Base water potential but not hydrot ime predicts seedling emergence of *Medicago sativa* under water stress conditions

Xianglai Chen, Zhichao Wei, Dali Chen and Xiaowen Hu

State Key Laboratory of Grassland Agro-Ecosystems; Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs; Engineering Research Center of Grassland Industry, Ministry of Education; College of Pastoral Agricult, Lanzhou, China

**ABSTRACT**

We determined the hydrot ime model parameters of 10 alfalfa seed lots by incubating seeds at various water potentials in the laboratory. Meanwhile, seedling emergence under drought and salinity conditions in a greenhouse pot experiment, and seedling establishment in the field were determined. Correlation analysis was utilized to detect the relationship between hydrot ime model parameters and seedling emergence under water stress conditions. The germination percentage did not differ significantly among seed lots when seeds were incubated at −0.1 MPa, while it differed significantly among seed lots at water potentials of −0.3 and −0.6 MPa. Compared to control conditions, drought and salinity decreased seedling emergence to different extents, depending on seed lots. Seedling emergence in the field differed significantly among seed lots and ranged from 30% to 80%. $\Psi_{b(50)}$ showed a significant correlation with seedling emergence under various conditions and with seedling establishment in the field, while no correlation was observed between $\theta_{H}$, $\sigma_{\phi b}$ and seedling emergence and establishment. These results suggest that $\Psi_{b(50)}$ can be used to rank the vigor of alfalfa seed lots and thus predict seedling emergence and establishment under water stress conditions.

**Subjects** Agricultural Science, Ecology, Plant Science

**Keywords** Drought, Hydrot ime model, *Medicago sativa*, Seedling emergence, Base water potential

**INTRODUCTION**

Alfalfa (*Medicago sativa* L.), an important perennial legume forage crop with high nutritional content, is one of the most important crops in China and is widely cultivated in arid and semi-arid regions (*Kong et al., 2020; Su, 2007*). However, poor establishment of this species has become one of the major constraints encountered by farmers (*Guo et al., 2005*), due to local harsh environments, e.g., water scarcity, salinization and soil erosion (*Wang et al., 2004b*). Thus, improving seed germination and seedling establishment under stressful conditions have become a key determinant of *M. sativa* cultivation and utilization.

Selection for high vigor alfalfa seeds is an effective way to enhance seedling establishment and consequently hay yield in the field. Previous studies (*Xia et al., 2020; Yacoubi et al., 2013*) showed that there was an advantage in using high vigor seeds to establish alfalfa fields because low vigor seeds resulted in limited seedling emergence.
Wang et al. (2004a) found a positive relationship between seed vigor and seedling emergence in the field. Selection for high vigor seeds also has proven to be beneficial for seedling emergence and yield of other crop species, e.g., maize, sorghum (Kim et al., 2013; Mondo et al., 2013).

There are many ways to evaluate seed vigor, such as seedling growth dynamics (Tohidloo & Kruse, 2009; Sharma et al., 2014; Wang, Liu & Song, 2015), electrical conductivity (EC) (Hampton, Leeks & McKenzie, 2009; Matthews et al., 2009; Sibelle et al., 2012; Jonathan et al., 2013), accelerated aging (Mcdonald & Phaneendranath, 1978; Souza et al., 2017), radicle emergence (Lv, Wang & Powell, 2016; Guan et al., 2018). Wang et al. (2004a) showed that EC and accelerating aging (AA) are more sensitive for evaluating alfalfa seed vigor than the standard germination test. Pan et al. (2017) showed that the standard germination test failed to detect differences in seed vigor, while EC and radicle emergence were highly sensitive ways to determine seed vigor.

Many studies have found that the germination rate (GRg, the reciprocal of time to a given germination fraction, 1/tg) is linearly related to water potential (Gummerson, 1986; Bradford, 1990; Dahal & Bradford, 1994). Thus, the hydrot ime model has been developed to evaluate the effect of water potential on progress towards germination (Gummerson, 1986; Bradford, 2002; Hu et al., 2015). The hydrot ime modeling approach can help predict seed germination under water deficit conditions, as reported by Patanè and Tringali for seeds of Brassica carinata (Patanè & Tringali, 2011). In particularly, the base or threshold water potential for a specific germination fraction has been reported to be closely related to seed vigor in many species, e.g., sugar beet, cotton, and rapeseed (Farzane & Soltani, 2011; Soltani & Farzaneh, 2014; Soltani et al., 2017). Castillo-Lorenzo et al. (2019) indicated that 10 Brassica seed lots had diverse seed germination phenotypes, with hydrotimes (θH) differing by 3 to 7-fold and base water potentials (Ψb) by −1.5 MPa, illustrating hydrot ime parameters can be used to explain seed vigor (Castillo-Lorenzo et al., 2019). Li et al. (2017) found that priming improved the Ψb(50) for alfalfa seeds and increase germination speed and seedling growth under high salinity conditions. This study implied that a change in hydrot ime model parameters induced by priming may be closely related to performance of seeds and seedlings under the stressful field conditions. However, the assumption that hydrot ime model parameters can be used to predict seed vigor and their performance under various stressful conditions has not been tested. In our study, we asked the following questions. (1) Does the hydrot ime model predict seed germination in response to water stress for M. sativa seed lots with various levels of vigor? (2) Are there any correlations between hydrot ime parameters and seedling emergence under stressful conditions?

**MATERIALS AND METHODS**

**Germination test**

Ten seed lots of alfalfa from different company and production sites were provided by the Forage and Turfgrass Seed Quality Inspection Test Center, Lanzhou, Ministry of Agriculture and Rural Affairs, China. According to a preliminary study, these ten seed lots
have similar germination percentage under standard germination test condition at 20 °C (ISTA, 2014).

A germination test for each seed lot was conducted by incubating seeds at 20 °C in 12/12 h light/dark at water potentials of −0.1, −0.3, −0.6 and −0.9 MPa. Polyethylene glycol 6000 (PEG) solutions were prepared according to Michel & Kaufmann (1973). In addition, as described in Hu et al. (2015), the water potential of solutions was calibrated using a Dew Point Microvoltmeter HR-33T (Wescor, Logan, UT, USA). For each treatment, three replicates of 50 seeds each were placed in 11 cm diameter Petri dishes on two sheets of filter paper moistened with 7 mL of PEG solution, then the Petri dishes were enclosed with parafilm to prevent evaporation of water. Seeds were transferred to new filter paper with new solution every 3 d to ensure relatively constant water potential in the treatments (Hu et al., 2015; Zhang et al., 2020). Seeds were counted for germination every 6, 18 and 24 h, depending on germination speed, for 20 d and seedlings removed at each counting. Seeds were considered to be germinated when the radicle had emerged to at least half the seed length. Final germination percentage was expressed as germinated seeds divided by total sown seeds. The germination rate was expressed as 1/t50. The t50 was the time to 50% of the maximum germination percentage in the most favorable environment for each alfalfa seedlot, it was estimated from the function according to Steinmaus, Prather & Holt (2000).

Greenhouse experiment
The pot experiment was conducted in the greenhouse at Yuzhong Campus of the Lanzhou University (35°85′N,104°12′E, 1,720 m a.s.l.), Gansu Province, China. Mean daily temperature is 20 °C (13–27 °C) and average air humidity is about 60% (30–90%). In addition, average photosynthetic photon flux densities (PPFD, 48.4–85.3 k lx) is 50–80% natural light. Seeds of the 10 alfalfa lots were sown in soil at control (90% relative field capacity), water stress (60% relative field capacity) and salinity stress (NaCl and soil were uniformly mixed at 1:1,000). Field capacity of the soil was determined according to Hu et al. (2018). Field capacity of soil was 21.21%. For each treatment, three replicates of 50 seeds each seed lot were sown pots (20 cm tall, 15 cm in diameter) that contained 3.00 kg soil dried at 105 °C. Based on the Chinese Soil Classification System (Gong, 1999), the soil used in this study was a cultivated loess soil. According to soil moisture characteristic curves from previous study (Ma et al., 2005), the soil water potential for 90% and 60% of field capacity is about −0.1 MPa and −0.6 MPa at 20 °C. No fertilizer was applied during the whole experimental period. To prevent water from escaping from the bottom of the pot, plastic bag was put inside the pot and a PVC tube was inserted into pot. Pots were observed daily and soil moisture content was controlled by weighing the pot and adding more water if needed until complete seedling emergence. The experiment applied completely random design. Final emergence percentage (EP) and emergence index (EI) were determined as follows:

\[
EP = \left( \frac{n}{N} \right) \times 100\%
\]

(1)
\[ EI = \sum (E_i / D_i) \]

where \( n \) is the number of emerged seeds, \( N \) was the total number of tested seeds; \( D_i \) is the days since sowing, \( E_i \) is the number of emerged seeds that day corresponding to \( D_i \).

**Field experiment**

The field experiment was carried out at the Yuzhong Campus, where mean annual precipitation and mean annual temperature are 350 mm and 6.7 °C, respectively. Four replicates of 200 seeds for each seed lot were sown in a block by drilling method in April 2018. The experiment applied completely random design. Seedlings were counted for field emergence every two days for two months, when final emergence percentage and emergence index (as indicated in Eq. (1)) were determined. During the whole experimental period, the temperature is typically ranged from 5–22 °C, and total rainfall is about 112 mm.

**Data analysis**

The effect of seed lot and water potential or environment conditions (the control, water stress and salt stress) on seed germination percentage, germination rate (1/t\(_{50}\)), seedling emergence percentage and emergence index were tested by fitting generalized linear mixed models (GLMMs). Seed lot and water potential or environment conditions were included as fixed effects and replicates as a random effect. Seed germination percentage and seedling emergence percentage was a probability ranging from 0 to 1, hence, for the GLMMs of these two parameters, a binomial estimation of the model with a logit link function were applied. Duncan’s test was used to compare means when significant differences were found. Pearson correlation analysis was used to detect the relationship between \( \theta_H \), \( \Psi_b(50) \), \( \sigma_{\Psi_b} \) and germination, germination rate, emergence percentage, emergence index.

The hydrot ime constant \( \theta_H \) (MPa·h) and base water potential \( \Psi_b(g) \) (MPa) for a specific germination fraction (g) were calculated using the hydrot ime model (Gummerson, 1986; Bradford, 1990), More details about hydrot ime model see supporting information.

\[ \theta_H = [\Psi - \Psi_b(g)]t_g \]

\[ \text{probit}(g) = [\Psi - (\theta_H / t_g) - \Psi_b(50)] / \sigma_{\Psi_b} \]

**RESULTS**

**Effect of water potential on seed germination**

Seed lot (SL), water potential (WP) and their interactions had significant effects on germination percentage (GP) (Table 1). Germination percentage differed significantly across seed lots, except −0.1 MPa. With decreasing WP, GP decreased to some extent depending on the seed lot. For example, GP of seeds lots 3 and 6 decreased from 89% to 3% and from 91% to 33%, respectively, when WP decreased from −0.1 to −0.9 MPa.
Seed lot, water potential and their interactions had significant effects on germination rate (Fig. 1). Germination rate differed across seed lots at all water potentials, except −0.9 MPa. Germination rate decreased in response to decreasing water potential, and no seed lots germinated to 50% when sown at −0.9 MPa.

Hydrotime analysis for seed germination in response to water potential

The predicted germination time courses at the four water potentials generally fit the observed germination data very well, with $R^2$ values of 0.82–0.97 (Fig. 2; Table 2). Estimated values of hydrotime ($\theta_H$), base water potential [$\Psi_{b(50)}$] and $\sigma_{\phi b}$ differed among seed lots (Table 2). The value of $\theta_H$ ranged from 13.37 to 22.23 MPa-h, the $\Psi_{b(50)}$ varied from −0.8 to −0.6 MPa, and $\sigma_{\phi b}$ varied from 0.17 to 0.25.

Effect of water and salinity stress on seedling emergence in the greenhouse

Seed lot, environmental condition and their interactions had significant effects on seedling emergence percentage (EP) in the greenhouse (Table 3). Emergence percentage decreased with water and salinity stress compared to the control and differed significantly across the seed lots at each environmental condition. For example, seedling emergence in the control, water stress and salinity stress was 55%, 23% and 17%, respectively, for lot 3, while

| Seed lot | Water potential (MPa) |
|----------|-----------------------|
|          | −0.1                  | −0.3                  | −0.6                  | −0.9                  |
| 1        | 93.96 ± 0.04 Aabcd    | 94.58 ± 0.71 Aab      | 84.32 ± 1.68 Ba       | 12.00 ± 3.06 Cbcd     |
| 2        | 87.48 ± 1.27 Ae       | 79.22 ± 2.82 Ad       | 34.67 ± 4.67 Bc       | 6.08 ± 0.08 Ccd       |
| 3        | 89.33 ± 4.06 Ade      | 86.67 ± 1.33 Ac       | 26.81 ± 1.90 Bc       | 3.33 ± 2.40 Cd        |
| 4        | 98.00 ± 1.15 Aa       | 94.61 ± 2.39 Aab      | 83.49 ± 2.26 Aa       | 16.88 ± 7.91 Bbc      |
| 5        | 97.33 ± 1.33 Aab      | 95.28 ± 1.82 Aab      | 56.39 ± 15.81 Bb      | 10.00 ± 3.06 Cbcd     |
| 6        | 91.33 ± 1.76 Acde     | 92.00 ± 1.15 Aabc     | 86.67 ± 1.76 Aa       | 32.88 ± 2.89 Ba       |
| 7        | 95.28 ± 1.82 Aabc     | 98.00 ± 2.00 Aa       | 55.36 ± 6.03 Bb       | 18.84 ± 4.81 Cb       |
| 8        | 91.93 ± 1.21 Abcde    | 90.00 ± 3.06 Abc      | 53.70 ± 0.91 Bb       | 8.75 ± 2.88 Cbcd      |
| 9        | 93.33 ± 1.33 Aabcbd   | 91.28 ± 1.75 Aabc     | 60.67 ± 5.93 Bb       | 6.00 ± 2.31 Ccd       |
| 10       | 97.33 ± 0.67 Aab      | 94.49 ± 3.02 Aab      | 62.49 ± 5.76 Bb       | 11.33 ± 2.67 Cbcd     |

**Note:** Different uppercase indicated significant difference among different water potential for given seed lot at the 0.05 level ($P < 0.05$), different lowercase indicated significant difference among different seed lots for given water potential at the 0.05 level ($P < 0.05$). The effect of seed lot and water potential on seed germination were analyzed using generalized linear mixed models. Three replications for each treatment of each seed lot. Duncan’s test was used to compare means when significant differences were found.
Figure 1  Seed germination rate ($1/t_{50}$) of 10 seed lots of alfalfa in response to water potentials. Three replications for each treatment of each seed lot.

Figure 2  Germination time courses of 10 alfalfa seed lots. The symbols are the observed data and the curves are the results of fitting by the hydrotime model. Three replications for each treatment of each seed lot.
it was 81%, 46% and 29%, respectively, for lot 8. Seed lot and environment condition had significant effects on emergence index, however, there was no significant effect for their interactions (Table 3).

Table 2 Estimated hydrotime model parameters for 10 alfalfa seed lots.

| Seed lot | θ_H (MPa·h) | Ψ_b(50) (MPa) | σ_φ_b | R² |
|----------|--------------|----------------|--------|----|
| 1        | 17.00        | −0.74          | 0.22   | 0.82|
| 2        | 22.23        | −0.57          | 0.23   | 0.97|
| 3        | 18.65        | −0.55          | 0.20   | 0.93|
| 4        | 18.32        | −0.77          | 0.21   | 0.89|
| 5        | 15.54        | −0.68          | 0.17   | 0.94|
| 6        | 20.56        | −0.78          | 0.25   | 0.85|
| 7        | 16.18        | −0.68          | 0.24   | 0.90|
| 8        | 15.26        | −0.64          | 0.21   | 0.95|
| 9        | 13.37        | −0.63          | 0.19   | 0.89|
| 10       | 14.60        | −0.67          | 0.17   | 0.91|

Note: The θ_H is constant hydrotime, Ψ_b(50) is base water potential for 50% of seeds to germinate, σ_φ_b is standard deviation of Ψ_b(50). Coefficients determination (R²) is showing the goodness of fitness of the model fitting. Three replications for each treatment of each seed lot.

Table 3 Effect of environmental conditions on seedling emergence percentage (EP) and emergence index (EI) of 10 alfalfa seed lots.

| Seed lot | Control | Water stress | Salinity stress |
|----------|---------|--------------|----------------|
|          | EP (%)  | EI           | EP (%)         | EI           |
| 1        | 93.33 ± 2.40 Aa | 7.15 ± 0.85 Aa | 42.00 ± 2.00 Bab | 1.64 ± 0.20 Ba |
| 2        | 74.67 ± 7.68 Ab | 4.25 ± 0.70 Abc | 36.67 ± 0.67 Bb | 1.36 ± 0.11 Ba |
| 3        | 54.67 ± 2.67 Ac | 2.73 ± 0.46 Ac | 23.33 ± 2.40 Bc | 0.78 ± 0.06 Bb |
| 4        | 90.00 ± 5.29 Aab | 5.66 ± 0.74 Aab | 42.67 ± 3.53 Bab | 1.85 ± 0.31 Ba |
| 5        | 85.33 ± 5.46 Aab | 5.25 ± 0.39 Aab | 46.00 ± 3.06 Ba | 1.86 ± 0.13 Ba |
| 6        | 90.67 ± 3.33 Aa | 6.49 ± 0.86 Aa | 42.67 ± 2.67 Bab | 1.90 ± 0.20 Ba |
| 7        | 87.33 ± 1.76 Aab | 5.57 ± 0.23 Aab | 38.67 ± 2.91 Bab | 1.48 ± 0.01 Ba |
| 8        | 81.33 ± 7.33 Aab | 4.02 ± 0.50 Abc | 46.00 ± 4.00 Ba | 1.63 ± 0.25 Bc |
| 9        | 88.00 ± 4.00 Aab | 5.44 ± 0.42 Aab | 40.67 ± 2.91 Bab | 1.54 ± 0.07 Bb |
| 10       | 88.67 ± 3.33 Aab | 5.70 ± 0.62 Aab | 43.33 ± 1.33 Bab | 1.69 ± 0.11 Ba |

Source of variation | DF | EP | Chisq | P   | EI | Chisq | P   |
|--------------------|----|----|-------|-----|----|-------|-----|
| Seed lot (SL)      | 9  | 109.933 | <0.001 | 137.120 | <0.001 |
| Environmental conditions (EC) | 2  | 73.432 | <0.001 | 182.878 | <0.001 |
| SL × EC            | 18 | 36.683 | 0.006  | 64.685  | <0.001 |

Note: Different uppercase indicated significant difference among different stress treatments for given seed lot at the 0.05 level (P < 0.05), different lowercase indicated significant difference among different seed lots for given stress treatment at the 0.05 level (P < 0.05). The effect of seed lot and environmental conditions on seedling emergence percentage and emergence index were analyzed using generalized linear mixed models. Three replications for each treatment of each seed lot. Duncan’s test was used to compare means when significant differences were found.
Effect of field sowing on seedling emergence

There were significant differences among the 10 alfalfa seed lots sown in the field for seedling emergence percentage and index (Fig. 3). The seedling emergence percentage ranged from 34% for lot 3 to 71% for lot 6.

Correlation between hydrotime parameters, seed germination and seedling emergence

The results indicated a correlation between traits of germination, emergence and hydrotime model parameters (Table 4). There was a negative significant relationship between base water potential $\Psi_{b(50)}$ and germination percentage ($r = -0.983, P < 0.001$), germination rate ($r = -0.746, P = 0.013$), emergence percentage ($r = -0.807, P = 0.005$; $r = -0.624, P = 0.054$; $r = -0.834, P = 0.003$), emergence index ($r = -0.841, P = 0.002$; $r = -0.813, P = 0.004$; $r = -0.927, P < 0.001$) under control condition, water stress and salinity stress, respectively, and between base water potential $\Psi_{b(50)}$, field emergence percentage ($r = -0.751, P = 0.012$) and index ($r = -0.728, P = 0.017$). On the other hand, there was no relationship between $\theta_{Hi}$, $\sigma_{\psi b}$ with germination, emergence traits.

**DISCUSSION**

Drought and salinity are the main factors affecting seedling establishment as well as crop production across the world (McMillan, 1971; Isselstein, Tallowin & Smith, 2002; Gu et al., 2018). Our study clearly showed that drought stress significantly decreased seed germination in the laboratory and seedling emergence in the greenhouse. Moreover, seedling emergence in the field was far less than the potential germination capacity of the seeds, suggesting that harsh environmental conditions play a key role in determining seedling establishment and consequently production of alfalfa hay on the Loess Plateau (Zhao, Liu & Li-Jie, 2005; Yang & Yong, 2010; Li, Zhang & Sun, 2015). However, the detrimental effect of stress can be alleviated by high quality seeds, e.g., seedling emergence of 10 lots ranged from 30–70% in the field, although all seed lots germinated to higher than 87% in the laboratory. Further, germination performance under moderately stressful conditions.
conditions in the lab failed to detect seed quality difference among seed lots, and thus germination percentage was not a good indicator of seed vigor in our study. This result is consistent with those of Pan et al. (2017) and Wang et al. (2003) who found that standard germination tests are poor in ranking seed quality, and they show no significant correlation with seed/seedling performance in the field. Interestingly, the germination percentage differed more as the water potential decreased from −0.1 to −0.6 MPa, suggesting germination at appropriate stressful conditions is helpful in distinguishing seed quality of alfalfa. In our study, −0.6 MPa was an ideal germination condition for seed quality evaluation, since the highest variation among seeds lots was observed under this level of stress.

Hydrotime models are used to describe the dynamics of seed germination in response to reduced water availability (Windauer, Altuna & Benech-Arnold, 2007; Cardoso & Bianconi, 2013), and the model allowed a better description of the seed germination time courses at reduced osmotic potential in carob and mangroves (Cavallaro et al., 2016; Torabi et al., 2016; Farahinia et al., 2017; Wijayasinghe et al., 2016). In agreement with these studies, our study clearly showed that hydrotime model also could describe the germination time course of different alfalfa seed lots at various water potentials very well, with R² values of 0.82–0.97 (Fig. 2; Table 2). A distinct advantage of the hydrotime model for seeds incubated across a range of water potentials is that variation or similarity in germination among seed lots can be ascribed to specific underlying factors such as $\theta_H$, $\Psi_{b(50)}$, and $\sigma_{\Psi_b}$ (Allen et al., 2000). Dahal & Bradford (1990) showed that seeds with lower

| Variable | $\theta_H$ (MPa•h) | $\Psi_{b(50)}$ (MPa) | $\sigma_{\Psi_b}$ |
|----------|------------------|----------------------|------------------|
| Laboratory | Germination (%) | −0.152 | −0.983 (<0.001) | 0.206 |
|          | Germination rate (h⁻¹) | −0.641 (0.046) | −0.746 (0.013) | −0.204 |
| Greenhouse | Control condition | Emergence (%) | −0.317 | −0.807 (0.005) | 0.086 |
|          | Emergence index | −0.136 | −0.841 (0.002) | 0.196 |
|          | Water stress | Emergence (%) | −0.368 | −0.624 (0.054) | −0.120 |
|          | Emergence index | −0.185 | −0.813 (0.004) | −0.011 |
|          | Salinity stress | Emergence (%) | −0.124 | −0.834 (0.003) | 0.280 |
|          | Emergence index | −0.039 | −0.927 (<0.001) | 0.320 |
| Field | Field experiment | Emergence (%) | −0.457 | −0.751 (0.012) | −0.043 |
|          | Emergence index | −0.424 | −0.728 (0.017) | 0.129 |

Note: The $\theta_H$ is constant hydrotime, $\Psi_{b(50)}$ is base water potential for 50% of seeds to germinate, $\sigma_{\Psi_b}$ is standard deviation of $\Psi_{b(50)}$. Numbers in parentheses indicate the significance of the coefficients. Three replications for each treatment of each seed lot.

Table 4 Pearson correlation coefficient between overall mean of seed germination percentage and rate (mean of four levels of water potential and three replicates), emergence percentage and index and hydrotime parameters ($\theta_H$, $\Psi_{b(50)}$, $\sigma_{\Psi_b}$) at different environmental conditions of control, water stress, salinity stress and field sowing for 10 alfalfa seed lots.
Ψ_b(g) and θ_H values generally had higher vigor than those with high values, which allowed seeds to germinate rapidly under stressful condition. Consistent with this, seed lot 6 with the lowest value of Ψ_b(50) had the highest seedling establishment, while seed lot 3 with the highest Ψ_b(50) had the lowest seedling establishment in the field. Moreover, our study showed significant correlations between Ψ_b(50) and seed germination and seedling emergence under water stress conditions, further supporting the assumption that tolerance of alfalfa seed lots to stressful conditions can be identified by a decrease in Ψ_b(50). Thus, Ψ_b(50) can be applicable for predicting early seed vigor and seedling establishment in the field.

On the other hand, no correlation between θ_H or σφ_b and seedling emergence was observed in our study. A possible reason is that a lower θ_H favors rapid and uniform germination; however, this occurs only when seeds are subjected to moist conditions. According to the hydrotime definition, when external water potential approaches or is lower than Ψ_b(50), seeds will accumulate the hydrotime units very slowly, which overrides the advantage of low θ_H. On the Loess Plateau, seedling emergence after sowing is completely dependent on the unpredictable rainfall, and drought frequently occurs even during the rainy season. This unpredictable drought stress may partly explain why Ψ_b(50) rather than θ_H contribute to the difference in seedling establishment among seed lots. Moreover, no correlation between θ_H and Ψ_b(50) was observed in our study, suggesting that the role of these two parameters may act independently in regulating seed germination and seedling emergence in the field. It seems reasonable to hypothesis that seeds with low θ_H will have an advantage in moist conditions, while seeds with low Ψ_b(50) will have an advantage during drought stress.

CONCLUSION
In brief, our study clearly showed that hydrotime model can describe the germination time course of alfalfa seeds in response to various water potentials very well. Ψ_b(50) can be used to rank alfalfa seed vigor and thus predict seedling emergence and speed under water stress conditions. However, it is also worth noting that current study only test seedling emergence in the field once, and a replicate experiment across sites and years is needed to confirm this conclusion.

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ADDITIONAL INFORMATION AND DECLARATIONS

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**Competing Interests**
The authors declare that they have no competing interests.

**Author Contributions**
- Xianglai Chen performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Zhichao Wei performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Dali Chen performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Xiaowen Hu conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

**Data Availability**
The following information was supplied regarding data availability:
The raw measurements are available in the Supplemental File.

**Supplemental Information**
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13206#supplemental-information.

**REFERENCES**
Allen PS, Thorne ET, Gardner JS, White DB. 2000. Is the barley endosperm a water reservoir for the embryo when germinating seeds are dried? *International Journal of Plant Sciences* 161(2):195–201 DOI 10.1086/314247.
Bradford KJ. 1990. A water relations analysis of seed germination rates. *Plant Physiology* 94(2):840–849 DOI 10.1104/pp.94.2.840.
Bradford KJ. 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science* 50:248–260 DOI 10.1614/0043-1745(2002)050[0248:AOHT]2.0.CO;2.
Cardoso VJM, Bianconi A. 2013. Hydrotime model can describe the response of common bean (Phaseolus vulgaris L.) seeds to temperature and reduced water potential. *Acta Scientiarum. Biological Sciences* 35(2):255–261 DOI 10.4025/actascibiolsci.v35i2.15393.
Castillo-Lorenzo E, Finch-Savage WE, Seal CE, Pritchard HW. 2019. Adaptive significance of functional germination traits in crop wild relatives of Brassica. Agricultural and Forest Meteorology 264:343–350 DOI 10.1016/j.agrformet.2018.10.014.

Cavallaro V, Barbera AC, Maucieri C, Gimma G, Scalisi C, Patanè C. 2016. Evaluation of variability to drought and saline stress through the germination of different ecotypes of carob (Ceratonia siliqua L.) using a hydrotime model. Ecological Engineering 95:557–566 DOI 10.1016/j.ecoleng.2016.06.040.

Dahal P, Bradford KJ. 1990. Effects of priming and endosperm integrity on seed germination rates of tomato genotypes: II. Germination at reduced water potential. Journal of Experimental Botany 41(11):1441–1453 DOI 10.1093/jxb/41.11.1441.

Dahal P, Bradford KJ. 1994. Hydrothermal time analysis of tomato seed germination at suboptimal temperature and reduced water potential. Seed Science Research 4(2):71–80 DOI 10.1017/s096025850000204x.

Farahinia P, Sadat-Noori SA, Mortazavian MM, Soltani E, Foghi B. 2017. Hydrotime model analysis of Trachyspermum ammi, (L.) Sprague seed germination. Journal of Applied Research on Medicinal and Aromatic Plants 5(6):88–91 DOI 10.1016/j.jarmap.2017.04.004.

Farzane S, Soltani E. 2011. Relationships between hydrotime parameters and seed vigor in sugar beet. Seed Science and Biotechnology 5:7–10.

Gong ZT. 1999. Chinese soil taxonomy: theory, method and application. Beijing: Science Press.

Gu RT, Zhou Y, Song XY, Xu SC, Zhang XM, Lin HY, Xu S, Zhu SY. 2018. Effects of temperature and salinity on, Ruppia sinensis, seed germination, seedling establishment, and seedling growth. Marine Pollution Bulletin 134(1):177–185 DOI 10.1016/j.marpolbul.2017.08.013.

Guan YJ, Yin MQ, Jia XW, An JY, Wang C, Pan RH, Song WJ, Hu J. 2018. Single counts of radicle emergence can be used as a vigour test to predict seedling emergence potential of wheat. Seed Science and Technology 46(2):349–357 DOI 10.15258/sst.2018.46.2.15.

Gummerson RJ. 1986. The effect of constant temperatures and osmotic potentials on the germination of sugar beet. Journal of Experimental Botany 37:729–741 DOI 10.1093/jxb/37.6.729.

Guo ZG, LiuHX, Wang SM, Tian FP, Cheng GD. 2005. Biomass, persistence and drought resistance of nine lucerne varieties in the dry environment of west China. Australian Journal of Experimental Agriculture 45(1):59–64 DOI 10.1071/EA03119.

Hampton JG, Leeks CRF, McKenzie BA. 2009. Conductivity as a vigour test for Brassica species. Seed Science and Technology 37:214–221 DOI 10.15258/sst.2009.37.1.24.

Hu XW, Ding XY, Baskin CC, Wang YR. 2018. Effect of soil moisture during stratification on dormancy release in seeds of five common weed species. Weed Research 58(3):210–220 DOI 10.1111/wre.12297.

Hu XW, Fan Y, Baskin CC, Baskin JM, Wang YR. 2015. Comparison of the effects of temperature and water potential on seed germination of Fabaceae species from desert and subalpine grassland. American Journal of Botany 102(5):649–660 DOI 10.3732/ajb.1400507.

Isselstein J, Tallowin JRB, Smith REN. 2002. Factors affecting seed germination and seedling establishment of fen-meadow species. Restoration Ecology 10(2):173–184 DOI 10.1046/j.1526-100x.2002.00045.x.

ISTA. 2014. International rules for seed testing. Bassersdorf, Switzerland: International Seed Testing Association.
Jonathan ME, Bárbara CFS, Silvia RSS, João AAG, Maria CJLA, Marcelo FP. 2013. Germination responses of *Jatropha curcas* L. seeds to storage and aging. *Industrial Crops and Products* 44:684–690 DOI 10.1016/j.indcrop.2012.08.035.

Kim SH, Lee MH, Kang KH, Park MW, Shim SI, Chung JS, Na YW. 2013. Verification of several seed vigor test methods to predict field emergence of sorghum (*Sorghum bicolor* L. Moench). *Journal of the Korean Society of International Agriculture* 25:56–61 DOI 10.12719/KSIA.2013.25.1.56.

Kong M, Kang J, Han CL, Gu YJ, Siddique KHM, Li FM. 2020. Nitrogen, phosphorus, and potassium resorption responses of alfalfa to increasing soil water and P availability in a semi-arid environment. *Agronomy* 10(2):310 DOI 10.3390/agronomy10020310.

Li R, Min DD, Chen LJ, Chen CY, Hu XW. 2017. Hydropriming accelerates seed germination of *Medicago sativa* under stressful conditions: a thermal and hydrotime model approach. *Legume Research* 40:741–747 DOI 10.18805/lr.v40i0.8404.

Li Z, Zhang W, Sun Z. 2015. Yield and water use efficiency of non- and single-irrigated alfalfa with ridge and furrow planting in northern china. *Agronomy Journal* 107(3):1039–1047 DOI 10.2134/agronj14.0419.

Lv Y, Wang Y, Powell A. 2016. Frequent individual counts of radicle emergence and mean just germination time predict seed vigour of *Avena sativa* and *Elymus nutans*. *Seed Science and Technology* 44(1):189–198 DOI 10.15258/sst.2016.44.1.08.

Ma AS, Liu SC, Lv JL, Quan DG, Guo WL, Zhao Y. 2005. Moisture characteristics and energy balance of several soils in the Loess Plateau. *Journal of Northwest A & F University* 11:123–126 (in Chinese with English abstract) DOI 10.13207/j.cnki.jnwafu.2005.11.023.

Matthews S, Demir I, Celikkol T, Kenanoglu BB, Mavi K. 2009. Vigour tests for cabbage seeds using electrical conductivity and controlled deterioration to estimate relative emergence in transplant modules. *Seed Science and Technology* 37(3):736–746 DOI 10.15258/sst.2009.37.3.20.

Mcdonald MB, Phaneendranath BR. 1978. A modified accelerated aging seed vigor test for soybeans. *Journal of Seed Technology* 3:27–37.

McMillan C. 1971. Environmental factors affecting seedling establishment of the black mangrove on the central Texas coast. *Ecology* 52(5):927–930 DOI 10.2307/1936046.

Michel BE, Kaufmann MR. 1973. The osmotic potential of polyethylene glycol 6000. *Plant Physiology* 51(5):914–916 DOI 10.1104/pp.51.5.914.

Mondo VHV, Cicero SM, Douradoneto D, Pupim TL, Dias MAN. 2013. Effect of seed vigor on intraspecific competition and grain yield in maize. *Agronomy Journal* 105(1):222–228 DOI 10.2134/agronj2012.0261.

Pan J, Li R, Guo YX, Hu XW. 2017. Comparison of methods for determination of alfalfa seed vigor. *Prataculturae Science* 34:1042–1048 (in Chinese with English abstract) DOI 10.11829/j.issn.1001-0629.2016-0259.

Patanè C, Tringali S. 2011. Hydrotime analysis of Ethiopian Mustard (*Brassica carinata* A. Braun) seed germination under different temperatures. *Journal of Agronomy and Crop Science* 197(2):94–102 DOI 10.1111/j.1439-037X.2010.00448.x.

Sharma AD, Rathore SVS, Srinivasan K, Tyagi RK. 2014. Comparison of several seed priming methods for seed germination, seedling vigour and fruit yield in okra (*Abelmoschus esculentus* L. Moench). *Scientia Horticulturae* 165:75–81 DOI 10.1016/j.scienta.2013.10.044.

Sibelle SDS, Roberval DV, Camila RDSG, Tereza CDC, Maristela P. 2012. Electrical conductivity of different common bean seeds genotypes. *Journal of Seed Science* 35(2):216–224 DOI 10.1590/S2317-15372013000200011.
Soltani E, Adeli R, Akbari GA, Ramshini H. 2017. Application of hydrot ime model to predict early vigour of rapeseed (Brassica napus L.) under abiotic stresses. *Acta Physiologiae Plantarum* 39:252 DOI 10.1007/s11738-017-2552-0.

Soltani E, Farzaneh S. 2014. Hydrot ime analysis for determination of seed vigour in cotton. *Seed Science and Technology* 42(2):260–273 DOI 10.15258/sst.2014.42.2.14.

Souza FFJ, Spehar CR, Souza NOS, Fagioli M, Souza RTG, Borges SRS. 2017. Accelerated ageing test for the evaluation of quinoa seed vigour. *Seed Science and Technology* 45(1):212–221 DOI 10.15258/sst.2017.45.1.18.

Steinmaus SJ, Prather TS, Holt JS. 2000. Estimation of base temperatures for nine weed species. *Journal of Experimental Botany* 51:275–286 DOI 10.1093/jexbot/51.343.275.

Su YZ. 2007. Soil carbon and nitrogen sequestration following the conversion of cropland to alfalfa forage land in northwest China. *Soil & Tillage Research* 92(1–2):181–189 DOI 10.1016/j.still.2006.03.001.

Tohidloo G, Kruse M. 2009. Development of an image analysis aided seedling growth test for winter oilseed rape and verification as a vigour test. *Seed Science and Technology* 37(1):98–109 DOI 10.15258/sst.2009.37.1.12.

Torabi B, Soltani E, Archontoulis SV, Rabii A. 2016. Temperature and water potential effects on Carthamus tinctorius L. seed germination: measurements and modeling using hydrothermal and multiplicative approaches. *Brazilian Journal of Botany* 39(2):427–436 DOI 10.1007/s40415-015-0243-x.

Wang YR, Ling YU, Liang LY, Xing SY. 2003. Vigour tests used to assess quality of seed lots and field emergence for several forage species. *Acta Pratacultural Science* 12:62–69 (in Chinese with English abstract) DOI 10.2135/cropsci2004.0535.

Wang WQ, Liu SJ, Song SQ. 2015. Proteomics of seed development, desiccation tolerance, germination and vigor. *Plant Physiology and Biochemistry* 86(6):1–15 DOI 10.1016/j.plaphy.2014.11.003.

Wijayasinghe MM, Jayasuriya KG, Gunatilleke CVS, Gunatilleke IAUN, Walck JL. 2016. Effect of salinity on seed germination of five mangroves from Sri Lanka: use of hydrot ime modelling for mangrove germination. *Seed Science Research* 29(1):55–63 DOI 10.1017/S0960258518000405.

Windauer L, Altuna A, Benech-Arnold R. 2007. Hydrot ime analysis of Lesquerella fendleri seed germination responses to priming treatments. *Industrial Crops and Products* 25(1):70–74 DOI 10.1016/j.indcrop.2006.07.004.

Xia FS, Wang YC, Wang F, Wang CC, Zhu HS, Liu M, Huai YM, Dong KH. 2020. Effect of boron priming on the seed vigour in different varieties of alfalfa (Medicago sativa L.). *Legume Research* 43(OF):415–420 DOI 10.18805/lr-484.

Yang B, Yong XU. 2010. Topographic differentiation simulation of alfalfa yield and soil and water loss in the loess plateau. *Progress in Geography* 29:530–534 (in Chinese with English abstract) DOI 10.11820/dlkxjz.2010.05.003.
Zhang R, Luo K, Chen DL, Baskin JM, Baskin CC, Wang YR, Hu XW. 2020. Comparison of thermal and hydrot ime requirements for seed germination of seven Stipa species from cool and warm habitats. *Frontiers in Plant Science* **11**:560714 DOI 10.3389/fpls.2020.560714.

Zhao YY, Liu WZ, Li-Jie PU. 2005. Effect of alfalfa growth on soil water environment in hill-gully area of the loess plateau. *Journal of Natural Resources* **20**:85–91 (in Chinese with English abstract) DOI 10.11849/zrzyxb.2005.01.012.