \pm 1.0\%$ ,  $99.0 \pm 0.7\%$ ,  $97.5 \pm 1.1\%$ ,  $99.5 \pm 0.5\%$ ,  $98.0 \pm 1.1\%$ ,  $98.5 \pm 1.1\%$  treated with pH 4, 5, 6, 7, 8, 9, 10 respectively, have nonsignificant difference with distilled water ( $98.5 \pm 0.7\%$ ) at 15 d after sowing (Figure 4). Hence, pH (4 to 10) was not a limiting factor for *P. paniculatum* germination. Furthermore, with this ability, *Phleum paniculatum* can survive with the change of pH caused by long-term use of chemical fertilizers, acid rain and pollution of industrial waste (Zeng et al., 2017).

### Effect of osmotic stress and salinity on germination

A logistic model used to describe *P. paniculatum* germination rates under different levels of water stress (Figure 5). Seed germination of *P. paniculatum* was not reduced with osmotic potentials ranging from 0 to -0.3 MPa. Germination decreased at a water potential of -0.35 MPa ( $73 \pm 8\%$ ), and germination was almost completely inhibited ( $3 \pm 6.1\%$ ) at a water potential of -0.5 MPa. Our results showed that germination of *P. paniculatum* declined as osmotic stress increased.

A logistic model was used to describe the germination percentages obtained at different salinity levels (Figure 6). *Phleum paniculatum* germinated under a relatively wide range of salt concentrations (0 to 130 mM), showing a moderate adaptation to saline areas. Germination rate was greater than 62% with NaCl concentration less than 120 mM. With increased NaCl concentrations, germination dropped to  $20 \pm 7.3\%$  or 0% at 160 or 320 mM NaCl, respectively. Hence, *P. paniculatum* was moderate adaptable to a relatively high salt concentration.

### Effect of burial depth on seedling emergence

A logistic model was used to describe the effect of burial depth on seedling emergence of *P. paniculatum* (Figure 7). The maximum seedling emergence occurred ( $97 \pm 5.5\%$ ) when seeds were placed on the surface. This emergence rate decreased slightly when seeds were buried at a depth of 0.5 cm ( $95.5 \pm 3\%$ ) and continued to decrease as burial depth increased with no seedlings emergence when seeds were buried at depths of 4 cm or more. We concluded that seedling emergence of *P. paniculatum* was quite sensitive to soil burial.

**Figure 4. Effect of pH on germination of *Phleum paniculatum* incubated at 20 °C/10 °C with a 12-h photoperiod for 15 d. Error bars represent 95% confidence interval of the means.**

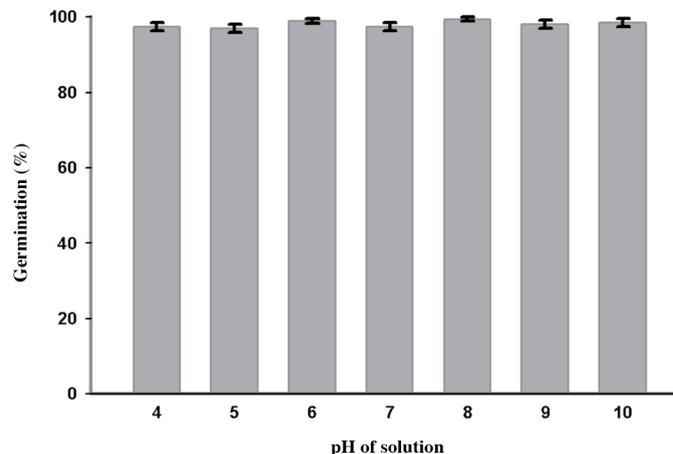


Figure 5. Effect of osmotic potential on germination of *Phleum paniculatum* seeds incubated at 15 °C with a 12:12 h photoperiod for 15 d. Error bars represent 95% confidence interval of the means.

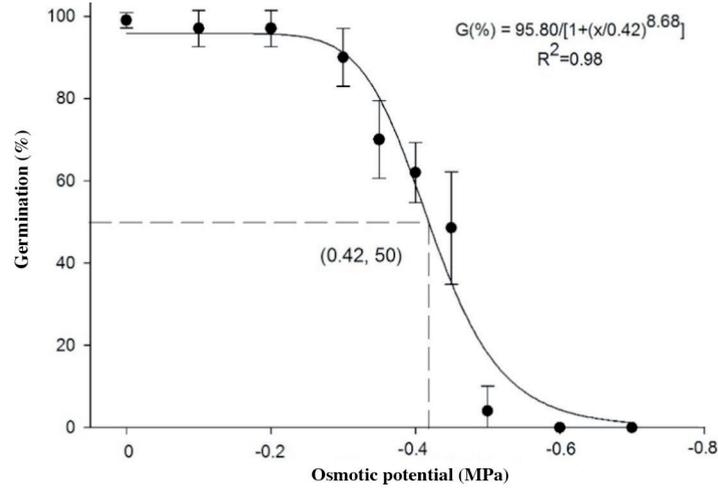


Figure 6. Effect of NaCl concentration on germination of *Phleum paniculatum* seeds incubated at 20 °C/10 °C with a 12:12 h photoperiod for 15 d. Error bars represent 95% confidence interval of the means.

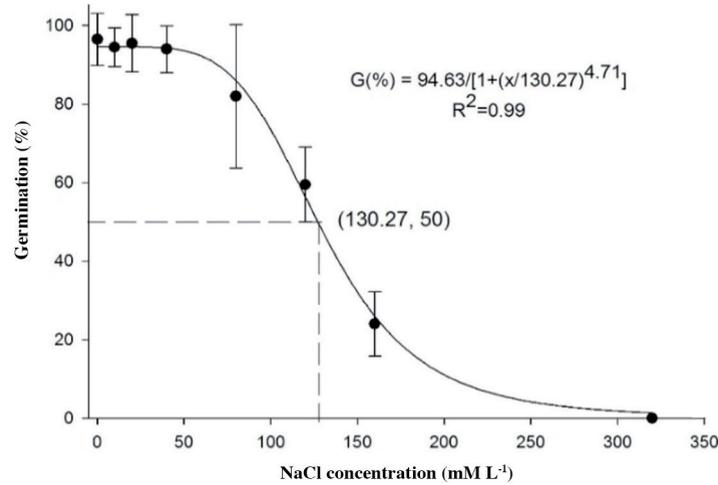
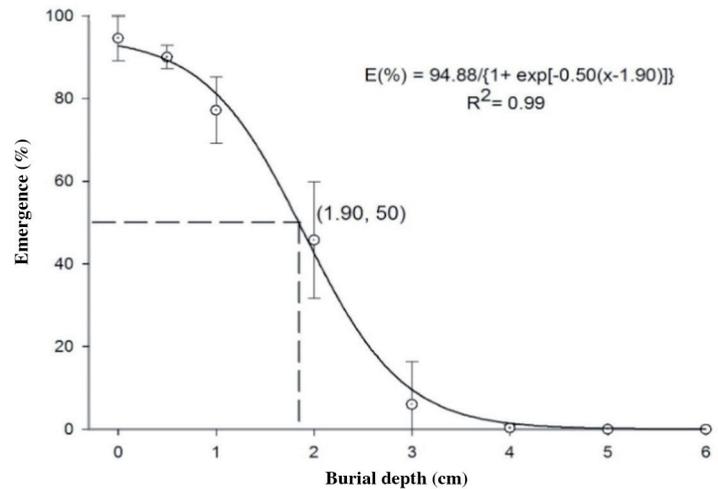


Figure 7. Effect of seed burial depth on seedling emergence of *Phleum paniculatum* incubated at 20 °C/10 °C with a 12:12 h photoperiod for 21 d. Error bars represent 95% confidence interval of the means.



## DISCUSSION

Dormancy period and adaptability to temperature of *P. paniculatum* seeds could help to protect its seedbank in wheat fields in south China. *Phleum paniculatum* seeds usually ripen in late-May and early-June in China, and the daily mean temperature of south China in June is above 25 °C (National Meteorological Information Center, <http://www.nmic.cn/en/>). Seed germination of *P. paniculatum* is adapted to temperature in autumn and spring, but not to summer (> 25 °C) and winter (< 10 °C). Moreover, its seeds have after ripening period of about 1-mo. These characteristics greatly benefit the maintaining of *P. paniculatum* seedbank in wheat fields during summer crop cultivation and allow *P. paniculatum* seeds to germinate readily when wheat is planted again.

*Phleum paniculatum* seeds were moderately adaptable to water potential and NaCl concentration. The water potential for 50% seed germination of *P. paniculatum* was -0.42 MPa, which was higher than some other principal winter weeds in China including *Polypogon fugax* Nees, -0.31 MPa (Wu et al., 2015) and *Galium aparine* L., -0.36 MPa (Wang et al., 2016), but similar to *Alopecurus japonicus* Steud., -0.42 MPa (Wu et al., 2016); *P. paniculatum* appears to be more adaptable to moisture stress compared with *P. fugax* and *G. aparine*. Soil moisture management to limit the germination of *P. paniculatum* may have nonsignificant effect, as this would also impede crop germination and growth.

The NaCl concentration that inhibited 50% germination was calculated to be 130 mM for *P. paniculatum*, which can be compared to wheat that has been reported to have 50% germination at 150 mM NaCl (Wang et al., 2017). This indicates that germination of *P. paniculatum* may be slightly more sensitive to high salt concentration than that of the wheat. In China, the Liao River estuary has a maximum total salt content of 1.6% (278.1 mM) followed by the Yellow and Yangtze River estuaries, which are 1.0% (173.8 mM) and 0.8% (139 mM), respectively (Ba and Zhao, 1997). Since 37.9% of *P. paniculatum* seeds germinated at the 140 mM NaCl concentration, the occurrence of *P. paniculatum* in Yangtze River regions may be problematic.

Deep plowing should be recommended to limit the infestation of *P. paniculatum*. Seed germination of *P. paniculatum* is not inhibited nor promoted by darkness or light, thus its seeds could germinate under soil tillage or under the dense crop canopy of wheat fields. However, the soil burial depth that inhibited 50% emergence for *P. paniculatum* was 1.9 cm, which was more sensitive compared to 2.6 cm for *Bromus japonicus* Houtt. (Li et al., 2015) and 2.5 cm for *Beckmannia syzigachne* (Steud.) Fernald (Rao et al., 2008). *Phleum paniculatum* seed is quite small, with a thousand-seed weight of only 0.17 to 0.23 g. Small seeds have limited nutritional storage and sustaining extensive seedling growth from germination stage to emergence may be the reason that seedling emergence of *P. paniculatum* is more sensitive to soil burial depth than other weed species (Baskin and Baskin, 1998). Many studies have reported that zero-tillage and reduced tillage often facilitate the seriousness of weed occurrence in croplands (Thomas et al., 2004; Chauhan and Johnson, 2009; Chen et al., 2017). In China, farmers either till lands to a depth of less 5 cm before seeding wheat or do not till the soil at all. Shallow tillage and zero tillage leave most of the weed seeds near the soil surface and may promote *P. paniculatum* infestation. Deep plowing is a possible recommendation for fields infested with *P. paniculatum* to bury the seed and limit its emergence.

## CONCLUSIONS

Our research demonstrated that several environmental factors, including temperature, light, pH, soil moisture, salt stress, and seed burial depth affected *Phleum paniculatum* germination and seedling emergence. The dormancy period and range of suitable temperature for *P. paniculatum* allow the seedbank to remain during summer crop cultivation. Light and soil pH had no effect on germination of *P. paniculatum*. *Phleum paniculatum* shows a moderate adaptation to salinity and water stress. No-till and shallow tillage may facilitate more *P. paniculatum* seed to germinate at the soil surface and promote infestation by this species. Proper drainage management and deep plowing are recommended to limit the detrimental effects of *P. paniculatum* on agricultural production.

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