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

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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_4^+ in the leaves of maize seedlings under drought stress increased significantly, while the content of NO_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

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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_2O_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 °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 °C for 15 min, the phase was separated by centrifugation (*g*, 10 min, 8 °C). The aqueous phase was transferred to a new tube containing 50 mg of activated carbon, fully mixed and centrifuged (*g*, 5 min, 8 °C). The supernatant obtained after charcoal treatment was diluted 1:1 (*v*/*v*) in 100 mM HCl and 20 µL the solution is mixed with 100 µL 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 °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_2HPO_4 and KH_2PO_4, pH 7.5), 5 mM cysteine and 5 mM EDTA-NA_2. The extract was centrifuged at 4000 rpm at 4 °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 °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 °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_2OH. The reaction was initiated by the addition of enzyme extract. After incubation at 30 °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 mL 20 mM L-glutamine, 0.05 mL 0.1 M 2-oxoglutarate, 0.1 mL 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_4Cl, 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 μmol NADH g^{-1} 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 α-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 × 30 cm × 2 cm). LA-S root analysis system (Hangxzhou 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_2SO_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* (leaf area index) = green leaf area (leaf length × leaf width × 0.75) × 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* at maturity/*GLA* 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 ± 2.21^{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 ^{ab}	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}
CK	Xianyu 335	NH_4^+ contents (μg g^{-1} FW)	294.24 ± 21.47 ^{a}	247.25 ± 20.88 ^{b}	261.93 ± 26.51 ^{b}	293.76 ± 26.22 ^{c}	281.52 ± 27.45 ^{c}
Spd			316.91 ± 17.07 ^{a}	267.65 ± 29.34 ^{ab}	272.14 ± 26.96 ^{b}	278.46 ± 21.95 ^{c}	247.86 ± 17.03 ^{c}
PEG			299.47 ± 25.48 ^{a}	303.14 ± 27.38 ^{a}	350.88 ± 29.89 ^{a}	397.80 ± 15.03 ^{a}	412.42 ± 15.04 ^{a}
Spd + PEG			321.51 ± 13.76 ^{a}	283.15 ± 30.24 ^{ab}	307.36 ± 20.94 ^{ab}	340.00 ± 26.92 ^{b}	345.44 ± 22.95 ^{b}
CK	Fenghe 1	NH_4^+ contents (μg g^{-1} FW)	246.68 ± 16.08 ^{a}	218.82 ± 21.08 ^{c}	197.60 ± 16.21 ^{c}	191.90 ± 16.21 ^{c}	203.30 ± 20.57 ^{c}
Spd			215.33 ± 15.79 ^{a}	192.53 ± 12.97 ^{c}	187.47 ± 20.65 ^{c}	207.73 ± 22.77 ^{c}	211.22 ± 20.93 ^{c}
PEG			243.11 ± 25.92 ^{a}	317.62 ± 13.35 ^{a}	363.22 ± 22.98 ^{a}	428.45 ± 26.48 ^{a}	472.47 ± 22.93 ^{a}
Spd + PEG			228.00 ± 21.52 ^{a}	264.73 ± 14.91 ^{b}	307.80 ± 20.11 ^{b}	340.10 ± 23.98 ^{b}	359.10 ± 20.55 ^{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.

3.3. 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	NR Activity (μg g^{-1} FW h^{-1})				
		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 ^{ab}	10.41 ± 1.02 ^{b}	8.56 ± 0.60 ^{b}	12.09 ± 1.13 ^{b}
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 ± 0.46 ^{a}	11.11 ± 0.78 ^{a}	10.37 ± 0.58 ^{a}	11.44 ± 1.08 ^{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 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 (μmol GHA g^{-1} FW h^{-1})	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			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			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			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 (μmol GHA g^{-1} FW h^{-1})	22.50 ± 1.20 ^{a}	24.23 ± 1.13 ^{a}	23.11 ± 1.90 ^{a}	25.44 ± 2.41 ^{a}	22.72 ± 2.03 ^{a}
Spd			22.65 ± 1.09 ^{a}	25.11 ± 2.19 ^{a}	25.40 ± 1.92 ^{a}	23.66 ± 1.13 ^{a}	23.42 ± 2.13 ^{a}
PEG			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}
Spd + PEG			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}
CK	Xianyu 335	GOGAT activity (μmol NADH g^{-1} FW h^{-1})	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}
Spd			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}
PEG			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}
Spd + PEG			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}
CK	Fenghe 1	GOGAT activity (μmol NADH g^{-1} FW h^{-1})	0.52 ± 0.03 ^{a}	0.59 ± 0.05 ^{a}	0.57 ± 0.04 ^{a}	0.58 ± 0.02 ^{a}	0.57 ± 0.03 ^{a}
Spd			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}
PEG			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}
Spd + PEG			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}
CK	Xianyu 335	GDH activity (μmol NAD g^{-1} FW h^{-1})	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}
Spd			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}
PEG			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}
Spd + PEG			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}
CK	Fenghe 1	GDH activity (μmol NAD g^{-1} FW h^{-1})	1.06 ± 0.06 ^{a}	0.97 ± 0.08 ^{a}	1.08 ± 0.07 ^{b}	1.08 ± 0.07 ^{a}	1.07 ± 0.07 ^{a}
Spd			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}
PEG			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}
Spd + PEG			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 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.

3.5. 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 ^{a}	0.28 ± 0.02 ^{a}	0.25 ± 0.02 ^{a}	0.28 ± 0.02 ^{a}	0.29 ± 0.02 ^{a}
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 ^{ab}	0.22 ± 0.02 ^{a}	0.21 ± 0.02 ^{b}	0.21 ± 0.01 ^{b}
CK	Fenghe 1	GOT 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			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			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			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}
CK	Xianyu 335	GPT activity (μmol mg^{-1} 30 min^{-1})	0.37 ± 0.04 ^{a}	0.31 ± 0.03 ^{ab}	0.36 ± 0.03 ^{a}	0.31 ± 0.04 ^{a}	0.30 ± 0.03 ^{a}
Spd			0.39 ± 0.03 ^{a}	0.34 ± 0.03 ^{a}	0.34 ± 0.03 ^{a}	0.30 ± 0.02 ^{a}	0.31 ± 0.03 ^{a}
PEG			0.36 ± 0.02 ^{a}	0.25 ± 0.04 ^{b}	0.23 ± 0.03 ^{b}	0.21 ± 0.02 ^{c}	0.18 ± 0.02 ^{c}
Spd + PEG			0.40 ± 0.03 ^{a}	0.31 ± 0.03 ^{ab}	0.29 ± 0.03 ^{a}	0.25 ± 0.03 ^{b}	0.23 ± 0.03 ^{b}
CK	Fenghe 1	GPT activity (μmol mg^{-1} 30 min^{-1})	0.30 ± 0.02 ^{a}	0.31 ± 0.02 ^{a}	0.33 ± 0.03 ^{a}	0.37 ± 0.04 ^{a}	0.33 ± 0.03 ^{a}
Spd			0.34 ± 0.03 ^{a}	0.33 ± 0.02 ^{a}	0.33 ± 0.02 ^{a}	0.35 ± 0.04 ^{a}	0.36 ± 0.03 ^{a}
PEG			0.35 ± 0.04 ^{a}	0.26 ± 0.02 ^{b}	0.20 ± 0.01 ^{c}	0.16 ± 0.02 ^{c}	0.14 ± 0.03 ^{c}
Spd + PEG			0.33 ± 0.03 ^{a}	0.29 ± 0.03 ^{ab}	0.25 ± 0.03 ^{b}	0.22 ± 0.03 ^{b}	0.20 ± 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 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.

3.6. 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).

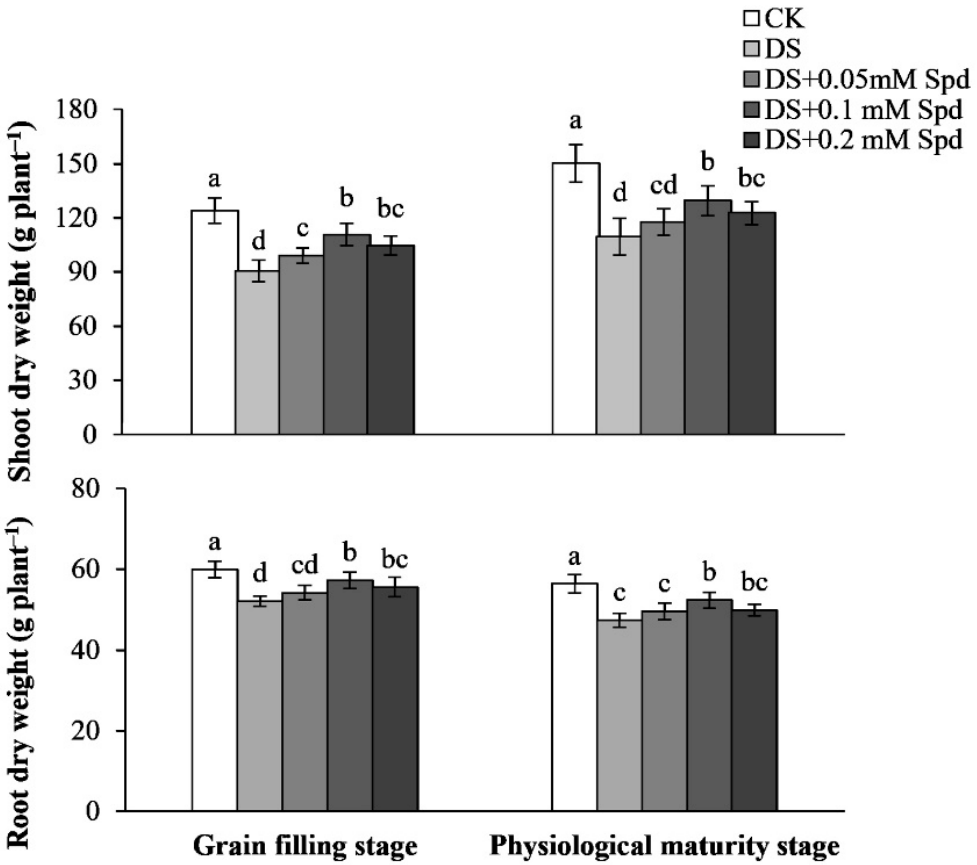

Figure 1. Effects of exogenous spermidine on dry matter accumulation of maize under drought stress. Different letters within the same column indicate significant difference at 5% level.

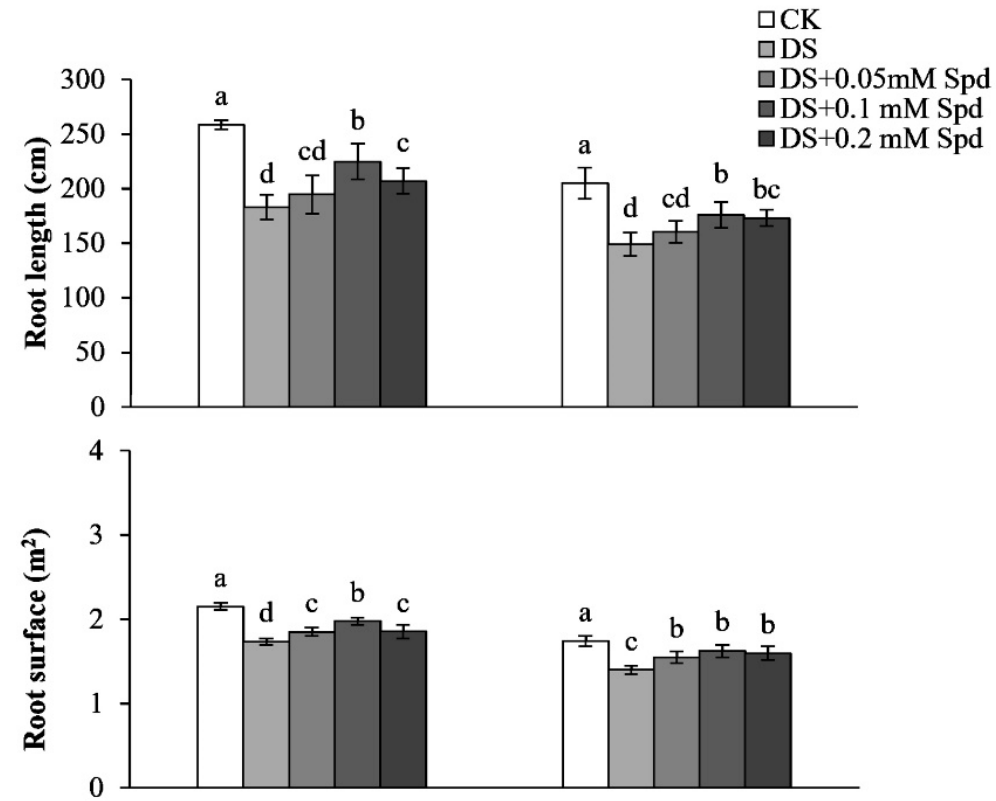

Figure 2. *Cont.*

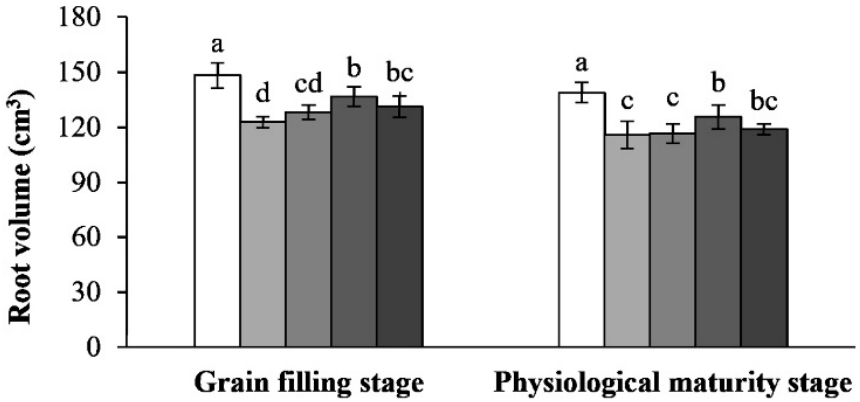

Figure 2. Effects of exogenous spermidine on root characteristic parameters of maize under drought stress. Different letters within the same column indicate significant difference at 5% level.

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 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).

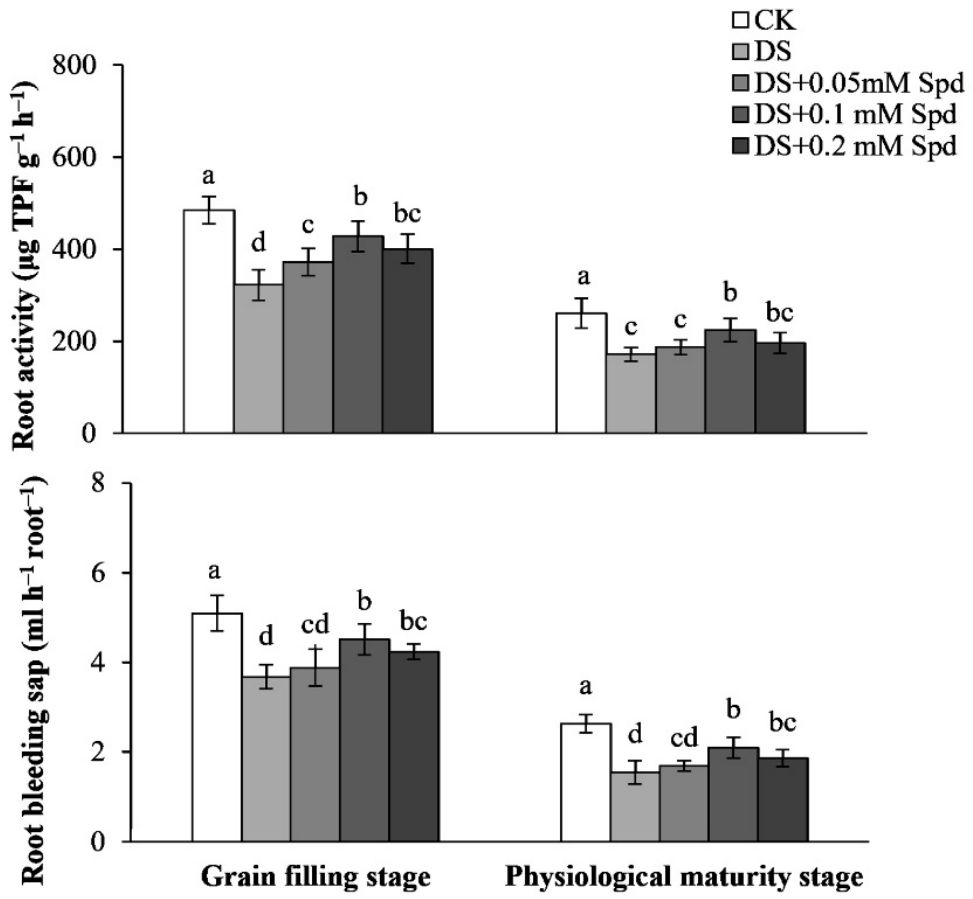

Figure 3. Effects of exogenous spermidine on root bleeding sap rate of maize under drought stress. Different letters within the same column indicate significant difference at 5% level.

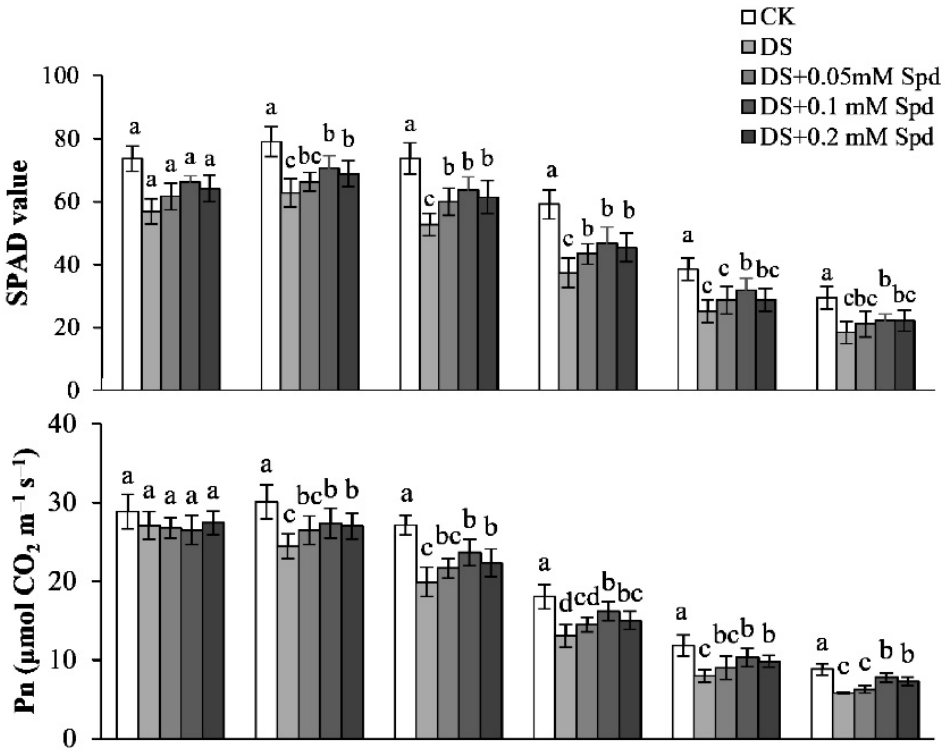

Figure 4. *Cont.*

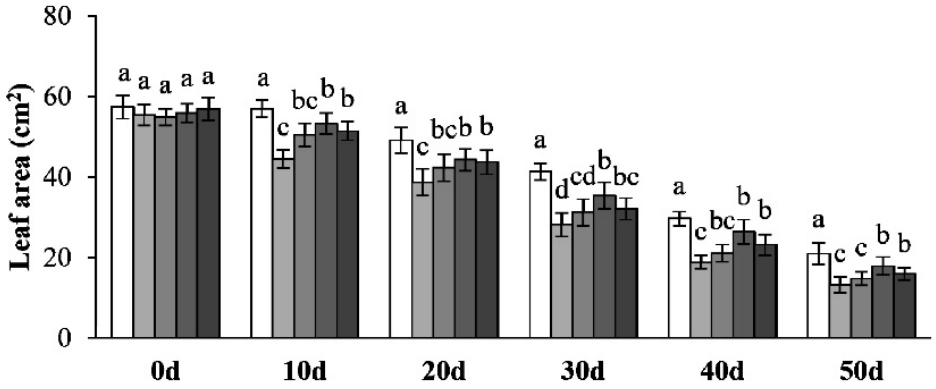

Figure 4. Effect of exogenous spermidine on SPAD value, P_n and leaf area of maize leaves at post-anthesis stage under drought stress. Different letters within the same column indicate significant difference at 5% level.

3.10. Leaf Senescence Characteristics

Compared with normal water treated plants, drought stressed plants had lower $RGLA_m$, LAD and T_{max} values, but higher V_m and V_{max} values. Under Spd treatment, $RGLA_m$, LAD and T_{max} values of stressed plants increased, V_m and V_{max} values decreased. Compared with simple drought treatment, the values of $RGLA_m$, 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	$RGLA_m$ (%)	V_m (%)	V_{max} (%)	T_{max} (d)	LAD (m^2 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).

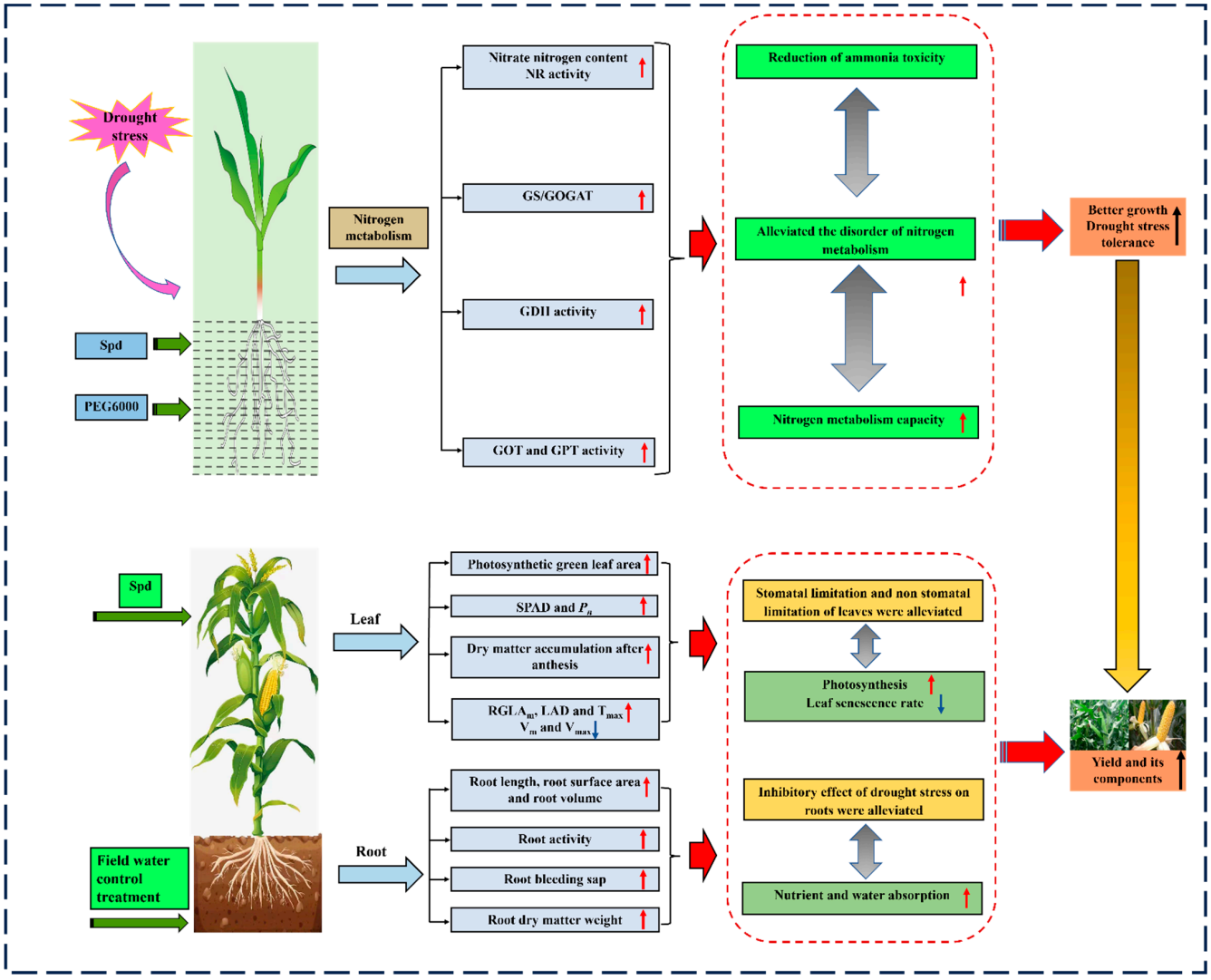

Figure 5. Schematic representation of the positive role of exogenous spermidine on nitrogen metabolism and transcriptome analysis in maize under drought stress and field verification. The red arrows (↑) and the blue arrows (↓) represent the positive and passive roles of spermidine, respectively.

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