

RESEARCH ARTICLE

# Comparison of prechilling stratification and sulfuric acid scarification on seed germination of *Panicum virgatum* under drought stress

Nan WANG (✉)<sup>1,2</sup>, Jing GAO<sup>3</sup>, Suiqi ZHANG<sup>2</sup>, Feng YAN<sup>2</sup>

**1** Shaanxi Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Shaanxi University of Chinese Medicine, Xianyang 712083, China

**2** Institute of Soil and Water Conservation, Northwest A&F University, Yangling 712100, China

**3** College of Pharmacy, Shaanxi University of Chinese Medicine, Xianyang 712046, China

**Abstract** In semi-arid regions of the Loess Plateau, water deficiency restricts plant performance. *Panicum virgatum* (switchgrass), which is a highly versatile grass, had been introduced to the Plateau as a restoration species. To determine if prechilling stratification (PCS) and sulfuric acid scarification (SAS) can optimize establishment, *P. virgatum* cvs Pathfinder, Trailblazer and Alamo were tested under different ambient water potentials by measuring germination and root and shoot growth along water potential gradients under laboratory conditions. Both PCS and SAS improved total germination percentage (TGP), with PCS being more beneficial. The effect of PCS and SAS on mean germination time (MGT) weakened gradually with increasing drought stress. Both PCS and SAS showed no obvious effect on promoting root and shoot growth. Both PCS and SAS reduced base water potential requirement for reaching 50% germination of Pathfinder and Trailblazer, with this effect greater for PCS. These results indicate that embryo dormancy may be a major factor limiting germination of *P. virgatum* under drought conditions. Pathfinder appears to be more suitable for a semi-arid environment, whereas Alamo appears to be unsuitable for drought conditions. Given the large difference between predicted value and measured value, the reliability and applicable scope of linear regression estimated  $\Psi_{50}$  needs further investigation, specification and optimization.

**Keywords** base water potential, data analysis method, embryo growth, germination

## 1 Introduction

*Panicum virgatum* (switchgrass), native to North America, is a highly versatile grass, used for ecology restoration, livestock forage feed and bioenergy<sup>[1]</sup>. In recent years, switchgrass has been introduced as a restoration species on the Loess Plateau of China<sup>[2]</sup>, where habitat destruction has increased due to the limited and erratic precipitation. Furthermore, the Loess Plateau is considered to have potential for bioenergy production using plants also useful as livestock forage<sup>[3]</sup>. Thus, increasing attention is being given to the cultivation of switchgrass on the Loess Plateau<sup>[4]</sup>.

Although switchgrass has been widely adopted, seed dormancy is a major factor limiting plant establishment, causing delayed and sporadic emergence, limiting the benefits of commercial adoption<sup>[5]</sup>. Research has focused on methods to effectively break switchgrass dormancy<sup>[6,7]</sup>, and has shown that breaking both seed coat dormancy and embryo dormancy can be effective<sup>[5]</sup>. However, on the Loess Plateau, soil water is often insufficient to support seed germination, resulting in reduced plant populations. Therefore, determining which method for breaking switchgrass seed dormancy is effective under drought stress has become a research priority for the Loess Plateau.

Switchgrass that is a polymorphic plant species with two distinct ecotypes, lowland and upland, and two main ploidy levels, tetraploid and octoploid<sup>[8]</sup>. Several studies have compared the practicality of methods for breaking seed dormancy of switchgrass ecotypes under benign conditions<sup>[5,9,10]</sup>. Others have examined how germination of one particular ecotype responds to various treatments<sup>[6,11,12]</sup>. However, relatively few studies have examined if and how germination of switchgrass cultivars differ under varied environmental conditions, even though such information is crucial for selecting effective

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Correspondence: [gousouth@hotmail.com](mailto:gousouth@hotmail.com)

techniques and suitable seed of switchgrass for specific situations.

Numerous mathematical models for data analysis and prediction of seed germination responses under varied environmental conditions have been reported<sup>[13,14]</sup>. A number of models for calculating the time taken to reach 50% emergence ( $t_{50}$ ) have been built and frequently used<sup>[15–17]</sup>. Recently,  $t_{50}$  was recognized as a key parameter for calculating the theoretical base water potential for obtaining 50% germination ( $\Psi_{50}$ ) of individual cultivars<sup>[17]</sup>. However, it is not known if  $t_{50}$  estimated by modeling provides an appropriate parameter for calculating  $\Psi_{50}$  for switchgrass, therefore the reliability of calculated  $\Psi_{50}$  for switchgrass needs to be tested.

In this study, a comparison of two seed treatments on germination and embryo growth at optimal temperature along water potential gradients were investigated for three switchgrass cultivars including both ecotypes. The aim was to verify if and how the treatments change plant growth during the early stages of development and to examine value of  $\Psi_{50}$  estimated from  $t_{50}$ .

## 2 Materials and methods

### 2.1 Seed treatment and experimental design

The study was conducted with seed of *P. virgatum* cvs Pathfinder (octoploid), Trailblazer (octoploid) and Alamo (tetraploid). The first two cultivars are classified as upland ecotype, and the last one as lowland ecotype. The seed was harvested from the Ansai Research Station of the Chinese Academy of Science. The mean 1000 seed weight for Pathfinder, Trailblazer and Alamo were  $1.68 \pm 0.05$ ,  $1.85 \pm 0.07$  and  $1.23 \pm 0.03$  g, respectively. The seed was one year old and had been stored in paper bags under cool and dry laboratory conditions. Before use, the seed was immersed in 1% sodium hypochlorite for 1 min then soaked in 75% ethanol for 3 min, and rinsed in distilled water and dried.

Prechilling stratification and sulfuric acid scarification were used to promote seed germination. Prechilling stratification was performed by refrigerating seed at 5°C for 14 days, after which the seed was washed in distilled water and air-dried. Sulfuric acid scarification was performed by soaking seed in 8 mol·L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> for 5 min, after which the seed was washed in distilled water and air-dried. Untreated seed was used as the control (CK).

Six water potentials (0, -0.1, -0.2, -0.3, -0.4 and -0.5 MPa) adjusted by polyethylene glycol (PEG 6000) were used. PEG solutions were prepared by different concentrations of PEG 6000 in deionized water according to the empirical equation<sup>[18]</sup>.

Samples of 90 seeds (three replicates of 30 seeds each) were placed on two filter papers in 90-mm Petri dishes with

PEG solutions. Seeds were allowed to germinate at a constant temperature of 30°C, the temperature considered the optimal for germination of switchgrass<sup>[19]</sup>, in a thermostatically controlled incubator ( $\pm 1^\circ\text{C}$ ). The Petri dishes were sealed with Parafilm and placed in the dark. Germinated seed were defined as those where the root reached approximately 2 mm length. Data were collected daily until no additional germination occurred. The length of the roots and shoots of germinated seeds were measured 3 d after germination.

### 2.2 Measurement and verification

Total germination percentage (TGP) and mean germination time (MGT) were calculated at the end of the experiment using the following equations:

$$\text{TGP (\%)} = \left(\frac{n}{N}\right) \times 100 \quad (1)$$

where  $n$  is the number of germinated seeds and  $N$  is the total number of seeds placed in the dish to germinate, and

$$\text{MGT (days)} = \sum \frac{(n_i \times t)}{n} \quad (2)$$

where  $n_i$  is the number of germinated seeds at day  $t$  and  $n$  is the number of germinated seeds.

The following three equations were used to calculate the theoretical time to 50% emergence ( $t_{50}$ ).

Equation (1), sigmoidal model:

$$y = \frac{a}{1 + \left(\frac{x}{x_0}\right)^b} \quad (3)$$

where  $a$  is the peak value of  $y$  (maximum seed germination),  $x$  is days after seed germination,  $x_0$  is days to achieve 50% of maximal seed germination,  $b$  is a calculated parameter for the curve in this sigmoidal model. Parameters were calculated by SigmaPlot 12.5 (Systat Software Inc., CA, USA). The  $x$  value on the curve, which corresponds to 50% of cumulative seed germination ( $y$  values of the curve), was considered the theoretical time to 50% germination ( $t_{50}$ ) in treatments<sup>[20]</sup>.

Equation (2), Farooq's model:

$$t_{50} = t_i + \frac{[(N/2) - n_i](t_j - t_i)}{n_j - n_i} \quad (4)$$

where  $N$  is the final germinated number of seeds,  $n_i$  and  $n_j$  are the cumulative number of seeds germinated between adjacent counts at times  $t_i$  and  $t_j$  when  $n_i < N/2 < n_j$ <sup>[16]</sup>.

Equation (3), Mo's model:

$$\text{Probit (GP)} = a + b \lg X \quad (5)$$

where Probit (GP) is probability unit of seed germination percentage and  $X$  is days of experiment. The  $a$  and  $b$  are

estimated from observed values of Probit (GP),  $\lg X$  and then  $t_{50}$  is predicted by the equation<sup>[15]</sup>.

### 2.3 Statistical analyses

A linear regression of the reciprocal of  $t_{50}$  versus water potential was used to calculate the theoretical base water potential to reach 50% germination ( $\Psi_{50}$ ) for each cultivar according to the value of abscissa intercept of regression line<sup>[17]</sup>.

The data were analyzed using the statistical package SPSS 13 (IBM, Armonk, NY, USA). One-way analysis of variance (ANOVA), followed by the least significant difference test, was applied to compare treatment means. The treatment effects were considered to be significant at  $P < 0.05$ .

## 3 Results

TGP was negatively correlated with decreasing water potential of the PEG solutions (Fig. 1; Table 1). Both prechilling stratification and sulfuric acid scarification increased TGP, and the increase with prechilling stratification on TGP was greater regardless of whether drought stress was imposed. TGP exhibited large variations among the three cultivars. Specifically, TGP of Pathfinder ranged from 88.4% to 26.7%. The highest TGP for Pathfinder corresponded to prechilling stratification without drought stress, and the lowest to  $-0.5$  MPa water potential stresses without seed priming. TGP of Trailblazer ranged from 71.1% to 18.9%. The highest TGP for Trailblazer corresponded to prechilling stratification without drought stress and prechilling stratification with  $-0.1$  MPa water potential stress, and the lowest to  $-0.5$  MPa water potential stresses without seed priming. TGP of Alamo ranged from 38.9% to 8.9%. The highest TGP for Alamo corresponded to prechilling stratification without drought stress, and the lowest to  $-0.5$  MPa water potential stresses without seed priming.

MGT was progressively delayed by increasing drought stress (Table 1). The effects of prechilling stratification and sulfuric acid scarification on MGT weakened gradually and then disappeared with increasing drought stress. There were significant effects of drought stress on root and shoot growth. The negative effects of drought stress were increased gradually with decreasing water potential. Both prechilling stratification and sulfuric acid scarification had no obvious effect on promoting root and shoot growth regardless of whether drought stress was applied. There was large difference between  $t_{50}$  estimated by three equations (Table 2).

The linear regression of  $t_{50}$  against  $\Psi$  was used to calculate the minimum  $\Psi$  for 50% germination in each treatment. For Pathfinder, thresholds of  $-1.34$ ,  $-1.47$  and  $-0.47$  MPa were estimated by the sigmoidal model for

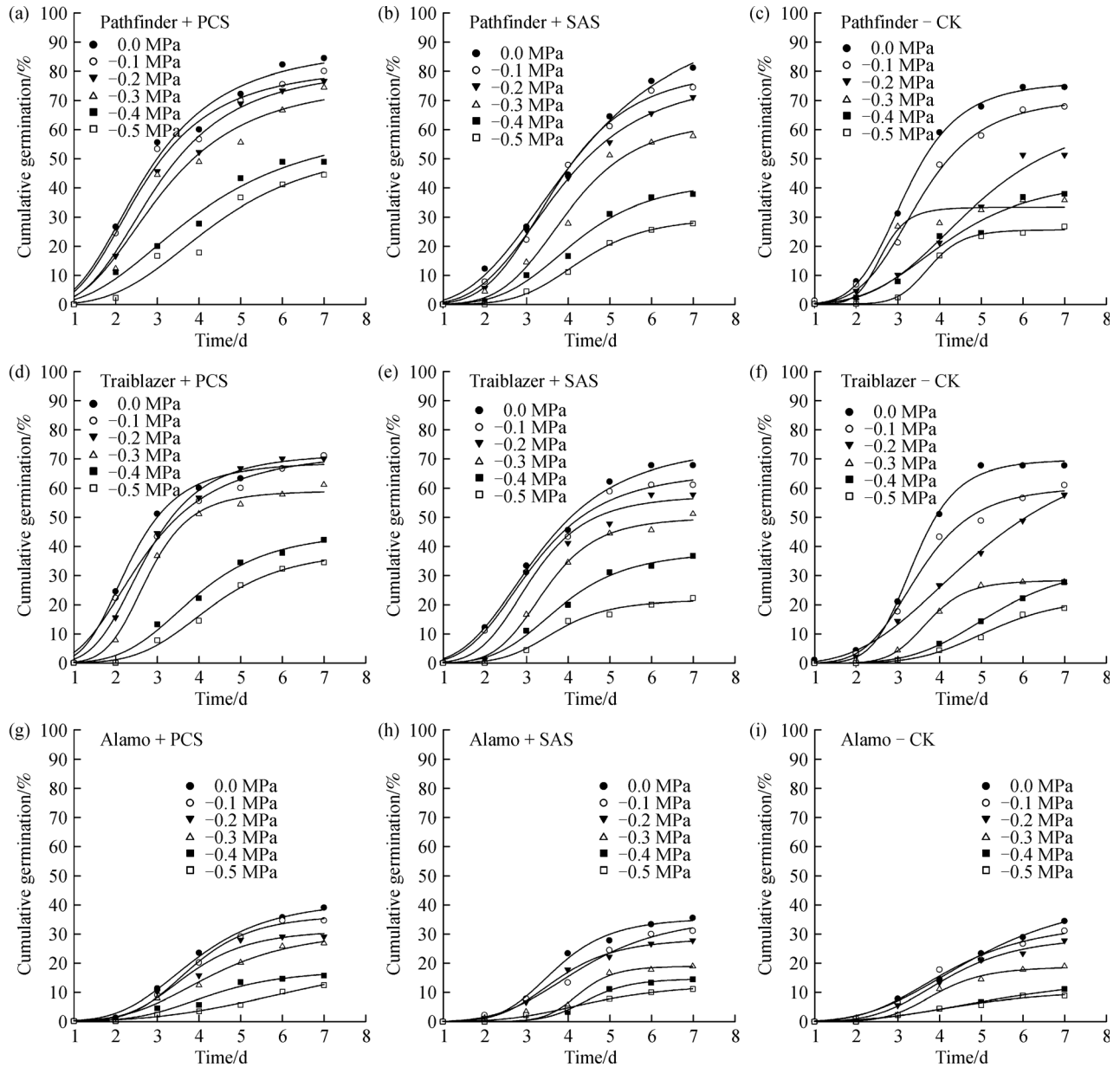
prechilling stratification treated group, sulfuric acid scarification treated group and CK, respectively,  $-2.79$ ,  $-4.47$  and  $-0.77$  MPa using Farooq's model, and  $-2.28$ ,  $-1.87$  and  $-1.15$  MPa using Mo's model (Fig. 2). For Trailblazer, thresholds of  $-1.46$ ,  $-0.81$  and  $-0.54$  MPa were estimated using the sigmoidal model for prechilling stratification treated group, sulfuric acid scarification treated group and CK, respectively,  $-2.47$ ,  $-2.36$  and  $-0.85$  MPa using Farooq's model, and  $-3.15$ ,  $-1.55$  and  $-1.33$  MPa using Mo's model (Fig. 2).

## 4 Discussion

This study focused on the variation in germination characteristics and early embryo growth of three cultivars of switchgrass, treated with prechilling stratification or sulfuric acid scarification and then exposed to reduced water potentials in PEG solution under laboratory conditions. A similar approach had been used to explore the effects of temperature stratification and acid scarification on seed germination in *Lupinus sulphureus* ssp. *kincaidii*<sup>[21]</sup>, *Prunus serotina*<sup>[22]</sup> and *Ribes multiflorum* ssp. *sandaliticum*<sup>[23]</sup>. However, relatively few reports have been published on the influence of water potential on the effects of temperature stratification and acid scarification.

Germination is known as a critical and sensitive stage in the life cycle of plants, and unsuitable environmental conditions, such as drought, greatly influence subsequent seedling establishment<sup>[24]</sup>. Our results showed that the TGP, and the length of root and shoot gradually declined with increasing drought intensity (Table 1). Drought restricts seed imbibition, reduces seed metabolism activation and delays seed germination<sup>[17]</sup>, and decreased elongation of root and shoot is caused by a progressively increased gap between optimal water potential for embryo growth and ambient water potential<sup>[25]</sup>. Among environmental variables, soil water availability is a critical factor controlling species habitat suitability and precipitation can partially determine whether the habitat is humid enough for a specific species. Soil moisture availability is low on much of the Loess Plateau, so planting more drought tolerant cultivars of switchgrass is appropriate without suitable priming treatment and irrigation.

The beneficial effects of seed priming on germination with increased drought stresses have been reported for various plant species, such as Chinese cabbage<sup>[26]</sup>, sunflower<sup>[27]</sup> and sweet sorghum<sup>[20]</sup>. Dormancy imposed by seed coats can be alleviated by acid scarification<sup>[28]</sup>, while stratification can also break seed embryo dormancy<sup>[29]</sup>. In the present study, prechilling stratification enhanced TGP for Pathfinder and Trailblazer under all treatments, and sulfuric acid scarification also enhanced TGP in Pathfinder and Trailblazer in most treatments (Table 1). These results indicate the existence of both embryo and seed coat dormancy in these cultivars. Similar



**Fig. 1** Cumulative germination time courses in polyethylene glycol (PEG 6000) with different treatments at 30°C for seeds of *Panicum virgatum* (switchgrass) cv. Pathfinder (a, b and c), cv. Trailblazer (d, e and f) and cv. Alamo (g, h and i). Symbols represent the observed percentages with time at each water potential. PCS, prechilling stratification; SAS, sulfuric acid scarification; CK, control.

conclusions also were drawn in previous studies with other switchgrass cultivars, Cave-in-Rock<sup>[30]</sup> and Kanlow<sup>[31]</sup>. Indeed, the promotion of TGP was better with stratification treatments in this study (Table 1). Furthermore, the effects of acid scarification treatment on TGP displayed a gradual decline with increasing osmotic stress (Table 1). Drought and the impermeable barrier imposed by seed coats can prevent root emergence due to insufficient embryo development water potential and water soluble inhibitors<sup>[5]</sup>, resulting in extended embryo dormancy. Thus, breaking embryo dormancy is the preferred method for promoting seed germination in arid environments. In

addition, Alamo had limited response to both methods tested in the present study (Table 1), which was consistent with a previous study<sup>[32]</sup>, suggesting that, for wet sites species, some seed priming methods may not offset the germination limiting factors provided by water deficiency.

MGT can be shortened by seed priming<sup>[27]</sup>. Our results were consistent with previous studies, showing that seed priming methods can shorten MGT, but the effect declined or/and disappeared with increasing osmotic stress (Table 1). Furthermore, prechilling stratification and sulfuric acid scarification were not found to give improvement in root and shoot growth (Table 1). Prechilling



**Table 1** Effect of seed treatments and water potential ( $\Psi$ ) on total germination percentage ( $n = 3$ ), mean germination time ( $n = 3$ ), and shoot ( $n = 10$ ) and root ( $n = 10$ ) length in germinating seeds for three cultivars of *Panicum virgatum* (switchgrass). *P*, cv. Pathfinder; *T*, cv. Trailblazer; *A*, cv. Alamo. PCS, prechilling stratification; SAS, sulfuric acid scarification

Variety	Treatment	Total germination/%				Mean germination time/d				Shoot length/mm				Root length/mm				
	$\Psi$ /MPa	PCS	SAS	CK	Mean	PCS	SAS	CK	Mean	PCS	SAS	CK	Mean	PCS	SAS	CK	Mean	
<i>P</i>	0.0	84.4	81.1	74.4	80.0a	3.5	4.2	3.8	3.8a	22.4	19.4	19.3	20.4a	7.2	6.8	8.2	7.4a	
	-0.1	80.0	74.4	67.8	74.1ab	3.5	4.1	4.0	3.9a	21.5	18.4	18.4	19.4a	7.0	6.7	8.1	7.3a	
	-0.2	76.7	71.1	51.1	66.3ab	3.7	4.3	4.7	4.2ab	20.2	16.7	17.4	18.1a	7.0	6.0	8.0	7.0a	
	-0.3	74.4	57.8	35.6	55.9bc	3.9	4.3	3.5	3.9a	12.5	11.0	12.5	12.0b	4.3	4.8	5.1	4.7b	
	-0.4	48.9	37.8	37.8	41.5cd	3.9	4.5	4.5	4.3a	6.8	5.8	9.5	7.4c	3.5	3.8	4.7	4.0b	
	-0.5	44.4	27.8	26.7	33.0d	4.4	4.8	4.5	4.6b	4.8	5.3	7.5	5.9c	3.3	3.7	4.3	3.8b	
	Mean		68.1	58.3	48.9		3.8	4.4	4.2		14.7	12.8	14.1		5.4	5.3	6.4	
	Sig.		$\Psi$ *				*				*				*			
		Treatment ns				ns				ns				ns				
		$\Psi$ x treatment ns				ns				ns				ns				
<i>T</i>	0.0	71.1	67.8	67.8	68.9a	3.3	3.7	3.8	3.6a	20.5	19.4	18.0	19.3a	9.5	8.3	8.3	8.7a	
	-0.1	71.1	61.1	61.1	64.4a	3.5	3.6	4.2	3.8a	20.0	18.1	17.6	18.6a	9.3	7.9	8.3	8.5a	
	-0.2	70.0	57.8	57.8	61.9ab	3.4	3.9	4.8	4.0ab	19.2	16.8	17.0	17.7a	9.2	7.8	8.1	8.4a	
	-0.3	61.1	51.1	27.8	46.7bc	3.6	4.2	4.2	4.0a	13.4	9.4	9.1	10.6b	6.4	5.7	5.8	6.0b	
	-0.4	42.2	36.7	27.8	35.5cd	4.4	4.4	5.4	4.7b	8.6	7.4	7.6	7.9c	5.7	5.2	5.7	5.5b	
	-0.5	34.4	22.2	18.9	25.2d	4.6	4.5	5.4	4.8b	6.3	5.6	5.5	5.8c	4.7	4.1	4.4	4.4c	
	Mean		58.3	49.4	43.5		3.8	4.1	4.6		14.7	12.8	12.5		7.5	6.5	6.8	
	Sig.		$\Psi$ *				*				*				*			
		Treatment ns				ns				ns				ns				
		$\Psi$ x treatment ns				ns				ns				ns				
<i>A</i>	0.0	38.9	35.6	34.4	36.3a	4.4	4.4	4.8	4.5a	30.3	29.1	28.3	29.2a	11.6	12.3	12.9	12.3a	
	-0.1	34.4	31.1	31.1	32.2ab	4.3	4.5	4.6	4.5a	26.9	26.5	26.3	26.6b	10.7	11.3	12.2	11.4ab	
	-0.2	28.9	27.8	27.8	28.2b	4.2	4.3	4.7	4.4a	23.7	23.6	21.5	22.9c	9.9	10.5	10.4	10.3bc	
	-0.3	26.7	18.9	18.9	21.5c	4.5	4.6	4.6	4.6a	18.7	16.3	14.8	16.6d	9.5	10.1	8.1	9.2cd	
	-0.4	15.6	14.4	11.1	13.7d	4.6	4.9	5.0	4.8a	11.1	10.9	11.8	11.3e	8.9	9.2	7.9	8.7d	
	-0.5	12.2	11.1	8.9	10.7d	5.3	4.8	4.6	4.9a	9.5	9.2	9.0	9.2f	8.5	8.3	6.9	7.9d	
	Mean		26.1	23.1	22.0		4.6	4.6	4.7		20.0	19.3	18.6		9.9	10.3	9.7	
	Sig.		$\Psi$ *				ns				*				*			
		Treatment ns				ns				ns				ns				
		$\Psi$ x treatment ns				ns				ns				*				

Note: \*, Significant at  $P < 0.05$ ; ns, not significant.

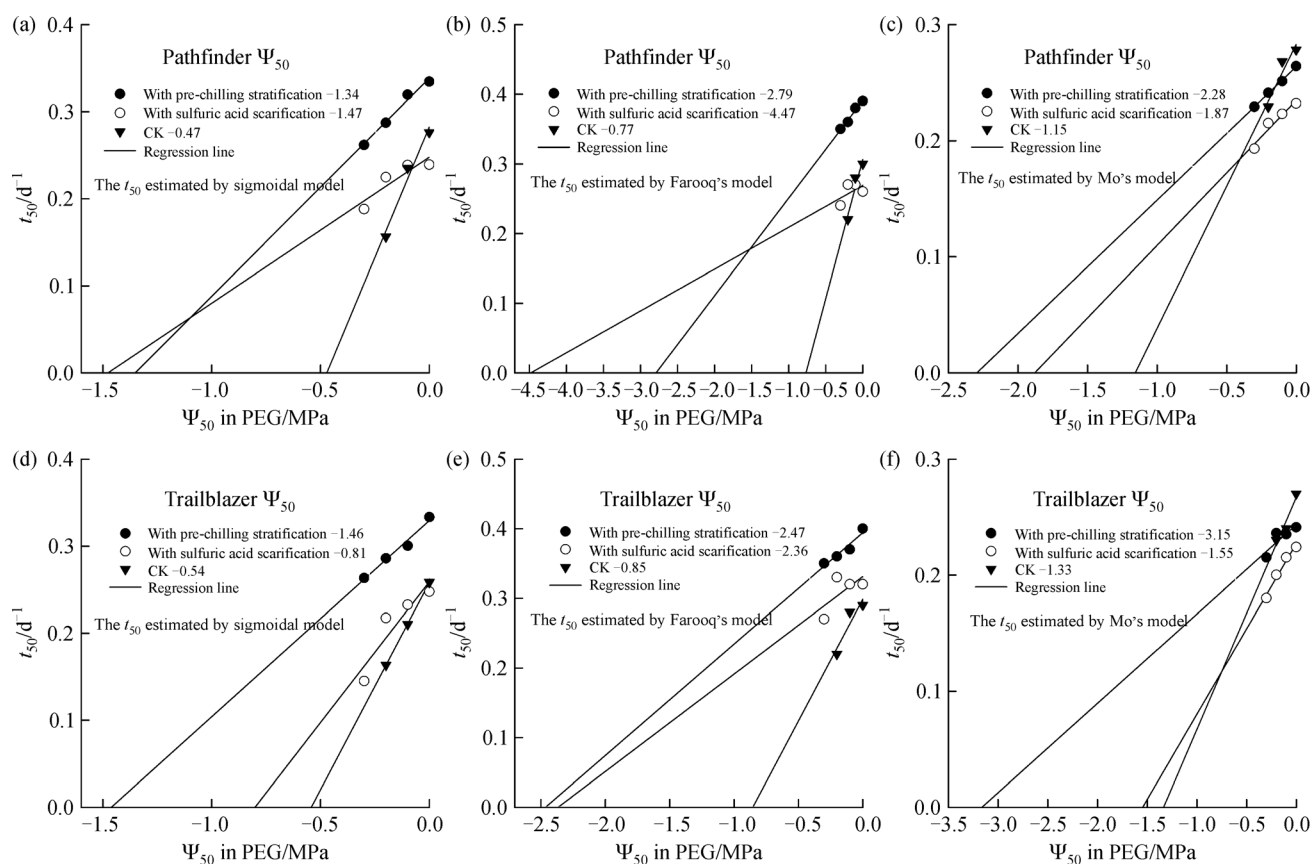
stratification can break seed dormancy by changes to physiologic traits<sup>[33]</sup>, while sulfuric acid scarification can dramatically alter seed coat physical properties, reducing thickness and strength thereby creating a weak area of root emergence<sup>[34]</sup>. Thus, prechilling stratification and sulfuric acid scarification mainly contribute to breaking seed coat or embryo dormancy, and their effect differs from hydro-priming, which has beneficial effects on MGT and root growth because of sufficient water and longer imbibition time<sup>[27]</sup>.

Linear regression of the reciprocal of  $t_{50}$  versus water

potential was used to calculate  $\Psi_{50}$  of each cultivar according to the value of abscissa intercept of the regression line<sup>[17]</sup>. This is a modification of the established seed analysis method. While the horizontal axis represented the temperature range of germination, the value of abscissa intercept of the regression line was used to estimate theoretical minimum temperature for germination<sup>[13]</sup>. Replacing temperature with water potential in the coordinate system, the value of abscissa intercept of regression line has been used to calculate  $\Psi_{50}$  of sorghum<sup>[17]</sup>. The present study showed that the range of

**Table 2** Time taken to reach 50% emergence ( $t_{50}$ ) for two cultivars of *Panicum virgatum* (switchgrass) estimated by three equations. *P*, cv. Pathfinder; *T*, cv. Trailblazer. PCS, prechilling stratification; SAS, sulfuric acid scarification

Variety	Treatment	Sigmoidal model			Farooq's model			Mo's model		
	$\Psi$ /MPa	PCS	SAS	CK	PCS	SAS	CK	PCS	SAS	CK
<i>P</i>	0.0	2.99	4.18	3.62	2.60	3.88	3.28	3.78	4.31	3.58
	-0.1	3.13	4.19	4.25	2.60	3.65	3.54	3.98	4.47	3.72
	-0.2	3.48	4.45	6.40	2.81	3.66	4.50	4.16	4.65	4.36
	-0.3	3.82	5.32	–	2.83	4.12	–	4.37	5.18	–
	-0.4	–	–	–	–	–	–	–	–	–
	-0.5	–	–	–	–	–	–	–	–	–
<i>T</i>	0.0	3.00	4.04	3.87	2.48	3.18	3.48	4.14	4.46	3.70
	-0.1	3.33	4.30	4.76	2.71	3.09	3.57	4.25	4.64	4.15
	-0.2	3.50	4.60	6.12	2.73	2.98	4.35	4.22	4.99	4.31
	-0.3	3.80	6.90	–	2.85	3.59	–	4.64	5.54	–
	-0.4	–	–	–	–	–	–	–	–	–
	-0.5	–	–	–	–	–	–	–	–	–

**Fig. 2** Relationships between water potential ( $\Psi$ ) of solution and theoretical time to 50% germination ( $t_{50}$ ) in seeds of *Panicum virgatum* (switchgrass) cv. Pathfinder (a, b and c) and cv. Trailblazer (d, e and f) at 30°C. Data were fitted by linear regression to predict the base water potential for reaching 50% germination ( $\Psi_{50}$ ) under treatments.

the lowest water potential requirements to achieve 50% germination of Pathfinder and Trailblazer under prechilling stratification and sulfuric acid scarification treatments was

between  $-0.3$  and  $-0.4$  MPa (Table 1). However, the linear regression estimated values of  $\Psi_{50}$  were far below this range regardless of which  $t_{50}$  was used (Fig. 2). Indeed,

seeds of Pathfinder and Trailblazer proved to be difficult to germinate under the estimated  $\Psi_{50}$  in subsequent attempts. Given the huge difference between predicted value and measured value, the reliability and applicable range of linear regression estimated  $\Psi_{50}$  needs further investigation, specification and optimization.

## 5 Conclusions

The greatest benefit of seed priming was achieved by prechilling stratification, indicating that embryo dormancy may be a major limitation to switchgrass germination under drought conditions. According to the findings of our research, Pathfinder is more suitable for semi-arid environment. In addition, Alamo did not reach 50% germination with any of the treatments, showing that this cultivar is not suitable for use under drought conditions.

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