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Exogenous Applications of Spermidine Improve Drought Tolerance in Seedlings of the Ornamental Grass *Hordeum jubatum* in Northeast China

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Abstract: *Hordeum jubatum* L. is a potential ornamental grass species. Spermidine (Spd) plays a regulatory role in plant stress. This study measured seedling growth, photosynthesis, chlorophyll fluorescence, osmotic regulatory substance contents, and antioxidant enzyme activities in *H. jubatum* seedlings pretreated with Spd (0–1.5 mM at 0.5 mM intervals) in drought treatments simulating natural water loss. The results indicated that the water content, photosynthetic parameters maximal quantum yield of PSII photochemistry (Fv/Fm), actual quantum yield of photochemical energy conversion in PSII (ϕPSII), and photochemical quenching coefficient (q_p) values of *H. jubatum* shoots decreased significantly with increasing drought intensity. Simultaneously, the contents of malondialdehyde (MDA) and the nonphotochemical quenching coefficient (NPQ) value increased. Spd improved growth and photosynthesis under drought stress. Spd also increased osmotic regulatory substance contents and antioxidant enzyme activities. These results suggest that the drought stress inhibited the growth of *H. jubatum* and damaged the photosynthetic systems, triggering a range of protective responses. Exogenous Spd mitigated the damage by promoting a variety of responses and adaptive mechanisms, such as adjusting stomatal conductance, promoting photosynthetic capacity, accelerating the synthesis of osmoregulatory molecules, and activating antioxidant enzyme systems. Additionally, 1.5 mM Spd-treated *H. jubatum* had the best drought tolerance. This study will help to develop an understanding of the effects of exogenous Spd on improving drought resistance and provide a strategy for the *H. jubatum* landscape effect to be achieved under water-limited conditions.

Keywords: *Hordeum jubatum*; spermidine; drought; photosynthesis; chlorophyll fluorescence

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Citation: Tian, J.; Zhao, Y.; Pan, Y.; Chen, X.; Wang, Y.; Lin, J.; Wang, J.; Yang, Q. Exogenous Applications of Spermidine Improve Drought Tolerance in Seedlings of the Ornamental Grass *Hordeum jubatum* in Northeast China. *Agronomy* **2022**, *12*, 1180. https://doi.org/10.3390/agronomy12051180

Academic Editor: Alfonso Albacete

Received: 31 March 2022
Accepted: 9 May 2022
Published: 14 May 2022

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1. Introduction

Drought stress is a serious environmental constraint to agricultural production and landscaping [1]. Approximately one-third of the world's land area suffers from drought stress, and drought severity is anticipated to be further aggravated [2]. Previous studies have reported that drought stress seriously affects photosynthesis by reducing the stability of the photosynthetic apparatus and impairing the function of the photosystem II (PSII) reaction centers [3]. Furthermore, drought stress has complex effects on growth and developmental delay [4], leaf yellowing and withering [5], the shortening of fluorescence [6], and reducing grain yield [7]. Hence, improving plant drought resistance is crucial for agricultural production and landscaping.

Polyamines (PAs), including putrescine (Put), spermidine (Spd), and spermine (Spm), are low molecular weight nitrogenous compounds that play regulatory roles in modulating plant growth, development, and productivity [8,9]. PAs have been demonstrated to modulate a suite of physiological processes, such as cell division and differentiation [10],

root elongation [11], floral development [12], fruit ripening [13], leaf senescence, and apoptosis [14,15]. Furthermore, many studies have reported that the exogenous application of PAs can improve resistance to drought stress [16–18]. Applying exogenous PAs could protect against the damaging effects of drought stress in *Cynodon dactylon* [19], *Zea mays* [20], *Tritimcum aestivus* [18], *Cerasus humilis* [21], *Hordeum vulgare* [22], and *Agrostis stolonifera* [23]. The physiological mechanisms of how exogenous PAs improve drought resistance include a range of metabolic pathways, such as photosynthesis, the tricarboxylic acid cycle, and hormone synthesis [8]. Kubis et al. revealed that stabilizing membrane structures, scavenging free radicals, and maintaining osmotic equilibrium are significant mechanisms of PAs in response to drought stress [22]. Peng et al. demonstrated that applying a biosynthetic PA inhibitor to white clover (*Trifolium repens*) impaired the physiological changes and stress defense [24]. Among the three major PAs, Spd is most closely associated with drought stress [25,26]. However, the underlying mechanism of how exogenous Spd modulates drought resistance in plants is poorly understood.

Hordeum jubatum L. (family Gramineae) is a perennial plant species native to the cold temperate zone of North America and Eurasia. It is also widely distributed in China, particularly in northeast China. Owing to its high adaptability and tolerance to saline soils, *H. jubatum* is considered a halophyte, indicating that this species can complete its life cycle in water-limited and saline environments [27]. *H. jubatum* is also an ornamental landscaping plant that has received considerable attention. Several of its traits, such as its unique green or purplish spikes and graceful culms that quiver with the breeze, are excellent for landscape design. However, the drought resistance mechanisms of this species have never been reported.

Therefore, the overall objectives of the present study were to clarify the mechanisms of the drought response in *H. jubatum* seedlings and explore the effects of exogenous Spd on drought resistance. Thus, we evaluated the effects of exogenous Spd on shoots of *H. jubatum* under drought stress by measuring seedling growth, photosynthesis, chlorophyll fluorescence, osmotic adjustments, and the activity of the antioxidant system.

2. Materials and Methods

2.1. Plant Material and Stress Conditions

The *Hordeum jubatum* seeds used in this study were obtained from the landscape plant research institute of the Northeast Forestry University in 2018 (126°68′ E, 45°72′ N, Harbin, China). After sterilization with 2% H_2O_2 solution, *Hordeum jubatum* seeds were sown in plastic pots (18 cm diameter × 15 cm depth) filled with vermiculite and peat soil (configuration ratio 1:2). The conditions in the growth house were controlled in a 12-h photoperiod with a photosynthetic photon flux density of 400 μmol·m^{-2}·s^{-1}, day/night temperature of 25/19 °C, and relative humidity of 60–70%.

Seedlings with uniform growth were selected at the two-leaf stage and randomly divided 25 days after sowing into four gradient drought stress treatments with four Spd application levels. The seedlings were tertian irrigated with Spd at four levels (0–1.5 mM at 0.5 mM intervals: S1, S2, S3, and S4) four times (50 mL each time) before the drought stress. Then, all seedlings were subjected to a drought treatment. The drought stress of natural water loss was simulated by stopping watering for 5, 10, and 15 days. Four moisture gradients (0–15 d at 5 d intervals: CK, D1, D2, and D3) were set and the soil relative water content (RWC) of each group was measured (CK: 90–95%, D1: 70–75%, D2: 50–55%, and D3: 30–35%). Soil moisture content was determined using the dry weighing method [7]. Three samples (replicates) were taken for each measurement. The shoots from each seedling were harvested to analyze changes in seedling growth, photosynthesis, chlorophyll fluorescence, osmotic regulators, and antioxidant systems. See Appendix A for the formulas and the manufacturers of the chemicals.

2.2. Growth Measurements

The shoots separated from each plant were immediately weighed for the determination of fresh weight (FW). To determine dry weight (DW), samples were oven-dried at 110 °C for 15 min and then dried at 65 °C to a constant weight. Water content (WC) was calculated using the following formula: WC = (FW − DW)/DW × 100%.

2.3. Gas Exchange Measurements

Gas exchange parameters were measured using a portable open flow LI-6400XT gas-exchange system (LI-COR Biosciences, Lincoln, NE, USA) with a Li-6400-02B red/blue LED chamber at 09:00–11:00 before harvesting. Shoots were randomly chosen to measure net photosynthetic rate (P_N), stomatal conductance (Gs), intracellular CO_2 concentration (Ci), and transpiration rate (Tr). All parameters were measured at 380 ± 10 μmol·mol^{-1} ambient CO_2 concentration, 25 °C air temperature, and 50% relative air humidity. The PAR was 1000 μmol·m^{-2}·s^{-1} (the ratio between the intensities of red (665 nm) and blue (447 nm) light was 9 to 1).

2.4. Chlorophyll Fluorescence Parameter Measurements

Chlorophyll fluorescence parameters were measured with a pulse amplitude modulation chlorophyll fluorescence imaging system (Open FluorCam FC 800-O, Photon System Instruments, Prague, the Czech Republic). The shoots from each treatment underwent dark adaptation for at least 1 h. The intensity of saturating light pulses was 40% (1767.00 μmol·s^{-1}·m^{-2}) and the intensity of actinic light was 39.12% (800 μmol·s^{-1}·m^{-2}) [28].

2.5. Malondialdehyde (MDA) Content Measurements

The level of membrane lipid peroxidation was based on the MDA levels using a slightly modified thiobarbituric acid (TBA) method [29]. The fresh shoots (0.5 g) were homogenized in 5 mL of 5% trichloroacetic acid (TCA) and centrifuged at 10,000× *g* for 10 min. Then, the reaction mixture (containing 2 mL of 0.67% TBA and 2 mL of supernatant) was placed in a boiling water bath for 30 min. After cooling, the mixture was centrifuged. The absorbance of the supernatant was recorded at 450 nm, 532 nm, and 600 nm by a UV-vis spectrophotometer (BioMate 3S UV–visible, Thermo Scientific, Waltham, MA, USA). The MDA content was calculated using the method described by Meng et al. [29].

2.6. Protein Content Measurements

Fresh shoots (0.5 g) were ground with liquid nitrogen. The homogenate powder was mixed with 50 mM sodium phosphate buffer (10 mL, pH 7.0) containing 0.2 mM ethylenediamine tetraacetic acid and 1% polyvinyl pyrrolidone. Homogenates were filtered and centrifuged at 8000× *g* for 15 min at 4 °C. The protein content was determined using bovine serum albumin (BSA) as a standard [30]. Absorbance was recorded at 595 nm.

2.7. Proline Content Measurements

Proline content in the shoots was determined by the acidic ninhydrin staining method [31]. The dried shoots (0.5 g) were homogenized with 5 mL of sulfosalicylic acid (3%) and centrifuged. A 2 mL aliquot of filtrate was mixed with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid. The mixture was placed in a water bath for 30 min at 98 °C and then cooled down to room temperature. The reaction mixture was extracted with 5 mL of toluene. Absorbance was measured at 520 nm with a spectrophotometer.

2.8. Soluble Sugar Content Measurements

Soluble sugar was extracted from oven-dried shoots (50 mg) with 80% ethanol and centrifuged, then the extract was dried via a water bath and resuspended in distilled water. The aliquot mixed with anthrone reagent was heated for 10 min, then cooled in an ice bath for 30 min. Absorbance was measured at 620 nm with a spectrophotometer [32].

2.9. Antioxidant Enzyme Activity Measurements

Fresh *H. jubatum* shoots (0.2 g) were homogenized at 4 °C for 10 min in 50 mM potassium phosphate buffer (1.6 mL, pH 7.8) containing 0.2 mM ethylenediaminetetraacetic acid and 1% polyvinylpyrrolidone. The homogenate was centrifuged and extracted to measure superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) activities.

SOD activity was assayed using the nitro blue tetrazolium (NBT) method [33]. The reaction was initiated by adding riboflavin (20 µM), and the tubes were shaken and stopped by switching off the light. One unit of SOD was defined as the amount of enzyme required to inhibit 50% of the photochemical reduction of NBT at 560 nm. POD activity was measured by the oxidation of guaiacol in the presence of H_2O_2 [34]. Peroxidase oxidizes guaiacol to produce a brown substance. Absorbance was measured at 470 nm with a spectrophotometer. A change of 0.01 in A_{470} per minute was defined as one peroxidase activity unit. CAT activity was estimated according to the consumption of H_2O_2 at 240 nm for 4 min. The reaction mixture consisted of 50 mM phosphate buffer containing 0.2 mM ethylenediaminetetraacetic acid and 1% polyvinyl pyrrolidone (pH 7.0), and 12.5 mM H_2O_2 [35]. One unit of CAT activity was taken as a decrease in absorbance at 240 nm by 0.1 unit per minute. APX activity was measured using the method described by D'Arcy-Lameta et al. [36]. The reaction buffer (1.0 mL) contained 50 mM phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.2 mM ethylenediaminetetraacetic acid, 1.0 mM H_2O_2, and 100 µL of enzyme extract. The reaction was initiated by adding H_2O_2 and measuring the oxidation of ascorbate at 290 nm. One unit of APX was defined as the amount of enzyme required to oxidize 1 µM ascorbate per mg of soluble protein per min.

2.10. Statistical Analysis

The data were analyzed using the SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA). SigmaPlot 10.0 was applied for mapping. A two-way analysis of variance (ANOVA) was used to test the effects of Spd application, drought, and their interactions on the physiological changes in the shoots of *Hordeum jubatum*. Post hoc comparisons with Duncan's multiple range test were used to identify differences between groups, with $p < 0.05$ as the significance cut-off.

3. Results

3.1. Growth

Although the dry weight (DW) of the *H. jubatum* shoots increased with increasing drought stress, this trend slowed down progressively (Figure 1A). The increases in DW were 55.53% (D1/CK), 29.37% (D2/D1), and 2.30% (D3/D2). Moreover, in S1, S2, and S3 shoots, the DW was much higher than that of the S0 shoots at all drought times ($p < 0.05$). For example, the DW of the S1, S2, and S3 shoots increased by 9%, 18%, and 27%, respectively, compared to the S0 shoots at the highest drought intensity (D3).

The WC of the *H. jubatum* shoots decreased significantly with increasing drought intensity ($p < 0.05$, Figure 1B). For example, the WC in the D1, D2, and D3 shoots decreased by 6.68%, 14.18%, and 20.54%, respectively, compared to CK. The WC of the shoots in the Spd treatment was much higher than those of the S0 shoots at any of the drought times. For example, the WC of the S1, S2, and S3 shoots increased by 6.97%, 11.67%, and 16.16%, respectively, at the highest drought intensity (D3) compared to the S0 shoots. Furthermore, the changes in the DW and WC of the shoots were more prominent at 1.0 and 1.5 mM Spd than at 0.5 mM under drought stress. The correlation analysis showed that the DW and WC of *H. jubatum* were positively correlated with POD in D1. In D2, DW and WC were positively correlated with Fv/Fm and SOD. In addition, Tr, NPQ, SOD, POD, CAT, and APX were positively correlated with the DW and WC of *H. jubatum* in D3 (Appendices B.1–B.3). The two-way analysis of variance (ANOVA) indicated that the DW and WC of *H. jubatum* shoots were affected by the drought time, Spd pretreatment, and their interaction (Table 1).

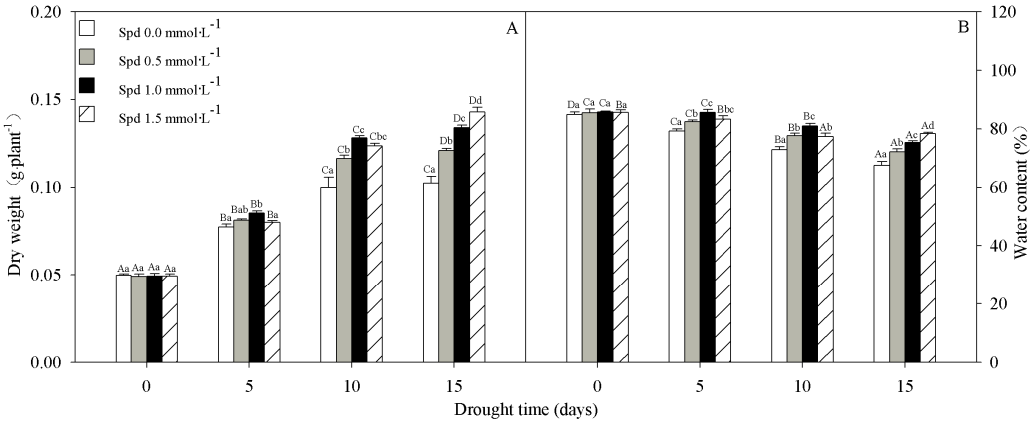

Figure 1. (**A**) Dry weight (DW) and (**B**) water content (WC) of *Hordeum jubatum* shoots not Spd treated (S0) or pretreated with 0.5 mM Spd (S1), 1.0 mM Spd (S2), and 1.5 mM Spd (S3) under drought stress. Days 0, 5, 10, and 15 correspond to CK, D1, D2, and D3 in the experimental treatment, respectively. The capital letters denote significant differences among the different drought intensities from the shoots under the same concentrations of Spd. The small letters denote significant differences among the different concentrations of Spd from the shoots under the same drought intensity. The differences in each parameter were tested by a one−way analysis of variance (ANOVA) at the $p < 0.05$ level. The bars represent the means ± SEs (n = 3).

Table 1. Two-way analysis of variance (ANOVA) of the effects of drought (D), Spd pretreatment (S), and their interactions on the growth, photosynthesis, chlorophyll fluorescence, lipid peroxidation, osmolyte accumulation, and antioxidant enzymes of *Hordeum jubatum* shoots.

Source of Variation	Variable		
	D	S	D × S
Dry weight	982.84 ***	51.01 ***	15.01 ***
Water content	134.10 ***	30.53 ***	5.00 ***
Net photosynthetic rate (P_N)	766.61 ***	11.33 ***	3.44 **
Stomatal conductance (Gs)	217.98 ***	15.92 ***	1.56 ^{NS}
Intercellular CO_2 concentration (Ci)	198.76 ***	6.66 **	1.45 ^{NS}
Transpiration rate (Tr)	240.12 ***	20.42 ***	2.91 *
Fv/Fm	13.59 ***	2.79 ^{NS}	1.13 ^{NS}
ϕPSII	457.15 ***	13.94 ***	1.99 ^{NS}
q_P	302.90 ***	26.96 ***	6.44 ***
NPQ	562.38 ***	28.95 ***	6.33 ***
Malondialdehyde (MDA)	2129.56 ***	115.45 ***	28.49 ***
Soluble protein	1546.63 ***	52.15 ***	11.61 ***
Proline	4465.64 ***	66.72 ***	7.51 ***
Soluble sugar	3359.01 ***	56.10 ***	6.61 ***
Superoxide dismutase (SOD)	1684.53 ***	45.89 ***	10.02 ***
Peroxidase (POD)	2276.98 ***	66.73 ***	12.50 ***
Catalase (CAT)	1863.74 ***	84.43 ***	16.72 ***
Ascorbate peroxidase (APX)	932.95 ***	32.54 ***	6.04 ***

The data represent *F*-values at the 0.05 level. *, **, ***, and ^{NS} indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p > 0.05$, respectively. Fv/Fm—maximal quantum yield of PSII photochemistry; ϕPSII—actual quantum yield of photochemical energy conversion in PSII; q_P—photochemical quenching coefficient; NPQ—nonphotochemical quenching coefficient.

3.2. Photosynthesis

The net photosynthetic rate (P_N), stomatal conductance (Gs), intracellular CO_2 concentration (Ci), and transpiration rate (Tr) of *H. jubatum* shoots decreased significantly with increasing drought stress ($p < 0.05$, Figure 2A–D). For example, the P_N of the D1, D2, and

D3 shoots decreased by 23.07%, 47.32%, and 77.05%, respectively, compared to the control (D0). The P_N, Gs, Ci, and Tr of the shoots treated with Spd were much higher than those of the S0 shoots under drought stress. For example, the Gs of the S0 shoots was only 0.34 mol H_2O $m^{-2}{\cdot}s^{-1}$, while the Gs of shoots treated with 1.0 mM Spd was 0.48 mol H_2O $m^{-2}{\cdot}s^{-1}$ during 10 d of drought stress (D2). Furthermore, these increases were much greater in the S2 and S3 shoots than in the S1 shoots. The increase in Tr in the S1 shoots was 72.97% at the highest drought intensity (D3), while it was 141.45% and 143.07% in the S2 and S3 shoots, respectively. In addition, exogenous Spd did not significantly increase P_N, Gs, Ci, or Tr in the shoots of the control group (D0). Two-way ANOVA indicated that the P_N and Tr of the *H. jubatum* shoots were affected by drought time, Spd, and their interaction (Table 1). Gs and Ci were affected by drought time and the Spd pretreatment but were not affected by the interaction between the two factors (Table 1). The P_N value of *H. jubatum* was positively correlated with Gs, Ci, Tr, proline, soluble sugar, POD, CAT, and APX and negatively correlated with MDA in D1. In addition, P_N was positively correlated with Tr, ϕPSII, q_p, proline, soluble protein, soluble sugar, SOD, and APX and was negatively correlated with MDA content in D2 (Appendices B.1–B.3).

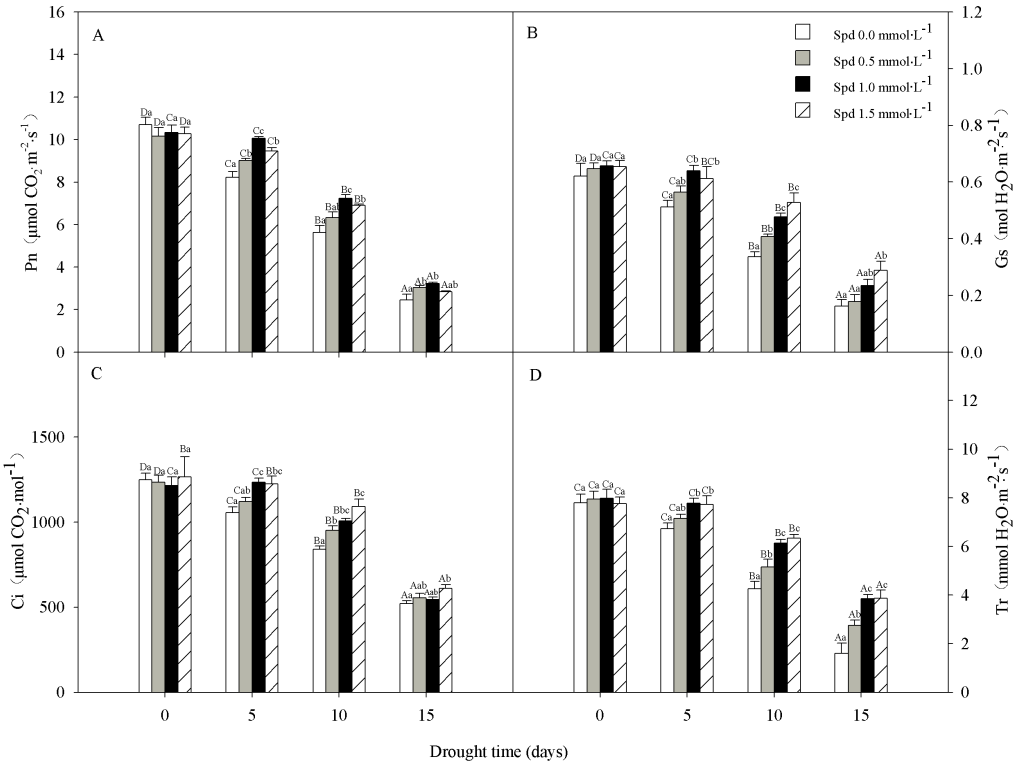

Figure 2. (**A**) Net photosynthetic rate (P_N), (**B**) stomatal conductance (Gs), (**C**) intracellular CO_2 concentration (Ci), and (**D**) transpiration rate (Tr) of *Hordeum jubatum* shoots not Spd treated (S0) or pretreated with 0.5 mM Spd (S1), 1.0 mM Spd (S2), and 1.5 mM Spd (S3) under drought stress. Days 0, 5, 10, and 15 correspond to CK, D1, D2, and D3 in the experimental treatment, respectively. The capital letters denote significant differences among the different drought intensities from the shoots under the same concentrations of Spd. The small letters denote significant differences among the different concentrations of Spd from shoots under the same drought intensity. The differences in each parameter were tested by a one−way analysis of variance (ANOVA) at the $p < 0.05$ level. The bars represent the means ± SEs (n = 3).

3.3. Chlorophyll Fluorescence

The maximal quantum yield of PSII photochemistry (Fv/Fm) and the actual quantum yield of photochemical energy conversion in the PS (ϕPSII) of all *H. jubatum* shoots decreased significantly with increasing drought stress ($p < 0.05$, Figure 3A,B). Spd treatments

significantly increased the Fv/Fm and ϕPSII compared to the S0 shoots under drought stress. Furthermore, the increases were much greater in the S2 shoots (1.0 mM Spd treatment). The increase in ϕPSII in the S2 shoots was 10.61% at D3, while that in the S1 and S3 shoots was only 3.97% and 7.29%, respectively.

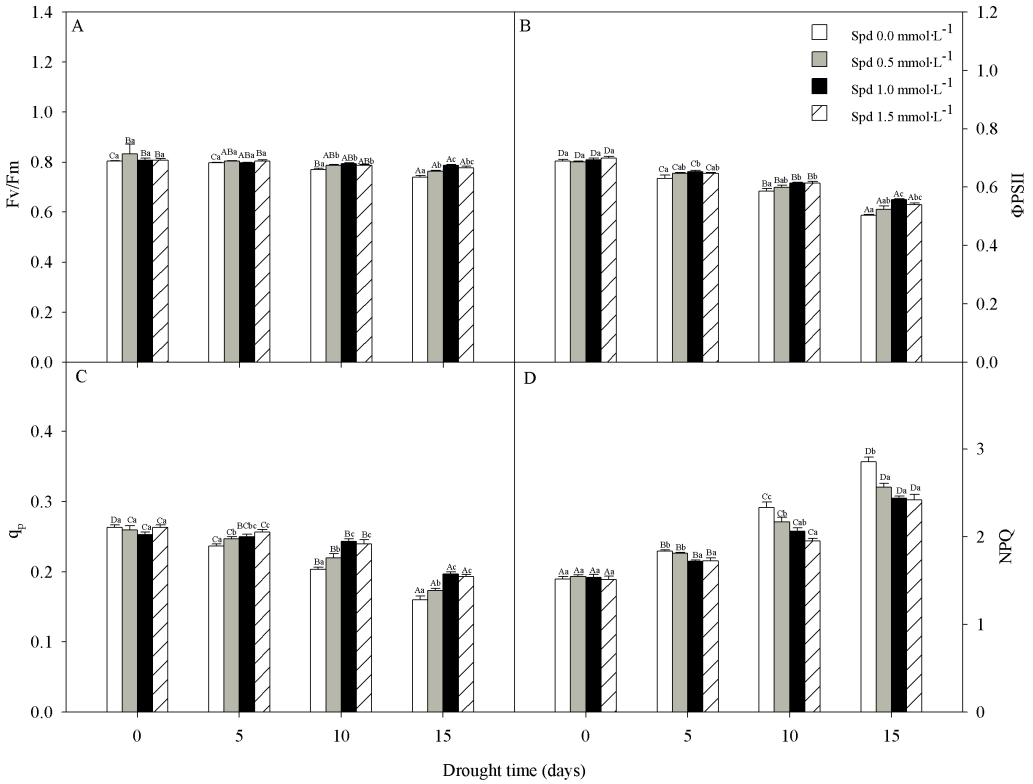

Figure 3. (**A**) Maximal quantum yield of PSII photochemistry (Fv/Fm), (**B**) actual quantum yield of photochemical energy conversion in PSII (ϕPSII), (**C**) photochemical quenching coefficient (q_p), and (**D**) nonphotochemical quenching coefficient (NPQ) of *Hordeum jubatum* shoots not Spd treated (S0) or pretreated with 0.5 mM Spd (S1), 1.0 mM Spd (S2), and 1.5 mM Spd (S3) under drought stress. Days 0, 5, 10, and 15 correspond to CK, D1, D2, and D3 in the experimental treatment, respectively. The capital letters denote significant differences among the different drought intensities from the shoots under the same concentrations of Spd. The small letters denote significant differences among the different concentrations of Spd from shoots under the same drought intensity. The differences in each parameter were tested by a one−way analysis of variance (ANOVA) at the $p < 0.05$ level. The bars represent the means ± SEs (n = 3).

The photochemical quenching coefficient (q_p) in *H. jubatum* shoots decreased significantly with increasing drought stress ($p < 0.05$, Figure 3C). Spd significantly increased q_p in the S1, S2, and S3 shoots under drought stress, and the increases in the S2 and S3 shoots were higher than those in the S1 shoots. The nonphotochemical quenching coefficient (NPQ) of the shoots increased significantly with increasing drought stress ($p < 0.05$, Figure 3D). Spd treatment significantly decreased the NPQ in the S1, S2, and S3 shoots compared to the S0 shoots under drought stress, and the NPQ value of the S2 and S3 shoots decreased more than the S1 shoots. For example, the NPQ values of the S0 and S1 shoots were 2.85 and 2.56, respectively, at the highest drought intensity (D3), and were 2.44 and 2.42 in the S2 and S3 shoots, respectively. In addition, Spd did not significantly affect the Fv/Fm, ϕPSII, q_p, or NPQ of the shoots in the control. Two-way ANOVA indicated that the Fv/Fm of *H. jubatum* shoots was only affected by drought time and ϕPSII was affected by drought time and the Spd pretreatment. The q_p and NPQ were affected by drought time, the Spd pretreatment, and their interaction (Table 1). The correlation analysis showed that the ϕPSII of *H. jubatum*

was positively correlated with q_p, proline, soluble protein, soluble sugar, CAT, and APX and negatively correlated with NPQ and MDA in D2. Moreover, a negative correlation was detected between NPQ, proline, and soluble protein in *H. jubatum* under drought stress (Appendices B.1–B.3).

3.4. Lipid Peroxidation and Osmolyte Accumulation

The MDA content of the shoots increased significantly with increasing drought intensity ($p < 0.05$, Figure 4A). Spd significantly decreased the MDA content compared to the S0 shoots under drought stress. For example, the MDA content in the S0 shoots was 23.5 mmol·g^{-1}, while it was 16.23 mmol·g^{-1} at the highest drought intensity (D3) in the S2 shoots.

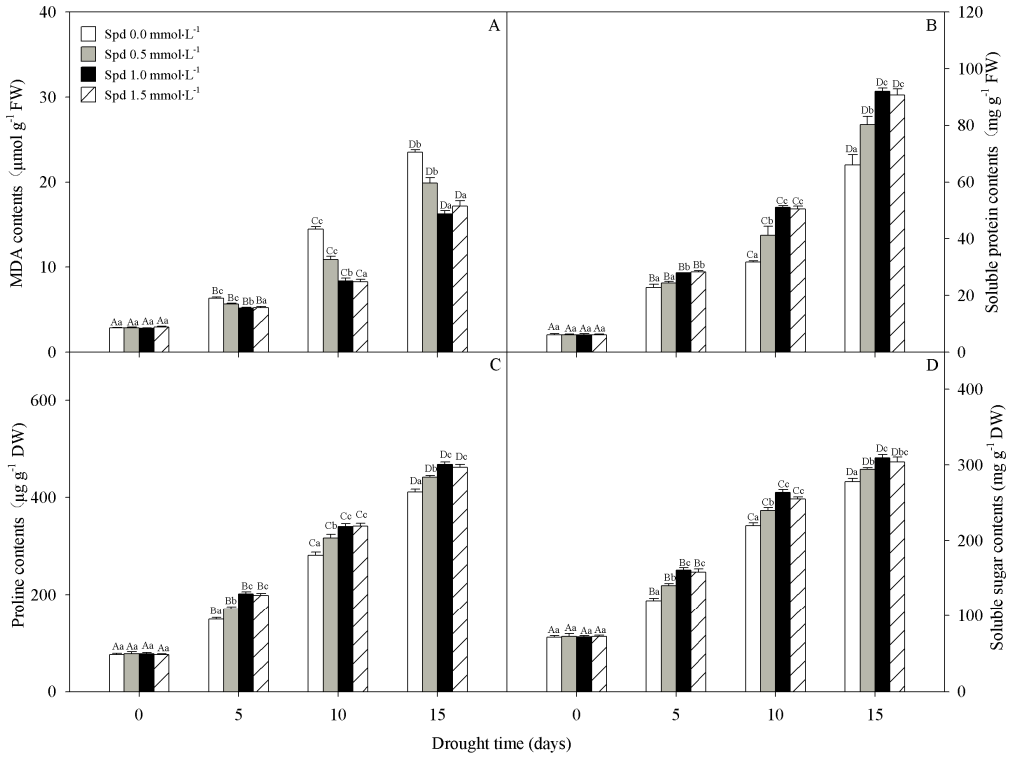

Figure 4. (**A**) Malondialdehyde (MDA), (**B**) soluble protein, (**C**) proline, and (**D**) soluble sugar contents of *Hordeum jubatum* shoots not Spd treated (S0) or pretreated with 0.5 mM Spd (S1), 1.0 mM Spd (S2), and 1.5 mM Spd (S3) under drought stress. Days 0, 5, 10, and 15 correspond to CK, D1, D2, and D3 in the experimental treatment, respectively. The capital letters denote significant differences among the different drought intensities from the shoots under the same concentrations of Spd. The small letters denote significant differences among the different concentrations of Spd from shoots under the same drought intensity. The differences in each parameter were tested by a one−way analysis of variance (ANOVA) at the $p < 0.05$ level. The bars represent the means ± SEs (n = 3).

The soluble protein, proline, and soluble sugar contents of the *H. jubatum* shoots increased significantly with increasing drought intensity ($p < 0.05$, Figure 4B–D). Moreover, Spd significantly increased the soluble protein, proline, and soluble sugar contents under drought stress. The soluble protein, proline, and soluble sugar contents in the S1, S2, and S3 shoots were much higher than those in the S0 shoots under drought stress (D1, D2, and D3), and no significant difference was found between S2 and S3. The soluble protein content of the S1, S2, and S3 shoots increased by 21.62%, 39.53%, and 37.51%, respectively, at the highest drought intensity (D3) compared to the S0 shoots. In addition, the Spd pretreatment did not significantly affect the MDA, soluble protein, proline, or soluble sugar contents of the shoots in the control (D0). Two-way ANOVA indicated that the MDA, soluble protein,

proline, and soluble sugar contents of *H. jubatum* shoots were affected by drought time, the Spd pretreatment, and their interaction (Table 1). In addition, MDA in *H. jubatum* was positively correlated with the NPQ under drought stress and negatively correlated with proline, soluble protein, and soluble sugar contents. Moreover, a positive correlation was detected between proline, soluble protein, and soluble sugar in *H. jubatum* under drought stress (Appendices B.1–B.3).

3.5. Antioxidant Enzyme Activities

The SOD and CAT activities in *H. jubatum* shoots increased first and then decreased with increasing drought intensity ($p < 0.05$). The highest SOD (183 U·mg^{-1}) and CAT (9.07 U·mg^{-1}) activities in the shoots occurred during the D2 drought stress (Figure 5A,C). Moreover, Spd significantly increased SOD and CAT activities compared to the S0 shoots under drought stress, except for CAT activity in S1 shoots at the highest drought intensity (D3). The SOD activity was only 149.33 U·mg^{-1} in S0 shoots, while it was 184.67 U·mg^{-1} in S3 shoots under the highest drought intensity (D3).

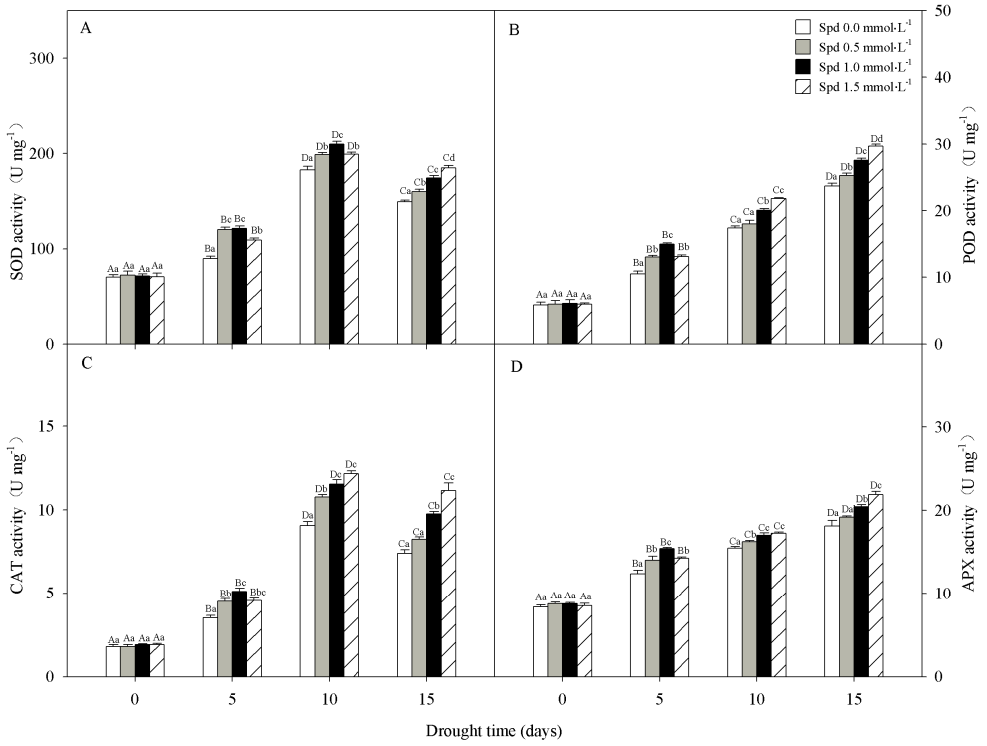

Figure 5. (**A**) Superoxide dismutase (SOD), (**B**) peroxidase (POD), (**C**) catalase (CAT), and (**D**) ascorbate peroxidase (APX) activity of *Hordeum jubatum* shoots not Spd treated (S0) or pretreated with 0.5 mM Spd (S1), 1.0 mM Spd (S2), and 1.5 mM Spd (S3) under drought stress. Days 0, 5, 10, and 15 correspond to CK, D1, D2, and D3 in the experimental treatment, respectively. The capital letters denote significant differences among the different drought intensities from the shoots under the same concentrations of Spd. The small letters denote significant differences among the different concentrations of Spd from shoots under the same drought intensity. The differences in each parameter were tested by a one−way analysis of variance (ANOVA) at the $p < 0.05$ level. The bars represent the means ± SEs (n = 3).

POD and APX activities in *H. jubatum* shoots increased significantly with increasing drought intensity ($p < 0.05$, Figure 5B,D). Spd significantly increased POD and APX activities compared to S0 shoots, except for POD activity in S1 shoots at the D2 drought time and APX activity at the D3 drought time. The POD activity of the S1, S2, and S3 shoots was increased by 6.61%, 16.32%, and 25.18%, respectively, at the highest drought intensity (D3) compared to the S0 shoots. Moreover, the POD and APX activities in the S2 shoots were

much greater; however, as the stress progressed to D2 and D3, the POD and APX activities of the S3 shoots were higher. In addition, Spd did not significantly affect SOD, POD, CAT, or APX activity in shoots of the control (D0). Two-way ANOVA indicated that the SOD, POD, CAT, and APX activities of the *H. jubatum* shoots were affected by drought time, the Spd pretreatment, and their interaction (Table 1). In addition, a positive correlation was detected between the POD, CAT, APX, WC, and P_N of *H. jubatum* under D1. As drought stress was extended, SOD, POD, CAT, and APX were positively correlated with DW, WC, and Gs in D3 (Appendices B.1–B.3).

4. Discussion

Drought stress affects seedling growth and triggers the response to insufficient water [37–39]. The results of the present study showed that the increasing trend of DW in the shoots slowed progressively as drought stress increased, indicating that the inhibitory effect of drought stress on the growth of shoots gradually intensified. Applying Spd significantly increased the DW compared to that in S0 shoots, suggesting that exogenous Spd alleviated the inhibition of growth caused by drought stress and conferred drought resistance in *H. jubatum* (Figure 1A). In addition, the WC decreased significantly with increasing drought intensity, and the Spd pretreatment alleviated the effects of water loss under drought stress (Figure 1B). The relatively high WC in the Spd-pretreated shoots indicated that the effects of osmotic stress caused by drought were mitigated by exogenous Spd. Similar results have been reported in rice (*Oryza sativa*) [40]. Moreover, with the increase in drought intensity, different concentrations of Spd also had different effects on alleviating the damage caused by exposure to drought stress, expressed by the changes in DW and WC. In the present study, when drought stress lasted for 5 or 10 days (D1 and D2), 1.0 mM Spd allowed the seedlings to grow better; however, the effects of S3 shoots pretreated with 1.5 mM Spd were better under the drought stress that lasted 15 days (D3; Figure 1).

Plants obtain energy through photosynthesis. Photosynthetic performance is a critical criterion to describe drought resistance in plants [41]. In the present study, the P_N value of the *H. jubatum* shoots decreased significantly with increasing drought intensity, indicating that the drought stress limited photosynthesis. Spd significantly increased the P_N of the shoots compared to the S0 shoots during drought stress (Figure 2A). This increase may have occurred because the exogenous Spd improved photosynthetic performance, which mitigated the inhibition caused by abiotic stress. This agrees with a previous study on bluegrass (*Poapratensis*) [26]. Better photosynthetic performance leads to a greater accumulation of photoassimilates; thus, the DW of the Spd-pretreated shoots was higher than that of the S0 shoots (Figure 1A). Photosynthesis responds to the osmotic shock caused by drought stress by modulating the stomata [42]. In the present study, the Gs of the shoots decreased gradually in the presence of increasing drought stress (Figure 2B). Stomatal closure regulates pivotal physiological processes in plants responding to drought stress, actively adjusts the absorption of CO_2 for photosynthesis, and controls transpiration to prevent excess water consumption [42]. In the present study, the Ci and Tr of the shoots decreased significantly under drought stress, indicating the effects of the stomata on photosynthesis, which may have limited carboxylation in *H. jubatum* (Figure 2B–D). A study on mulberry (*Morus indica*) seedlings under drought stress reported similar results [43]. The Spd pretreatment significantly enhanced the Gs, Ci, and Tr of the shoots, suggesting that exogenous Spd alleviated photosynthetic damage in the shoots caused by the inhibition of stomata during drought stress (Figure 2B–D). The P_N, Gs, Ci, and Tr values of the shoots pretreated with 1.0 or 1.5 mM Spd were greater than those of the 0.5 mM Spd treatment under drought stress, suggesting that a relatively high concentration of Spd modulated photosynthesis to avoid the negative effects caused by drought stress (Figure 2).

PSII is a central component of photosynthesis and the most vulnerable part of the photosynthetic apparatus in plants subjected to abiotic stress. Inhibited energy conversion and electron transport in PSII is the first manifestation of photosynthesis [44]. Measuring chlorophyll fluorescence, the efficiency of PSII photochemistry, and effective PSII quantum

yield indicates the status of PSII. Chlorophyll fluorescence analysis has emerged as a rapid non-destructive method to determine photosynthetic performance and has been broadly applied to dissect the mechanisms related to photosynthesis in plants suffering from abiotic stress [45,46]. The maximum efficiency of PSII photochemistry, Fv/Fm, is a sensitive coefficient used to estimate the intrinsic quantum efficiency on the premise that all PSII centers are open to reduction of Q_A (the primary quinone electron acceptor in PSII) [45]. In the current study, the Fv/Fm ratio of *H. jubatum* shoots decreased progressively with increasing drought stress. In particular, the ratio under 15-day drought stress was lower than 0.75 (optimal value is ~0.83 in most plant species) (Figure 3A) [45]. These results suggest that the *H. jubatum* shoots exposed to drought stress were suffering from severe stress-induced perturbations, which may have deactivated the D1 proteins in the chloroplasts [47]. Similar results were reported in a barley (*Hordeum vulgare*) drought tolerance study [48]. The apparent quantum yield, ϕPSII, assesses the proportion of light energy absorbed by LHCII to drive and participate in photochemical reactions. The extent of openness of the PSII centers, q_p, is an indicator of the potential for photochemistry in the PSII reaction centers [49]. The ϕPSII and q_p values in the shoots decreased progressively with increasing drought stress, indicating an overall reduction in photochemical efficiency (Figure 3B,C). These results could be due to a reduction in downstream electron acceptors, such as plastoquinone, caused by the drought stress. This reduction, in turn, leads to inhibited photochemical electron transfer [50]. Apart from being re-emitted as chlorophyll fluorescence, excess excitation energy that is not utilized by photochemistry is dissipated as heat [45]. The NPQ represents the quenching of excess excitation energy [51]. In the present study, the NPQ progressively increased with the increasing drought stress, indicating that the inhibition of the photochemical reaction in PSII was aggravated, leading to excess energy being dissipated as heat (Figure 3D).

In the present study, Spd significantly alleviated the inhibition of PSII under drought stress. Spd acted as a permeable buffer and stimulated ATP synthesis, such that the PSII centers were held open. Thereby, the *H. jubatum* photosynthetic reaction centers maintained the diversion of electrons and produced sufficient NADPH and ATP for carbon assimilation [52], which may also explain why the Spd pretreatment optimized photosynthetic performance and maintained plant growth during drought stress (Figures 1 and 2). Moreover, PSII in the shoots pretreated with 1.0 or 1.5 mM Spd performed better under drought stress, suggesting that a relatively high concentration of Spd promoted energy conversion and accelerated the diversion of electrons to maintain the photochemical reactions in PSII.

Plants actively accumulate osmotic substances (as solutes in the cytochylema) to reduce osmotic potential, maintain osmotic equilibrium, and cope with oxidative stress, a process which is defined as osmotic adjustment. This adaptive mechanism is a significant plant drought-resistance strategy to maintain cell turgor and stabilize the macromolecular structures [6,53]. A range of substances are known to accumulate and are involved in osmotic adjustment, such as soluble proteins, proline, and soluble sugars [6,54]. Here, soluble protein, proline, and soluble sugar contents accumulated in the *H. jubatum* shoots with increasing drought intensity (Figure 4B–D). Thus, *H. jubatum* accumulated osmotic regulatory substances in response to drought stress. Blum et al. showed that osmotic adjustments triggered by the dehydration signal ameliorate the deleterious effects of stress on growth and photosynthesis under drought stress conditions [55]. Prominent evidence is available regarding Spd as a growth regulator that promotes the production of metabolites from several osmotically active substances [17,56]. The Spd pretreatment facilitated the accumulation of soluble protein, proline, and soluble sugar in *H. jubatum* shoots during drought stress (Figure 4B–D). This accumulation led to an increase in the osmotic adjustment ability of the shoots in response to drought stress, which maintained water equilibrium and protected the membranes and macromolecules. These results suggest that Spd controls redox homeostasis and maintains normal cellular metabolic processes [14,23]. A previous study in wheat (*T. aestivum*) seedlings showed that exogenous Spd effectively facilitated the accumulation of osmoprotectants to protect against oxidative

damage in plants under drought stress [17]. Moreover, the degree of accumulation of soluble protein, proline, and soluble sugar in the shoots pretreated with 1.0 or 1.5 mM Spd was more than that in the 0.5 mM Spd treatment under drought stress, suggesting that a relatively high concentration of Spd promoted osmotic adjustments to improve *H. jubatum* drought resistance (Figure 4B–D).

The balance between the generation and utilization of electrons is broken when plants suffer from drought stress. Excessive reactive oxygen species (ROS) subsequently accumulate to attack the most sensitive biological macromolecules, giving rise to the peroxidation of membrane lipids and ultimately leading to oxidative deterioration and cell death [57]. MDA is a major lipid peroxidation product and an indicator of the degree of oxidative damage. SOD, POD, CAT, and APX are pivotal antioxidant defense enzymes that cooperatively defend by scavenging ROS in response to oxidative stress [58]. In the present study, the *H. jubatum* shoots exhibited oxidative damage, as indicated by the progressive increase in MDA levels with increasing drought stress (Figure 4A). Concomitantly, protective responses must be executed in the shoots to prevent damage from over-accumulating ROS. The level of antioxidant enzyme activity increased significantly as the drought stress increased. Among them, the POD and APX activities increased progressively with increased drought intensity, whereas the SOD and CAT activities increased first and then decreased, suggesting that drought stress damaged the antioxidant systems in the shoots (Figure 5). It has been shown that enhanced abiotic stress resistance can be achieved by exogenous Spd treatment, which detoxifies ROS and activates the antioxidative machinery [26,56,59]. The antioxidant enzymes (SOD, POD, CAT, and APX) were further activated in the shoots in response to the Spd pretreatment, and MDA content decreased compared to that in untreated shoots (S0) under drought stress (Figure 5). These results suggest that Spd improved the drought resistance of *H. jubatum* by modulating antioxidative capacity, which is in line with a previous report [23]. Furthermore, the 1.0 and 1.5 mM Spd treatments revealed enhanced effects. Increasing the concentration of Spd within limits better protected the plants against ROS.

5. Conclusions

This study revealed the effects of exogenous Spd on conferring drought resistance in *H. jubatum* seedlings. Drought stress inhibited the growth of *H. jubatum* and damaged the photosynthetic system, triggering a range of protective responses (e.g., reducing the openness of the PSII centers, inhibiting photochemical electron transfer, and increasing heat dissipation). Spd mitigated the drought damage by promoting a variety of responses and adaptive mechanisms, such as the adjustment of stomatal movement, promotion of photosynthetic capacity, acceleration of the synthesis of osmoregulatory molecules, and activation of antioxidant enzyme systems. Additionally, *H. jubatum* under the 1.5 mM Spd treatment showed the best drought tolerance (Figure 6). These findings illustrate that the Spd pretreatment is a potential method to improve drought resistance in *H. jubatum* and enable the establishment of lush landscapes under water-limited conditions.

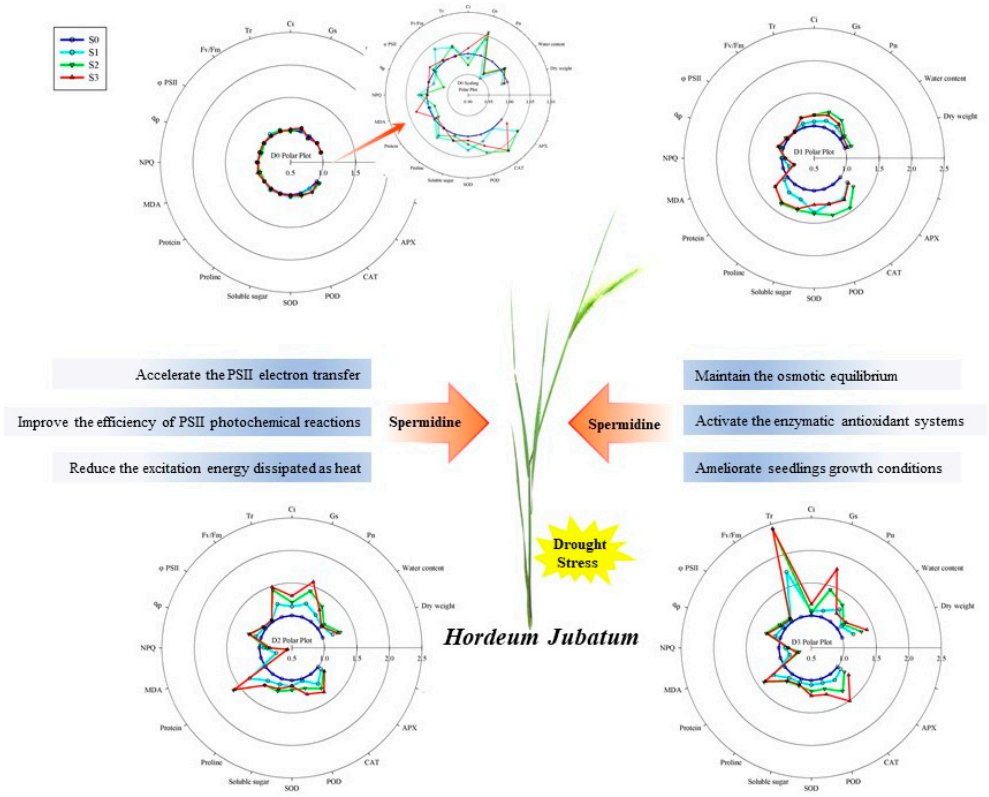

Figure 6. Diagram of the multiple roles of exogenous Spd in the drought tolerance of *Hordeum jubatum* shoots. The polar plots show the effects of Spd pretreatment on the parameters under different drought times (D0, D1, D2, and D3 represent 0–15 d drought intensity with 5 d intervals). S0, S1, S2, S3 corresponds to exogenous spd concentration of 0, 0.5 mM, 1.0 mM, 1.5 mM respectively. All the data were normalized to those of the reference treatment (no pretreatment with Spd), and each reference variable was standardized by receiving a numerical value of 1. In order to facilitate observation, the D0 scaling polar plot represents D0 with refinement from 0.5~2.5 to 0.9~1.1.

Author Contributions: Conceptualization, J.T. and Y.Z.; methodology, J.T., Y.Z., J.L. and Y.P.; software, J.T., Y.Z., J.W., X.C. and Q.Y.; validation, Y.W. and J.W.; formal analysis, J.T. and Y.Z.; investigation, Y.P., Q.Y. and J.L.; resources, J.W. and J.L.; data curation, J.W., Q.Y. and J.L.; writing—original draft preparation, J.T. and Y.W.; writing—review and editing, J.T. and X.C.; visualization, J.T. and Y.Z.; supervision, J.W., Q.Y. and J.L.; project administration, J.W., Q.Y. and J.L.; funding acquisition, J.W., Q.Y. and J.L. All authors have read and agreed to the published version of the manuscript.

Funding: The study was supported by the Fundamental Research Funds for the Central Universities (2572019CP06, 2572018DB01), the Natural Science Foundation of China (32072666), and the Science and Technology Fundamental Resources Investigation Programme from the Ministry of Science and Technology (2019FY100506-05).

Data Availability Statement: Not applicable.

Acknowledgments: The authors sincerely acknowledge the technical assistance provided by Yuanhong Sun from College of Finance and Public Administration, Harbin University of Commerce for the data statistics and analysis.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. List of primary chemicals used in this study.

Chemicals	Formula	Manufacturers
Hydrogen peroxide	H_2O_2	Merck
Spermidine	$C_7H_{19}N_3$	Crovell
Trichloroacetic acid	$C_2HCl_3O_2$	Mayao
Thiobarbituric acid	$C_4H_4N_2O_2S$	Amitychem
Ethylenediamine tetraacetic acid	$C_{10}H_{16}N_2O_8$	Damao
Polyvinyl pyrrolidone	$C_{13}H_{16}O_4$	Crovell
Ninhydrin	$C_9H_6O_4$	Amitychem
Sulfosalicylic acid	$C_7H_6O_6S$	Crovell
Glacial acetic acid	$C_2H_4O_2$	Yingruida
Toluene	$C_6H_5CH_3$	Merck
Ethanol	C_2H_6O	Jiaoze
Anthrone	$C_{14}H_{10}O$	Beierka
Guaiacol	$C_7H_8O_2$	Longfei
Nitro blue tetrazolium	$C_{40}H_{30}C_{l2}N_{10}O_6$	Amitychem

Appendix B

Appendix B.1

Table A2. Correlation analysis of physiological indexes under drought stress lasting for 5 days (D1).

Correlations	DW	WC	P_N	Gs	Ci	Tr	Fv/Fm	ϕPSII	q_p	NPQ	MDA	SP	Proline	SS	SOD	POD	CAT	APX
DW	1																	
WC	0.938	1																
P_N	0.905	0.994 **	1															
Gs	0.842	0.974 *	0.991 **	1														
Ci	0.736	0.921	0.954 *	0.985 *	1													
Tr	0.733	0.921	0.953 *	0.984 *	0.999 **	1												
Fv/Fm	−0.113	0.09	0.075	0.133	0.191	0.221	1											
ϕPSII	0.971 *	0.912	0.862	0.797	0.687	0.691	0.087	1										
q_p	0.496	0.758	0.79	0.851	0.905	0.916	0.579	0.529	1									
NPQ	−0.668	−0.866	−0.915	−0.956 *	−0.987 *	−0.983 *	−0.099	−0.586	−0.869	1								
MDA	−0.763	−0.938	−0.956 *	−0.979 *	−0.982 *	−0.987 *	−0.327	−0.757	−0.935	0.94	1							
SP	0.644	0.861	0.908	0.954 *	0.991 **	0.989 *	0.193	0.583	0.912	−0.995 **	−0.958 *	1						
Proline	0.755	0.934	0.962 *	0.989 *	0.999 **	0.999 **	0.219	0.716	0.911	−0.977 *	−0.990 **	0.984 *	1					
SS	0.768	0.942	0.964 *	0.988 *	0.992 **	0.995 **	0.271	0.746	0.922	−0.959 *	−0.998 **	0.971 *	0.997 **	1				
SOD	0.875	0.865	0.811	0.762	0.67	0.683	0.355	0.961 *	0.631	−0.547	−0.777	0.57	0.707	0.751	1			
POD	0.960 *	0.994 **	0.977 *	0.946	0.878	0.88	0.106	0.950 *	0.72	−0.81	−0.911	0.806	0.896	0.911	0.909	1		
CAT	0.933	0.991 **	0.975 *	0.951 *	0.892	0.896	0.192	0.939	0.771	−0.819	−0.935	0.824	0.911	0.93	0.921	0.996 **	1	
APX	0.949	0.998 **	0.985 *	0.959 *	0.898	0.9	0.116	0.936	0.745	−0.834	−0.927	0.831	0.914	0.928	0.896	0.999 **	0.997 **	1

* Significant correlation ($p < 0.05$); ** extremely significant correlation ($p < 0.01$). DW—dry weight; WC—water content; P_N—net photosynthetic rate; Gs—stomatal conductance; Ci—intercellular CO_2 concentration; Tr—transpiration rate; Fv/Fm—maximal quantum yield of PSII photochemistry; ϕPSII—actual quantum yield of photochemical energy conversion in PSII; q_p—photochemical quenching coefficient; NPQ—nonphotochemical quenching coefficient; MDA—malondialdehyde; SP—soluble protein; SS—soluble sugar; SOD—superoxide dismutase; POD—peroxidase; CAT—catalase; APX—ascorbate peroxidase.

Appendix B.2

Table A3. Correlation analysis of physiological indexes under drought stress lasting for 10 days (D2).

Correlations	DW	WC	P_N	Gs	Ci	Tr	Fv/Fm	ϕPSII	q_p	NPQ	MDA	SP	Proline	SS	SOD	POD	CAT	APX
DW	1																	
WC	0.945	1																
P_N	0.989 *	0.931	1															
Gs	0.903	0.712	0.902	1														
Ci	0.879	0.677	0.860	0.991 **	1													
Tr	0.956 *	0.811	0.960 *	0.987 *	0.966 *	1												
Fv/Fm	0.970 *	0.980 *	0.935	0.787	0.775	0.862	1											
ϕPSII	0.980 *	0.864	0.980 *	0.968 *	0.944	0.995 **	0.904	1										
q_p	0.977 *	0.876	0.991 **	0.949	0.913	0.987 *	0.897	0.995 **	1									
NPQ	−0.899	−0.705	−0.888	−0.998 **	−0.998 **	−0.980 *	−0.791	−0.961 *	−0.937	1								
MDA	−0.984 *	−0.873	−0.972 *	−0.963 *	−0.949	−0.989 *	−0.924	−0.996 **	−0.984 *	0.962 *	1							
SP	0.983 *	0.874	0.985 *	0.961 *	0.935	0.993 **	0.909	1.000 **	0.997 **	−0.954 *	−0.995 **	1						
Proline	0.985 *	0.874	0.970 *	0.961 *	0.949	0.987 *	0.927	0.995 **	0.982 *	−0.961 *	−1.000 **	0.994 **	1					
SS	0.992 **	0.935	1.000 **	0.903	0.864	0.961 *	0.942	0.982 *	0.990 **	−0.891	−0.975 *	0.986 *	0.974 *	1				
SOD	0.964 *	0.998 **	0.951 *	0.756	0.723	0.848	0.986 *	0.895	0.905	−0.750	−0.903	0.904	0.904	0.955 *	1			
POD	0.783	0.547	0.810	0.966 *	0.945	0.930	0.615	0.891	0.881	−0.955 *	−0.869	0.883	0.864	0.807	0.600	1		
CAT	0.941	0.783	0.916	0.983 *	0.987 *	0.981 *	0.866	0.975 *	0.949	−0.989 *	−0.984 *	0.969 *	0.985 *	0.921	0.821	0.902	1	
APX	0.952 *	0.800	0.951 *	0.991 **	0.974 *	0.999 **	0.857	0.993 **	0.981 *	−0.987 *	−0.989 *	0.990 *	0.987 *	0.952 *	0.837	0.933	0.987 *	1

* Significant correlation ($p < 0.05$); ** extremely significant correlation ($p < 0.01$). DW—dry weight; WC—water content; P_N—net photosynthetic rate; Gs—stomatal conductance; Ci—intercellular CO_2 concentration; Tr—transpiration rate; Fv/Fm—maximal quantum yield of PSII photochemistry; ϕPSII—actual quantum yield of photochemical energy conversion in PSII; q_p—photochemical quenching coefficient; NPQ—nonphotochemical quenching coefficient; MDA—malondialdehyde; SP—soluble protein; SS—soluble sugar; SOD—superoxide dismutase; POD—peroxidase; CAT—catalase; APX—ascorbate peroxidase.

Appendix B.3

Table A4. Correlation analysis of physiological indexes under drought stress lasting for 15 days (D3).

Correlations	DW	WC	PN	Gs	Ci	Tr	Fv/Fm	ϕPSII	qp	NPQ	MDA	SP	Proline	SS	SOD	POD	CAT	APX
DW	1																	
WC	0.998 **	1																
P_N	0.663	0.618	1															
Gs	0.933	0.948	0.373	1														
Ci	0.829	0.860	0.226	0.864	1													
Tr	0.980 *	0.966 *	0.772	0.878	0.702	1												
Fv/Fm	0.915	0.889	0.875	0.764	0.538	0.976 *	1											
ϕPSII	0.869	0.839	0.853	0.738	0.445	0.949	0.989 *	1										
q_p	0.949	0.933	0.750	0.873	0.622	0.987 *	0.976 *	0.973(*)	1									
NPQ	−0.970 *	−0.956 *	−0.819	−0.820	−0.723	−0.983 *	−0.962 *	−0.912	−0.944	1								
MDA	−0.947	−0.926	−0.836	−0.817	−0.608	−0.992 **	−0.996 **	−0.979(*)	−0.987 *	0.975 *	1							
SP	0.967 *	0.949	0.817	0.840	0.664	0.997 **	0.987 *	0.960(*)	0.983 *	−0.988 *	−0.997 **	1						
Proline	0.952 *	0.932	0.840	0.816	0.626	0.993 **	0.994 **	0.971(*)	0.982 *	−0.983 *	−0.999 **	0.999 **	1					
SS	0.931	0.907	0.862	0.785	0.573	0.984 *	0.999 **	0.983(*)	0.980 *	−0.971 *	−0.999 **	0.993 **	0.998 **	1				
SOD	0.985 *	0.990 **	0.547	0.980 *	0.846	0.953 *	0.868	0.834	0.937	−0.918	−0.909	0.928	0.910	0.886	1			
POD	0.975 *	0.983 *	0.491	0.990 *	0.869	0.931	0.833	0.797	0.914	−0.892	−0.879	0.901	0.881	0.853	0.998 **	1		
CAT	0.964 *	0.974 *	0.457	0.995 **	0.868	0.917	0.814	0.782	0.904	−0.873	−0.862	0.885	0.863	0.835	0.995 **	0.999 **	1	
APX	0.975 *	0.985 *	0.482	0.988 *	0.889	0.924	0.821	0.779	0.901	−0.892	−0.869	0.895	0.872	0.842	0.996 **	0.999 **	0.998 **	1

* Significant correlation ($p < 0.05$); ** extremely significant correlation ($p < 0.01$). DW—dry weight; WC—water content; P_N—net photosynthetic rate; Gs—stomatal conductance; Ci—intercellular CO_2 concentration; Tr—transpiration rate; Fv/Fm—maximal quantum yield of PSII photochemistry; ϕPSII—actual quantum yield of photochemical energy conversion in PSII; q_p—photochemical quenching coefficient; NPQ—nonphotochemical quenching coefficient; MDA—malondialdehyde; SP—soluble protein; SS—soluble sugar; SOD—superoxide dismutase; POD—peroxidase; CAT—catalase; APX—ascorbate peroxidase.

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