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Effect of environmental factors on the germination and emergence of drunken horse grass (*Achnatherum inebrians*)

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Research Article

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Abstract

Drunken horse grass [*Achnatherum inebrians* (Hance) Keng] is a perennial poisonous weed in western China. A comprehensive understanding of the ecological response of *A. inebrians* germination to environmental factors would facilitate the formulation of better management strategies for this weed. Experiments were conducted under laboratory conditions to assess the effects of various abiotic factors, including temperature, light, water, pH, and burial depth, on the germination and seedling emergence of *A. inebrians*. The seeds germinated at constant temperatures of 15, 20, 25, 30, and 35 C and in alternating-temperature regimes of 15/5, 20/10, 25/15, 30/20, 35/25, and 40/30 C, and the germination percentages under constant and alternating temperatures ranged from 51% to 94% and 15% to 93%, respectively. Maximum germination occurred at a constant temperature of 25 C, and germination was prevented at 45/35 C. Light did not appear to affect germination. The germination percentage of seeds was more than 75% in the pH range of 5 to 10, with the highest germination percentage at pH 6. The seeds germinated at osmotic potentials of 0 MPa to −1.0 MPa, but decreasing osmotic potential inhibited germination, with no germination at −1.2MPa. After 21 d of low osmotic stress, the seeds that did not germinate after rehydration had not lost their vitality. The seedling emergence percentage was highest (90%) when seeds were buried at 1 cm, but declined with increasing burial depth, with no emergence at 9 cm. Deep tillage may be effective in limiting the germination and emergence of this species. The results of this study provide useful information on the conditions necessary for *A. inebrians* germination and provide a theoretical basis for science-based prediction, prevention, and control of this species.

Introduction

Grasslands, one of the most important ecosystems in the world, comprise 40% of the global land surface and are not being utilized effectively due to topography and climate (Squires 2009). In recent years, due to overgrazing and human activities, the stability of grassland ecosystems has been greatly weakened, resulting in grassland degradation (Asner et al. 2004; Squires 2009). Overgrazing of grasslands causes flora and fauna biodiversity to decline (Hilker et al. 2014). China has the second-largest area of grazing grasslands in the world and plays an important role in global ecology (Hua and Squires 2015). Due to overgrazing, grasslands in northwest China are being invaded by poisonous and harmful plants (Lu et al. 2012). One of these inedible plants is drunken horse grass [*Achnatherum inebrians* (Hance) Keng], which is toxic to grazing animals. (Miles et al. 1996).

Achnatherum inebrians is a clumping perennial poisonous herb of the Poaceae family. This species is native to Europe and Asia and grows in high mountains, slopes, roadsides, and valleys at altitudes of 1,700 to 2,400 m (Ji 2009). Mature *A. inebrians* can reach a height of 60 to 150 cm, and each compact inflorescence can produce approximately 700 small, easily shed seeds (Miles et al. 1996). Spreading by seeds is one of the important ways of population dispersal (Dilixiati et al. 2017). Because this grass contains toxic alkaloids (Miles et al. 1996), livestock fed upon it display symptoms of intoxication such as sluggishness, tottering, drooping, and glaring (Ji 2009). These alkaloids are produced by a seed-transmitted symptomless fungal endophyte, *Epichloë gansuensis* (C.J. Li & Nan) Schardl (Bruehl et al. 1994). Previous research has shown that almost 100% of *A. inebrians* plants in natural rangeland are infected by the endophyte *E. gansuensis* (Li et al. 2004).The endophytic fungi improves resistance to biotic (pest, nematode, etc.) and abiotic (drought, cold, barren, etc.) factors and promotes rapid growth of *A. inebrians* (Nan et al. 2016; Wang et al. 2018). However, *A. inebrians* is poisonous to livestock and has come

to dominate degraded grasslands in Xinjiang, Qinghai, and other northwestern areas (Li et al. 1996). For example, the total area of natural grassland in Xinjiang is 57 million ha, and the area dominated by *A. inebrians* is as high as 450,000 ha; its coverage can reach 85% in lush areas, greatly affecting local animal husbandry development (Yan et al. 2015).

The expansion and reproduction of most plants depend on the spread of seeds and successful establishment of seedlings to build new populations. Germination is a key stage of the plant life cycle (Xu et al. 2001). Some research has shown that germination and emergence ability are positively correlated with the establishment and spread of poisonous weeds (Chauhan et al. 2006d). Germination and seedling emergence are affected not only by seed properties but also by abiotic environmental factors, including temperature, light, pH, water, and depth of burial (Baskin and Baskin 1998; Chachalis and Reddy 2000; Chauhan and Johnson 2010; Chauhan et al. 2006a; Javaid and Tanveer 2014; Koger et al. 2004). Previously published studies on *A. inebrians* have focused mainly on its endophytic association (Chen et al. 2016), toxicological mechanism (Liang et al. 2017), production and degradation of alkaloids (Zhang et al. 2011; Zhu et al. 2017), and germination (Duan et al. 2012; Wang et al. 2010; Yu et al. 2009). However, extreme tolerance-range information for environmental factors affecting *A. inebrians* germination and emergence is not clear in the published literature. Detailed information on the ecological requirements of *A. inebrians* germination may facilitate the development of effective control measures. Therefore, the objectives of this study were to systematically determine the effects of environmental factors such as temperature, light, pH, moisture, and burial depth on the germination and emergence of *A. inebrians*.

Materials and Methods

Seed Collection and Preparation

Seeds (0.32 kg total) were collected randomly from 300 individuals belonging to the same natural population of *A. inebrians* in July 2017 from Saerdaban village in Urumqi, Xinjiang, China (43.47°N, 87.25°E). The tests of individual samples (conducted 5 mo after collection; unpublished data) revealed that germination among samples was similar. After being combined, the seeds were air-dried and stored in paper bags in a cool (20 ± 5 C), ventilated, dry environment before use. The 1,000-seed weight (with awns) was 1.527 g.

General Germination Test

This experiment was conducted in May 2018 at the College of Grass and Environmental Sciences, Xinjiang Agricultural University, Urumqi, China. Germination of *A. inebrians* was assessed in a laboratory by placing 30 seeds on two layers of filter paper (Whatman No. 1, Maidstone, UK) in a 9-cm-diameter petri dish. The seeds were moistened with 5 ml of distilled water or test solution for the experiment (solutions of different pH or levels of osmotic stress), and petri dishes were sealed with parafilm to prevent moisture loss and kept in controlled-environment growth chambers set at a constant temperature of 25 C with alternating 12-h light and dark conditions for all experiments unless specified otherwise. Fluorescent lamps produced a photosynthetic photon flux density of 200 µmol m^{-2} s^{-1} for all experiments. Germination was considered successful when the radicle broke through the seed coat. (Chauhan and Johnson 2008). Germinated seeds were counted daily for 21 d (from the beginning of experiment to the time germination stabilized). The germination values were defined as the ratio of the number of germinated seeds to the total number of seeds per petri dish.

Effect of Temperature on Germination

To determine the optimal germination temperature and influence of temperature on the germination of *A. inebrians*, 30 seeds per replicate of each treatment were placed at seven constant temperatures (10, 15, 20, 25, 30, 35, and 40 C) and in seven fluctuating temperature (day/night) regimes (15/5, 20/10, 25/15, 30/20, 35/25, 40/30, and 45/35 C). The fluctuating temperature regimes were selected to reflect the temperature variation from spring to summer of the collection habitat in Saerdaban village in Urumqi, Xinjiang, China. To determine whether exposure to high or low temperatures and low temperature affects seed viability, the ungerminated seeds from specific constant temperatures (10, 35, and 40 C) and fluctuating temperatures (15/5, 40/30, and 45/35 C) and blank control seeds were transferred to a growth chamber with the temperature set at optimal temperature (25 and 30/20 C, respectively). The number of germinated seeds was determined after 21 d.

The following equation was used to estimate the time required for the germination percentage to reach 90% (t_{90}) at different temperatures (Li et al. 2012):

$$t_{90} = \left(H_p - L_p\right)^{-1} + L \quad [1]$$

where L is the day before germination percentage reaches 90%, L_p is the germination percentage on day L, and H_p is the germination percentage when it reaches or exceeds 90%.

Germination percentages obtained at different constant temperatures and fluctuating temperatures were fit to a functional three-parameter sigmoid model (SigmaPlot v. 13, Systat Software, San Jose, USA) (Chauhan and Johnson 2008). The fitted model was as follows:

$$G(\%) = G_{max}/\{1 + \exp[-(t - t_{50})/G_{rate}]\} \quad [2]$$

where G is the cumulative germination percentage (%) at time t, G_{max} is the maximum germination percentage (%), t_{50} is the time required to achieve 50% germination, and G_{rate} is the slope of the curve.

Effect of Light on Germination

To evaluate the influence of photoperiod on germination, petri dishes containing *A. inebrians* seeds were incubated under 0/24, 6/18, 12/12, 18/6, and 24/0-h light/dark regimes at a constant temperature of 25 C. In the dark treatment, petri dishes were wrapped in double layers of aluminum foil to avoid any effects of light on the experiment. In the treatments with 0/24, 6/18, 12/12, 18/6, and 24/0-h light/dark regimes, petri dishes were allowed light exposure for 24, 18, 12, 6, and 0 h, respectively. Additionally, the addition of water to the petri dishes and daily germination counts were conducted under green safe light in a darkroom.

Effect of pH on Germination

To determine the influence of pH on the germination of *A. inebrians*, seeds were placed in buffer solutions with pH values ranging from 4 to 10 configured in advance according to the methods described by Reddy and Singh (1992) and Wu et al. (2015). Three acid solutions (pH < 7), one neutral solution (pH = 7), and three alkaline

solutions (pH > 7) were used. Other experimental conditions were the same as those described in the general germination test.

Effect of Osmotic Stress on Germination

To study the influence of drought stress on the germination of *A. inebrians*, seeds were tested in aqueous solutions with osmotic potentials of 0, −0.1, −0.2, −0.4, −0.6, −0.8, −1.0, −1.2, and −1.3 MPa. Polyethylene glycol 6000 was used as a drought stimulator and prepared following published methods (Chachalis and Reddy 2000; Michel 1983; Michel and Radcliffe 1995). Other experimental conditions were the same as those described in the general germination test. To determine whether exposure to low osmotic potential would affect seed viability, the remaining ungerminated seeds under low osmotic potential (−0.8, −1.0, −1.2, and −1.3 MPa) were rinsed with distilled water five times and transferred to a growth chamber at 25 C after 5 ml of distilled water was added. The number of germinated seeds was counted after 21 d.

Germination percentages (%) obtained at different osmotic potential conditions were fit to a three-parameter sigmoid model (SigmaPlot software) (Chauhan and Johnson 2008). The fitted model was as follows:

$$G(\%) = G_{max}/\{1 + \exp[-(x - x_{50})/G_{rate}]\} \quad [3]$$

where G is the cumulative germination percentage (%) at osmotic potential x, G_{max} is the maximum germination percentage (%), x_{50} is the osmotic potential required to achieve 50% germination, and G_{rate} is the slope of the curve.

Effect of Burial Depth on Germination

To study the effects of burial depth on the emergence of *A. inebrians* seeds, experiments were conducted in controlled-environment growth chambers. The soil (pH 7.34, organic matter 33.93 g kg^{-1}, electrical conductivity 206.33 μs cm^{-1}) used for this experiment was collected from the site where the seeds were collected. The soil was passed through a 3-mm sieve and autoclaved before the experiment to ensure that there were no living seeds in the soil. In each plastic pot (height: 12 cm; diameter: 15 cm), 30 seeds were placed evenly on the soil surface (0 cm) or covered with soil at depths of 1, 2, 4, 6, 8, and 9 cm. Pots were watered every other day to maintain adequate soil moisture. All pots were placed randomly inside a growth chamber at a constant temperature of 25 C with a photoperiod of 12 h. The seedlings were considered to have emerged when the coleoptile was visible above the soil surface. Emergence was counted daily for 21 d until emergence stabilized. At the end of the experiment, pots with no plant emergence were checked to identify whether the coleoptile failed to reach the soil surface or the seeds failed to germinate.

Variation trends of seedling emergence percentage (%) obtained at different burial depths were fit to a three-parameter sigmoid model (SigmaPlot software) (Mahmood et al. 2016). The fitted model was as follows:

$$E(\%) = E_{max}/\{1 + \exp[-(x - x_{50})/E_{rate}]\} \quad [4]$$

where E is the final seedling emergence (%) at depth x, E_{max} is the maximum seedling emergence percentage (%), x_{50} is the depth at which 50% seedling emergence is achieved, and E_{rate} is the slope of the curve.

Statistical Analysis

All experiments were arranged in a randomized complete block design with four replications. Experiments were repeated over time, and the second run of experiments was started within a month of termination of the first run. There was no statistical difference ($P > 0.05$) between the two runs for all experiments, and data were pooled across runs and used for subsequent analyses. Data from all experimental sets from repeated experiments were subjected to ANOVA with the general linear model procedure using SPSS software v. 19.0 (IBM, Armonk, NY, USA), and a mean comparison was performed using Fisher's protected LSD test at $P \leq 0.05$. (Chauhan et al. 2006b; Hanif et al. 2017; Zhao et al. 2018). Regression analysis was conducted where appropriate using SigmaPlot software.

Results and Discussion

Effect of Temperature on Germination

The effect of constant-temperature conditions on germination is shown in Table 1. *Achnatherum inebrians* seeds germinated in the temperature range of 15 to 35 C. In this range, the germination percentages of the seeds first increased and then decreased with increasing temperature. The germination percentages ranged from 50.83% (35 C) to 94.44% (25 C) in the constant temperature range of 15 to 35 C, but the seeds did not germinate at constant temperatures of 10 and 40 C (Table 1). *Achnatherum inebrians* germinated within 2 d at 25 and 30 C, which may reflect that the effective accumulated temperature is reached more quickly at these temperatures than at higher or lower temperatures. Germination was completely inhibited at 40 C, which indicates that even though the effective accumulated temperature was reached at high temperatures, the seeds failed to germinate. This result indicates that temperature is an important factor for germination of this species, as has been shown previously for other species (Chauhan and Johnson 2008). A reasonable explanation is that such a high temperature may affect the activity of enzymes needed for germination.

Seeds took longer to start germinating at 15/5 C and did not germinate at the 45/35 C temperature regime (Tables 1 and 2). Compared with constant temperatures, the same average fluctuating temperatures had no significant effect on the germination percentage of *A. inebrians*, except that the germination percentage was significantly higher at 15/5 C than at 10 C. (Table 1). Nevertheless, the germination trends under fluctuating temperatures (20/10, 25/15, 30/20, 35/25, or 40/30 C) are similar to those under constant temperatures (15, 20, 25, 20, or 35 C). These results are consistent with those from previous studies on field brome (*Bromus arvensis* L.), which is a gramineous weed (Li et al. 2015). Based on this study, the optimum temperature range (20, 25, 30, 25/15, 30/20 C) of *A. inebrians* is wider than was previously reported (Yu et al. 2009). This may be caused by the different places where the experimental materials were collected.

The seeds that did not germinate at constant temperature (10, 35, and 40 C) and fluctuating temperatures (15/5, 40/30, and 45/35 C) were transferred to 25 and 30/20 C, respectively. After 21 d of high- and low-temperature stress, both the constant temperatures (10, 35, and 40 C) and the fluctuating temperatures (15/5, 40/30, and 45/35 C) inhibited germination ($P < 0.05$). These data suggest that high-temperature stress had a greater effect on germination than low-temperature stress ($P < 0.05$) (Figures 1

Table 1. The germination of *Achnatherum inebrians* under constant- and alternating-temperature regimes and t_{90} (the time required to reach 90% of the germination percentage).[a]

Temperature	*T*	Total germination, mean ± SE	t_{90}[b]
C	d	%	d
10	—	0 ± 0 e	NA
15	6	84.16±3.69 ab	NA
20	3	93.33 ± 3.04 a	16.10
25	2	94.44 ± 2.94 a	15.17
30	2	93.33 ± 1.92 a	16.30
35	3	50.83 ± 3.33 c	NA
40	—	0 ± 0 e	NA
15/5	13	15 ± 0.96 d	NA
20/10	7	74.16 ± 6.14 b	NA
25/15	3	89.16 ± 1.59 a	NA
30/20	3	92.5 ± 1.59 a	9.16
35/25	3	83.33 ± 1.36 ab	NA
40/30	3	45.83 ± 5.15 c	NA
45/35	—	0 ± 0 e	NA

Abbreviations: *T*, time at which germination began; t_{90}, day on which the germination percentage reached 90%; NA, the germination rate did not reach 90% in all replicates; SE, standard error of all replicates for each treatment. Within a column, means followed by the same letter indicate no significant difference in the mean value by Fisher's protected LSD test ($P \leq 0.05$).

[b]t_{90} was calculated using Equation 1: $t_{90} = (H_p - L_p)^{-1} + L$, where L is the last day before 90% germination was reached, L_p is the observed germination percentage on day L, and H_p is the observed germination percentage on the day when germination reached or exceeded 90%.

Table 2. Parameters of the functional three-parameter sigmoid model used to fit *Achnatherum inebrians* germination percentages (%) resulting from different constant- and alternating-temperature regimes.[a]

	Parameter characteristics[b]			
Temperature	G_{max}	G_{rate}	R^2	t_{50}
C				
10/10	ND	ND	ND	ND
15/15	89.32	3.21	0.98	10.38
20/20	90.79	2.12	0.98	8.96
25/25	94.81	2.52	0.99	6.77
30/30	86.26	1.13	0.96	3.21
35/35	43.06	3.79	0.91	7.79
40/40	ND	ND	ND	ND
15/5	14.99	1.25	1	14.39
20/10	70.12	1.58	0.99	10.60
25/15	89.16	0.65	0.99	3.54
30/20	92.86	0.42	0.99	3.01
35/25	46.36	1.69	0.99	7.29
40/30	27.79	1.24	0.99	6.56
45/35	ND	ND	ND	ND

[a]$G(\%) = G_{max}/\{1 + \exp[-(t - t_{50})/G_{rate}]\}$, where G is the total germination (%) at time t, G_{max} is the maximum germination (%), t_{50} is the time required for 50% inhibition of the maximum germination, and G_{rate} is the slope.

[b]ND indicates that the seeds did not germinate at 10/10, 40/40, and 45/35 C.

and 2). Moreover, seeds in the fluctuating-temperature treatment of 45/35 C completely lost their viability.

Effect of Light on Germination

Under continuous light (24/0), the germination percentage was 89%, whereas exposure to continuous dark (0/24 h) increased the germination percentage to 96% (Figure 3). The germination percentages were above 89% under continuous illumination or continuous darkness, and the mean value was 93%, which indicated that germination was not light sensitive. These results further suggest that the seeds can germinate both in soil and on the soil surface. The insensitivity to light makes *A. inebrians* more competitive in grassland habitats, thus explaining to some extent the spreading of this plant.

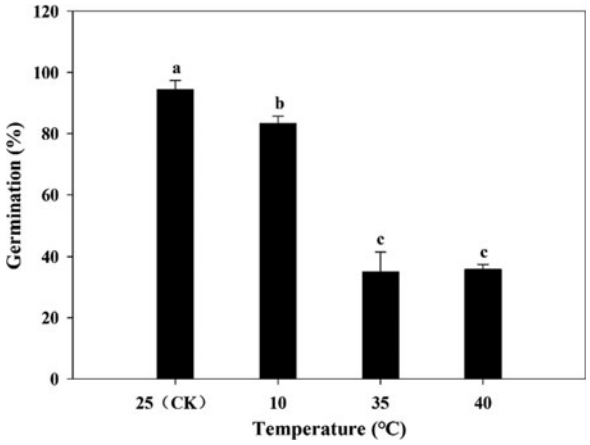

Figure 1. Effect of rewarming on the germination of *Achnatherum inebrians* seeds at 25 C. Rewarming refers to the transfer of ungerminated seeds kept under a constant temperature of 10, 35, or 40 C to a growth chamber set at the optimal temperature, 25 C (CK). The vertical bars represent the standard error of the mean. Bars with the same

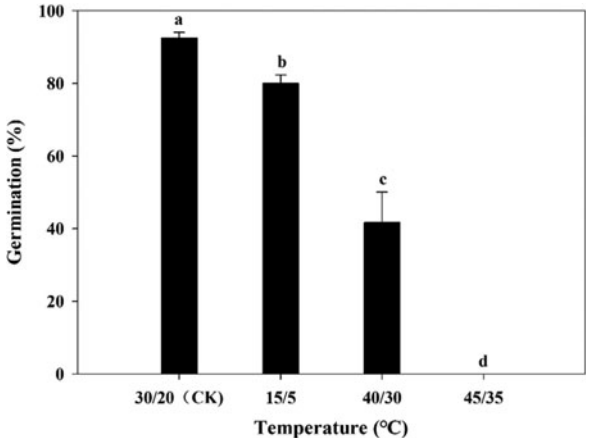

Figure 2. Effect of rewarming on the germination of *Achnatherum inebrians* seeds at 30/20 C. Rewarming refers to the transfer of ungerminated seeds kept under a constant temperature of 10, 35, or 40 C to a growth chamber set at the optimal temperature, 25 C (CK). The vertical bars represent the standard error of the mean. Bars with

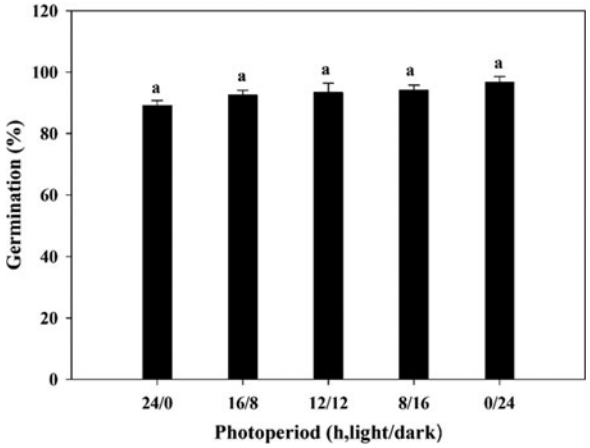

Figure 3. Effects of different photoperiods on the germination of *Achnatherum inebrians* seeds under 25 C culture conditions. Bars with the same letters indicate that there are no significant differences in the mean values by Fisher's protected LSD test ($P \leq 0.05$).

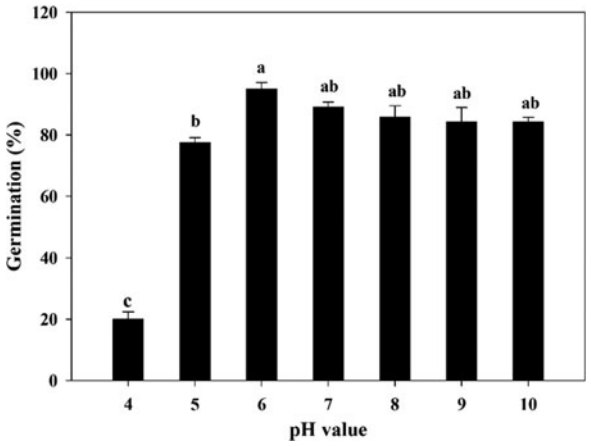

Figure 4. Effect of buffered pH solutions on the germination of *Achnatherum inebrians* seeds at 25 C. The vertical bars represent the standard error of the mean. Bars with the same letters indicate that there are no significant differences in the mean values by Fisher's protected LSD test ($P \leq 0.05$).

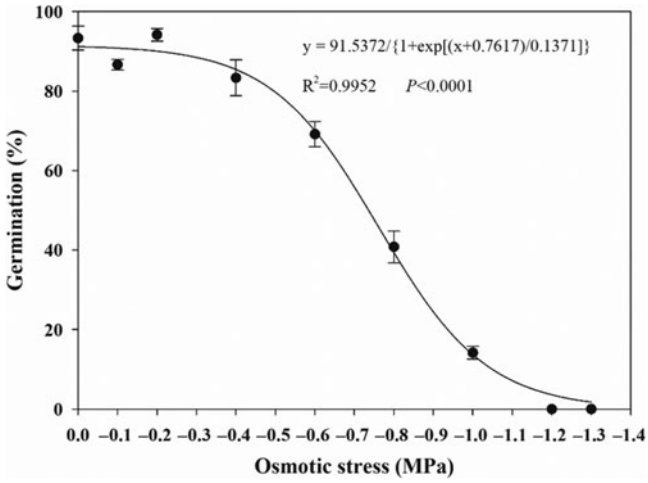

Figure 5. Effect of osmotic potential on the germination of *Achnatherum inebrians* seeds at 25 C. Vertical bars represent the standard error of the mean, and a logistic sigmoidal regression model is fit to the data.

Light is an important environmental signal regulating germination (Pons 1991), but the effect of light on the germination is species specific. Many studies have shown that light can increase the germination percentage of some species (Eslami 2011; Tang et al. 2010). However, studies have also found that light does not promote or may even harm the germination of certain weed seeds (Li et al. 2007). Research showed that tall morningglory [*Ipomoea purpurea* (L.) Roth] (Singh et al. 2012) germination was not affected by light. Similar to our study, the germination of seeds of American sloughgrass [*Beckmannia syzigachne* (Steud.) Fernald] (Rao et al. 2008), Japanese brome (*Bromus japonicus* Thunb.) (Li et al. 2015), musk weed (*Myagrum perfoliatum* L.) (Honarmand et al. 2016), tropical signalgrass [*Urochloa distachya* (L.) T.Q. Nguyen] (Teuton et al. 2004), and Tausch's goatgrass (*Aegilops tauschii* Coss.) (Fang et al. 2012) were not affected by light.

Effect of pH on Germination

The germination of *A. inebrians* was ≥20% in the pH range of 4 to 10 (Figure 4). The highest (95%) and lowest (20%) germination percentages were reached at pH values of 6 and 4, respectively (Figure 4). While the germination percentage was highest at pH 6, it was relatively stable between pH 7 and pH 10. The results showed that *A. inebrians* could germinate at a wide range of pH values, a strongly acidic environment could inhibit the germination of *A. inebrians* seeds, and a strongly alkaline environment had no impact on *A. inebrians* seeds. Different plants require different pH environments for their germination and growth; however, some plant germination can tolerate extreme pH levels. (Evetts and Burnside 1972). Studies have reported that the germination percentage of common sowthistle (*Sonchus oleraceus L.*) seeds was more than 90% in the pH range of 5 to 8 (Chauhan et al. 2006b). Many invasive weeds have this feature, such as *A. tauschii* (Fang et al. 2012), *B. syzigachne* (Rao et al. 2008), and *B. japonicus* (Li et al. 2015). The ability of *A. inebrians* to germinate over a wide range of pH levels indicates that it may adapt to a wide range of soil conditions. The pH range of most soils in Xinjiang is between 5 and 10, and most of these soils are alkaline (pH > 7) (Feng et al. 2017). The wide range of germination in either acid or base conditions may make *A. inebrians* adaptable to many environments and may increase its competitive advantage. This may be one of the reasons why degraded grasslands in Xinjiang were severely invaded by *A. inebrians*.

Effect of Osmotic Stress on Germination

Germination was greatly affected by osmotic potential (Figure 5). The germination percentage decreased from 93% to 14% as the osmotic potential decreased from 0 to −1.0 MPa, with no seeds germinating at −1.2 MPa. The osmotic potential necessary for a 50% reduction in the maximum germination percentage was estimated at approximately −0.76 MPa. Therefore, low osmotic potentials had a strong effect on germination. The response of *S. oleraceus* (Chauhan et al. 2006b), *B. syzigachne* (Rao et al. 2008), *I. purpurea* (Singh et al. 2012), and false daisy [*Eclipta prostrata* (L.) L.] germination to osmotic potential was similar to the response of *A. inebrians* germination observed in this study. Other studies have reported that turnip weed [*Rapistrum rugosum* (L.) All.] (Chauhan et al. 2006c) and *B. japonicus* (Li et al. 2015) can withstand −1.2 MPa low osmotic potentials.

The germination percentages of seeds decreased gradually with the decrease in osmotic potential. However, when seeds that failed to germinate at −0.8, −1.0, −1.2, and −1.3 MPa were transferred to distilled water, the germination percentages of the rehydrated seeds were more than 85%, which was similar to the control group ($P > 0.05$). (Figure 6). The results suggest that germination was inhibited, but the vitality of the seeds was not adversely affected after 21 d of water stress. The seeds would be able to germinate if a timely rainfall event occurred shortly after drought stress, which may explain the distribution of *A. inebrians* in desert areas.

Effect of Burial Depth on Germination

Seedling emergence decreased as burial depth increased from 0 to 9 cm (Figure 7) and fit the three-parameter sigmoid model. The maximum seedling emergence percentage (90%) was observed for seeds at 1-cm depth. The minimum seedling emergence percentage (2%) occurred with seeds sown at 8 cm, whereas no seedlings emerged from seeds buried at 9 cm. The seedling emergence percentage decreased sharply when seeds were planted deeper than 4 cm. The depth required for a 50%

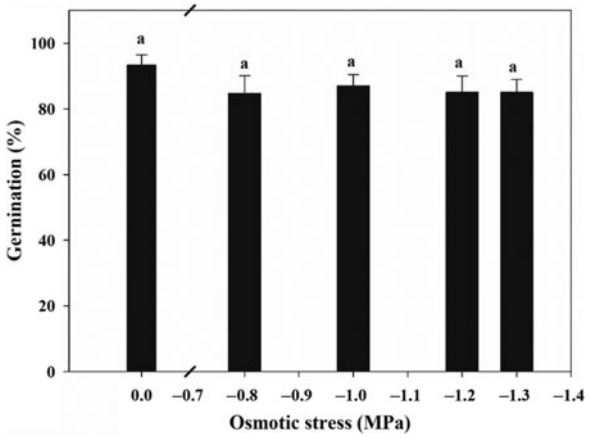

Figure 6. Germination of *Achnatherum inebrians* seeds at low osmotic potential. The vertical bars represent the standard error of the mean. Bars with the same letters indicate that there are no significant differences in the mean values by Fisher's protected LSD test ($P \leq 0.05$).

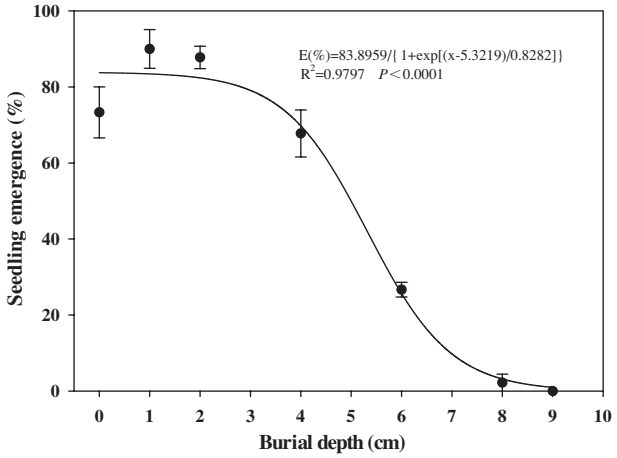

Figure 7. Effect of burial depth on the emergence of *A. inebrians* seeds at 25 C. Vertical bars represent the standard error of the mean, and a logistic sigmoidal regression model is fit to the data.

reduction in the maximum seedling emergence percentage was estimated to be 5.32 cm. Similar to our findings, the emergence of many species decreases with increased burial depth (Li et al. 2015; Schutte et al. 2014).

In this study, *A. inebrians* seedling emergence was inversely related to burial depth. Studies have shown that Asia Minor bluegrass (*Polypogon fugax* Nees ex Steud.) emergence was completely inhibited at 4-cm depth, and seeds planted deeply failed to emerge because the small seeds could not provide enough nutrition for the coleoptiles to reach the soil surface (Wu et al. 2015). Because of the absence of light, the seedling emergence behavior of seeds buried deeply may completely depend on seed reserves (Mennan and Ngouajio 2006). This mechanism of germination and emergence inhibition may be an important survival strategy for *A. inebrians*, resulting in an underground seedbank (Benvenuti et al. 2001). Most of the seeds of mature *A. inebrians* remain on the soil surface or shallowly buried after being subjected to external forces. The seed emergence percentage was highest on the surface and in the shallow soil layers, which indicated that the seeds of *A. inebrians* perform best under a natural, no-tillage system (natural grassland). No seed emergence was observed from a depth of 9 cm, indicating that deep tillage, with seeds buried at this depth, could be a method to limit the emergence of *A. inebrians*. Deep-tillage operations might be an option in an area being totally restored to native grassland, but the potential adverse effects on native species that are already established on an invaded site need further study.

In summary, the seeds of *A. inebrians* showed strong tolerance to variable temperature, light, moisture, pH, and burial depth during germination. The results of this study indicate that *A. inebrians* has adapted to germinate under a wide range of environmental conditions commonly found in the Xinjiang region. These characteristics of *A. inebrians* seed can also partly explain the successful expansion of this species in Xinjiang, China. *Achnatherum inebrians* has become a significant poisonous weed in natural grasslands. Whether deep tillage can be applied in invasive sites where native species have been already established needs to be further explored.

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