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Alleviating Effects of 2,4-Epibrassinolide Priming in Seed Germination of Proso Millet Under Drought Stress

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Abstract

Drought stress is a major factor limiting seed germination and seedling establishment in crops. Here, two proso millet (Panicum miliaceum L.) varieties were used to evaluate the effects of 2,4-epibrassinolide (EBR) seed priming on germination under polyethylene glycol (PEG)-simulated drought stress. Seeds primed with different EBR concentrations were assessed for germination traits, seedling growth, water absorption, and α-amylase activity. Drought stress markedly reduced germination potential, germination rate, germination index, and vigor index, while prolonging mean germination time. EBR priming alleviated these adverse effects, with 0.01 μmol·L^{-1} showing the most consistent and effective promotion of germination and early seedling vigor under drought conditions. The two varieties exhibited different response patterns to EBR, indicating genotype-dependent sensiortivity. Moreover, EBR priming enhanced seed water uptake and maintained higher α-amylase activity, suggesting improved reserve mobilization and energy supply during germination. In conclusion, optimal EBR priming effectively mitigates drought-induced inhibition of proso millet germination and provides a useful strategy for improving seedling establishment under water-limited conditions.

Keywords: proso millet; 2,4-epibrassinolide (EBR); seed priming; drought stress; seed germination

1. Introduction

Drought is widely recognized as one of the most important abiotic factors restricting global agricultural productivity, with particularly severe consequences during sensitive stages of crop growth and development [1]. As the initial step of the plant life cycle, seed germination is especially vulnerable to fluctuations in environmental water availability. When drought stress is prolonged or intense, germination rates are markedly reduced and seedling establishment becomes difficult, ultimately resulting in poor stand establishment and considerable yield losses [2–5]. Consequently, developing effective approaches to enhance drought tolerance during the germination stage is critical for sustaining agricultural production in arid and semi-arid regions.

Proso millet is a typical C4 cereal crop with short-day growth characteristics. Owing to its relatively strong drought tolerance and ability to adapt to infertile soils, it is an important food and forage crop in arid and semi-arid regions of China [6–8]. Nevertheless, despite its inherent stress tolerance, the germination and early seedling stages of proso millet remain highly sensitive to water deficit [9]. At present, research on drought tolerance in proso millet has focused mainly on physiological responses at the adult plant stage, whereas studies addressing stress resistance and recovery during seed germination are still limited. Moreover, potential differences in drought tolerance among proso millet varieties at the germination stage have not been systematically evaluated, and their regulatory mechanisms remain insufficiently understood.

The concept of seed priming was first proposed by Heydecker et al. [10] and refers to a pre-sowing treatment in which seeds are partially hydrated under controlled conditions to activate early

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metabolic processes without visible radicle emergence, followed by controlled re-drying [11]. Numerous studies have demonstrated that seed priming can improve germination rate and percentage, promote seedling growth, and enhance tolerance to environmental stresses [12–16]. In recent years, increasing attention has been given to hormonal priming strategies. Brassinosteroids (BRs) are a class of steroidal phytohormones widely distributed in plants and are regarded as the "sixth major hormone" after auxin, cytokinins, and other classical plant hormones [17]. Among them, 2,4-epibrassinolide (EBR) is one of the most biologically active BRs and plays an important role in regulating plant growth and development, as well as in improving tolerance to abiotic stresses such as drought, salinity, and high temperature [18–20]. Previous studies have shown that EBR can alleviate stress-induced damage by enhancing antioxidant defense, regulating osmoprotectant accumulation, and maintaining membrane stability [21–23]. However, compared with major crops such as wheat and rice, the application of EBR as a seed priming agent in proso millet, particularly during germination, has received little attention. In addition, systematic investigations into varietal differences in response to EBR priming under different drought intensities remain scarce.

In the present study, two proso millet varieties were selected as experimental materials, and gradient drought stress was simulated using polyethylene glycol (PEG-6000) solutions at different concentrations. Based on this experimental system, the effects of seed priming with 2,4-epibrassinolide (EBR) on germination characteristics of proso millet were systematically examined. The objectives of this study were to provide a theoretical basis for developing effective strategies to enhance drought tolerance and ensure successful seedling establishment during germination, and to lay the groundwork for further elucidation of the physiological mechanisms underlying EBR-mediated regulation of stress tolerance at the seed germination stage.

2. Materials and Methods

2.1. Materials

Red and yellow proso millet varieties cultivated in the northern Shaanxi region of China were used as experimental materials.

2.2. Experimental Methods

2.2.1. Priming Treatment

Seeds with uniform size, good plumpness, and consistent coloration were selected for the experiment. The seeds were surface-sterilized with 75% ethanol for 30 s and subsequently rinsed thoroughly with distilled water. They were then incubated in darkness at 15 °C and soaked for 24 h in distilled water (H_2O) or in solutions containing 0.01, 0.1, 0.5, and 1 μmol·L^{-1} 2,4-epibrassinolide (EBR). After soaking, the solutions were discarded, and the seeds were air-dried at room temperature for 48 h. The treated seeds were then used for subsequent germination experiments. Seeds that did not receive any priming treatment served as the control.

2.2.2. Determination of Germination Parameters

Germination tests were conducted using the between-paper (BP) method. Seeds from each treatment were placed in Petri dishes lined with two layers of filter paper, with 50 seeds per dish and three replicates per treatment. Subsequently, 5 mL of polyethylene glycol (PEG) solutions at concentrations of 0 (distilled water), 15%, and 20% were added to each dish. The dishes were then incubated in a growth chamber under controlled conditions of 25 ± 2 °C, 85% relative humidity, and a 16/8 h (light/dark) photoperiod with a light intensity of 10,000 lx. Seed germination was defined as radicle protrusion, and germination counts were recorded daily for seven consecutive days. These data were used to calculate germination potential, germination index, vigor index, mean germination time, and final germination rate. At the end of the experiment, sprout length and root length were measured. For clarity, the priming treatments were designated as P0 (0 μmol·L^{-1}), P0.01 (0.01

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μmol·L⁻¹), P0.1 (0.1 μmol·L⁻¹), P0.5 (0.5 μmol·L⁻¹), and P1 (1 μmol·L⁻¹). Similarly, the PEG-induced drought stress levels were designated as D0 (distilled water), D15 (15% PEG), and D20 (20% PEG).

$$Germination\ potential\ (GP)=\frac{number\ of\ germinated\ seeds\ in\ the\ first\ 3d}{total\ number\ of\ seeds\ for\ testing}\times 100\% \quad (1)$$

$$Germination\ rate\ (GR)=\frac{total\ number\ of\ germinated\ seeds}{total\ number\ of\ seeds\ for\ testing}\times 100\% \quad (2)$$

$$Germination\ index\ (GI)=\sum\frac{Gt}{Dt} \quad (3)$$

Where, Gt represents the number of seeds germinated by day t, and Dt denotes the corresponding germination time (day t);

$$Vigor\ index\ (VI)\ =S\times\sum\frac{Gt}{Dt} \quad (4)$$

Where, S represents the seedling length;

$$Mean\ germination\ time\ (MGT)=\frac{Gt\times Dt}{\sum Gt} \quad (5)$$

2.2.3. Determination of Seed Water Absorption Rate

For each treatment, 50 seeds were used, with three replicates per treatment. The seeds were imbibed in distilled water at 25 °C and sampled at 2, 4, 6, 8, 10, 24, and 48 h after the onset of imbibition. At each sampling time, the seeds were removed, gently blotted with filter paper to eliminate surface moisture, and immediately weighed. The seed water absorption rate at each time point was calculated according to the following formula.

$$Water\ absorption\ rate(\%)=\frac{(w1\text{-}w2)}{w2}\times 100\% \quad (6)$$

Where, w_1 represents the seed mass after water absorption (g), and w_2 represents the initial seed mass before water absorption (g).

2.2.4. Determination of α-Amylase Activity

α-Amylase activity was determined using the 3,5-dinitrosalicylic acid (DNS) colorimetric method. For each treatment, 0.1 g of proso millet seeds germinated for 4 days were weighed and homogenized with 10 mL of distilled water. The homogenate was extracted for 15 min with gentle shaking at 2-min intervals. The mixture was then centrifuged at 4 °C and 4000 × g for 10 min, and the resulting supernatant was collected as the crude enzyme extract. A 2 mL aliquot of the crude extract was diluted with distilled water to a constant volume to obtain the total amylase solution. To determine α-amylase activity, a separate portion of the crude extract was heated at 70 °C to inactivate β-amylase. Subsequently, the appropriate reagents were added according to the standard protocol. The reaction mixture was incubated in a boiling water bath for 10 min and then rapidly cooled. Absorbance was measured at 540 nm, and enzyme activity was calculated using the following formula.

$$\alpha-Amylase\ Activity=\frac{(A-A')\times Vt}{FW\times Vs\times t} \quad (7)$$

Where A represents the amount of maltose (mg) produced by α-amylase-mediated starch hydrolysis; A′ denotes the maltose content in the α-amylase control tube; Vs represents the volume of enzyme solution used for colorimetric analysis; Vt represents the total volume of the diluted sample solution; FW denotes the fresh weight of the sample (g); and t represents the enzyme reaction time (min).

2.3. Data Analysis

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Germination rate, germination potential, germination index, and other related parameters were compiled and statistically analyzed using Microsoft Excel. Analysis of variance (ANOVA) was performed using SPSS software (version 27.0.1). All figures were generated using OriginLab OriginPro (version 2026).

3. Results

3.1. Two-Way ANOVA

To clarify how drought stress intensity and EBR priming concentration jointly influence seed germination of proso millet, a two-way analysis of variance (two-way ANOVA) was performed. In this analysis, drought stress and priming concentration were considered as independent factors, and multiple germination-related parameters were subjected to statistical evaluation to determine both their individual effects and their combined interaction. Detailed outcomes of the two-way ANOVA are summarized in Tables 1 and 2.

For both red and yellow proso millet varieties, the two main factors—drought stress and priming concentration—showed highly significant influences on germination rate (GR), germination potential (GP), vigor index (VI), germination index (GI), and mean germination time (MGT) ($P < 0.01$). The strong main effect of drought stress highlights the decisive role of water availability in restricting seed germination and early seedling establishment, with changes in moisture conditions consistently affecting the onset of germination, completion of germination, and subsequent seedling vigor. Likewise, the significant main effect of priming concentration indicates that EBR priming modulates germination behavior in a concentration-dependent manner, exerting substantial regulatory effects on germination speed, uniformity, and seedling vigor.

Regarding interaction effects, a highly significant interaction between drought stress and priming concentration was detected for vigor index (VI) and germination index (GI) ($P < 0.01$), whereas no significant interactions were observed for the remaining germination parameters. This pattern suggests that the effectiveness of EBR priming in improving overall germination performance is partially conditioned by drought stress intensity, with such dependence being most evident in integrative indicators related to germination rate and early seedling growth. In other words, the extent to which EBR priming preserves seed vigor and optimizes germination differs across water conditions, indicating a pronounced environmental dependency. Overall, the two-way ANOVA results confirm that drought stress constitutes the primary limiting factor for proso millet seed germination, while EBR priming acts as an important regulatory measure that enhances germination performance under different drought levels. The significant interactions observed for VI and GI provide a statistical foundation for subsequent analyses focusing on germination initiation, germination progression, and related physiological and biochemical mechanisms.

Table 1. Two-way ANOVA of seed germination parameters for red proso millet under varying drought stress and priming concentrations.

Source	SS	df	Mean Square	*F*	*P*
Germination rate(GR)					
Priming concentration(P)	4534.81	5.00	906.96	10.62	2.54E-06
Drought stress centration(D)	1321.04	2.00	660.52	7.73	1.61E-03
P*D	426.52	10.00	42.65	0.50	8.79E-01
Germination potential(GP)					
Priming concentration(P)	6770.44	5.00	1354.09	15.13	5.21E-08
Drought stress centration(D)	1241.33	2.00	620.67	6.94	2.83E-03
P*D	138.22	10.00	13.82	0.15	9.98E-01
Germination index(GI)					
Priming concentration(P)	954.79	5.00	190.96	35.15	6.88E-13
Drought stress centration(D)	888.58	2.00	444.29	81.78	4.10E-14

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P*D	155.48	10.00	15.55	2.86	9.94E-03
Vigor index(VI)					
Priming concentration(P)	6293.63	5.00	1258.73	41.21	6.41E-14
Drought stress centration(D)	26381.00	2.00	13190.50	431.88	6.91E-26
P*D	2701.33	10.00	270.13	8.84	4.10E-07
Mean germination time(MGT)					
Priming concentration(P)	2.94	5.00	0.59	10.81	2.12E-06
Drought stress centration(D)	3.08	2.00	1.54	28.30	4.12E-08
P*D	1.02	10.00	0.10	1.87	8.21E-02

Table 2. Two-way ANOVA of seed germination parameters for yellow proso millet under varying drought stress and priming concentrations.

Source	SS	df	Mean Square	*F*	*P*
Germination rate(GR)					
Priming concentration(P)	5166.81	5.00	1033.36	29.12	1.04E-11
Drought stress centration(D)	5604.15	2.00	2802.07	78.97	6.84E-14
P*D	159.41	10.00	15.94	0.45	9.11E-01
Germination potential(GP)					
Priming concentration(P)	7897.48	5.00	1579.50	30.42	5.59E-12
Drought stress centration(D)	7218.37	2.00	3609.19	69.51	4.35E-13
P*D	210.07	10.00	21.01	0.40	9.36E-01
Germination index(GI)					
Priming concentration(P)	1251.27	5.00	250.25	53.41	1.18E-15
Drought stress centration(D)	1617.31	2.00	808.65	172.60	3.57E-19
P*D	138.80	10.00	13.88	2.96	8.04E-03
Vigor index(VI)					
Priming concentration(P)	4267.14	5.00	853.43	20.39	1.31E-09
Drought stress centration(D)	27372.33	2.00	13686.17	326.94	8.23E-24
P*D	1540.92	10.00	154.09	3.68	1.85E-03
Mean germination time(MGT)					
Priming concentration(P)	6.72	5.00	1.34	10.45	3.00E-06
Drought stress centration(D)	6.34	2.00	3.17	24.65	1.80E-07
P*D	1.09	10.00	0.11	0.85	5.85E-01

3.2. Effects of EBR Priming on Germination Initiation and Early Seedling Morphology of Proso Millet

Figures 1 and 2 illustrate the germination responses of the two proso millet varieties subjected to PEG-simulated drought stress following different priming treatments, including the untreated control (CK), hydro-priming (P0), and EBR priming at 0.01, 0.1, 0.5, and 1 μmol·L^{-1} (P0.01, P0.1, P0.5, and P1). Under non-stress conditions (D0), all EBR-primed seeds initiated germination earlier than the CK, with acceleration evident as early as day 3. As drought stress intensified, germination was generally suppressed, and the inhibitory effect was more pronounced in yellow proso millet than in red proso millet. Nevertheless, earlier radicle protrusion was consistently observed in EBR-primed treatments, indicating that EBR facilitates the onset of germination. Among the concentrations tested, P0.01 produced the strongest response, with seeds showing the earliest initiation and greatest uniformity. Across all drought levels, seedlings from the P0.01 treatment also exhibited superior vigor, characterized by coordinated radicle and plumule development and more uniform growth. Notably, under severe drought stress (D20), P0.01 resulted in the highest final germination count, demonstrating its overall effectiveness in promoting germination and enhancing drought tolerance.

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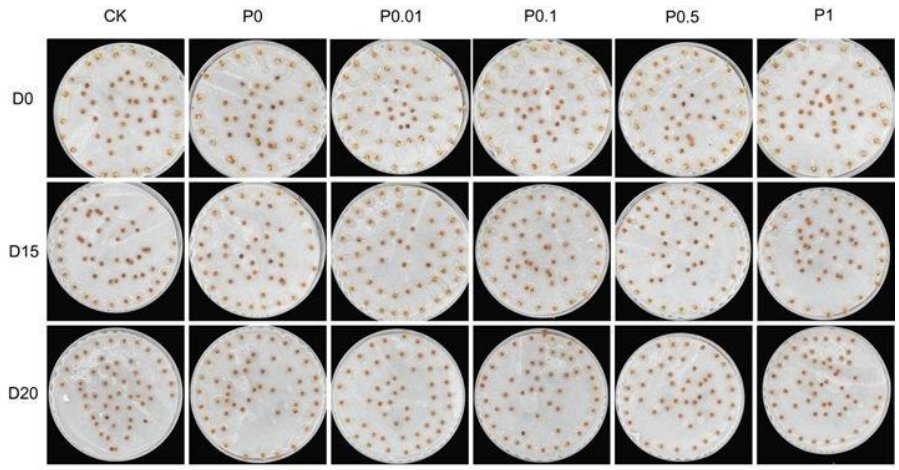

Figure 1. Effects of EBR priming on seed germination of red proso millet under drought stress.

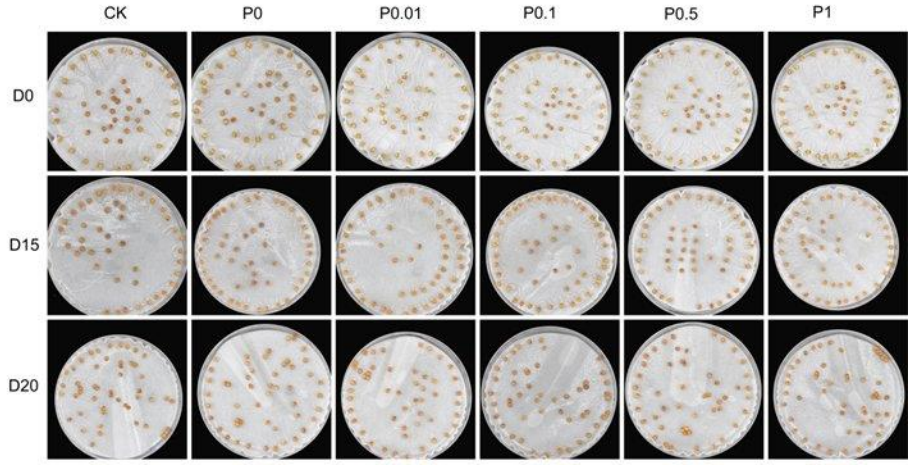

Figure 2. Effects of EBR priming on seed germination of yellow proso millet under drought stress.

As shown in Figure 3, drought stress markedly inhibited early germination in both proso millet varieties. Increasing stress intensity from D0 to D20 led to a consistent decline in germination potential across all treatments, indicating strong constraints on both the rate and uniformity of germination initiation. Under moderate drought stress (D15), EBR priming alleviated this inhibition, although the response patterns differed between varieties. In red proso millet, germination potential exceeded that of the CK only under P0.01 and P0.1. In contrast, yellow proso millet exhibited significantly higher germination potential than the CK at all EBR concentrations, with P0.01 producing the strongest effect and reaching 82%. Under severe drought stress (D20), both varieties showed their highest germination potential with the P0.01 treatment, which was significantly higher than that of the CK and P0. Comparative analysis further revealed that, except for P1, germination potential of yellow proso millet remained lower than that of red proso millet under D20, indicating weaker early germination vigor. However, under the highest EBR concentration (P1), yellow proso millet surpassed red proso millet, suggesting a greater capacity for germination initiation when exposed to elevated exogenous EBR.

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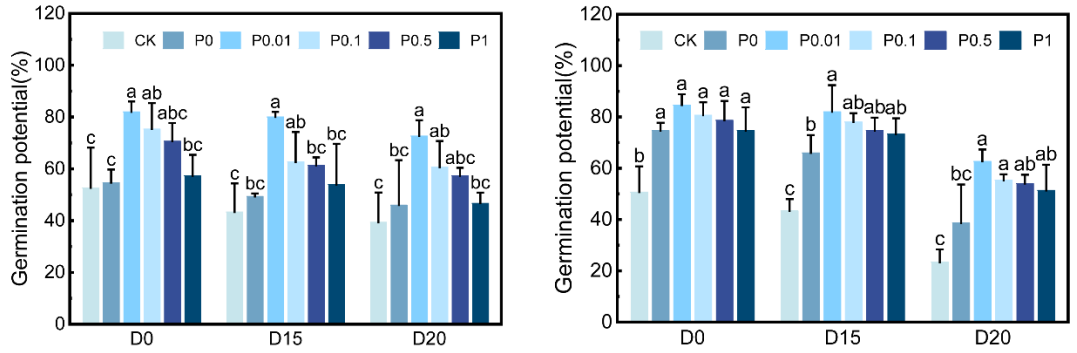

Figure 3. Effects of EBR priming on germination potential of proso millet. (a) Red proso millet; (b) Yellow proso millet. Different lowercase letters indicate significant differences among treatments under drought stress (P< 0.05).

3.3. Effects of EBR Priming on the Germination Process of Proso Millet

Figure 4 shows that germination rate declined significantly in both varieties as drought stress increased, regardless of whether seeds were primed or not, confirming the broad inhibitory effect of drought on germination. Even under D0 conditions, differences in germination rate were evident among EBR treatments relative to the CK, implying that EBR promotes germination under favorable conditions. Under D15 and D20 stress, germination rates of EBR-primed seeds were generally higher than those of the CK, demonstrating the alleviating role of EBR priming. At D15, red proso millet reached 82% germination only under P0.01, whereas yellow proso millet exceeded the CK at all EBR concentrations, with P0.01 reaching 87%. Under D20, both varieties achieved their highest germination rates with P0.01, but yellow proso millet remained significantly higher than the CK across all EBR treatments, while red proso millet responded significantly only at P0.1 and P0.5. This pattern indicates that the response range of red proso millet to EBR broadens as drought stress intensifies.

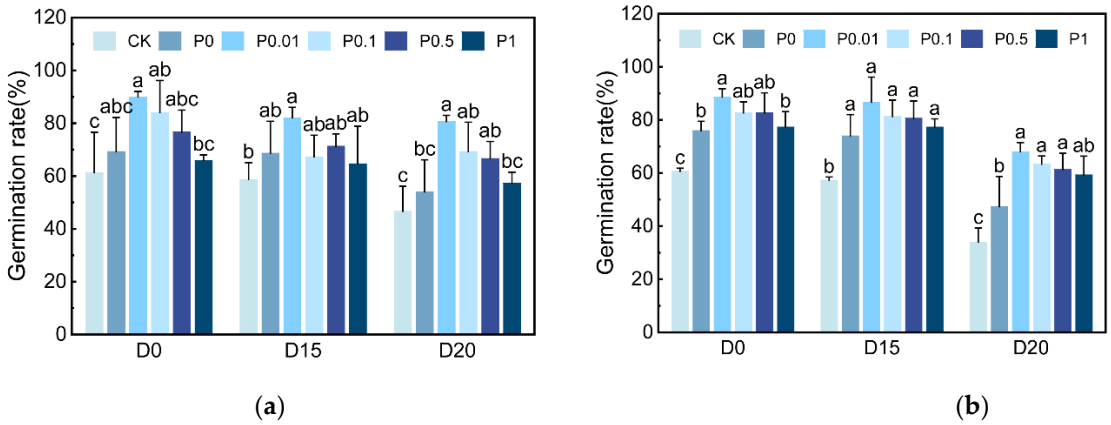

Figure 4. Effects of EBR priming on germination rate of proso millet. (a) Red proso millet; (b) Yellow proso millet. Different lowercase letters indicate significant differences among treatments under drought stress (P < 0.05).

Under D0 conditions, both red and yellow proso millet achieved their maximum germination index with the P0.01 treatment, which was significantly higher than all other treatments (Figure 5), indicating that this concentration is optimal for enhancing germination vigor. This result suggests that EBR can improve seed physiological status even under non-stress conditions by increasing germination uniformity and speed. Under D15 and D20 stress, the two varieties exhibited distinct response patterns. Red proso millet responded selectively to EBR, as the germination index under P1 did not differ from that of the CK. In contrast, yellow proso millet showed significantly higher

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germination indices than the CK at all EBR concentrations, indicating a stronger dependence on exogenous EBR regulation under drought conditions.

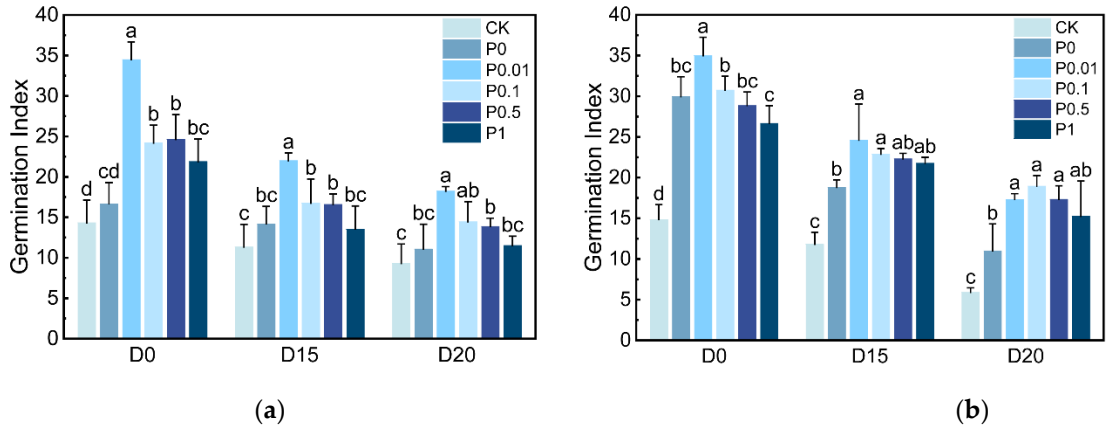

Figure 5. Effects of EBR priming on germination index of proso millet. (a) Red proso millet; (b) Yellow proso millet. Different lowercase letters indicate significant differences among treatments under drought stress (P< 0.05).

Figure 6 illustrates the effects of EBR priming on mean germination time (MGT). Relative to the CK, all EBR treatments reduced MGT in both varieties. The P0.01 treatment produced the most stable effect, significantly shortening MGT of red proso millet under D0 and D15. Red proso millet reached its shortest MGT with P0.01, with reductions of 0.68, 0.95, and 0.38 days under D0, D15, and D20, respectively. In yellow proso millet, the concentration yielding the shortest MGT shifted with stress intensity (P0 at D0, P0.01 at D15, and P0.1 at D20). Faster germination favors rapid seedling establishment during periods of limited water availability. Under D15, red proso millet responded only to P0.01 and P0.1, whereas yellow proso millet showed reduced MGT at all EBR concentrations. Under D20, red proso millet exhibited no significant MGT differences among treatments, suggesting a tolerance threshold, while yellow proso millet maintained significantly lower MGT at P0.01, P0.1, and P0.5, indicating greater regulatory flexibility.

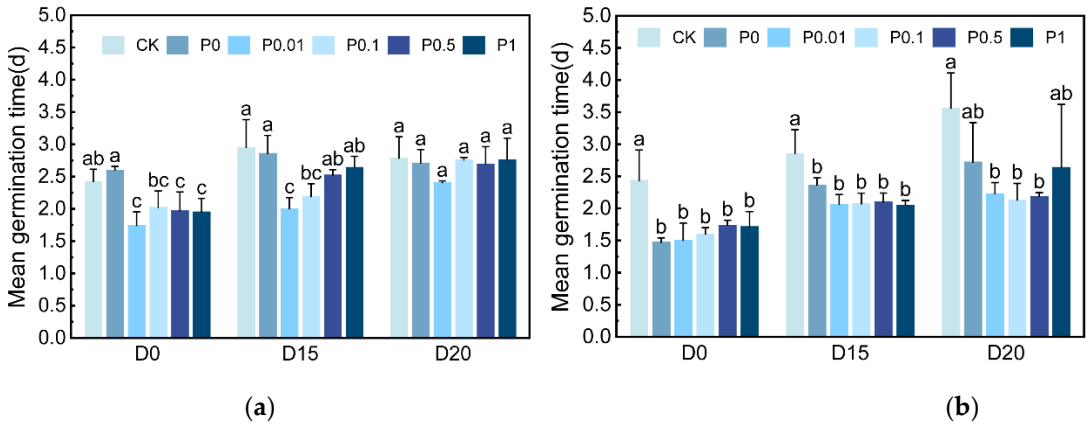

Figure 6. Effects of EBR priming on mean germination time (MGT) of proso millet. (a) Red proso millet; (b) Yellow proso millet. Different lowercase letters indicate significant differences among treatments under drought stress (P< 0.05).

3.4. Effects of EBR Priming on Seedling Growth and Vigor of Proso Millet

As shown in Figure 7, the promotive effect of EBR priming on sprout and root length was limited compared with its effects on germination parameters, and overall differences among treatments were not significant. Sprout length declined sharply with increasing drought stress. Under D0 and D15, sprout length of red proso millet was significantly greater under P0.01 than under the CK, whereas

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yellow proso millet showed no significant response to EBR. Under D20, sprout length did not differ among treatments in either variety, indicating severe inhibition of aboveground growth and loss of morphological alleviation by EBR. Root length patterns differed: under D0, the CK exhibited longer roots than both EBR- and hydro-primed seeds, suggesting adverse effects of exogenous treatments without stress. Under D15, red proso millet showed enhanced root length under P0.01 and P0.1, whereas yellow proso millet showed no significant differences. Under D20, red proso millet achieved maximum root length under P0.5, while yellow proso millet had the lowest root length under P0, indicating contrasting varietal responses under severe stress.

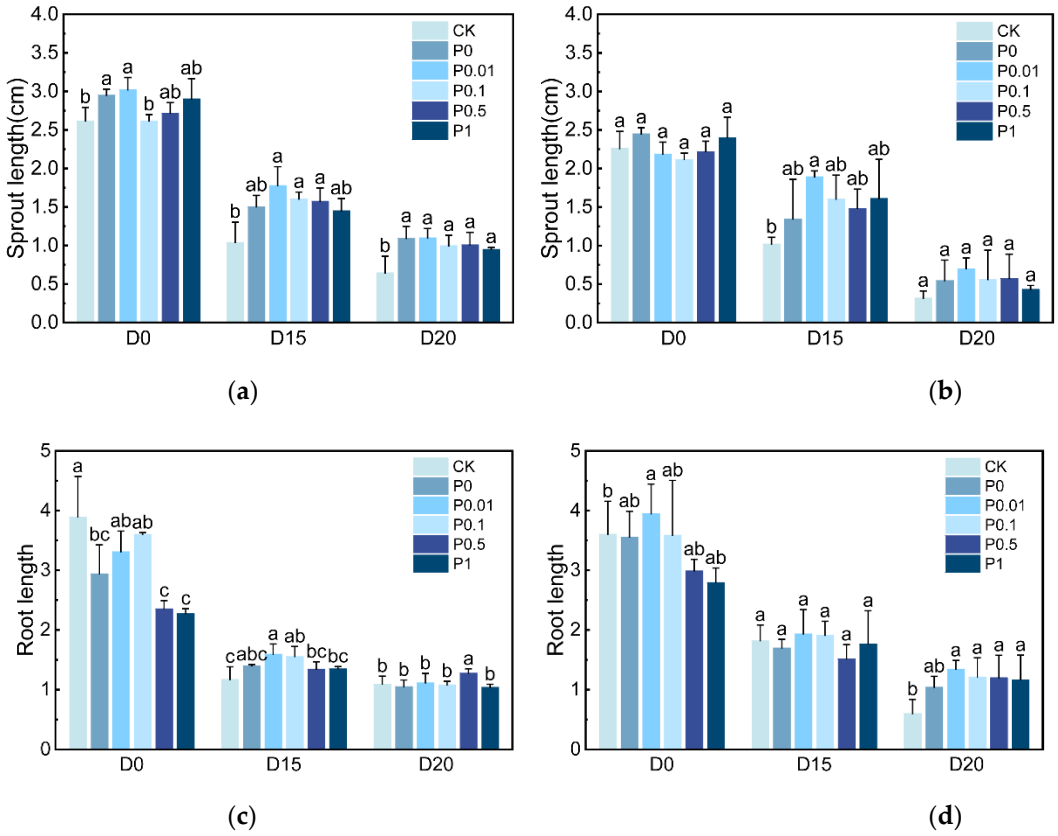

Figure 7. Effects of EBR priming on sprout and root length of proso millet seedlings. (a) Sprout length of red proso millet; (b) Sprout length of yellow proso millet; (c) Root length of red proso millet; (d) Root length of yellow proso millet. Different lowercase letters indicate significant differences among treatments under drought stress ($P< 0.05$).

Figure 8 shows that seedling vigor index declined sharply with increasing drought stress in both varieties. In red proso millet, vigor index decreased by 70.76% from D0 to D15 and by a further 49.15% from D15 to D20. Yellow proso millet showed even greater reductions of 64.17% and 84.35%, respectively, indicating higher sensitivity during seedling establishment. EBR priming mitigated this decline to varying degrees. Under D0, vigor index of both varieties exceeded that of the CK across all EBR treatments. Under D0 and D15, P0.01 produced the highest vigor index in red proso millet. Under D20, although P0.01 remained higher than CK, P0, and P1, it did not differ from P0.1 and P0.5, suggesting a requirement for broader EBR signaling under extreme stress. In yellow proso millet, vigor index peaked at P0.1 and P0.5 under D15 but could not be effectively enhanced by EBR under D20, indicating saturation of the alleviating capacity.

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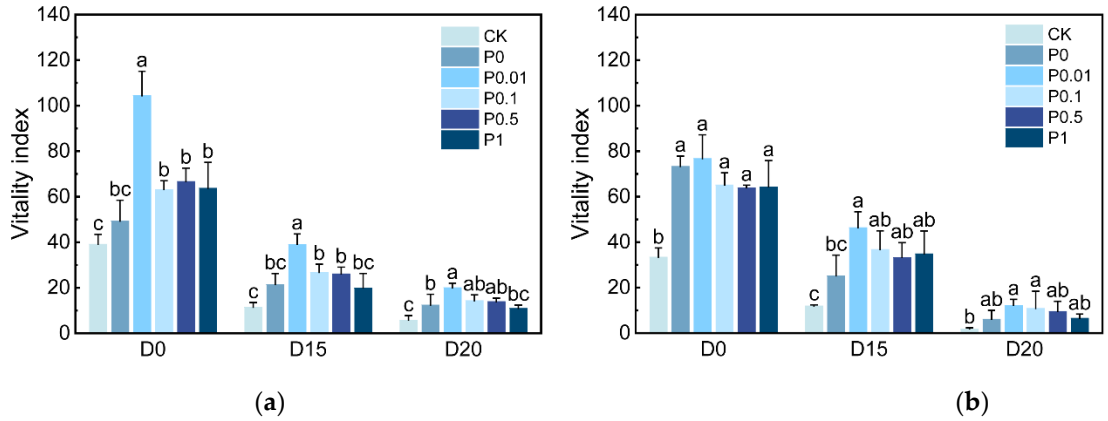

Figure 8. Effects of EBR priming on vigor index of proso millet. (a) Red proso millet; (b) Yellow proso millet. Different lowercase letters indicate significant differences among treatments under drought stress ($P < 0.05$).

3.5. Effects of EBR Priming on the Physiological and Biochemical Characteristics of Proso Millet During Seed Germination

Figure 9 shows that seed water absorption increased over the 48-h imbibition period, with a rapid rise during the first 6 h followed by a slower increase. Compared with the CK, all EBR priming treatments significantly enhanced water absorption in both varieties, with the strongest effect observed under P0.01. This effect was particularly evident in red proso millet, where water absorption increased by 90.64% at 24 h and reached the highest value among treatments, indicating enhanced early water uptake during germination.

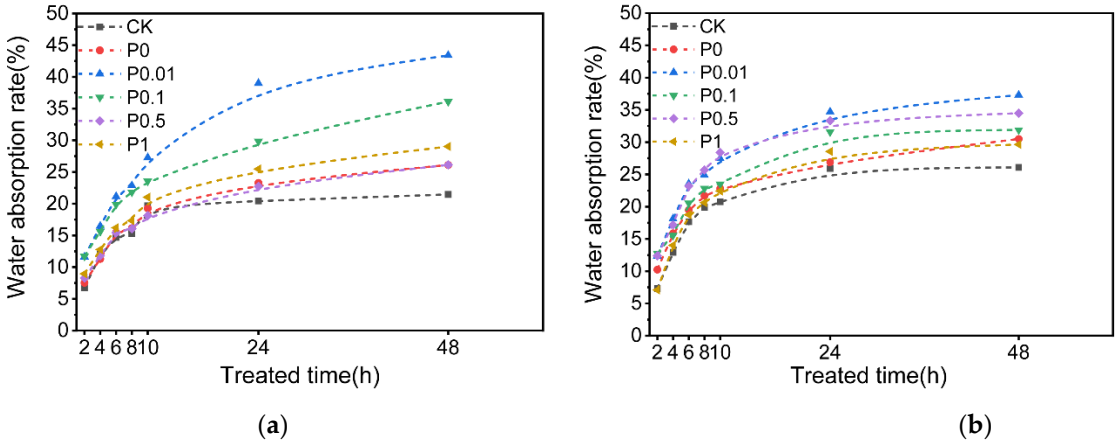

Figure 9. Effects of EBR priming on water absorption rate of proso millet. (a) Red proso millet; (b) Yellow proso millet. Different lowercase letters indicate significant differences among treatments under drought stress ($P< 0.05$).

As shown in Figure 10, EBR priming increased α-amylase activity in both proso millet varieties, with a greater enhancement observed in yellow proso millet. Under D0, α-amylase activity was significantly higher in all EBR treatments than in the CK, with P0.01 producing the highest activity. Although enzyme activity declined with increasing drought stress, EBR-primed seeds maintained higher activity levels than the CK. Under D15, yellow proso millet exhibited the strongest response under P0.01, and under D20, α-amylase activity in EBR-treated seeds—especially in yellow proso millet—remained significantly higher than in the CK.

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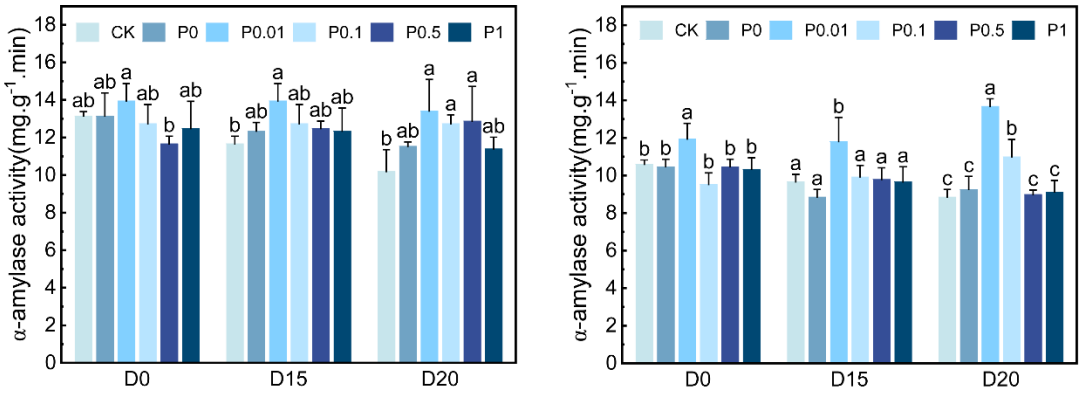

Figure 10. Effects of EBR priming on α-amylase activity of proso millet. (a) Red proso millet; (b) Yellow proso millet. Different lowercase letters indicate significant differences among treatments under drought stress ($P<0.05$).

4. Discussion

The germination stage is widely regarded as the most stress-sensitive period in the plant life cycle, and damage typically becomes more severe as stress intensity increases [24–26]. In this study, PEG-simulated drought stress clearly suppressed germination rate, germination potential, germination index, and seedling vigor, while extending mean germination time in both proso millet varieties. Taken together, these shifts suggest that water deficit first constrains the speed and uniformity of germination initiation, which in turn becomes a major bottleneck for stand establishment under drought conditions. Earlier work has attributed drought-related loss of seed vigor to restricted water uptake, inhibited metabolic reactivation, and disturbances in hormonal homeostasis [24,25]. Accordingly, exogenous application of gibberellins, melatonin, and abscisic acid (ABA) has been reported to partially offset abiotic stress-induced inhibition of germination [27–29]. Brassinosteroids (BRs), as steroidal plant hormones, have similarly been shown to alleviate salt- and drought-related germination inhibition when used as priming agents, while also participating broadly in growth, development, and stress-response regulation [18–20]. In line with these reports, EBR priming in the present work improved germination performance under multiple drought levels, reflected in higher germination rate and germination potential together with a shortened germination process. Such responses agree well with findings in peanut, soybean, and wheat, where BR/EBR priming has been linked to improved drought tolerance during germination and enhanced stress adaptation [26–29].

Across the tested concentration range, 0.01 μmol·L^{-1} EBR produced the most stable alleviating effect in both varieties under different drought stress levels, as indicated by earlier initiation, greater uniformity, and stronger maintenance of vigor index, consistent with an optimal concentration pattern. A growing body of literature suggests that BRs often display a hormetic response under stress, with clear benefits at low doses but reduced—or sometimes negligible—effects at higher doses; this has been associated with signaling thresholds, receptor saturation, and increased metabolic costs at elevated hormone exposure [30,31]. In addition, the two varieties did not respond identically. Under moderate drought stress, yellow proso millet showed strong dependence across multiple EBR concentrations, whereas red proso millet responded in a more selective manner. Under severe drought stress, the alleviating effect tended to weaken or approach saturation in both varieties. This "variety × concentration × stress intensity" pattern is consistent with observations in wheat and soybean, where genotype-dependent sensitivity to EBR priming has been reported [32–34]. It also accords with evidence that proso millet varieties differ intrinsically in drought tolerance, particularly with respect to antioxidant regulation, hormone signaling, and recovery capacity under stress [35,36].

From the physiological standpoint, the mitigation conferred by EBR priming likely reflects coordinated regulation across several pathways. In this study, EBR priming increased seed water

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absorption rate, and the enhancement was most evident at 0.01 μmol·L^{-1}, providing an important material basis for germination initiation and radicle protrusion under drought. At the same time, α-amylase activity was markedly elevated, which helped sustain starch mobilization during drought stress and thus supported early energy supply. Mechanistic support for this interpretation is available from rice, where BRs were shown to promote α-amylase expression via the BZR1–RAmy3D transcriptional module, accelerating endosperm starch degradation and increasing germination rate [37]. Moreover, in wheat and soybean, EBR has been linked to improved hormonal balance, strengthened antioxidant defenses, and enhanced accumulation of protective proteins such as dehydrins during drought stress at germination [38,39]. In summary, priming with 0.01 μmol·L^{-1} EBR alleviated drought-induced inhibition of proso millet germination by promoting water uptake, strengthening starch metabolism, and improving seed physiological vigor, thereby offering a theoretical basis for rapid establishment and stable production under drought conditions.

5. Conclusions

This study investigated whether 2,4-epibrassinolide (EBR) priming mitigates drought-induced inhibition of proso millet seed germination and its physiological mechanisms. PEG-simulated drought stress significantly delayed germination initiation, reduced germination potential and index, and prolonged mean germination time. EBR priming (especially at 0.01 μmol·L^{-1}) significantly improved germination speed, uniformity and final germination percentage under moderate and severe drought. Genotypic responses to EBR varied among varieties. Mechanistically, EBR priming enhanced water uptake and maintained higher α-amylase activity, promoting starch mobilization and energy supply. In conclusion, EBR seed priming effectively alleviates drought stress during seed germination by pre-activating water absorption and reserve mobilization, which provides a physiological basis for stable seedling emergence under drought.

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