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Foliar applications of spermidine improve foxtail millet seedling characteristics under salt stress

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Abstract

This study investigated the mitigating effects of spermidine (Spd) application on salinity-induced ion imbalance, physiological properties, and the expression of some genes in foxtail millet (*Setaria italica* L.). We observed 30-d-old seedlings maintained at a half-strength Hoagland solution (control), 1.0 % NaCl, 10, 20, and 40 μ M Spd, and 10, 20, and 40 μ M Spd + 1.0 % (m/v) NaCl for 14 d. The results show that salt stress significantly inhibited plant growth, and this was significantly ameliorated by Spd. The mass of the shoots and roots, content of chlorophyll *a* and chlorophyll *b*, root activity, and K^+ content were higher whereas Na^+ content, Na^+/K^+ ratio, relative electrolyte leakage, glutathione content, H_2O_2 content, activity of glutathione reductase (GR), and catalase (CAT) were lower after application of Spd in comparison with NaCl alone. The expression of *GR*, *ascorbate peroxidase*, *CAT*, and *superoxide dismutase* genes also significantly decreased in salt stressed plants with Spd. This study has proved the role of Spd in alleviating salt stress in foxtail millet and identified that 20 μ M Spd was most effective.

Additional key words: antioxidant enzymes, gene expression, reactive oxygen species, root activity, soil salinity.

Introduction

Soil salinity affects plant growth, crop productivity, and quality, and hence, is one of the most important problems for the future of global agriculture (Shahbaz and Ashraf 2013). Approximately 20 % of irrigated lands worldwide are affected by salinity (Zhao *et al.* 2017). Salt stress leads to the disruption of enzymes and other macromolecules, the damage to cell organelles, and the changes in photosynthesis and respiration (Porcel *et al.* 2016). Furthermore, drought stress and nutrient imbalances in plants are also induced by soil salinity, and lead to the accumulation of Na^+ and the impairment of K^+ uptake (Ruiz-Lozano *et al.* 2012). Abnormally high cytosolic Na^+/K^+ ratio triggers the inhibition of protein synthesis and enzymatic activities (Porcel *et al.* 2016). The saline conditions have also resulted in the generation of excessive reactive oxygen species (ROS) that leads to chlorophyll (Chl) degradation, membrane lipid peroxidation, and membrane fluidity reductions (Taïbi *et al.* 2016). The ROS also exert effects on several other macromolecules,

such as proteins and nucleic acids (Ahmad *et al.* 2010). However, plants have developed several protective mechanisms and both enzymatic and non-enzymatic antioxidants are involved in scavenging ROS (Abd Allah *et al.* 2015). Research has widely documented that superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), detoxify ROS (Farhangi-Abri and Torabian 2017). In addition, the accumulation of osmolytes, such as proline, glycine betaine, soluble proteins, and soluble sugars, is another strategy to mitigate osmotic stress provoked by salinity (Lou *et al.* 2019). To date, various methods have been used to improve cultivation of plants in saline-alkali soils and some systems for their efficient exploitation have been established. One approach is the selection of more salt-tolerant crops.

Foxtail millet (*Setaria italica* L.), a perennial C4 grass that belongs to the *Poaceae* family, subfamily *Panicoideae*, tribe *Paniceae* (Daverdin *et al.* 2015), was recognized as a particularly important cereal and fodder crop that is cultivated widely in arid and semi-arid

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Abbreviations: APX - ascorbate peroxidase; CAT - catalase; Chl - chlorophyll; EL - electrolyte leakage; GR - glutathione reductase; GSH - glutathione; O_2^- - superoxide anion; ROS - reactive oxygen species; Spd - spermidine; SOD - superoxide dismutase; TP - total protein; TTC - 2,3,5-triphenyltetrazolium chloride; TTF - triphenylformazan.

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regions (Zhang and Liu 2015). With excellent drought and salinity tolerance, foxtail millet has the potential to be cultivated in saline-alkali soils (Sudhakar *et al.* 2015). Furthermore, the demand for foxtail millet has increased significantly as societies focus on healthier diets, and it offers a high nutrient content, including starch, amylose, protein, vitamins, and fatty acids (Wang *et al.* 2017). Veeranagamallaiah *et al.* (2008) reported that the seedling growth of foxtail millet was inhibited significantly with 100 mM NaCl and then linearly decreased with increasing salt concentrations, and similar results were found for their dry mass. Unfortunately, there is little documented how to improved its salt tolerance.

Spermidine (Spd) acts as a growth regulator and is a ubiquitous low molecular mass aliphatic nitrogen compound that is present in animals, plants, and bacteria (Sang *et al.* 2016). Application of Spd is known to elicit several physiological, histochemical, and biochemical effects. Fariduddin *et al.* (2018) revealed the ameliorative role of Spd on *Lycopersicon esculentum*, as 1.0 mM Spd successfully restored the negative effects induced by salt stress. Similar results with the application of Spd were also found for rice under heat stress (Tang *et al.* 2018), *Boehmeria nivea* under Cd²⁺ stress (Gong *et al.* 2016), creeping bentgrass under drought stress (Zhou *et al.* 2015), and mung bean under low temperature stress (Nahar *et al.* 2015). However, the optimum concentration of Spd for damage alleviation in plants under abiotic stress varied in individual crops (Lou *et al.* 2018), and little is known about the regulatory mechanisms of Spd on plant physiological and biochemical responses (Li *et al.* 2015).

In the present study, different concentration of Spd were applied to foxtail millet seedlings to investigate whether exogenous Spd could improve foxtail millet resistance to salt stress by analyzing plant growth, antioxidant enzyme activity, root activity, ion balance, and gene expression. The findings of this work could provide a new theoretical approach for understanding the physiological basis of the Spd-alleviation of saline damage and could promote the development and expansion of the foxtail millet cultivation on saline-alkali soils.

Material and methods

Plants and treatments: The seeds of foxtail millet (*Setaria italica* L.) cultivar Jingu No. 2 were obtained from the Center of Crop Germplasm Resources, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China. Disposable plastic cups (upper diameter 6.8 cm, lower diameter 4.8 cm, height 7.4 cm) were filled with moistened sterile sand (< 1 mm particle size). To drain the excess water and maintain good soil aeration, five holes (diameter of 5 mm) were drilled in the base of each cup, and forty seeds were sown. The cups were arranged randomly in a greenhouse and irrigated with 10 cm³ of a half-strength Hoagland solution every 2 d. After 30 d of cultivation, the seedlings were transferred to a growth chamber (*Adaptis-A1000*, *Conviron*, Shanghai, China), with a 16-h photoperiod, temperatures of 25/20 °C (day/night),

a 75 % relative humidity, and a photon flux density of 300 μmol m⁻² s⁻¹ at plant height, and cultivated 4 d for adaption. Then, eight different treatments were applied: CK - only a half-strength Hoagland solution; T1 - 1.0 % (m/v) NaCl; T2 - 10 μM Spd; T3 - 10 μM Spd + 1.0 % (m/v) NaCl; T4 - 20 μM Spd; T5 - 20 μM Spd + 1.0 % (m/v) NaCl; T6 - 40 μM Spd; and T7 - 40 μM Spd + 1.0 % (m/v) NaCl. For all treatments, NaCl was dissolved in a half-strength Hoagland nutrient solution. Spermidine was mixed with water and applied *via* spray (30 cm³ per cup, and equal amounts of double distilled H₂O were applied to the treatments without Spd). All seedlings were arranged randomly and cultivated for 14 d. Each treatment was performed in triplicate. Ten seedlings were selected randomly and their shoot heights, root lengths, and fresh masses were individually determined.

Ion content: Fresh shoots and roots were oven dried at 105 °C for 30 min, and then at 80 °C to a constant mass. The dried samples were ground into a powder and then passed through a sieve (≤ 0.25 mm). Sub-samples (0.1 g) were weighed and digested with concentrated H₂SO₄ (98 %, m/m) and H₂O₂ (30 %, v/v), 2:1 v/v, at 370 °C until a clear solution was obtained. The K⁺ and Na⁺ content were determined by flame photometry (*API500*, *AoPu*, Shanghai, China) following the method of Kalhor *et al.* (2016); the Na⁺/K⁺ ratio was also calculated.

Chlorophyll content: For Chl content determination, fresh leaf material (0.1 g) was transferred into a 20 cm³ cuvette filled with 10 cm³ of dimethyl sulfoxide (DMSO). All cuvettes were kept in the dark at 20 °C for 3 d and shaken every 24 h. The supernatants were diluted 3 × with DMSO and absorptions measured at 663 and 645 nm using a spectrophotometer (*UV-2600*, *UNICO Instruments*, Shanghai, China) according to Rasool *et al.* (2013).

Electrolyte leakage (EL) was assessed using an electrical conductivity meter, as described by Jungklang *et al.* (2017). Fresh leaf material (0.1 g) was cut into 0.5 cm segments. Then, all segments were immersed in 10 cm³ of deionized water and shaken at 25°C for 12 h. The initial conductivity (C_i) of the soaking solution was measured using a conductance meter (*JENCO-3173*, *Jenco Instruments*, San Diego, CA, USA). The samples were then heated in a boiling water bath for 30 min, cooled to room temperature, shaken, and the final electrical conductivity (C_{max}) measured. Relative EL was calculated as follows: EL = (C_i/C_{max}) × 100 %.

Hydrogen peroxide, total protein, and glutathione content, CAT and GR activities, and O₂⁻ production were determined using a spectrophotometer (*UV-2600*), as described by Han *et al.* (2019). Fresh leaf material (0.2 g) was shredded and grounded with 1.8 cm³ of physiological saline in liquid nitrogen in an ice bath. The homogenate was transferred into 2-cm³ centrifuge tubes and centrifuged at 12 000 g and 4 °C for 15 min. The supernatants were collected and analyzed with *A 064-1*, *A 045-2*, *A 006-1*, *A 007-1*, *A 062*, and *A 052* assay kits (*Nanjing Jiancheng*

Bioengineering Institute, Nanjing, China). The absorbance of the reaction solution was determined at 405 nm for hydrogen peroxide, at 595 nm for total protein (TP), at 420 nm for glutathione (GSH), at 240 nm for CAT, at 340 nm for GR, and at 550 nm for O_2^- production. One unit of GR activity was defined as 1 mmol of NADPH consumed per minute per gram of protein, one unit of CAT activity was defined as 1 μ mol of H_2O_2 consumed per minute per gram of protein, and the O_2^- production rate was calculated as the amount of O_2^- (μ g) evolved per gram of tissue protein.

Root activity was assessed according to the method described by Zhang *et al.* (2016) with slight modifications. The roots were washed and dried with filter paper. Thereafter, 0.5 g roots samples were placed in 50-cm³ beakers where 5 cm³ of 0.1 M phosphate buffer saline (PBS; pH 7.0) and 5 cm³ of 0.4 % (m/v) 2,3,5-triphenyltetrazolium chloride (TTC) were added. The roots were fully immersed at 37 °C for 1 h. Then, to stop the reaction, 2 cm³ of 1 M sulfuric acid was added, and the root sample removed from the beaker and dried with filter paper. The roots were ground with 10 cm³ ethyl acetate, a small amount of quartz sand was added for the extraction of TTC, the red triphenylformazan (TTF) was transferred into a test tube, and the absorption at 485 nm was measured using a spectrophotometer (UV-2600). Ethyl acetate was employed as the blank reference, and the absorbance was determined. A standard curve was then generated to determine the amount of reduced tetrazole which characterizes root activity.

Spermidine content: For Spd content analysis, dried samples (1.0 g) were weighed, transferred into tubes, and 1.0 cm³ of 5 % (m/v) ice perchloric acid was added. Then, the mixture was immersed in an ice bath and shaken for 2 h and then centrifuged at 12 000 g for 30 min. Then, 0.5 cm³ of the supernatant was removed and added to 1.0 cm³ of 2 M NaOH solution followed by the addition of 0.015 cm³ of benzoyl chloride, vortexed for 20 s and then kept in a warm bath at 30 °C for 20 min. Next, 2.0 cm³ of saturated NaCl and 2.0 cm³ of ice diethyl ether were added and vortexed for 20 s and centrifuged for 5 min. Next, 1.0 cm³ of the ether phase was evaporated to dryness in a 37 °C water bath, and then 0.2 cm³ of chromatographic methanol was added and passed through a 0.22 μ m filter.

The LC20 (Shimadzu, Kyoto, Japan) liquid chromatograph VWD detector was employed to determine Spd, and the high performance liquid chromatography conditions were: *Eclipse Plus C18* (250 mm $\times$ 4.6 mm, 5 μ m) reversed phase column; methanol:acetonitrile:water 60.5:2.5:37 (volume ratio); flow rate 0.8 cm³ min⁻¹; temperature 30 °C; detection wavelength 254 nm; injection volume 0.01 cm³.

Gene expression analysis with reverse-transcription quantitative PCR: Two segments of fresh leaves were harvested after Spd was applied for 0, 2, 4, 6, 8, 10, and 12 h and pulverized with liquid nitrogen. Total RNA was extracted with *TRIzol* reagent (Invitrogen, Carlsbad, CA, USA) according to the method of Hu *et al.* (2016). The first-strand cDNA was synthesized and gDNA removed using the *AQ141* kit. The reaction system consisted of a template (10 pg⁻¹ μ g), primer, 2 $\times$ *TransSart Tip Green qPCR SuperMix*, passive reference dye, and ddH₂O. Reverse transcription PCR amplification was executed in two steps using the following conditions: amplification at 94 °C for 30 s, then 40 - 45 cycles of 94 °C for 15 s, and 60 °C for 20 s. All PCR amplification data were analyzed in *Opticon Monitor v. 2.03* (MJ Research, Cambridge, MA, USA). The primer sequences are shown in Table 1 Suppl.

Statistical analyses: Experimental data are the averages of three replications and were subjected to ANOVA in *SPSS v. 21.0* (IBM Corp, Armonk, NY, USA). Statistical significance was assessed using the least significant difference (LSD) test at $P \leq 0.05$. Diagrams were generated using *OriginPro 2019* software (Originlab Corp., USA) and *GraphPad* software (GraphPad Corp., USA).

Results

Significant reductions were observed in the shoot mass (34.63 %), root mass (44.71 %), plant height (35.89 %), and root length (26.19 %) when the plants were exposed to the 1.0 % (m/v) NaCl alone, relative to those in the non-NaCl treatments (Figs. 1 and 2). Meanwhile, no negative effects were detected on these parameters when Spd alone was applied. However, higher shoot mass, root mass, and plant height were detected at NaCl + Spd treatments than at NaCl alone. There was notably lower root mass (a 11.17 % decrease) under the T7 treatment compared with that under

Fig. 1. Photographs of foxtail millet. Thirty-day-old seedlings were maintained in eight treatments: CK -- a half-strength Hoagland solution, T1 - 1.0 % (m/v) NaCl, T2 - 10 μ M Spd, T3 - 10 μ M Spd + 1.0 % (m/v) NaCl, T4 - 20 μ M Spd, T5 - 20 μ M Spd + 1.0 % (m/v) NaCl, T6 - 40 μ M Spd, and T7 - 40 μ M Spd + 1.0 % (m/v) NaCl for 14 d.

Fig. 2. Effects of spermidine (Spd) on growth of foxtail millet. A - Shoot mass and root mass, B - plant height and root length. Means $\pm$ SDs, $n = 3$; different letters indicate significant differences at $P \leq 0.05$.

the T5 treatment. Meanwhile, no significant differences in root length were determined among T1, T3, T7 treatments, and higher root length at T5 relative to T1 (with 22.48 % increase).

The Chl *a*, Chl *b*, and Chl *a+b* content was significantly reduced when the plants were exposed to NaCl alone (Fig. 3A). However, only 20 and 40 μM Spd in combination with NaCl decreased the Chl *a* (12.44 - 16.54 %) and Chl *a+b* (10.11 - 10.95 %) content, relative to the control treatment. Meanwhile, no significant differences in the Chl *a* and Chl *a+b* content were observed between the salt and non-salt treatments when using the 10 μM Spd applications, and there were no remarkable effects on Chl *b* content under the salt stress conditions relative to the non-salt stress treatments, when using various Spd concentrations (10, 20, and 40 μM). Notably, Chl *a* and Chl *a+b* content in treatments T4 and T5 was higher than that in CK and T1. Furthermore, Chl *b* and Chl *a+b* content in T3 and T7 was higher than in T1 treatment.

Significant reduction in TP content in T1 (30.02 %) relative to CK was detected, and similar results were observed between salt treated and non-salt treated plants when they were exposed to the various concentrations

of Spd (Fig. 3B). The 20 μM Spd application increased TP content significantly compared with T1, and no significant differences were determined among T1, T3, and T5.

Under salt stress, relative electrolyte leakage (REL) in the leaves of the foxtail millet increased significantly compared with that in the control, and the increase was observed also after Spd application (Fig. 3C). Compared to T1, the Spd application treatments T3, T5, and T7 reduced REL by 21.86, 37.29, and 23.71 %, respectively. Meanwhile, the REL in T5 was lower than in T3 and T7, and there were no significant differences between T3 and T7. Furthermore, no significant effects on REL were detected among the various Spd treatments (CK, T2, T4, and T6) in the absence of salinity.

The 1.0 % (m/v) NaCl alone reduced the root activity significantly (by 37.42 %) compared with that in the CK treatment (Fig. 3D). Fortunately, the root activities in T3, T5, and T7 were higher (33.35, 47.78, and 37.11 % respectively) in comparison to T1 treatment. In addition, the root activity in the T5 was higher than in T3 and T7, and no significant differences were found between T3 and T7.

To study the adverse effects of NaCl on foxtail millet, we

Fig. 3. Effects of spermidine (Spd) on chlorophyll content (A, Cha - chlorophyll *a*, Chb - chlorophyll *b*, Cht - total chlorophyll); total protein (B); relative electrolyte leakage (C), and root activity (D) in foxtail millet. Means $\pm$ SDs, $n = 3$; different letters indicate significant differences at $P \leq 0.05$.

also determined the Na^+ and K^+ content and found that the K^+ content in the leaves was significantly lower (36.12 %) and the Na^+ content was significantly higher (85.49 %) in the T1 treatment compared with the CK (Fig. 4A-C). The Spd applications decreased the Na^+ content, and no significant differences were observed among the treatments T3, T5, and T7. However, the K^+ content in T3, T5, and T7 was increased markedly compared with the T1 treatment. No significant differences were detected among Spd concentrations. The excessive absorption of Na^+ , and the inhibition of K^+ uptake by salinity led to higher Na^+/K^+ ratio in T1 relative to CK. However, the Na^+/K^+ ratios decreased with the Spd applications (28.05, 35.83, and 33.12 % for T3, T5, and T7, respectively), when compared with T1. The Na^+/K^+ ratios were lower in T5 and T7 than in T3, and no significant differences were detected between T5 and T7. However, the Spd applications led to higher Na^+/K^+ ratios also when exposed to the non-salt stress conditions.

Under salt stress, Spd was synthesized by plants to cope with the detrimental damage, and as such, a higher Spd content of 32.80 % was detected under the T1 treatment than in the CK (Fig. 5). Higher Spd content was also observed in the plants treated with the Spd, both without and with 1.0 % (m/v) NaCl. Furthermore, there was a higher Spd content under the T5 and T7 treatments than under T3, and under T4 and T6 than under T2. There were no significant differences in Spd content between T5 and T7, and T4 and T6, respectively.

The GSH content, H_2O_2 content, and $\text{O}_2^{\cdot -}$ activity were significantly increased in T1 treatment by 65.43, 122.92, and 36.68 %, respectively, compared to those in the CK treatment (Fig. 4D-F). However, the GSH content decreased by 18.79 - 27.96 %, and the H_2O_2 content by 20.12 - 25.33% after Spd application, relative to the T1 treatment. Regarding the $\text{O}_2^{\cdot -}$ activity, only that in T5 was higher than in T1, and there were no significant differences among T1, T3, and T7 treatments. In addition,

Fig. 4. Effects of spermidine (Spd) on Na⁺ content (A), K⁺ content (B), and Na⁺/K⁺ ratio (C), H₂O₂ (D), O₂⁻ (E), and glutathione (GSH) (F) in foxtail millet. Means $\pm$ SDs, $n = 3$; different letters indicate significant differences at $P \leq 0.05$.

Fig. 5. Effects of spermidine (Spd) on Spd content in foxtail millet. Means $\pm$ SDs, $n = 3$; different letters indicate significant differences at $P \leq 0.05$.

the Spd application alone had no significant effects on GSH content, H_2O_2 content, and $O_2^{\cdot -}$ activity.

Compared with those under CK treatment, the activities of GR and CAT were significantly increased due to the 1.0 % (m/v) NaCl stress and increased by 57.35 and 64.39 % in T1 relative to CK (Fig. 6). The Spd application decreased the GR and CAT activities by 10.42 - 17.80 % and 9.03 - 22.70 %, respectively. The lower CAT activities were in T5 and T7 than in T2, and the difference between T5 and T7 were not significant. No significant differences in GR activity were observed among the T3, T5, and T7 treatments, and no remarkable effects on the activities of GR and CAT by the Spd alone.

The expression of *GR*, *APX*, *CAT*, and *SOD* genes were significantly increased in T1 compared with those in CK (by 1.39 - 2.73, 3.80 - 8.42, 3.48 - 4.73, and 2.85 - 5.35 times, respectively (Fig. 7). Lower *GR* and *SOD* expressions were detected after various Spd concentrations (T3, T5, and T7) than in T1 at 4, 6, 8, 10, and 12 h. The *SOD* expressions in T5 and T7 were lower than in T1 and T3 at 2 h, and *APX* and *CAT* expressions decreased after Spd application relative to T1 at 2, 4, 6, 8, 10, and 12 h. Notably, no significant effects Spd applications on the *GR*, *APX*, *CAT*, and *SOD* expressions were found under non-saline conditions at 2 - 12 h. There was a lower expression of *GR* in T5 than in T3 at 10 h, and in T3 and T7 at 12 h. Among various Spd application treatments, T5 exposed to the 1.0 % (m/v) NaCl regime induced the lowest expression of *APX* and *SOD*. Furthermore, *CAT* expression was lower in T5 than in T3 with no significant difference between T5 and T7 at 10 and 12 h.

Discussion

Foxtail millet is widely cultivated for its high yield, outstanding quality, and economic value, and it can be

Fig. 6. Effects of spermidine (Spd) on antioxidant activity of foxtail millet. A - glutathione reductase (GR); B - catalase (CAT). Means $\pm$ SDs, $n = 3$; different letters indicate significant differences at $P \leq 0.05$.

widely grown in saline-alkali soils owing to its excellent salt tolerance (Veeranagamallaiah *et al.* 2008). However, the growth and yield of foxtail millet are limited by high osmotic pressure and adverse effects of Na^+ and Cl^- (Talaat *et al.* 2015), which induce imbalances in various physiological and biochemical processes. The toxicity of Na^+ accumulation in plants is considered a major problem associated with salinity stress. Previous reports have demonstrated that Na^+ in substrate causes K^+ deficiencies by inhibiting K^+ uptake by plants (Liu *et al.* 2000), but maintaining a lower Na^+/K^+ ratio is important. In the present study, we observed that the Na^+/K^+ ratio increased by up to 1.68-times when the plant was exposed to 1.0 % NaCl. However, we noted that after addition of Spd, the Na^+/K^+ ratios decreased due to increased K^+ absorption maybe influencing competition with Na^+ at both the transport site and the binding site (Maathuis *et al.* 1999). Similar results were obtained in wheat by Zhang *et al.* (2016), where Spd treatments significantly increased K^+ content

Fig. 7. Effects of spermidine on the *glutathione reductase* (GR) (A), *ascorbate peroxidases* (APX) (B), *catalase* (CAT) (C), and *superoxide dismutase* (SOD) (D) gene expressions in foxtail millet.

and decreased Na^+ content.

As a comprehensive response index, biomass decrease is a reliable indicator for assessing the degree of damage and the tolerance ability of plants to salt stress. This study showed that the growth indexes (plant height, root length, shoot mass, and root mass) decreased significantly with salt stress. Meanwhile, this detrimental damage was alleviated by foliar applications of Spd, and the amelioration was non-dose dependent. Therefore, greater shoot mass, root mass, plant height, and root length were observed in the Spd-treated plants, compared with those not treated with Spd. Similar results were also found with *Calendula officinalis* (Baniyadi *et al.* 2018), maize, *Arabidopsis* (Chen *et al.* 2017), chrysanthemum (Zhang *et al.* 2016), and zoysiagrass (Li *et al.* 2016).

The photosynthetic pigment content of leaves is also an important indicator, reflecting the plant's photosynthetic capacity. However, chloroplast pigment content varies according to changing of various environmental factors. Present results showed that the Chl content decreased significantly under salt stress, consistent with the research of Dąbrowski *et al.* (2017) on perennial ryegrass. The Chl content increased with Spd application, especially Chl *a+b*

content.

Spermidine is a small aliphatic polyamine that occurs as ubiquitous polycation in higher plants and is recognized as a plant growth regulator (Satish *et al.* 2016). The content of Spd in plants can reflect the resistance of plants to abiotic stresses. To deal with salt stress, plants synthesize excess Spd, and higher Spd content was observed in plants treated with 1.0 % (m/v) NaCl, than in the control. We also found that spraying with Spd increased the content of Spd in foxtail millet and proved that the foliar application was an effective method to increase Spd content in plants.

In the present study, the TP content was reduced significantly in response to the salt stress conditions and was attributed to the oxidation of Chl, damage to the chloroplast ultrastructure, and absorption of nutrients (Cherif-Silini *et al.* 2016). Fortunately, Spd applications increased the TP content. Similar results were obtained by Radhakrishnan *et al.* (2013) in cucumber plants treated with Spd, and the reason for this phenomenon is attributed to the effects of Spd application on the activities of enzymes responsible for protein metabolism.

It is well documented that excessive ROS are produced directly *via* the Haber-Weiss and Fenton reactions and can

destroy proteins, nucleic acids, and enzymes (Buapet *et al.* 2019). The maintenance of an efficient antioxidant system is crucial for plants to protect themselves also under salinity (Saxena *et al.* 2016). Our results also demonstrated that excessive H_2O_2 was produced in plants under salt stress, nearly 2.23 times more under NaCl treatments than in the control. However, the GSH content, and CAT and GR activities were increased significantly and attributed to the defense mechanisms of plants to alleviate the oxidative stress-related damage. These results are consistent with the reports by Abd Allah *et al.* (2015) who suggested that the GSH content and CAT in *Sesbania sesban* increased with increasing salt concentrations. Nevertheless, the increased GR activity induced by salt stress in foxtail millet was significantly decreased by Spd, regardless of its concentration. In addition, the CAT activity was also reduced under Spd application. These results disagreed with those of Puyang *et al.* (2015), who indicated that the CAT activity in Kentucky bluegrass increased with Spd application. This phenomenon may be due to species differences that resulted in different Spd protection mechanisms. It is well-known that exposure to higher concentrations of NaCl induces metabolic imbalances in plant cells, thereby altering the amount and activities of different enzymes. Researchers have illustrated that salt stress can also lead to changes in respective gene expressions. By comparing the expressions of *APX*, *CAT*, *GR*, and *SOD* genes under salt stress alone and under addition of Spd, we concluded that Spd may play an important role in improving the salt-tolerance of foxtail millet. Our results indicated that the expressions of *APX*, *CAT*, *GR*, and *SOD* were up-regulated under salt stress conditions, similar to the results from Hu *et al.* (2011), who found that gene expressions were up-regulated with saline conditions in perennial ryegrass. Importantly, the expressions of *APX*, *CAT*, *GR*, and *SOD* were lowered by the application of Spd.

In summary, application of Spd of a certain concentration was effective at ameliorating the detrimental damage of foxtail millet caused by salt stress. The beneficial effects of 20 μ M Spd application may be attributed to inhibition of Na^+ absorption, promotion of K^+ absorption, and reduction of Na^+/K^+ ratio. Spd also promoted the increase of total biomass, TP content, Chl content, and root activity. However, it decreased H_2O_2 , and GSH content, GR, CAT, and O_2^- activity as well as expressions of *CAT*, *APX*, *GR*, and *SOD* genes.

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