Article

Exogenous Spermidine Optimizes Nitrogen Metabolism and Improves Maize Yield under Drought Stress Conditions

Ling Dong1,†, Lijie Li2,†, Yao Meng3, Hongliang Liu3, Jing Li1, Yang Yu4, Chunrong Qian4, Shi Wei1 and Wanrong Gu1,*

1 College of Agriculture, Northeast Agricultural University, Harbin 150030, China
2 Henan Collaborative Innovation Center of Modern Biological Breeding, Henan Institute of Science and Technology, Xinxiang 453003, China
3 Heilongjiang Academy of Land Reclamation Sciences, Harbin 150030, China
4 Institute of Tillage and Cultivation, Heilongjiang Academy of Agricultural Sciences, Harbin 150086, China
* Correspondence: wanronggu@neau.edu.cn
† These authors contributed equally to this work.

Abstract: This study was to explore the nitrogen metabolism and transcriptome mechanism of spermidine (Spd) under drought stress conditions. Firstly, maize variety Xianyu 335 (drought insensitive type) and Fenghe 1 (drought sensitive type) were chosen as experimental materials under hydroponic conditions. The effects of PEG-6000 combined with Spd application on nitrogen metabolism were studied. Secondly, we chose maize variety Xianyu 335 for the field experiment. At the flowering stage, normal water treatment and moderate drought stress were carried out, respectively. The results showed that: (1) Hydroponics experiment showed that the content of NH\textsuperscript{4+} in the leaves of maize seedlings under drought stress increased significantly, while the content of NO\textsuperscript{3−} and nitrate reductase (NR), glutamine synthetase (GS), glutamate synthase (GOGAT), glutamine dehydrogenase (GDH), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) increased significantly. Spd can promote the assimilation of excess ammonia by enhancing the activities of ammonia assimilating enzymes GS/GOGAT and GDH, and transaminase (GOT and GPT), effectively alleviate the ammonia toxicity and nitrogen metabolism disorder induced by drought stress. (2) Pot experiment showed that Spd significantly promoted the root growth of maize under drought stress, so as to improve the absorption and utilization of water and nutrients. In addition, Spd can improve the chlorophyll content and photosynthetic rate of maize leaves under drought stress. After the application of exogenous Spd, the photosynthetic green leaf area increased, the leaf senescence rate slowed down, and the dry matter accumulation increased after anthesis, resulting in the increase of grain weight and grain number per ear, and finally improve the maize yield.

Keywords: maize; spermidine; drought stress; nitrogen metabolism; transcriptome analysis; yield

1. Introduction

Drought stress has been affecting crop production due to its high frequency, wide range, and long duration [1]. Drought stress can hinder crop growth, and the annual yield loss due to drought has reached 30 billion kg [2–6]. Maize (Zea mays L.) is the main source of animal feed and industrial raw materials [5,6]. Drought stress is the main restrictive factor of maize production, which will reduce maize yield by 25–30% [7–9]. Even in the major maize production area of the United States (US), despite the improvement of varieties and agronomic management, drought stress had a negative impact on maize production in the past 20 years [10]. It is expected that global climate warming will further exacerbate the adverse effects of drought, which may lead to a significant decline in maize production [7,11]. Therefore, under the pressure of escalating environmental conditions, the physiological adaptation strategies of maize to drought stress and the regulation...
mechanism have become important research topics [12–15]. Polyamines (PAs) are aliphatic nitrogen-containing bases with strong biological activity [16]. There are three forms of PAs, which are diamine putrescine (Put), triamine spermidine (Spd) and tetramine spermine (Spm), respectively. PAs participate in metabolic processes related to maize growth, such as cell division, leaf senescence, protein translation and so on [17]. Spd can not only regulate osmotic potential as a direct stress-protective substance, but also participate in the construction of plant stress resistance mechanism as a signal molecule in stress signal transduction. Former studies showed Spd can effectively alleviate the persecution of abiotic stresses on maize, such as temperature stress, salt stress, drought stress, hypoxia stress, flooding stress, heavy metal [18–21].

The process of nitrogen (N) absorption depends largely on the mobility of water in the soil. NH$_4^+$ or NO$_3^-$ is initially dissolved in the water, then absorbed by the roots and then transported to the aboveground part of the plant [22]. Drought stress reduces the absorption of ammonium and nitrate by plants [23]. After NH$_4^+$ and NO$_3^-$ are absorbed into the roots, a large amount of NH$_4^+$ is locally assimilated. In contrast, only a limited amount of NO$_3^-$ is assimilated in roots [24]. During this assimilation, NO$_3^-$ is converted to NH$_4^+$ by nitrate reductase (NR) and nitrite reductase (NiR) [24]. NH$_4^+$ is assimilated with glutamine and glutamate through glutamine synthetase (GS) and glutamate synthase (GOGAT) [25]. Drought stress also affects enzyme activity and transcriptional abundance of genes involved in N metabolism [26,27]. The potential role of N metabolism on plant photosynthesis under drought stress may involve the following aspects. First, higher N increased stomatal sensitivity to drought stress and maintained high photosynthetic capacity [28]. Studies have shown that NO$_3^-$ content in leaves is positively correlated with GS, indicating that NO$_3^-$ content can be used as a regulator of stomatal movement [29]. GS increased accompanied with the increase of NO$_3^-$ concentration in the matrix even under drought stress [30]. Secondly, N metabolism consumes too much ATP energy. Therefore, N metabolism can partially dissipate the excessive captured light energy to reduce the photoinhibition of photosynthesis caused by drought stress [31–34].

The changes of PAs levels were generally observed in various plant species subjected to a series of abiotic stresses [35–37]. Recent studies have shown that constitutive or inducible overexpression of polyamine biosynthesis genes from different plant and animal sources, leading to an increase in the level of at least one endogenous polyamine and enhances the tolerance of plants to various abiotic stresses [17,38–40]. In contrast, AtADC1/2 knockout mutants showed less Put accumulation and reduced tolerance to salt and freezing, and AtSPMS/AtACL5 knockout mutants showed less Spm accumulation and reduced tolerance to salt, drought and heat stress [17,41]. In addition, other genes involved in polyamine metabolism can also regulate plant tolerance to abiotic stress by affecting polyamine metabolism [40]. The expression of arginase can regulate the accumulation of Put and Spm and enhance the tolerance of plants to stress [39].

Higher plants have special strategies to deal with drought stress [42,43]. Studies have shown that PAs inhibits the ability of rectifier K$^+$ channel in broad bean guard cell membrane, which indicates that PAs is related to inhibiting stomatal opening and inducing stomatal closure [44–46]. Drought stress induced increase in ABA content may promote the accumulation of PAs, where they are oxidized by apoplast amine oxidase to produce H$_2$O$_2$ for signal cascade reaction [47,48]. On the other hand, PAs significantly enhances enzymatic activity and non-enzymatic antioxidants [49–51]. Higher PAs content can activate SOD and CAT enzyme activities [52]. There is a lot of evidence showing the importance of the coordinated role of synthesis and catabolism of PAs in plant adaptation and response to drought stress [53,54]. Several attempts have been made to produce drought tolerant strains by overexpression of PA synthesis genes. Induced by the stress response promoter RD29A from Arabidopsis, ADC gene was expressed in Lotus tenuis plants, resulting in a significant increase in the content of Put in plants, but there was no change in the contents of Spd and Spm, which showed a direct correlation between ADC expression level and drought tolerance [45,55]. The NCED gene (encoding a key enzyme involved in ABA biosynthesis)
was also up-regulated, suggesting that this phenotype may also depend on the activation of ABA pathway [55]. In general, former studies have shown that PAs is a key regulator of plant antioxidant enzyme activity and antioxidant balance under drought stress [46,54]. However, the relationship between polyamine metabolism and plant drought tolerance and its physiological regulation function on plants have not been clarified so far. Therefore, the study on the mitigation effect of exogenous Spd on drought stress and the mechanism of improving plant drought tolerance will help to further improve the regulation network of plant drought tolerance, and provide a certain reference for stress resistant and efficient cultivation of maize, which has important theoretical value and practical significance.

2. Materials and Methods

2.1. Plant Materials and Growth Condition

This experiment was divided into two parts. The hydroponic experiment was conducted in the artificial climate and light culture room of the college of agriculture of Northeast Agricultural University. Based on the preliminary experiment, the maize varieties Xianyu 335 (drought insensitive type) and Fenghe 1 (drought sensitive type) were selected as the experimental materials. The seeds were sterilized with 10% (v/v) sodium hypochlorite (NaClO) for 10 min. The germinated seeds were sown in 45 cm × 12 cm plastic tray, vermiculite is used as the matrix. When the seedlings grow to one leaf, we select neat and consistent seedlings and plant them in a plastic water tank containing 25 L 1/2 Hoagland nutrient solution (pH 6.3). Regular ventilation (40 min h⁻¹) was controlled by regular intercalation and controlled air pump, and the nutrient solution was changed every three days. When the maize seedlings grow to three leaves stages, they are divided into four groups, they were divided into four groups, and 60 seedlings in each group were selected for the following rhizosphere test treatment: (1) Control group (CK) (2) Spd treatment (Spd), Hoagland nutrient solution containing 0.1 mmol L⁻¹ Spd. (3) Drought stress (PEG), Hoagland nutrient solution containing 15% (w/v) PEG-6000 was applied (osmotic potential was −0.8 MPa). (4) Drought stress and Spd combined treatment (Spd + PEG). Spd (purchased from Sigma, Roedermark, Germany) was added to the nutrient solution to make its concentration reach 0.1 mmol L⁻¹ after PEG stress 24 h treatment. According to the pre-test results, 0.1 mmol L⁻¹ Spd had the best effect on the growth of maize seedlings of the two tested varieties, and had the most obvious protective effect on maize seedlings under drought stress [36]. During the treatment, adjust the pH once a day. The conditions for seedling growth are as follows: photoperiod is 12/12 (day/night), temperature is 28°C/25°C (day/night), and light intensity is 400 µmol m⁻² s⁻¹, relative humidity 60–70%.

The pot experiment site is located in Northeast Agricultural University (126°55′E, N45°45′N). The annual average temperature in this area is 23.0°C, the average annual sunshine hours are 1606 h, and the average annual precipitation is 569 mm. It belongs to the continental monsoon climate in the middle temperate zone. The tested maize (Zea mays L.) variety was Xianyu 335. The experiment was carried out in a rainproof shed. The maize grains were planted in a large barrel pot. The maize grains were sown in a plastic barrel (the barrel height was 45 cm, the barrel bottom diameter was 30 cm, and the volume was about 20.5 L). The matrix in the barrel was a mixture of soil and vermiculite (the ratio of soil to vermiculite was 1:1). The soil basic fertility values were as follows—total nitrogen (0.42 g kg⁻¹), total phosphorus (0.18 g kg⁻¹), total potassium (2.48 g kg⁻¹), organic matter (1.62 g kg⁻¹), alkali hydrolysable nitrogen (200.11 mg kg⁻¹), available phosphorus (53.14 mg kg⁻¹), available potassium (112.01 mg kg⁻¹) and pH 6.6. Water control treatment was carried out at flowering stage, which was divided into two water treatments: normal water treatment and moderate drought stress treatment. The corresponding water content was controlled at 75 ± 5% and 50 ± 5% of the maximum field water capacity, respectively. Meanwhile, the plants from each drought stress treatment were sprayed with distilled water, 0.05 mM Spd, 0.1 mM Spd and 0.2 mM Spd, respectively. Tween-20 (0.05%) was added as surfactant during treatment. Water control continued until 28 August. We choose
to take 5 samples for each determination and analysis, and each treatment was repeated 3 times.

2.2. Determination of NH$_4^+$ and NO$_3^-$

We cut off the false stem with a sharp blade 2 cm away from the root stem junction of maize seedlings, and ensure that the incision is flat. The bleeding fluid collection bag was prepared in advance and tighten it with rubber hoop [56]. The collection bag is an intact small transparent plastic bag containing dry absorbent cotton balls, numbered before use, and collected from 18:00 to 8:00 the next day. Remove the collection bag and store the collected bleeding fluid in the refrigerator at $-80^\circ C$ for the determination of NO$_3^-$ Concentrations of NO$_3^-$ was measured using 1 mol L$^{-1}$ KCl solution by using the ultra-violet spectro-photo-meter method [57].

The content of NH$_4^+$ in leaves was determined by the method of Natali [58]. Extract 100 mg of leaf powder sample in 1 mL of 100 mM HCl, and then add 500 µL chloroform. After shaking at 4 $^\circ C$ for 15 min, the phase was separated by centrifugation (g, 10 min, 8 $^\circ C$). The aqueous phase was transferred to a new tube containing 50 mg of activated carbon, fully mixed and centrifuged (g, 5 min, 8 $^\circ C$). The supernatant after charcoal treatment was diluted 1:1 (v/v) in 100 mM HCl and 20 µL the solution is mixed with 1% (w/v) phenol, 0.005% (w/v) sodium nitroprusside solution. Subsequently, add 100 µL 1% (v/v) sodium hypochlorite and 0.5% (w/v) sodium hydroxide solution. The mixture was incubated at 37 $^\circ C$ for 30 min and the light absorption was measured at 620 nm.

2.3. Determination of Nitrate Reductase (NR) Activity

NR activity in plant tissues was determined according to the method of Yu [59]. Grind 0.5 g leaves into homogenate in 4 mL extraction buffer under ice bath. The buffer consists of 25 mM phosphate buffer (K$_2$HPO$_4$ and KH$_2$PO$_4$, pH 7.5), 5 mM cysteine and 5 mM EDTA-NA$_2$. The extract was centrifuged at 4000 rpm at 4 $^\circ C$ for 15 min. 0.4 mL of enzyme extract sample was mixed with 1.2 mL of 0.1 M KNO$_3$ phosphate buffer and 0.4 mL of 2.0 mg mL$^{-1}$ NADH and incubated at 25 $^\circ C$ for 30 min. For control care, 0.4 mL phosphate buffer was used instead of 0.4 mL NADH. 1 mL of 3N HCl dissolved with 1% (w/v) sulfonamide and 0.02% N-naphthyl ethylenediamine were added to the mixture to terminate the reaction. After incubation for 15 min, all samples were centrifuged at 4000 rpm for 5 min, and the nitrite concentration in the supernatant was measured by spectrophotometry at 540 nm.

2.4. Determination of Glutamine Synthetase (GS), Glutamate Synthase Activity (GOGAT) and Glutamate Dehydrogenase (GDH) Activity

In order to extract crude enzyme solution, 0.5 g leaves were ground into homogenate with precooled pestle and mortar in 10 mM Tris HCl buffer. The culture was centrifuged at g for 30 min at 4 $^\circ C$. The supernatant was used to determine the enzyme activity.

GS activity was determined according to the method of Singh [60]. The reaction mixture (1 mL, pH 8.0) contained 80 µmol Tris-HCl buffer, 40 µmol L-glutamate, 8 µmol ATP, 24 µmol MgSO$_4$ and 16 µmol NH$_2$OH. The reaction was initiated by the addition of enzyme extract. After incubation at 30 $^\circ C$ for 30 min, 2 mL of acidic FeCl$_3$ (2% HCl solution containing 2% TCA and 3.5% FeCl$_3$) was added to terminate the reaction.

The activity of GOGAT was determined according to the method of Magalhaes [61]. The reaction mixture (3 mL) was composed of 0.4 mM Tris-HCl buffer, 0.05 mL 0.1 M 2-oxoglutarate, 0.1 mM 10 mM KCl, 0.2 mL 3 mM NADH, 1.75 mL 25 mM Tris HCl (pH 7.6) and 0.5 mL enzyme extract. L-Glutamine was added immediately after enzyme preparation to initiate the reaction. The change of absorbance within 3 min at 340 nm was measured.

The activity of glutamate dehydrogenase (GDH) was determined according to the method of Magalhaes [61]. The reaction mixture (3 mL) contained 0.3 mL 0.1 M α-oxoglutarate, 0.3 mL 1 M NH$_4$Cl, 0.2 mL 3 mM NADH, 1.2 mL 0.2 M Tris HCl buffer (pH 8.0) and 1 mL enzyme extract. The reaction was initiated by the addition of enzyme extract. Replace the blank with 0.2 M Tris HCl buffer α-ketoglutarate. The change of
absorbance within 3 min at 340 nm was measured. The activity of GDH unit is \( \mu \text{mol NADH g}^{-1} \text{FM min}^{-1} \).

2.5. Glutamate Oxaloacetate Aminotransferase (GOT) and Glutamate Pyruvate Aminotransferase (GPT) Activities

The enzyme extract was added to the solution containing 2 mM \( \alpha \)-Oxoglutarate and 200 mM DL aspartic acid (GOT) or 200 mM DL-alanine (GPT) in substrate solution (pH 7.4). The mixture was incubated at 37 °C for 1 h and the reaction was terminated by the addition of 2,4-dinitrophenylhydrazine. After the mixture was incubated again at 37 °C for 20 min, 5 mL of 0.4 M NaOH was added, and the absorbance of the solution was measured by chromatography at 500 nm.

2.6. Dry Matter Accumulation and Determination of Root Characteristic Parameters

Six maize plants were selected from each plot in the filling stage and physiological maturity stage, respectively, the aboveground parts and roots of the plants were separated, and the roots were cleaned with tap water and placed on a transparent glass resin tray (40 cm \( \times \) 30 cm \( \times \) 2 cm). LA-S root analysis system (Hangzhou wanshen Testing Technology Co., Ltd., Hangzhou, China) was used to analyze the root length, root surface area and root volume. The plant was killed at 105 °C for 30 min, and then dry it at 80 °C to constant weight before weighing.

2.7. Determination of Root Activity and Root Exudate

Measure the root activity according to the triphenyltetrazole chloride (TTC) method. Wash the fresh roots thoroughly with distilled water and cut them into 3–4 mm small pieces. Place 0.5 g root samples in a graduated glass tube containing 5 mL 0.4% TTC solution and 5 mL 0.1 mol L\(^{-1}\) phosphate buffer (pH 7.0) and place them at 37 °C for 3 h. Then add 2 mL sulfuric acid (H\(_2\)SO\(_4\)) to the tube to terminate the chemical reaction. The root activity is expressed by the amount of TPF (triphenylmethyl) deoxidized by TTC.

Five plants were sampled at silking stage, filling stage and physiological maturity, and each plant was cut at about 12 cm above the soil surface at 18:00 P.M. in order to determine the amount of stem bleeding fluid, then cover the residual stem with 500 mL plastic bottle containing degreasing cotton to determine the amount of stem bleeding fluid and calculate the flow rate of bleeding fluid (mL h\(^{-1}\) root\(^{-1}\)).

2.8. Determination of SPAD Value and Net Photosynthetic Rate

At 0, 10, 20, 30, 40 and 50 days after flowering, the relative chlorophyll content (SPAD value) of clover was measured by hand-held SPAD-502 chlorophyll meter produced by Minolta company of Japan, and the net photosynthetic rate (\( P_n \)) of clover was measured by LI-6400 portable photosynthetic instrument (LI-COR Biotechnology, Lincoln, NE, USA).

2.9. Determination of Leaf Area Index and Leaf Senescence Characteristics

At the beginning of flowering period, five plants in each plot are marked with red lines to measure the length and width of each green leaf at 0, 10, 20, 30, 40 and 50 days after flowering. The length width method is used to measure the leaf area, and the leaf area index is calculated: \( LAI = \text{green leaf area} \times \frac{0.75}{\text{number of plants per unit area}} \). The leaf senescence process is described by the curve equation \( y = ae^{b-cx} / (1 + e^{b-cx}) \), where \( y \) is the relative green leaf area (RGLA, %), \( x \) is the number of days after flowering, \( a \) is the theoretical initial value of RGLA (RGLA\(_s\)), \( b \) is related to the beginning of leaf senescence, and \( c \) is related to the rate of leaf senescence. RGLA at maturity: \( RGLA_m (\%) = GLA \text{ at maturity} / GLA \text{ at flowering} \). Average decline rate of RGLA (\( V_m \)) = (RGLA\(_s\)–RGLA\(_m\))/T (duration from flowering to maturity). Maximum reduction rate of RGLA (\( V_{max} \)) = C/4. The day on which \( V_{max} \) occurred (\( T_{max} \)) = b/c. Duration of leaf area (\( LAD \)) = (GLA\(_s\) + GLA\(_m\)) × T/2.
2.10. Determination of Yield

At the maturity stage, 20 plants were taken from each plot (except boundary plants) to determine the number of grains per panicle. The 100-grain weight was determined by drying the 100 grain sample to constant weight at 80 °C.

2.11. Data Analysis

The data were expressed by the measured mean value, analyzed by SPSS19.0 (IBM SPSS Statistics, 2010, Armonk, NY, USA), and compared by Duncan’s new complex difference method ($\alpha = 0.05$), and origin 8 is used for drawing.

3. Results

3.1. NO$_3^-$ Concentration in Xylem Bleeding Sap

Under drought stress, the content of NO$_3^-$ in the sap of maize seedlings of the two varieties decreased significantly. Spd treatment significantly increased the content of NO$_3^-$ in the sap of the two varieties of maize seedlings on the 4th day of stress, and significantly alleviated the decline of NO$_3^-$ in the sap of Fenghe 1 on the second day of stress. Compared with drought treatment, the content of NO$_3^-$ in sap of Xianyu 335 and Fenghe 1 seedlings increased by 28.18% and 46.04%, respectively, under Spd treatment for 4 days (Table 1).

Table 1. Effects of exogenous spermidine on NO$_3^-$ concentration in xylem bleeding sap of maize seedlings under drought stress (µg L$^{-1}$).

| Treatment          | Xianyu 335 | Fenghe 1 |
|--------------------|------------|----------|
|                    | 2d         | 4d       | 2d         | 4d         |
| CK                 | 28.83 ± 2.13$^{ab}$ | 27.08 ± 1.63$^a$ | 30.00 ± 2.21$^a$ | 31.13 ± 2.22$^a$ |
| Spd                | 31.31 ± 1.49$^a$ | 26.88 ± 1.73$^a$ | 32.27 ± 1.76$^a$ | 31.43 ± 2.73$^a$ |
| PEG                | 23.69 ± 2.14$^c$ | 15.71 ± 1.28$^c$ | 17.23 ± 2.37$^c$ | 10.93 ± 1.56$^c$ |
| Spd + PEG          | 26.16 ± 1.56$^{bc}$ | 20.13 ± 1.24$^b$ | 22.13 ± 1.96$^b$ | 15.97 ± 1.98$^b$ |

Note: Data are expressed as mean ± standard deviation. Different letters within the same column indicate significant difference at 5% level. CK represents control group; Spd represents Hoagland nutrient solution containing 0.1 mmol L$^{-1}$ Spd; PEG represents Hoagland nutrient solution containing 15% (w/v) PEG-6000; Spd + PEG represents Hoagland nutrient solution containing 0.1 mmol L$^{-1}$ Spd combined 15% (w/v) PEG-6000. Different letters within the same column indicate significant difference at 5% level.

3.2. Contents of NO$_3^-$ and NH$_4^+$ in Leaves

The content of NO$_3^-$ in leaves of maize seedlings decreased gradually under drought stress. The decrease of NO$_3^-$ content in Fenghe 1 was higher than that in Xianyu 335. Compared with CK, the content of NO$_3^-$ in the leaves of Xianyu 335 and Fenghe 1 decreased by 40.95% and 69.73%, respectively. Under drought stress, Spd alleviated the decline of NO$_3^-$ content in the leaves of maize seedlings, and had a stronger mitigation effect on the decline of NO$_3^-$ content in Fenghe 1 under drought stress. After 4 days of drought stress treatment, the content of NO$_3^-$ in Xianyu 335 and Fenghe 1 increased by 30.46% and 82.68%, respectively. Compared with CK, the content of NH$_4^+$ in the leaves of Xianyu 335 and Fenghe 1 increased by 46.49% and 132.39%, respectively. Under normal conditions, Spd had no significant effect on the content of NH$_4^+$ in maize leaves, but it could significantly reduce the content of NH$_4^+$ in maize leaves under drought stress. After 4 days of drought stress treatment, the content of NH$_4^+$ in Xianyu 335 and Fenghe 1 decreased by 16.24% and 23.99%, respectively (Table 2).
Table 2. Effects of exogenous spermidine on the content of NO$_3^-$ and NH$_4^+$ in leaves of maize seedlings under drought stress.

| Treatment | Varieties | Parameters | 0d | 1d | 2d | 3d | 4d |
|-----------|-----------|------------|----|----|----|----|----|
| CK        | Xianyu 335 NO$_3^-$ contents (mg g$^{-1}$ FW) | 3.60 ± 0.27$^a$ | 3.18 ± 0.23$^ab$ | 3.23 ± 0.18$^a$ | 3.39 ± 0.13$^a$ | 3.21 ± 0.22$^a$ |
| Spd       |          | 3.91 ± 0.19$^a$ | 3.36 ± 0.22$^a$ | 3.06 ± 0.26$^a$ | 3.13 ± 0.25$^a$ | 3.33 ± 0.24$^a$ |
| PEG       |          | 3.67 ± 0.22$^a$ | 2.46 ± 0.28$^c$ | 1.96 ± 0.25$^c$ | 1.67 ± 0.10$^c$ | 1.89 ± 0.10$^c$ |
| Spd + PEG |          | 3.94 ± 0.17$^a$ | 2.93 ± 0.15$^b$ | 2.70 ± 0.18$^b$ | 2.35 ± 0.20$^b$ | 2.47 ± 0.20$^b$ |
| CK        | Fenghe 1 NO$_3^-$ contents (mg g$^{-1}$ FW) | 3.00 ± 0.22$^a$ | 3.11 ± 0.22$^a$ | 2.58 ± 0.22$^a$ | 2.71 ± 0.16$^a$ | 2.96 ± 0.19$^a$ |
| Spd       |          | 3.23 ± 0.18$^a$ | 3.14 ± 0.27$^a$ | 2.72 ± 0.25$^a$ | 2.93 ± 0.15$^a$ | 2.70 ± 0.18$^a$ |
| PEG       |          | 3.06 ± 0.26$^a$ | 1.73 ± 0.22$^c$ | 1.52 ± 0.16$^c$ | 1.31 ± 0.11$^c$ | 0.90 ± 0.13$^c$ |
| Spd + PEG |          | 3.28 ± 0.14$^a$ | 2.60 ± 0.20$^b$ | 2.22 ± 0.30$^b$ | 2.70 ± 0.18$^b$ | 1.64 ± 0.15$^b$ |

Note: Data are expressed as mean ± standard deviation. Different letters within the same column indicate significant difference at 5% level. CK represents control group; Spd; PEG represents Hoagland nutrient solution containing 0.1 mmol L$^{-1}$ Spd; PEG represents Hoagland nutrient solution containing 15% (w/v) PEG-6000; Spd + PEG represents Hoagland nutrient solution containing 0.1 mmol L$^{-1}$ Spd combined 15% (w/v) PEG-6000. Different letters within the same column indicate significant difference at 5% level.

3.3. NR Activity in Leaves

The activity of nitrate reductase (NR) in maize seedling leaves decreased gradually under drought stress. The decline rate of NR activity in Fenghe 1 was higher than that in Xianyu 335. Compared with CK, the NR activity in the leaves of Xianyu 335 and Fenghe 1 decreased by 37.19% and 63.43%, respectively. Under drought stress, Spd alleviated the decline of NR activity in the leaves of maize seedlings, and had a stronger slowing effect on the decline of NR activity in Xianyu 335 under drought stress. After 4 days of drought stress treatment, the activity of NR in Xianyu 335 and Fenghe 1 decreased by 28.99% and 44.32%, respectively (Table 3).

Table 3. Effects of exogenous spermidine on nitrate reductase (NR) activity in leaves of maize seedlings under drought stress.

| Treatment | Varieties | 0d | 1d | 2d | 3d | 4d |
|-----------|-----------|----|----|----|----|----|
| CK        | Xianyu 335 | 12.50 ± 0.91$^a$ | 14.03 ± 1.07$^a$ | 13.19 ± 1.15$^a$ | 13.52 ± 0.89$^a$ | 14.92 ± 1.11$^a$ |
| Spd       |          | 13.47 ± 0.74$^a$ | 14.86 ± 0.80$^a$ | 14.27 ± 0.99$^a$ | 13.80 ± 1.25$^a$ | 14.09 ± 0.62$^a$ |
| PEG       |          | 12.72 ± 1.09$^a$ | 11.63 ± 1.08$^b$ | 8.00 ± 0.84$^c$ | 6.02 ± 0.61$^c$ | 9.37 ± 0.45$^c$ |
| Spd + PEG |          | 13.68 ± 0.59$^a$ | 13.04 ± 1.15$^b$ | 10.41 ± 1.02$^a$ | 8.56 ± 0.60$^b$ | 12.09 ± 1.13$^a$ |
| CK        | Fenghe 1 | 10.40 ± 0.69$^a$ | 11.48 ± 0.86$^a$ | 9.88 ± 0.89$^a$ | 9.61 ± 0.70$^a$ | 10.80 ± 0.82$^a$ |
| Spd       |          | 10.62 ± 0.96$^a$ | 10.84 ± 0.94$^a$ | 11.11 ± 0.78$^a$ | 10.37 ± 0.58$^a$ | 11.44 ± 1.10$^a$ |
| PEG       |          | 9.65 ± 0.48$^a$ | 7.23 ± 0.37$^c$ | 4.69 ± 0.52$^c$ | 3.46 ± 0.31$^c$ | 3.95 ± 0.84$^c$ |
| Spd + PEG |          | 10.42 ± 0.66$^a$ | 9.30 ± 0.87$^b$ | 7.63 ± 0.69$^b$ | 5.36 ± 0.49$^b$ | 5.70 ± 0.69$^b$ |

Note: Data are expressed as mean ± standard deviation. Different letters within the same column indicate significant difference at 5% level. CK represents control group; Spd; PEG represents Hoagland nutrient solution containing 0.1 mmol L$^{-1}$ Spd; PEG represents Hoagland nutrient solution containing 15% (w/v) PEG-6000; Spd + PEG represents Hoagland nutrient solution containing 0.1 mmol L$^{-1}$ Spd combined 15% (w/v) PEG-6000. Different letters within the same column indicate significant difference at 5% level.

3.4. Activities of GS, GOGAT and GDH in Leaves

The activities of GS, GOGAT and GDH in maize seedling leaves decreased gradually under drought stress. On the 4th day, compared with CK, the activities of GS, GOGAT and GDH in the leaves of Xianyu 335 treated with drought stress decreased by 41.31%,
34.97% and 26.46%, respectively, and the activities of GS, GOGAT and GDH in the leaves of Fenghe 1 decreased by 66.36%, 55.77% and 65.64%, respectively. Spd did not change the decreasing trend of GS, GOGAT and GDH activities, but slowed down the decreasing range of GS, GOGAT and GDH activities in the leaves of maize seedlings. The changes of GS, GOGAT and GDH activities were different in different maize varieties. Under normal water conditions, Spd treatment had no significant effect on GS, GOGAT and GDH activities of maize seedlings (Table 4).

### Table 4. Effects of exogenous Spd on activities of glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) in leaves of maize seedlings under drought stress.

| Treatment | Varieties | Parameters | 0d  | 1d  | 2d  | 3d  | 4d  |
|-----------|-----------|------------|-----|-----|-----|-----|-----|
| CK        | Xianyu 335| GS activity | 26.64 ± 1.75 a | 28.79 ± 1.54 a | 29.55 ± 2.33 a | 26.50 ± 2.18 a | 25.89 ± 1.78 a |
| Spd       | Xianyu 335| GS activity | 24.82 ± 1.76 a | 30.66 ± 2.27 a | 30.75 ± 2.24 a | 25.31 ± 2.23 a | 26.77 ± 2.08 a |
| PEG       | Xianyu 335| GS activity | 24.33 ± 2.17 a | 25.04 ± 2.00 b | 20.30 ± 1.59 c | 16.29 ± 1.15 c | 15.20 ± 1.30 c |
| Spd + PEG | Xianyu 335| GS activity | 27.48 ± 1.49 a | 29.16 ± 1.91 a | 24.93 ± 1.80 b | 20.76 ± 1.87 b | 21.59 ± 1.18 b |
| CK        | Fenghe 1  | GS activity | 22.65 ± 1.09 a | 25.11 ± 2.19 a | 25.40 ± 1.92 c | 25.66 ± 1.13 a | 23.42 ± 2.13 a |
| Spd       | Fenghe 1  | GS activity | 20.79 ± 1.84 a | 15.53 ± 1.82 c | 11.26 ± 1.19 c | 10.38 ± 1.55 c | 7.64 ± 0.93 c  |
| PEG       | Fenghe 1  | GS activity | 22.43 ± 1.02 a | 20.29 ± 1.42 b | 16.59 ± 1.37 b | 15.83 ± 2.36 b | 12.93 ± 1.63 b |
| Spd + PEG | Fenghe 1  | GS activity | 0.65 ± 0.05 a  | 0.58 ± 0.04 a  | 0.59 ± 0.05 ab | 0.52 ± 0.05 a  | 0.56 ± 0.03 a  |
| CK        | Xianyu 335| GOGAT activity | 0.71 ± 0.06 a  | 0.64 ± 0.04 a  | 0.63 ± 0.04 a  | 0.56 ± 0.03 a  | 0.59 ± 0.05 a  |
| Spd       | Xianyu 335| GOGAT activity | 0.62 ± 0.06 a  | 0.45 ± 0.03 b  | 0.41 ± 0.03 c  | 0.28 ± 0.02 c  | 0.36 ± 0.03 c  |
| PEG       | Xianyu 335| GOGAT activity | 0.68 ± 0.05 a  | 0.59 ± 0.04 a  | 0.54 ± 0.04 b  | 0.36 ± 0.03 b  | 0.46 ± 0.05 b  |
| Spd + PEG | Xianyu 335| GOGAT activity | 0.52 ± 0.03 a  | 0.59 ± 0.05 a  | 0.57 ± 0.04 a  | 0.57 ± 0.02 a  | 0.57 ± 0.03 a  |
| CK        | Fenghe 1  | GOGAT activity | 0.50 ± 0.05 a  | 0.57 ± 0.03 a  | 0.58 ± 0.03 a  | 0.60 ± 0.05 a  | 0.61 ± 0.02 a  |
| Spd       | Fenghe 1  | GOGAT activity | 0.56 ± 0.04 a  | 0.37 ± 0.02 c  | 0.30 ± 0.03 c  | 0.22 ± 0.03 c  | 0.25 ± 0.02 c  |
| PEG       | Fenghe 1  | GOGAT activity | 0.54 ± 0.04 a  | 0.47 ± 0.03 b  | 0.45 ± 0.02 b  | 0.35 ± 0.03 b  | 0.37 ± 0.04 b  |
| Spd + PEG | Fenghe 1  | GOGAT activity | 1.19 ± 0.07 a  | 1.22 ± 0.08 b  | 1.09 ± 0.09 b  | 1.22 ± 0.08 c  | 1.12 ± 0.09 c  |
| CK        | Xianyu 335| GDH activity  | 1.31 ± 0.10 a  | 1.31 ± 0.07 ab | 1.18 ± 0.06 ab | 1.26 ± 0.07 bc | 1.18 ± 0.05 c  |
| Spd       | Xianyu 335| GDH activity  | 1.25 ± 0.10 a  | 1.37 ± 0.06 a  | 1.29 ± 0.07 a  | 1.39 ± 0.06 b  | 1.42 ± 0.06 b  |
| PEG       | Xianyu 335| GDH activity  | 1.16 ± 0.10 a  | 1.44 ± 0.09 a  | 1.31 ± 0.07 a  | 1.54 ± 0.07 a  | 1.61 ± 0.07 a  |
| Spd + PEG | Xianyu 335| GDH activity  | 1.06 ± 0.06 a  | 0.97 ± 0.08 a  | 1.08 ± 0.07 a  | 1.08 ± 0.07 a  | 1.07 ± 0.07 a  |
| CK        | Fenghe 1  | GDH activity  | 1.00 ± 0.07 a  | 1.00 ± 0.06 a  | 1.09 ± 0.07 b  | 1.18 ± 0.08 a  | 1.13 ± 0.04 a  |
| Spd       | Fenghe 1  | GDH activity  | 0.93 ± 0.06 a  | 0.77 ± 0.03 b  | 0.66 ± 0.06 c  | 0.48 ± 0.05 c  | 0.37 ± 0.04 c  |
| PEG       | Fenghe 1  | GDH activity  | 1.06 ± 0.07 a  | 0.89 ± 0.07 a  | 0.85 ± 0.07 a  | 0.78 ± 0.07 b  | 0.69 ± 0.07 b  |

Note: Data are expressed as mean ± standard deviation. Different letters within the same column indicate significant difference at 5% level. CK represents control group; Spd represents Hoagland nutrient solution containing 0.1 mmol L⁻¹ Spd; PEG represents Hoagland nutrient solution containing 0.1 mmol L⁻¹ PEG; Spd + PEG represents Hoagland nutrient solution containing 0.1 mmol L⁻¹ Spd combined with 0.1 mmol L⁻¹ PEG. Different letters within the same column indicate significant difference at 5% level.

#### 3.5. Activities of GOT and GPT in Leaves

Compared with CK, the activities of GOT and GPT under drought stress treatment continue to decrease with the extension of drought stress time, and the decline range in Fenghe 1 is greater than that in Xianyu 335. Compared with drought stress treatment, the activities of GOT and GPT in maize seedlings treated with Spd increased significantly, and the effect of Spd on Fenghe 1 was more obvious. On the 4th day of drought stress, the GOT and GPT activities of Xianyu 335 seedling leaves treated with Spd were significantly higher than those treated with simple drought stress in the same period by 36.35% and 28.21%, and the GOT and GPT activities of Fenghe 1 seedling leaves were significantly higher than those treated with simple drought stress in the same period by 65.12% and 47.15% (Table 5).
Table 5. Effects of exogenous spermidine on the activities of glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) in maize seedlings under drought stress.

| Treatment | Varieties | Parameters | 0d       | 1d       | 2d       | 3d       | 4d       |
|-----------|-----------|------------|---------|---------|---------|---------|---------|
| CK        | Xianyu 335| GOT activity (µmol mg⁻¹ 30 min⁻¹) | 0.27 ± 0.02 a | 0.26 ± 0.02 a | 0.23 ± 0.02 a | 0.26 ± 0.02 a | 0.27 ± 0.01 a |
| Spd       |          |            | 0.27 ± 0.02 b | 0.28 ± 0.02 b | 0.25 ± 0.02 b | 0.28 ± 0.02 b | 0.29 ± 0.02 b |
| PEG       |          |            | 0.29 ± 0.02 a | 0.22 ± 0.01 b | 0.18 ± 0.01 b | 0.17 ± 0.01 c | 0.15 ± 0.02 c |
| Spd + PEG |          |            | 0.27 ± 0.03 a | 0.24 ± 0.01 b | 0.22 ± 0.02 a | 0.21 ± 0.02 b | 0.21 ± 0.01 b |
| CK        | Fenghe 1  | GPT activity (µmol mg⁻¹ 30 min⁻¹) | 0.26 ± 0.02 a | 0.28 ± 0.03 a | 0.29 ± 0.02 a | 0.26 ± 0.02 a | 0.24 ± 0.02 a |
| Spd       | Fenghe 1  |            | 0.27 ± 0.03 a | 0.29 ± 0.03 a | 0.31 ± 0.03 a | 0.26 ± 0.03 a | 0.27 ± 0.03 a |
| PEG       | Fenghe 1  |            | 0.29 ± 0.03 a | 0.20 ± 0.02 c | 0.16 ± 0.02 c | 0.14 ± 0.02 c | 0.10 ± 0.01 c |
| Spd + PEG | Fenghe 1  |            | 0.26 ± 0.02 a | 0.25 ± 0.02 b | 0.22 ± 0.03 b | 0.20 ± 0.02 b | 0.17 ± 0.02 b |

Note: Data are expressed as mean ± standard deviation. Different letters within the same column indicate significant difference at 5% level. CK represents control group; Spd represents Hoagland nutrient solution containing 0.1 mM Spd; PEG represents Hoagland nutrient solution containing 0.15% (w/v) PEG-6000; Spd + PEG represents Hoagland nutrient solution containing 0.1 mM Spd combined with 0.15% (w/v) PEG-6000. Different letters within the same column indicate significant difference at 5% level.

3.6. Dry Matter Accumulation

Compared with normal water treatment, drought stress treatment inhibited the accumulation of maize biomass and significantly reduced the aboveground dry weight and root dry weight. Spd alleviated the inhibitory effect of drought stress on maize growth. Among them, 0.1 mM Spd had the best effect on promoting maize growth under drought stress, and increased shoot dry weight and root dry weight to a significant level. Compared with the simple drought stress treatment, the shoot dry weight and root dry weight of maize plants treated with 0.1 mM Spd increased by 22.17% and 10.13%, respectively, in the filling stage and 18.13% and 10.44%, respectively, in the physiological maturity stage (Figure 1).

3.7. Root Characteristic Parameters

Spd alleviated the inhibitory effect of drought stress on maize root growth. Among them, 0.1 mM Spd had the best effect on the growth of maize under drought stress, and increased root length, root surface area and root volume to a significant level. Compared with the simple drought stress treatment, the root length, root surface area and root volume of maize plants treated with 0.1 mM Spd increased by 22.95%, 14.01% and 11.29%, respectively, at the filling stage, and 17.85%, 16.07% and 8.35% at the physiological maturity stage, respectively (Figure 2).
3.7. Root Characteristic Parameters

Spd alleviated the inhibitory effect of drought stress on maize root growth. Among them, 0.1 mM Spd had the best effect on the growth of maize under drought stress, and increased root length, root surface area, and root volume to a significant level. Compared with the simple drought stress treatment, the root length, root surface area, and root volume of maize plants treated with 0.1 mM Spd increased by 22.95%, 14.01%, and 11.29% respectively, at the filling stage, and 17.85%, 16.07%, and 8.35% respectively at the physiological maturity stage (Figure 2).

Figure 1. Effects of exogenous spermidine on dry matter accumulation of maize under drought stress. Different letters within the same column indicate significant difference at 5% level.

Figure 2. Effects of exogenous spermidine on root characteristic parameters of maize under drought stress. Different letters within the same column indicate significant difference at 5% level.

3.8. Root Activity and Root Bleeding Sap Rate

Compared with normal water treatment, drought stress treatment significantly reduced TTC activity of maize roots. Compared with the simple drought stress treatment, Spd enhanced the TTC activity of maize roots, in which 0.05–0.2 mM Spd enhanced the TTC activity of maize roots at grain filling stage and 0.1 mM Spd at physiological maturity stage. The effect of 0.1 mM Spd was the most significant. Compared with plants treated with drought stress alone, the TTC of plants treated with 0.1 mM Spd increased by 32.65% and 30.67% at grain filling stage and physiological maturity stage respectively.

Drought stress treatment significantly reduced root bleeding. Compared with normal water treatment, the root bleeding of maize under drought treatment decreased by 27.89% and 41.29% at grain filling stage and physiological maturity stage respectively. Under drought stress, Spd increased maize bleeding, and the increasing effects of 0.1 mM and 0.2 mM Spd on maize bleeding reached a significant level. Compared with the...
10 days after anthesis, and then decreased gradually. Drought stress treatment significantly reduced TTC activity of maize roots. Compared with the simple drought stress treatment, Spd enhanced the TTC activity of maize roots, in which 0.05–0.2 mM Spd enhanced the TTC activity of maize roots at grain filling stage and 0.1 mM Spd at physiological maturity stage. The effect of 0.1 mM Spd was the most significant. Compared with plants treated with drought stress alone, the TTC of plants treated with 0.1 mM Spd increased by 32.65% and 30.67% at grain filling stage and physiological maturity stage, respectively. Drought stress treatment significantly reduced root bleeding. Compared with normal water treatment, the root bleeding of maize under drought treatment decreased by 27.89% and 41.29% at grain filling stage and physiological maturity stage, respectively. Under drought stress, Spd increased maize bleeding, and the increasing effects of 0.1 mM and 0.2 mM Spd on maize bleeding reached a significant level. Compared with the plants treated with drought stress alone, the root bleeding of the plants treated with 0.1 mM Spd and 0.2 mM Spd increased by 22.93% and 35.49%, respectively, in the filling stage and 15.25% and 20.98%, respectively, in the physiological maturity stage (Figure 3).

3.8. Root Activity and Root Bleeding Sap Rate

Compared with normal water treatment, drought stress treatment significantly reduced TTC activity of maize roots. Compared with the simple drought stress treatment, Spd enhanced the TTC activity of maize roots, in which 0.05–0.2 mM Spd enhanced the TTC activity of maize roots at grain filling stage and 0.1 mM Spd at physiological maturity stage. The effect of 0.1 mM Spd was the most significant. Compared with plants treated with drought stress alone, the TTC of plants treated with 0.1 mM Spd increased by 32.65% and 30.67% at grain filling stage and physiological maturity stage, respectively. Drought stress treatment significantly reduced root bleeding. Compared with normal water treatment, the root bleeding of maize under drought treatment decreased by 27.89% and 41.29% at grain filling stage and physiological maturity stage, respectively. Under drought stress, Spd increased maize bleeding, and the increasing effects of 0.1 mM and 0.2 mM Spd on maize bleeding reached a significant level. Compared with the plants treated with drought stress alone, the root bleeding of the plants treated with 0.1 mM Spd and 0.2 mM Spd increased by 22.93% and 35.49%, respectively, in the filling stage and 15.25% and 20.98%, respectively, in the physiological maturity stage (Figure 3).

3.9. Leaf Photosynthesis and Leaf Area at Post-Anthesis Stage

SPAD values of maize leaves changed in a single peak curve after anthesis, peaked at 10 days after anthesis, and then decreased gradually. Drought stress treatment significantly reduced the SPAD value of maize leaves at all stages. Under Spd treatment, the SPAD values of maize leaves were higher than those of pure drought treatment. 0.05 mM Spd, 0.1 mM Spd and 0.2 mM Spd were 8.35–13.26%, 11.83–39.38% and 10.44–27.10% higher than those of simple drought treatment, respectively. 0.1 mM Spd has the best effect on the increase of SPAD value of maize leaves under drought stress. From 20 days after anthesis, \( P_n \) of maize leaves under normal water treatment decreases steadily, while that under drought treatment decreases rapidly. Under Spd treatment, the \( P_n \) of maize leaves was higher than that of simple drought treatment. 0.05 mM Spd, 0.1 mM Spd and 0.2 mM were 9.26–11.90%, 14.35–40.44% and 12.78–23.12% higher than those of simple drought treatment, respectively, indicating that Spd treatment could maintain a high net photosynthetic rate after flowering, the photosynthetic rate decreased slowly, and the leaves could maintain a long photosynthetic function period, so as to assimilate and synthesize more carbohydrates to supply grain filling and increase the accumulation of photosynthetic products (Figure 4).
Figure 3. Effects of exogenous spermidine on root bleeding sap rate of maize under drought stress. Different letters within the same column indicate significant difference at 5% level.

Figure 4. Cont.
3.10. Leaf Senescence Characteristics

Compared with normal water treated plants, drought stressed plants had lower \( RGLAm \), \( LAD \) and \( T_{max} \) values, but higher \( V_m \) and \( V_{max} \) values. Under Spd treatment, \( RGLAm \), \( LAD \) and \( T_{max} \) values of stressed plants increased, \( V_m \) and \( V_{max} \) values decreased. Compared with simple drought treatment, the values of \( RGLAm \), \( LAD \) and \( T_{max} \) under 0.1 mM Spd treatment increased by 53.22%, 20.15% and 21.19%, respectively, and the values of \( V_m \) and \( V_{max} \) decreased by 18.56% and 20.68%, respectively (Table 6).

Table 6. Effect of exogenous spermidine on leaf senescence characteristics of maize leaves under drought stress.

| Treatment         | \( RGLAm \) (%) | \( V_m \) (%) | \( V_{max} \) (%) | \( T_{max} \) (d) | \( LAD \) (m² d⁻¹) |
|-------------------|-----------------|---------------|-------------------|------------------|-------------------|
| CK                | 43.05           | 1.10          | 1.87              | 45.91            | 21.70             |
| DS                | 18.30           | 1.67          | 2.95              | 32.04            | 14.74             |
| DS + 0.05 mM Spd  | 22.67           | 1.41          | 2.41              | 37.52            | 17.96             |
| DS + 0.1 mM Spd   | 28.04           | 1.36          | 2.34              | 38.83            | 17.71             |
| DS + 0.2 mM Spd   | 25.08           | 1.50          | 2.60              | 34.70            | 15.69             |

3.11. Maize Yield and It’s Components

Drought stress treatment significantly reduced the number of grains per ear and 100 grain weight of maize, thus reducing the yield of maize. Compared with normal water treatment, the number of grains per ear, 100 grain weight and yield of maize under drought conditions decreased by 8.63%, 13.79% and 25.61%, respectively. The spraying of Spd alleviated the effect of drought on yield, and the effect of 0.1 mM Spd was the best. Compared with simple drought treatment, the number of grains per ear, 100 grain weight and yield of maize plants treated with 0.1 mM Spd increased by 3.77%, 6.95% and 8.36%, respectively (Table 7).

Table 7. Effect of exogenous spermidine on maize yield and it’s components under drought stress.

| Treatment          | Kernels (No Ear⁻¹) | 100-Kernel Weight (g) | Ear Number (No m⁻²) | Yield (kg ha⁻¹) |
|--------------------|---------------------|-----------------------|----------------------|-----------------|
| CK                 | 486.69 ± 10.85 a    | 33.72 ± 0.49 a        | 7.2 ± 0.5 a          | 11,816.81 ± 200.56 a |
| DS                 | 444.71 ± 11.43 c    | 29.07 ± 1.06 c        | 6.8 ± 0.2 a          | 8790.63 ± 124.58 c |
| DS + 0.05 mM Spd   | 452.21 ± 14.56 bc   | 30.23 ± 0.75 bc       | 6.8 ± 0.4 a          | 9295.23 ± 178.43 bc |
| DS + 0.1 mM Spd    | 461.47 ± 9.17 b     | 31.09 ± 1.04 b        | 7.0 ± 0.2 a          | 9841.26 ± 126.60 b |
| DS + 0.2 mM Spd    | 457.49 ± 6.79 bc    | 30.62 ± 0.85 bc       | 6.8 ± 0.4 a          | 9525.89 ± 132.24 b |

Different letters within the same column indicate significant difference at 5% level.
4. Discussion

NH$_4^+$ or NO$_3^-$ can only be absorbed by roots and then transported to the aboveground part of plants when dissolved in water [22,23]. Drought stress significantly reduced the content of NO$_3^-$ in the leaves of maize seedlings, while Spd significantly increased the content of NO$_3^-$ in drought maize seedlings. In this study, it was found that Spd increased the rate of NO$_3^-$ transport in xylem, indicating that Spd can increase the content of NO$_3^-$ in leaves by promoting the absorption of NO$_3^-$. NO$_3^-$ regulates plant growth and development and adapting to fluctuating environments [30,62,63]. Higher NO$_3^-$ content leads to depolarization of guard cells. Under drought stress, the stomatal conductance of plants treated with Spd is less affected [63]. NR is the rate limiting enzyme in NO$_3^-$ assimilation and drought stress reduces NR activity [64–66]. In this experiment, we also found that drought stress reduced NR activity of maize seedlings. Under drought stress, the increase of NO$_3^-$ in plant leaves with Spd is consistent with the increase of NR activity. Spd plays an important role in regulating the binding of 14-3-3 protein to H$^+$-ATPase and helps to activate NR activity, which is conducive to alleviate the inhibition of NR activity induced by drought stress [67]. In addition, the higher photosynthetic capacity induced by Spd can activate NR activity by increasing the availability of reducing agents. The enhancement of NO$_3^-$ reduction may play an important role in dissipating excess energy [68,69]. The reduction of NO$_3^-$ in leaves can use excess energy from photosynthetic organs. Under drought stress, NO$_3^-$ assimilation as an effective electron absorption can reduce the photoinhibition of photosynthesis, which indicates that enhancing the reduction of NO$_3^-$ is an important mechanism to deal with drought stress.

Avoiding excessive accumulation of NH$_4^+$ in plant tissues is considered to be an important ability to resist drought stress [70]. NH$_4^+$ can be produced by nitrate reduction, protein hydrolysis and photorespiration, and can be assimilated to GS/GOGAT cycle, or by GDH assimilation to glutamate [71]. Glutamate (Glu) can be used as the source of C and N in most other biosynthesis, and plays a central signal and metabolic role at the interface of C and N assimilation pathway. In this experiment, we found that under drought stress, the content of NH$_4^+$ in maize seedlings was very high, but the increase of NH$_4^+$ in Xianyu 335 was lower than that of Fenghe 1, indicating that the ammonia toxicity of drought stress to Xianyu 335 plant was less. Under drought stress, NR activity decreased significantly, and protein hydrolysis was not affected by drought stress. Therefore, the accumulation of NH$_4^+$ may be due to the large amount of NH$_4^+$ released by photorespiration and the inhibition of GS/GOGAT pathway. The stability of leaf photosynthetic system caused by Spd application can reduce the release of NH$_4^+$ caused by photorespiration induced by drought stress. Previous studies have found that Spd can improve the activities of GS, GOGAT and GDH in cucumber seedling leaves under Ca(NO$_3$)$_2$ stress, so as to reduce the toxic effect of NH$_4^+$ on cucumber seedlings [72]. Therefore, Spd can reduce the toxic effect of NH$_4^+$ on maize seedlings under drought treatment by increasing the activity of ammonia assimilation enzyme. Glutamate (Glu) can be transformed into aspartic acid (ASP) and alanine (ALA) through glutamate deoxyribonucleic acid aminotransferase (GOT) and glutamate pyruvate aminotransferase (GPT) [73–77]. In this experiment, under drought stress, the GOT and GPT activities in the leaves gradually decreased. This may be due to the decrease of glutamate content caused by the weakening of GS/GOGAT pathway, and then inhibit a series of amino transfer reactions using it as substrate in plants.

Root morphology can affect the water extraction and nutrient absorption capacity [78–82]. Root distribution and extension can be expressed as root length, root surface area or root volume [81]. Higher root length, root surface area and root volume are benefit for the increase of nutrient supply [79]. The results showed that under drought stress, Spd had higher root characteristics than those without Spd in silking, filling and physiological maturity. Previous experiments of soil culture, sand culture and water culture showed that 0.1–0.5 mmol L$^{-1}$ polyamines (Put and Spd) could significantly promote the occurrence of tobacco lateral roots, increase the number of roots, root length, root body and root dry weight, and enhance root activity, and the promoting effect of Spd was greater than that of
Put [83]. Root exudate is another essential root characteristic, and its content reveals the potential growth and activity of roots [81]. Bleeding fluid is consistent with root activity in field experiments [84,85]. The results showed that the root activity and root bleeding of Spd were higher than those of control treatment, and the best results were observed in 0.1 mM Spd. Root activity and bleeding reached the peak at silking stage, and then decreased gradually. Since root bleeding is the expression of root pressure, the improvement of root bleeding under Spd may be due to the increase of root growth and root activity [86]. The rate of root bleeding is related to the active water absorption of roots, reflecting the physiological root activity [87,88]. Spd can enhance the root activity of wheat under drought stress [89,90]. Therefore, Spd can improve the root absorption of water and nutrients by improving root activity and root bleeding under drought stress.

Photosynthesis is the basis of biomass production and yield [91,92]. \( P_n \) is an index used to directly evaluate the photosynthetic performance of a single leaf [93,94]. LAI is the area of canopy photosynthesis, which reflects the canopy structure and nutritional status in different growth stages. In this study, in the process of grain filling, the function of leaves gradually decreased, the leaves lost green and gradually aged, and the effective photosynthetic leaf area decreased. Therefore, maintaining high photosynthetic area during post anthesis is conducive to increasing dry matter accumulation (DMA), which helps to improve yield [95,96]. In this experiment, we found that the maize plants treated with Spd had higher SPAD, \( P_n \) and \( RGLA_m \) values, and the leaf senescence rate was slower. Therefore, plants sprayed with Spd have higher DMA at the post flowering stage, which may be mainly due to the delay of leaf senescence and prolonging the accumulation time of photosynthetic substances to ensure sufficient carbohydrate supply for grain filling [93]. Maintaining green leaves areas at maturity contributes greatly to the genetic increase of maize yield [97–99]. In this study, Spd could significantly increase the plant dry weight under drought stress. Compared with the simple drought treatment, the plants treated with Spd maintained higher LAI and \( P_n \) values and effectively delayed the leaf senescence at the post flowering stage, which may be attributed to higher bleeding flow, more active root activity and larger root structure. Higher LAI and photosynthetic capacity ensure high dry matter accumulation [94]. Therefore, Spd improved canopy photosynthetic capacity to obtain higher dry matter accumulation in post flowering.

The main reason why drought stress affects yield is that it destroys the formation of grain “sink”, and the reduction of grain number per ear leads to the final reduction of maize yield [100–102]. The results showed that drought stress significantly reduced the number and weight of grains per ear, and finally led to the decrease of yield. Spd significantly increased the number of grains per panicle and grain weight. This may be because Spd improves canopy function and delays leaf senescence to enhance grain filling, thereby increasing grain number and grain weight per panicle. Foliar spraying Spd can effectively improve the physiological functions of maize roots and leaves, so as to reduce the yield reduction of maize under drought stress [86]. In conclusion, these results showed that Spd increased maize yield by enhancing source intensity (grain filling) and sink intensity (grain number) significantly.

5. Conclusions

Spd can promote the assimilation of excess ammonia in maize by enhancing ammonia assimilating enzymes GS/GOGAT and GDH, and transaminases (GOT and GPT), effectively alleviate the ammonia toxicity and nitrogen metabolism disorder induced by drought stress. Spd can improve the absorption and utilization of water and nutrients by promoting the root growth, root activity and root bleeding of maize under drought stress. At the same time, Spd could enhance photosynthesis, delay leaf senescence, increase dry matter accumulation after anthesis, increase grain weight and grain number per ear, and finally improve maize yield. Furthermore, Schematic representation of the positive role of exogenous spermidine on nitrogen metabolism and transcriptome analysis in maize under drought stress and field verification were listed as follows (Figure 5).
Figure 5. Schematic representation of the positive role of exogenous spermidine on nitrogen metabolism and transcriptome analysis in maize under drought stress and field verification. The red arrows (↑) and the blue arrows (↓) represent the positive and passive roles of spermidine, respectively.

Author Contributions: Conceptualization, C.Q., S.W. and W.G.; formal analysis, L.D., Y.Y. and L.L.; funding acquisition, W.G. and C.Q.; methodology, L.D., Y.M. and J.L.; software, H.L., Y.M. and J.L.; supervision, C.Q. and W.G.; validation, L.D. and L.L.; writing—original draft, L.D. and L.L. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Agricultural Science and Technology Innovation Spans Project of Heilongjiang Academy of Agricultural Sciences (HNK2019CX12), the National Natural Science Foundation of China (32172112) and the Special Project of Collaborative Innovation and Extension System of Green Organic Agriculture in Heilongjiang Province (GOAS2022).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: We are grateful for Chemical Control Research Center, College of Agriculture, China Agricultural University for technical guidance and assistance in the methods and data analysis.

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

1. Zhou, G.S. Research prospect on impact of climate change on agricultural production in China. Meteor. Environ. Sci. 2015, 38, 80–94. [CrossRef]

2. Song, H.; Jiang, Y.L.; Xu, Z.Z.; Zhou, G.S. Response of photosynthetic physiological parameters of maize to drought during the whole growth period and after the jointing stage. Acta Ecol. Sin. 2019, 39, 2405–2415. [CrossRef]

3. Talaat, N.B.; Shawky, B.T. Dual application of 24-epibrassinolide and spermine confers drought stress tolerance in maize (Zea mays L.) by modulating polyamine and protein metabolism. J. Plant Growth Regul. 2016, 35, 518–533. [CrossRef]

4. Al-Suhaibani, N.; Ali, N.; Akmal, M.; Alotaibi, M.; Refay, Y.; Dindaroglu, T.; Abdul-Wajid, H.H.; Battaglia, M.L. Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects. Plants 2021, 10, 259. [CrossRef]

5. O’Connell, E. Towards adaptation of water resource Systems to climatic and socio-economic Chang. Water Resour. Manag. 2017, 31, 2965–2984. [CrossRef]

6. Meng, Q.; Hou, P.; Wu, L.; Chen, X.; Cui, Z.; Zhang, F. Understanding production potentials and yield gaps in intensive maize production in China. Field Crop. Res. 2013, 143, 91–97. [CrossRef]

7. Jiao, H.Y.; Zhou, G.S.; Chen, Z.L. Blue paper about agricultural issues on climate change. In Assessment Report on Effect of Climate Change on China agriculture (No.1); Social Science Literature: Beijing, China, 2014; pp. 1–6.

8. Avramova, V.; Abdelgawad, H.; Zhang, Z.; Fotschki, B.; Vergauwen, L.; Knapen, D.; Taleisnik, E.; Guisez, Y.; Asard, H. Drought induces distinct growth response, protection, and recovery opportunities in the maize leaf growth zone. Plant Physiol. 2015, 169, 1382–1396. [CrossRef]

9. Bai, X.L.; Sun, S.X.; Yang, G.H.; Liu, M.; Zhang, Z.P.; Qi, H. Effect of water stress on maize yield during different growing stages. J. Maize Sci. 2009, 17, 60–63.

10. Benari, T.; Adrian, J.; Klein, T.; Calanca, P.; Van der Velde, M.; Makowski, D. Identifying indicators for extreme wheat and maize yield losses. Agric. Forest Meteorol. 2016, 220, 130–140. [CrossRef]

11. Lobell, D.B.; Roberts, M.J.; Wolfram, S.; Braun, N.; Little, B.B.; Rejesus, R.M.; Hammer, G.L. Greater sensitivity to drought accompanies maize yield increase in the U.S. Midwest. Science 2014, 344, 516–519. [CrossRef]

12. Ribaut, J.M.; Betran, J.; Monneveux, P.; Setter, T. Handbook of Maize: Its Biology. In Drought Tolerance in Maize; Bennetzen, J.L., Hake, S.C., Eds.; Springer: New York, NY, USA, 2009; pp. 311–344.

13. Jin, Z.N.; Zhuang, Q.L.; Wang, J.L.; Archontoulis, S.V.; Zobel, Z.; Kotamarthi, V.R. The combined and separate impacts of climate extremes on the current and future US rainfed maize and soybean production under elevated CO2. Glob. Chang. Biol. 2017, 23, 2687–2704. [CrossRef] [PubMed]

14. Myers, S.S.; Smith, M.R.; Guth, S.; Golden, G.D.; Vaitha, B.; Mueller, N.D.; Dangour, A.D.; Huybers, P. Climate change and global food systems: Potential impacts on food security and undernutrition. Annu. Rev. Public Health 2017, 38, 259–277. [CrossRef] [PubMed]

15. Majeed, S.; Nawaz, F.; Naeeem, M.; Ashraf, M.Y. Effect of exogenous nitric oxide on sulfur and nitrate assimilation pathway enzymes in Arabidopsis (Zea mays L.) under drought stress. Acta Physiol. Plantarum. 2018, 40, 206. [CrossRef]

16. Sezgin, A.; Altuntas, C.; Demiray, M.; Cinemre, S.; Terzi, S. Exogenous alpha lipoic acid can stimulate photosystem II activity and the gene expressions of carbon fixation and chlorophyll metabolism enzymes in maize seedlings under drought. J. Plant Physiol. 2019, 232, 65–73. [CrossRef] [PubMed]

17. Liu, J.H.; Wang, W.; Wu, H.; Gong, X.; Takaya, M. Polyamines function in stress tolerance: From synthesis to regulation. Front. Plant Sci. 2015, 6, 827. [CrossRef]

18. Tavladoraki, P.; Cona, A.; Federico, R.; Tempera, G.; Viceconte, N.; Saccoccio, S.; Saccoccio, S.; Battaglia, V.; Toninello, A.; Agostinelli, E. Polyamine catabolism: Target for antiproliferative therapies in animals and stress tolerance strategies in plants. Amino Acids 2012, 42, 411–426. [CrossRef] [PubMed]

19. Alcázar, R.; Bitrián, M.; Bartels, D.; Koncz, C.; Altabella, T.; Tiburcio, A.F. Polyamine metabolic canalization in response to drought stress in Arabidopsis and the resurrection plant Craterostigma plantagineum. Plant Signal. Behav. 2011, 6, 243–250. [CrossRef] [PubMed]

20. Su, G.X.; Zhang, W.H.; Liu, Y.L. Involvement of hydrogen peroxide generated by polyamine oxidative degradation in the development of lateral roots in soybean. J. Integr. Plant Biol. 2010, 48, 426–432. [CrossRef]

21. Wimalasekera, R.; Tebertz, F.; Scherer, G.F.E. Polyamines, polyamine oxidases and nitric oxide in development, abiotic and biotic stresses. Plant Sci. 2011, 181, 593–603. [CrossRef]

22. Alet, A.I.; Sánchez, D.H.; Cuevas, J.C.; Marina, M.; Carrasco, P.; Altabella, T.; Tiburcio, A.F.; Ruiz, O.A. New insights into the role of spermine in Arabidopsis thaliana under long-term salt stress. Plant Sci. 2012, 182, 94–100. [CrossRef]

23. Garwood, E.A.; Williams, T.B. Growth, water use and nutrient uptake from the subsoil by grass swards. J. Agric. Sci. 1967, 69, 125–130. [CrossRef]

24. Rennenberg, H.; Loreto, F.; Polle, A.; Brilli, F.; Fares, S.; Beniwal, R.S.; Gessler, A. Physiological responses of forest trees to heat and drought. Plant Biol. 2006, 8, 556–571. [CrossRef] [PubMed]

25. Xu, G.H.; Fan, X.R.; Miller, A.J. Plant nitrogen assimilation and use efficiency. Annu. Rev. Plant Biol. 2012, 63, 153–182. [CrossRef] [PubMed]

26. Huang, L.L.; Li, M.J.; Zhou, K.; Sun, T.T.; Hu, L.Y.; Li, C.Y.; Ma, F.W. Uptake and metabolism of ammonium and nitrate in response to drought stress in malus prunifolia. Plant Physiol. Biochem. 2018, 127, 185–193. [CrossRef]
27. Huang, L.; Li, M.J.; Shao, Y.; Sun, T.T.; Li, C.Y.; Ma, F.W. Ammonium uptake increases in response to PEG-induced drought stress in Malus hupehensis Rehd. *Environ. Exp. Bot.* **2018**, *151*, 32–42. [CrossRef]

28. Meng, S.; Zhang, C.X.; Su, L.; Li, Y.M.; Zhao, Z. Nitrogen uptake and metabolism of Populus simonii in response to PEG-induced drought stress. *Environ. Exp. Bot.* **2016**, *123*, 78–87. [CrossRef]

29. Oaks, A.; Long, D.M.; Zoumadakis, M.; Li, X.Z.; Hertig, C. The Role of NO$_3^-$ and NH$_4^+$ Ions in the Regulation of Nitrate Reductase in Higher Plants; Springer-Verlag: Berlin/Heidelberg, Germany, 1990; pp. 165–170. [CrossRef]

30. Xiong, D.L.; Yu, T.T.; Liu, X.; Li, Y.; Peng, S.B.; Huang, J.L. Heterogeneity of photosynthesis within leaves is associated with alteration of leaf structural features and leaf N content per leaf area in rice. *Funct. Plant Biol.* **2015**, *42*, 687–696. [CrossRef]

31. Wilkinson, S.; Bacon, M.A.; Davies, W.J. Nitrate signalling to stomata and growing leaves: Interactions with soil drying, ABA, and xylem sap pH in maize. *J. Exp. Bot.* **2007**, *58*, 1705–1716. [CrossRef]

32. Bloom, A.J.; Sukrapanna, S.S.; Warner, R.L. Root respiration associated with ammonium and nitrate absorption and assimilation by barley. *Plant Physiol.* **1992**, *99*, 1294–1301. [CrossRef]

33. Yi, X.P.; Zhang, Y.L.; Yao, H.S.; Zhang, X.J.; Luo, H.H.; Guo, L.; Zhang, W.F. Alternative electron sinks are crucial for conferring photoprotection in field-grown cotton under water deficit during flowering and boll setting stages. *Func. Plant Biol.* **2014**, *41*, 737. [CrossRef]

34. Cai, K.Z.; Wu, X.Z.; Luo, S.M. Effects of water stress on osmolytes at different growth stages in staces in rice leaves and roots. *J. Integr. Plant Biol.* **2014**, *56*, 114–121. [CrossRef]

35. Ashraf, M.; Foolad, M.R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* **2007**, *59*, 206–216. [CrossRef]

36. Li, L.J.; Gu, W.R.; Li, J.; Li, C.F.; Xie, T.L.; Qu, D.Y.; Meng, Y.; Li, C.F.; Wei, S. Exogenously applied spermidine alleviates photosynthetic inhibition under drought stress in maize (*Zea mays* L.) seedlings associated with changes in endogenous polyamines and phytohormones. *Plant Physiol. Biochem.* **2018**, *129*, 35–55. [CrossRef] [PubMed]

37. Fu, G.F.; Song, J.; Li, Y.R.; Yue, M.K.; Angelini, R.; RoubelakisAngelakis, K.A. The polyamines and their catabolic products are significant players in the turnover of nitrogenous molecules in plants. *Functional Plant Biology*. **2019**, 46, 1–17. [CrossRef]

38. Shi, H.T.; Chan, Z.L. Improvement of plant abiotic stress tolerance through modulation of the polyamine pathway. *J. Integr. Plant Biol.* **2014**, *56*, 114–121. [CrossRef]

39. Cai, K.Z.; Wu, X.Z.; Luo, S.M. Effects of water stress on osmolytes at different growth stages in staces in rice leaves and roots. *J. Integr. Plant Biol.* **2014**, *56*, 114–121. [CrossRef]

40. Shi, H.T.; Ye, T.T.; Chen, F.F.; Cheng, Z.M.; Wang, Y.P.; Yang, P.F.; Zhang, Y.S.; Chan, Z.L. Manipulation of arginase expression alters the root urinary excretion of urea and nitrate in maize (*Zea mays* L.). *Environ. Exp. Bot.* **2014**, *102*, 161–170. [CrossRef] [PubMed]

41. Moschou, P.N.; Wu, J.; Cona, A.; Tavladoraki, P.; Cona, A.; Angelini, R.; RoubelakisAngelakis, K.A. The polyamines and their catabolic products are significant players in the turnover of nitrogenous molecules in plants. *Environ. Exp. Bot.* **2014**, *102*, 161–170. [CrossRef] [PubMed]

42. Cuevas, J.C.; López-Cobollo, R.; Alcázar, R.; Zarza, X.; Kaczmarski, E.; Libuda, T.; Salinas, J.; Tiburcio, A.F.; Ferrando, A. Putrescine is involved in arabidopsis freezing tolerance and cold acclimation by regulating abscisic acid levels in response to low temperature. *Plant Physiol.* **2008**, *148*, 1094–1105. [CrossRef]

43. Pottosin, I.; Shabala, S. Polyamines control of cation transport across plant membranes: Implications for ion homeostasis and abiotic stress signaling. *Front. Plant Sci.* **2014**, *5*, 154. [CrossRef]

44. Blatt, M.R. Cellular signaling and volume control in stomatal movements in plants. *Annu. Rev. Cell Dev. Biol.* **2000**, *16*, 221–241. [CrossRef] [PubMed]

45. Liu, K.; Fu, H.; Bei, Q.X.; Luan, S. Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements. *Plant Physiol.* **2000**, *124*, 1315–1325. [CrossRef] [PubMed]

46. Shi, J.; Fu, X.Z.; Peng, T.; Huang, X.S.; Liu, J.H. Spermine pretreatment confers dehydration tolerance of citrus in vitro plants via modulation of antioxidant capacity and stomatal response. *Tree Physiol.* **2010**, *30*, 914–922. [CrossRef] [PubMed]

47. Yamaguchi, T.; Aharon, G.S.; Sottosanto, J.B.; Blumwald, E. Vacuolar Na$^+$ /H$^+$ antiporter cation selectivity is regulated by calmodulin from within the vacuole in a Ca$^{2+}$- and pH-dependent manner. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 16107–16112. [CrossRef] [PubMed]

48. Miller, G.; Suzuki, N.; Ciftciylmaz, S.; Mittler, R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ.* **2010**, *33*, 453–467. [CrossRef] [PubMed]

49. Tourni, I.; Moschou, P.N.; Paschalidis, K.A.; Bouamama, B.; Ben, S.A.; Ghorbel, A.W.; Miliki, A.; Roubelakis-Angelakis, K.A. Abscisic acid signals reorientation of polyamine metabolism to orchestrate stress responses via the polyamine exodus pathway in grapevine. *J. Plant Physiol.* **2010**, *167*, 519–525. [CrossRef]

50. Wang, B.Q.; Zhang, Q.F.; Liu, J.H.; Li, G.H. Overexpression of PtADC1 confers enhanced dehydration and drought tolerance in transgenic tobacco and tomato: Effect on ROS elimination. *Biochem. Biophys. Res. Commun.* **2011**, *413*, 10–16. [CrossRef]

51. Radhakrishnan, R.; Lee, I.J. Spermine promotes acclimation to osmotic stress by modifying antioxidant, abscisic acid, and jasmonic acid signals in soybean. *J. Plant Growth Regul.* **2013**, *32*, 22–30. [CrossRef]

52. Zhang, Y.; Li, Z.; Li, Y.P.; Zhang, X.Q.; Ma, X.; Huang, L.K.; Yan, Y.H.; Peng, Y. Chitosan and spermine enhance drought resistance in white clover, associated with changes in endogenous phytohormones and polyamines, and antioxidant metabolism. *Func. Plant Biol.* **2018**, *45*, 1205–1222. [CrossRef]
53. Sánchez-Rodriguez, E.; Romero, L.; Ruiz, J.M. Accumulation of free polyamines enhances the antioxidant response in fruits of grafted tomato plants under water stress. *J. Plant Physiol.* 2016, 190, 72–78. [CrossRef]
54. Li, Z.; Zhou, H.; Peng, Y.; Zhang, X.Q.; Xiao, Y.H. Exogenously applied spermidine improves drought tolerance in creeping bentgrass associated with changes in antioxidant defense, endogenous polyamines and phytohormones. *Plant Growth Regul.* 2015, 76, 71–82. [CrossRef]
55. Espasandin, F.D.; Maiale, S.J.; Calzadilla, P.; Ruiz, O.A.; Sansberro, R.A. Transcriptional regulation of 9-cis-epoxycarotenoid dioxygenase (NCED) gene by putrescine accumulation positively modulates ABA synthesis and drought tolerance in Lotus tenuis plants. *Plant Physiol. Biochem.* 2016, 94, 29–35. [PubMed]
56. Wu, Y.X.; Li, Q.; Dou, P.; Kong, F.L.; Ma, X.J.; Cheng, Q.B.; Yuan, J.C. Effect of low nitrogen stress on bleaching sap characters and root activity of maize cultivars with different low N tolerance. *J. Plant Nutr. Fertil.* 2017, 23, 278–288. [CrossRef]
57. Jia, Q.M.; Yang, L.Y.; An, H.Y.; Dong, S.; Chang, S.H.; Zhang, C.; Liu, Y.J.; Hou, F.J. Nitrogen fertilization and planting models regulate maize growth, nitrate and root distributions in semi-arid regions. *Soil Tillage Res.* 2020, 200, 104636. [CrossRef]
58. Natali, S.M.; Sanudo-Wilhelmy, S.A.; Lerdau, M.T. Effects of elevated carbon dioxide and nitrogen fertilization on nitrate reductase activity in sweetgum and loblolly pine trees in two temperate forests. *Plant Soil.* 2009, 314, 197–210. [CrossRef]
59. Yu, X.Z.; Zhang, F.Z. Activities of nitrate reductase and glutamine synthetase in rice seedlings during cyanide metabolism. *J. Hazard. Mater.* 2012, 225–226, 190–194. [CrossRef]
60. Singh, R.P.; Srivastava, H.S. Increase in glutamate synthase (GOGAT) activity in maize seedlings in response to nitrate and ammonium nitrogen. *Physiol. Plant.* 1986, 66, 413–416. [CrossRef]
61. Magalhaes, J.R.; Huber, D.M. Response of ammonium assimilation enzymes to nitrogen form treatments in different plant species. *J. Plant Nutr.* 1991, 14, 175–185. [CrossRef]
62. Krouk, G.; Crawford, N.M.; Coruzzi, G.M.; Tsay, Y.F. Nitrate signaling: Adaptation to fluctuating environments. *Curr. Opin. Plant Biol.* 2010, 13, 265–272. [CrossRef]
63. Linkohr, B.L.; Williamson, L.C.; Fitter, A.H.; Leyser, O. Nitrate and phosphate availability and distribution have different effects on root system architecture of Arabidopsis. *Plant J.* 2002, 29, 751–760. [CrossRef]
64. Guo, F.Q.; Young, J.; Crawford, N.M. The nitrate transporter AtNRT1.1 (CHL1) functions in stomatal opening and contributes to drought susceptibility in Arabidopsis. *Plant Cell.* 2003, 15, 107–117. [CrossRef]
65. Foyer, C.H.; Valadier, M.H.; Migge, A.; Becker, T.W. Drought-induced effects on nitrate reductase activity and mRNA and on the coordination of nitrogen and carbon metabolism in maize leaves. *Plant Physiol.* 1998, 117, 283–292. [CrossRef] [PubMed]
66. Plaut, Z. Nitrate Reductase activity of wheat seedlings during exposure to and recovery from water stress and salinity. *Physiol. Plantarum.* 2010, 30, 212–217. [CrossRef]
67. Miura, K. Nitrogen and phosphorus nutrition under salinity stress. In *Ecophysiology and Responses of Plants under Salt Stress*; Ahmad, P.; Azooz, M.; Prasad, M., Eds.; Springer: New York, NY, USA, 2013; pp. 425–441. [CrossRef]
68. Sunil, B.; Talla, S.K.; Aswani, V.; Raghavendra, A.S. Optimization of photosynthesis by multiple metabolic pathways involving interorganellae interactions: Resource sharing and ROS maintenance as the bases. *Photosynth. Res.* 2013, 117, 61–71. [CrossRef] [PubMed]
69. Gonzalez-Dugo, V.; Durand, J.L.; Gastal, F. Water Deficit and Nitrogen Nutrition of Crops. In *Sustainable Agriculture*; Lichtfouse, E., Hamelin, M., Navarrete, M., Debaeke, P., Eds.; Springer: Dordrecht, The Netherlands, 2011; Volume 2, pp. 557–575. [CrossRef]
70. Hoai, N.T.T.; Shim, I.S.; Kobayashi, K.; Kenji, U. Accumulation of some nitrogen compounds in response to salt stress and their relationships with salt tolerance in rice (*Oryza sativa* L. seedlings). *Plant Growth Regul.* 2003, 41, 159–164. [CrossRef]
71. Márquez, A.J.; Betti, M.; García-Calderón, M.; Estivill, G. Nitrate and ammonium assimilatory enzymes. In *Lotus Japonicus Handbook*; Márquez, A.J., Ed.; Springer: Dordrecht, The Netherlands, 2005; pp. 315–328. [CrossRef]
72. Du, J.; Shu, S.; Shao, Q.S.; An, Y.H.; Zhou, H.; Guo, S.R.; Sun, J. Mitigative effects of spermidine on photosynthesis and carbon–nitrogen balance of cucumber seedlings under Ca(NO₃)₂ stress. *J. Plant Res.* 2016, 129, 79–91. [CrossRef]
73. Forde, B.G.; Lea, P.J. Glutamate in plants: Metabolism, regulation, and signalling. *J. Exp. Bot.* 2007, 58, 2339–2358. [CrossRef]
74. Labboun, S.; Terce-Laforgue, T.; Roscher, A.; Bedu, M.; Restivo, F.M.; Velanis, C.N.; Skopelitis, D.S.; Moschou, P.N.; RoubelakisAngelakis, K.A.; Suzuki, A.; et al. Resolving the role of plant glutamate dehydrogenase. I. in vivo real time nuclear magnetic resonance spectroscopy experiments. *Plant Cell Physiol.* 2009, 50, 1761–1773. [CrossRef]
75. King, J.; Waygood, E.R. Glyoxylate aminotransferases from wheat leaves. *Can. J. Biochem.* 1968, 46, 771–779. [CrossRef]
76. Masclaux-Daubresse, C.; Reisdorf-Cren, M.; Pageau, K.; Lelandais, M.; Grandjean, O.; Kronenberger, J.; Valadier, M.H.; Feraud, M.; Jouglet, T.; Suzuki, A. Glutamine synthetase-glutamate synthase pathway and glutamate dehydrogenase play distinct roles in the sink-source nitrogen cycle in tobacco. *Plant Physiol.* 2006, 140, 444–456. [CrossRef]
77. Joshi, V.; Joung, J.G.; Fei, Z.; Jander, G. Interdependence of threonine, methionine and isoleucine metabolism in plants: Accumulation and transcriptional regulation under abiotic stress. *Amino Acids* 2010, 39, 933–947. [CrossRef] [PubMed]
78. Wei, W.; Li, Q.T.; Chu, Y.N.; Reiter, R.J.; Yu, X.M.; Zhu, D.H.; Zhang, W.K.; Ma, B.; Lin, Q.; Zhang, J.S.; et al. Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. *J. Exp. Bot.* 2015, 66, 695–707. [CrossRef] [PubMed]
79. Guan, D.H.; Al-Kaisi, M.M.; Zhang, Y.S.; Duan, L.S.; Tan, W.M.; Zhang, M.C.; Li, Z.H. Tillage practices affect biomass and grain yield through regulating root growth, root-bleeding sap and nutrients uptake in summer maize. *Field Crop. Res.* 2014, 157, 89–97. [CrossRef]
80. Fageria, N.K. Influence of dry matter and length of roots on growth of five field crops at varying soil zinc and copper levels. J. Plant Nutr. 2004, 27, 1517–1523. [CrossRef]
81. Gao, Y.; Duan, A.W.; Qiu, X.Q.; Liu, Z.G.; Sun, J.S.; Zhang, J.P.; Wang, H.Z. Distribution of roots and root length density in a maize/soybean strip intercropping system. Agr. Water Manage. 2010, 98, 199–212. [CrossRef]
82. Wasson, A.P.; Rebetezka, G.J.; Kirkegaard, J.A.; Christopher, J.; Richards, R.A.; Watt, M. Soil coring at multiple field environments can directly quantify variation in deep root traits to select wheat genotypes for breeding. J. Exp. Bot. 2014, 65, 6231–6249. [CrossRef]
83. Han, J.; Zhu, D.; Lin, X.; Han, W. Effects of polyamines on tobacco root growth and development. Plant Physiol. Commun. 1992, 28, 124–126. [CrossRef]
84. Peuke, A.D. The chemical composition of xylem sap in Vitis vinifera L. cv. Riesling during vegetative growth on three different Franconian vineyard soils and as influenced by nitrogen fertilizer. Am. J. Enol. Vitic. 2000, 51, 329–339. [CrossRef]
85. Amos, B.; Walters, D.T. Maize root biomass and net rhizodeposited carbon. Soil Sci. Soc. Am. J. 2006, 70, 1489–1503. [CrossRef]
86. Morita, S.; Okamoto, M.; Abe, J.; Yamagishi, J. Bleeding rate of field-grown maize with reference to root system development. Jpn. J. Crop Sci. 2000, 69, 80–85. [CrossRef]
87. Noguchi, A.; Kageyama, M.; Shimimachi, F.; Schmidhalter, U.; Hasegawa, I. Potential for using plant xylem sap to evaluate inorganic nutrient availability in soil. Soil Sci. Plant Nutr. 2005, 51, 343–350. [CrossRef]
88. Ansari, T.H.; Yamamoto, Y.; Yoshida, T.; Sakagami, K. Relation between bleeding rate during panicle formation stage and sink size in rice plant. Soil Sci. Plant Nutr. 2004, 50, 57–66. [CrossRef]
89. Ji, B.; Li, J.; Xin, L.; Wang, F.; Li, B. Effects of spermidine on antioxidant properties and root activity of rice stubble wheat seedlings under waterlogging stress. J. Henan Agric. Sci. 2018, 47, 29–33. [CrossRef]
90. Seng, S.S.; Wang, Q.; Zhang, Y.E.; Li, C.H.; Liu, T.X.; Zhao, L.F.; Liu, H.P. Effects of exogenous spermidine on physiological regulatory of maize after waterlogging stress. Acta Agron. Sin. 2012, 38, 1042–1050. [CrossRef]
91. Ding, L.; Wang, K.J.; Jiang, G.M.; Liu, M.Z.; Gao, L.M. Photosynthetic rate and yield formation in different maize hybrids. Biol. Plant 2007, 51, 165–168. [CrossRef]
92. Marchiori, P.E.R.; Machado, E.C.; Ribeiro, R.V. Photosynthetic limitations imposed by self-shading in field-grown sugarcane varieties. Field Crop. Res. 2014, 155, 30–37. [CrossRef]
93. Chen, X.C.; Chen, F.J.; Chen, Y.L.; Gao, Q.; Yang, X.L.; Yuan, L.X.; Zhang, F.S.; Mi, G.H. Modern maize hybrids in Northeast China exhibit increased yield potential and resource use efficiency despite adverse climate change. Glob. Chang Biol. 2013, 19, 923–936. [CrossRef]
94. Sun, X.; Ding, Z.; Wang, X.; Ming, Z. Subsoiling practices change root distribution and increase post-anthesis dry matter accumulation and yield in summer maize. PLoS ONE 2017, 12, e174952. [CrossRef]
95. Sun, X.F.; Ding, Z.S.; Hou, H.P.; Ge, J.Z.; Tang, L.; Zhao, M. Post-anthesis photosynthetic assimilation and the changes of carbon and nitrogen in different varieties of spring maize. Acta Agron. Sin. 2013, 39, 1284–1292. [CrossRef]
96. Martinez, D.; Guiamet, J. Distortion of the SPAD 502 chlorophyll meter readings by changes in irradiance and leaf water status. Agronomie 2004, 24, 41–46. [CrossRef]
97. Duvick, D.N. The contribution of breeding to yield advances in maize (Zea mays L.). Adv. Agron. 2005, 86, 83–145. [CrossRef]
98. Zhou, B.Y.; Yue, Y.; Sun, X.F.; Wang, X.B.; Wang, Z.M.; Ma, W.; Zhao, M. Maize grain yield and dry matter production responses to variations in weather conditions. Agron. J. 2015, 108, 196–204. [CrossRef]
99. Khan, N.A.; Murayama, S.; Ishimine, Y.; Tsuzuki, E.; Nakamura, I. Physio-morphological studies of F1 hybrids in rice (Oryza sativa L.): Photosynthetic ability and yield. Plant Prod. Sci. 1998, 1, 233–239. [CrossRef]
100. Schussler, J.R.; Westgate, M.E. Assimilate flux determines kernel set at low water potential in maize. Crop Sci. 1995, 35, 1074–1080. [CrossRef]
101. Zhang, J.P.; Sun, J.S.; Liu, Z.G.; Gao, Y. Effect of moisture and mulching on filling characteristics and yield of summer maize. Chin. J. Eco.-Agric. 2010, 18, 501–506. [CrossRef]
102. Bräutigam, A.; Gagneul, D.; Weber, A.P.M. High-throughput colorimetric method for the parallel assay of glyoxylic acid and ammonium in a single extract. Anal. Biochem. 2007, 362, 151–153. [CrossRef] [PubMed]