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Research Article

Exogenous Spermidine (Spd) alleviates NaCl-induced injury effects by improving photosynthesis and oxidative stress tolerance in *Brassica napus* seedlings

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Abstract

To better understand the mechanism of exogenous application of Spermidine (Spd) to enhance the salt tolerance of rapeseed (*Brassica napus* L.). The seedlings of rapeseed cultivar 'Zheyou-18' were treated with different concentrations of spermidine under 200 mM NaCl. The portable photosynthetic system flame, photometer and portable fluorometer were used to determine the gas-exchange parameters, ion content and Chlorophyll fluorescence. A spectrophotometer was used to determine the activities of SOD (Superoxide Dismutase), POD (Peroxidase), CAT (Catalase), and APX (Ascorbate Peroxidase). Our study revealed that different concentrations of exogenous Spd can alleviate the harmful effect caused by NaCl stress in rapeseed, and inhibition in rapeseed seedling growth was significantly alleviated with the application of 160 mg·L^{-1} spermidine under 200 mM NaCl, which is reflected in dry and fresh weight. The Spd treatment further enhanced the photosynthetic efficiency of rapeseed leaves, which is reflected in the changes in gas exchange parameters Pn (Photosynthetic rate), Ci (Intercellular CO_2 concentration), Tr (Transpiration rate), Gs (Stomatal conductance), Ls (stomatal limitation), WUE (Water Use Efficiency) and chlorophyll fluorescence parameters (Fv/Fm, ΦPSII, qP, NPQ), and antioxidant enzymes activity (SOD, POD, CAT, APX) which in turn reduced the level of active oxygen (H_2O_2, O_2^-). This study also indicated the Spd treatment reduced the absorption of Na^+ and increased the absoraption of K^+ and Ca^{2+}. The physiological experiments demonstrate that exogenous Spd enhances salt tolerance in rapeseed under NaCl stress through multiple mechanisms, including improved photosynthetic efficiency, maintenance of cellular ion homeostasis, and reduced reactive oxygen species (ROS) levels.

Keywords: *Brassica napus*; photosynthesis; salt stress; spermidine; stomatal conductance

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Introduction

Soil salinity is a common limiting environmental factor affecting global crop productivity by decreasing net photosynthesis, stomatal conductance, and protein synthesis (Zhu, 2001; Meloni *et al*., 2003; Munns and Tester, 2008; Jin *et al*., 2011). Current estimates, near 20% of cultivated land and 50% of irrigated land are affected by soil salinity, which is increasing (Shrivastava and Kumar, 2015).

Salinity stress inhibits the normal growth and development of crops and ultimately affects the yield of crops. First of all, the high concentration of salt solubility reduces water potential in the soil, which in turn causes root dehydration (Munns and Tester, 2008). Secondly, excessive Na+ produces ion toxicity in plant tissues and cells suffering salt damage (Parida and Das, 2005; Agarwal *et al*., 2012). High concentration of Na+ in saline soil inhibit the absorption of K+ by roots, resulting in abnormally low K+ content in plants(Passricha *et al*., 2019). Plants accumulate more reactive oxygen species under salt stress, such as superoxide radicals (O_2^-), hydroxyl radicals (·OH), and hydrogen peroxide (H_2O_2)(Ijaz *et al*., 2019). Excessive reactive oxygen species will cause damage to DNA structure and lead to cell senescence even death (Hasegawa *et al*., 2000; Zhang *et al*., 2016). To accommodate salt stress, plants evolved a variety of mitigation strategies, among which the following three approaches are the main ones. The first way is to synthesize osmotic regulators such as proline, betaine, choline, organic acids (Ashraf and Foolad, 2007). The second way is to improve the antioxidant capacity of enzymes including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) (Zhang *et al*., 2016). They mainly protect the enzyme system by removing active oxygen in the cell, participate in cell photosynthesis, respiration, and lignin formation, and play an important role in chloroplasts, mitochondria, and cytoplasm (Rangani *et al*., 2016). The third way is selective ions absorption. Under salt stress, the accumulation of Na+ will damage the cell membrane system, and K+ is essential for reducing osmotic potential and maintaining the balance of water and pH in plant cells as an important inorganic solute. Plants maintain high K^+/Na^+ values of tissue cells by restricting Na^+ entering and selectively K^+ absorbing to ensure normal physiological metabolism (Zhu *et al*., 1998).

Polyamines (PAs) are ubiquitous low molecular weight organic polycations, carrying out vital function in plant response to abiotic stress, such as salinity, drought, heavy metals, oxidative stress (Alcázar *et al*., 2006; Groppa and Benavides, 2007; Mostofa *et al*., 2013; Pál *et al*., 2015). These compounds mainly exist in three forms in plant cells including diamine putrescine (Put), triamine spermidine (Spd), and tetraamine spermine (Spm) (Li *et al*., 2016). Many evidence to date shows that Spd treatment enhances tolerance to salinity stress in plants by stabilizing membrane, modulating ion channels, maintaining the cation-anion balance, and scavenging reactive oxygen species （ROS) (Ndayiragije and Lutts, 2006; Siddiqui *et al*., 2017; Islam *et al*., 2020; Jiang *et al*., 2020; Wu *et al*., 2020).

Rapeseed (*Brassica napus* L.), as a globally important oilseed crop, requires in-depth research on its salinity tolerance mechanisms. While studies have shown that external additives such as salicylic acid (SA) can alleviate the negative effects of salt stress on rapeseed (Mirza Hasanuzzaman, 2014; Ahmadi *et al*., 2018; Zeng *et al*., 2018), significant gaps remain in understanding the role of spermidine (Spd)-a polyamine confirmed to enhance salinity tolerance in other plants by regulating redox balance and ion homeostasis (Alharbi *et al*., 2025). Specifically, (1) existing research primarily focuses on model plants(Zhu *et al*., 2006; Saleethong *et al*., 2013), whereas the unique lipid metabolism of oilseed crops may lead to distinct Spd regulatory pathways; and (2) systematic studies on the effects of Spd concentration gradients on rapeseed salt stress mitigation-particularly the coordinated responses of photosynthetic efficiency and ion transport-are still lacking. This study aims to investigate the photosynthetic parameters (e.g., net photosynthetic rate, Pn), ion distribution (Na+/K+ ratio), and antioxidant enzyme activities (SOD, POD) in rapeseed under varying Spd concentrations to elucidate (1) whether Spd operates through distinct pathways in B. napus compared to other crops, and (2) the optimal Spd

concentration threshold for alleviating salt stress during the seedling stage. The findings will provide targeted theoretical and practical strategies for improving salinity tolerance in oilseed crops.

Materials and Methods

Plant materials and seedlings culture

Rapeseed cultivar 'Zheyou-18' was provided by the Oil Crop Research Institute, Chinese Academy of Agricultural Sciences, Wuhan, China. Four hundred seed samples were sterilized for 10 min with 5% sodium hypochlorite solution and washed 5 times. The samples were soaked for 4 h in deionized water and germinated in a seed tray with full strength Hoagland's nutrient solution under the artificial climate chest at 24 ± 1 °C, relative humidity 85-90%. During germination, the nutrient solution was renewed every 2 d. 30 seedlings of comparable size and bearing one or two true leaves were transplanted in the hole of foam-board suspending on the top of nutrient solution of pots (diameter 20 cm) in a greenhouse. The environmental conditions were controlled under relative humidity 70-80%, day/night temperature was 25/15 °C, and 16 h photoperiod using a photosynthetic photon flux density (PPFD) of 350 μmol m-2 s^{-1} (measured at the top of the plants with a 190 SB quantum sensor, LI-COR, Lincoln, NE, USA). The nutrient solution was renewed every three days.

The exogenous induction of Spd and exposure to salinity stress

The concentration of Spd was varied from 0 mg·L^{-1} to 200 mg·L^{-1}, including the following seven treatments: (1) 0 mg·L^{-1} Spd / 0 mM NaCl, (2) 0 mg·L^{-1} Spd / 200 mM NaCl, (3) 40 mg·L^{-1} Spd / 200 mM NaCl, (4) 80 mg·L^{-1} Spd / 200 mM NaCl, (5) 120 mg·L^{-1} Spd / 200 mM NaCl, (6) 160 mg·L^{-1} Spd /200 mM NaCl, (7)200 mg·L^{-1} Spd / 200 mM NaCl. When the third true leaf of seedlings first unfolded, seven concentrations of Spd treatments were used to induce rhizosphere (Spd was added to the full-strength Hoagland's nutrient solution to maintain seven concentrations, respectively). 1 week after induction, salt treatment was increased daily in stepwise aliquots of 50 mM in Hoagland's nutrient solution until the appropriate salt treatments were reached(Ashraf, 2001). After 7 days of 200 mM NaCl stress, the following physiological parameters were measured.

Biomass determination

Six rapeseed seedlings with relatively uniform growth were selected. The shoots and roots were washed with tap water three times and with distilled water four times. Then, the washed seedlings were blotted dry with napkins for fresh weighing. Dry weight was measured after placing at 70 °C in a laboratory oven for 72 h.

Estimation of K, Ca, Na

Dry leaves and roots of each plant were digested in triacid (H_2SO_4 + HNO_3 + $HClO_4$ in 9:3:1 ratio). The volume of white colorless digested material was made up to 100 ml with distilled water and filtered. The filtrate was read directly on digital flame photometers using K, Na, and Ca filters respectively (Jatav *et al.*, 2014; Ahanger *et al.*, 2015).

Measurement of gas-exchange parameters

Six rapeseed seedlings with relatively uniform growth were selected. Gas-exchange parameters were measured using a portable photosynthetic system (LI-6400, LI-COR Inc., USA). Net photosynthesis rate (Pn), intercellular CO_2 concentration (Ci), stomatal conductance (Gs), transpiration rate (Tr), and intercellular CO_2 concentration (Ci) were measured under the following conditions: external CO_2 concentration of 400 μmol·mol^{-1}, supplied from CO_2 steel gas cylinder, and photosynthetic active radiation (PAR) of 1200 μmol·m^{-}

^{2}·s^{-1}. The stomatal limitation to photosynthesis (Ls) was calculated according to the methods of (Farquhar and Sharkey, 1982). The water use efficiency (WUE) was calculated as the Pn/Tr ratio (Nieva *et al*., 1999).

Measurement of Chlorophyll fluorescence parameters

Three latest fully expanded leaves were scored for each plant and an average value was calculated. Chlorophyll fluorescence was measured using a portable fluorometer (PAM 2100, Walz, Germany). According to the method of Zhang *et al*. (2019), the main chlorophyll fluorescence parameters are e calculated as (1) the maximum quantum efficiency of PSII: Fv/Fm = (Fm-Fo)/Fm; (2) the coefficient of photochemical quenching: qP = (Fm- Fs)/ (Fm' - Fo'); (3) the coefficient of non-photochemical quenching: NPQ = Fm/Fm'-1 and; (4) quantum efficiency of electron transfer in PSII: ΦPSII = （Fm'-Fs） / Fm'. It should be noticed that gas exchange and fluorescence measured together on one portion of the leave.

Determination of membrane damage and reactive oxygen species production

MDA level was measured according to the method of thiobarbituric acid reaction (Heath and Packer, 1968). Electrolyte leakage percentage was determined according to the method of Achary *et al* (2012). H_2O_2 level was measured according to the method of Orozco-Cardenas et al. O_2^- production rate was assayed according to the protocol of Orozco-Cárdenas *et al* (2001).

Evaluation of antioxidant enzyme

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) according to the method of Bayer and Fridovich (Beyer and Fridovich, 1987). POD (EC 1.11.1.7) activity was measured by following the change of absorption at 436 nm due to guaiacol oxidation (extinction coefficient, 6.39 $mM^{-1}cm^{-1}$) following Pütter (1974). CAT (EC 1.11.1.6) activity was determined by the consumption of H_2O_2 (extinction coefficient, 39.4 mM^{-1}·cm^{-1}) at 240 nm for 3 min (Aebi, 1974). APX (EC 1.11.1.11) activity was determined in 1 ml reaction mixture containing 50 mM K-phosphate (pH 7.0), 0.1 mM ascorbate (extinction coefficient, 2.8 mM^{-1}·cm^{-1}), and 0.3 mM H2O2. The decrease in absorbance was recorded at 290 nm for 3 min (Chen and Asada, 1989).

Statistical analysis

All experiments were repeated three times, and statistical procedures were performed using the PC-SAS software package. The least significant difference (LSD) was calculated for the significant data at $p < 0.05$. Data followed by the same letter are not significantly different by LSD test at $p < 0.05$. Data represent means ± SD, n = 6. The same letter do not differ statistically at $p < 0.05$ (Duncan Multiple Range Test) (Cabusora, 2024).

Results

The biomass of Brassica napus seedlings under NaCl stress

The growth variables including fresh weight and dry weight of rape seedlings were markedly decreased under NaCl stress compared with the control (Table 1). The shoot fresh weight and dry weight were decreased by 59.9% and 55.3%. The root fresh weight and dry weight were decreased by 56.6% and 41.5%. Interestingly, Spd addition significantly altered the growth of rape seedlings at different concentrations. Specifically, the application of 160 mg·L^{-1} Spd increased shoot fresh and dry weight by 1.8, 1.7, 1.5, and 1.7 times, respectively. The ratio of root/shoot increased remarkably under salt stress and Spd treatments suggesting that the range of improving root salt resistanc capacity is more productive than that of shoot. Under salt stress, the dry weights of both shoots and roots treated with other Spd concentrations were lower than those treated with 160 mg·L^{-1}

Spd. Therefore, Spd is beneficial to the growth of salt-stressed rapeseed seedlings and this effect depends on the applied concentrations of Spd.

Table 1. Effects of Spd with different concentrations on shoot weight, root weight and root / shoot ratio of rapeseed seedlings

Treatments	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	Root fresh weight (g/plant)	Root dry weight (g/plant)	Root/ Shoot ratio
1	6.566 ± 0.711 a	0.783 ± 0.025 a	0.838 ± 0.093 a	0.065 ± 0.005 a	0.083 ± 0.005 b
2	2.632 ± 0.179 d	0.350 ± 0.030 e	0.364 ± 0.053 d	0.038 ± 0.003 c	0.108 ± 0.004 c
3	3.054 ± 0.317 d	0.370 ± 0.036 e	0.385 ± 0.020 d	0.041 ± 0.004 c	0.111 ± 0.002 a
4	3.931 ± 0.355 c	0.437 ± 0.025 d	0.421 ± 0.040 cd	0.049 ± 0.003 b	0.112 ± 0.001 a
5	4.537 ± 0.169 bc	0.533 ± 0.025 bc	0.483 ± 0.010 bc	0.060 ± 0.003 a	0.113 ± 0.002 a
6	4.732 ± 0.253 b	0.580 ± 0.030 b	0.530 ± 0.044 b	0.064 ± 0.002 a	0.110 ± 0.003 ab
7	4.162 ± 0.177 bc	0.490 ± 0.036 c	0.417 ± 0.029 cd	0.051 ± 0.004 b	0.104 ± 0.004 ab

1: 0 mg·L^{-1} Spd / 0 mM NaCl; 2: 0 mg·L^{-1} Spd / 200 mM NaCl; 3: 40 mg·L^{-1} Spd / 200 mM NaCl; 4: 80 mg·L^{-1} Spd / 200 mM NaCl; 5: 120 mg·L^{-1} Spd / 200 mM NaCl; 6: 160 mg·L^{-1} Spd /200 mM NaCl; 7: 200 mg·L^{-1} Spd / 200 mM NaCl. Data represent means ± SD, n=6. The same letter do not differ statistically at $p < 0.05$ (Duncan Multiple Range Test)

The gas exchange characteristics of Brassica napus leaves under NaCl stress

The rate of net photosynthesis (Pn) and intercellular CO_2 concentration (Ci) of *B. napus* leaves significantly decreased under NaCl stress ($p < 0.05$). In NaCl only treatment Pn and Ci decreased 58.55% and 28.47% relative to the control (Figure1 A and B) respectively. However, the Pn and Ci value improved with different concentrations of Spd application. Exogenous application of Spd lead to increase in the Pn by 36.31%, 39.93%, 65.4%, 93.32% and 77.78%, and the Ci by 23.76%, 19.6%, 38.61%, 41.13% and 41.96% over NaCl only treatment plants. The protective effect of Spd on the Pn under salt stress was not the normal level (Figure 1 A), there is a significant difference among the NaCl treated with the five concentrations of Spd and the control. However, the Ci of the treatments of 120-160 mg·L^{-1} Spd under the salt stress could return to the normal because of no difference among the NaCl treated with the three concentrations of Spd and the control.

As shown in Figure 1 C and D, the transpiration rate (Tr) and stomatal conductivity (Gs) of *B. napus* leave in NaCl only treatment reduced sharply compared with the control. However, the Tr and Gs increased with the application of Spd could markedly increase, then an increase in Tr and Gs was observed with increasing concentration of Spd. When the concentration of Spd was 200 mg·L^{-1}, the Tr and Gs reached 3.112 mmol $H2O{\cdot}m^{-2}$ s^{-1} and 0.5726 mmol $H2O{\cdot}m^{-2}$ s^{-1}, respectively, much higher than the corresponding control. Moreover, NaCl stress significantly increased stomatal limitation (Ls) by 248.61% relative to the control (Figure 1 E). The application of low concentrations of Spd reduced the Ls under salt stress, such as the treatment of 40 and 80 mg·L^{-1} Spd. The LS decreased markedly with the concentration of Spd exceeding 80 mg·L^{-1}, however, there was no significant difference in the treatment of 120, 160 and, 200 mg·L^{-1}.

Water use efficiency (WUE) is an important physiological index reflecting the relationship between water use and photosynthesis in plants under limited water conditions, which depends on the relative changes of Pn and Tr. The changing trend of WUE is similar to Tr. Under the same concentration of NaCl stress, as Spermidine concentration increases, the WUE of its leaves shows a trend of first rising and then falling (Figure 1 F). When the concentration of spermidine is 40 mg L^{-1}, WUE holds the maximum value of 16.3615. When the concentration is 200 mg L^{-1}, WUE holds the minimum value of 6.6054, lower than the value of the control 11.2036. Compared with the control, under salt stress, the WUE value of 'Zheyou-18' increased slightly.

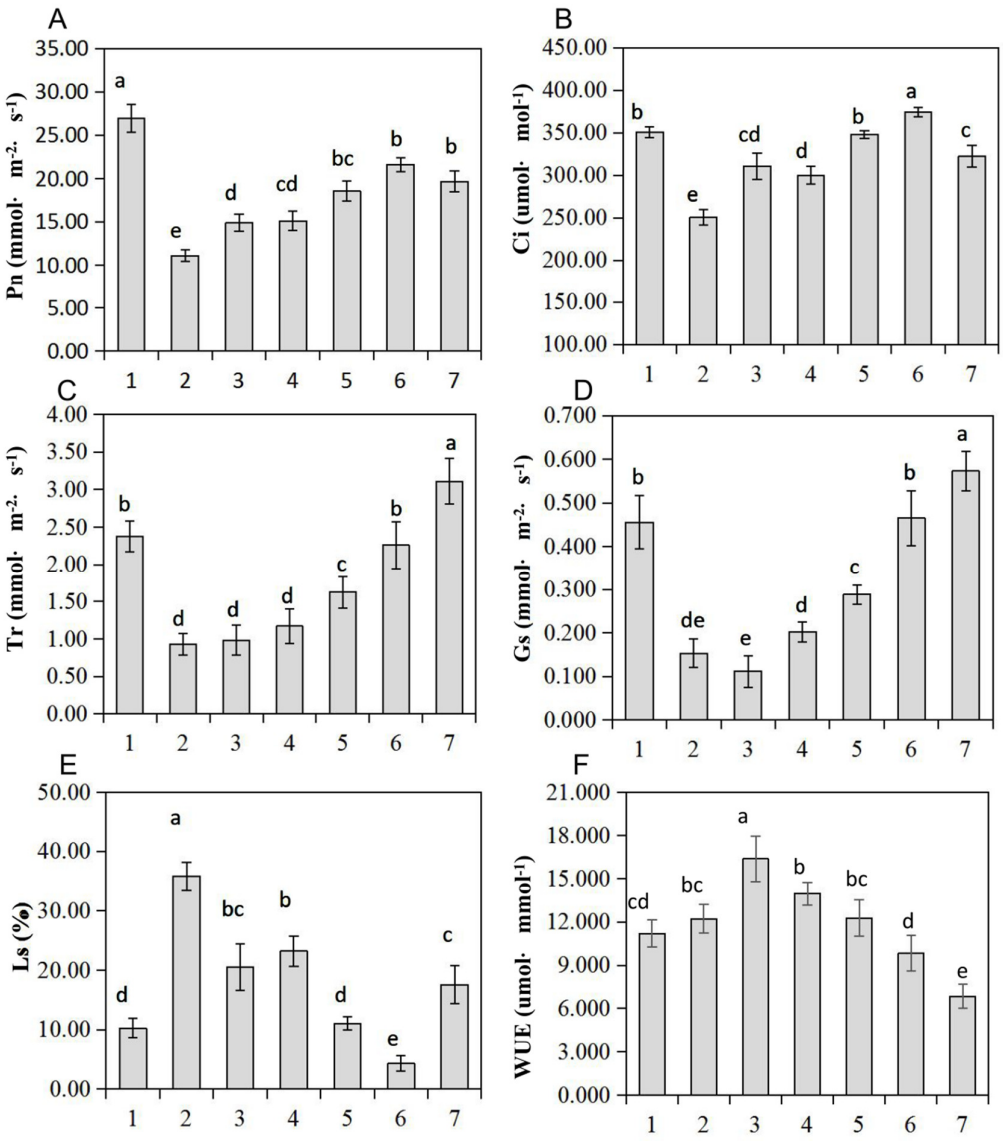


Figure 1. Effect of exogenous spermidine on gas exchange characteristics of rapeseed seedlings under NaCl stress. A, net photosynthesis rate (Pn); B, intercellular CO_2 concentration (Ci); C, transpiration rate (Tr); D, stomatal conductance (Gs); E, stomatal limitation (Ls) and F, water use efficiency (WUE)
Values are means ± standard error (n=6). 1: 0 mg·L^{-1} Spd / 0 mM NaCl; 2: 0 mg·L^{-1} Spd / 200mM NaCl; 3: 40 mg·L^{-1} Spd / 200 mM NaCl; 4: 80 mg·L^{-1} Spd / 200 mM NaCl; 5: 120 mg·L^{-1} Spd / 200 mM NaCl; 6: 160 mg·L^{-1} Spd /200 mM NaCl; 7: 200 mg·L^{-1} Spd / 200 mM NaCl. Bars followed by the same letter do not differ statistically at $p < 0.05$ (Duncan Multiple Range Test)

Chlorophyll fluorescence characteristics of B. napus seedlings under NaCl stress.

Fv/Fm is the maximum photochemical quantum yield of PSII, using for measuring the original light energy conversion efficiency of the PSII reaction center. With the increase of spermidine concentration, the Fv/Fm of 'Zheyou-18' leaves first increases and then decreases (Figure 2 A). The highest Fv/Fm, 0.8033, achieved when treatment with 120 mg L^{-1} Spd under NaCl stress, where the Fv/Fm decreases significantly compared to the control group.

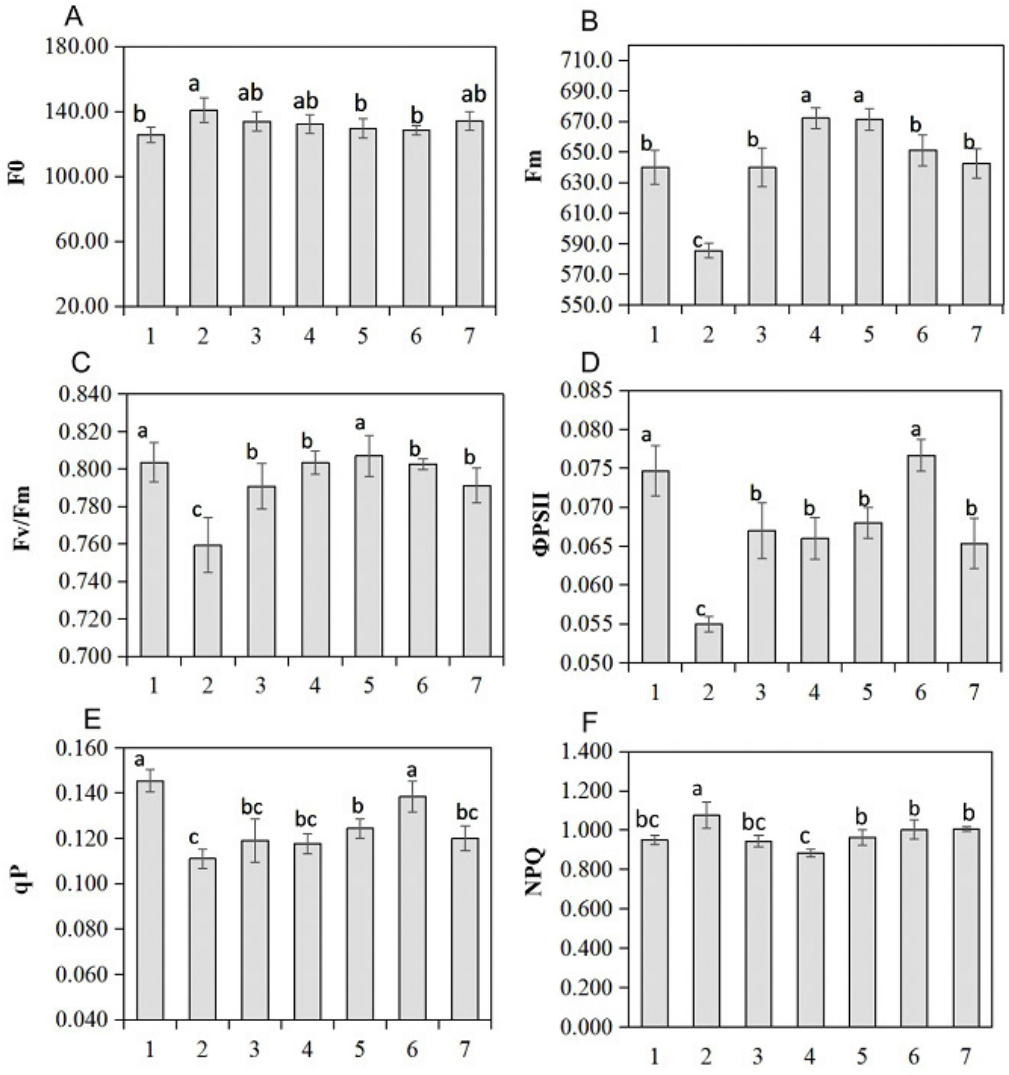


Figure 2. Effects of exogenous spermidine on chlorophyll fluorescence characteristics of rapeseed seedlings under NaCl stress. A. Initial Fluorescence (Fo), B, Maximum Fluorescence (Fm), C, maximum photochemical efficiency (Fv/Fm); D, the efficiency of excitation energy capture by open photosystem II efficiency (ΦPSII); E, the coefficient of photochemical quenching (qP) and F, the coefficient of non-photochemical quenching (NPQ)

1: 0 mg·L^{-1} Spd / 0 mM NaCl, 2: 0 mg·L^{-1} Spd / 200 mM NaCl, 3: 40 mg·L^{-1} Spd / 200 mM NaCl,4: 80 mg·L^{-1} Spd / 200 mM NaCl, 5: 120 mg·L^{-1} Spd / 200mM NaCl, 6: 160 mg·L^{-1} Spd /200 mM NaCl, 7: 200 mg·L^{-1} Spd / 200 mM NaCl. Values are means ± standard error (n = 6). Bars followed by the same letter do not differ statistically at $p < 0.05$ (Duncan Multiple Range Test)

ΦPSII is the actual photochemical quantum efficiency of PSII, reflecting the actual photochemical reaction efficiency of PSII reaction center accurately. With the increase of Spd concentration, 'Zheyou-18' ΦPSII first increases and then decreases (Figure 2 B). The highest ΦPSII, 0.077, achieved when treatment with 120 mg L^{-1} Spd.

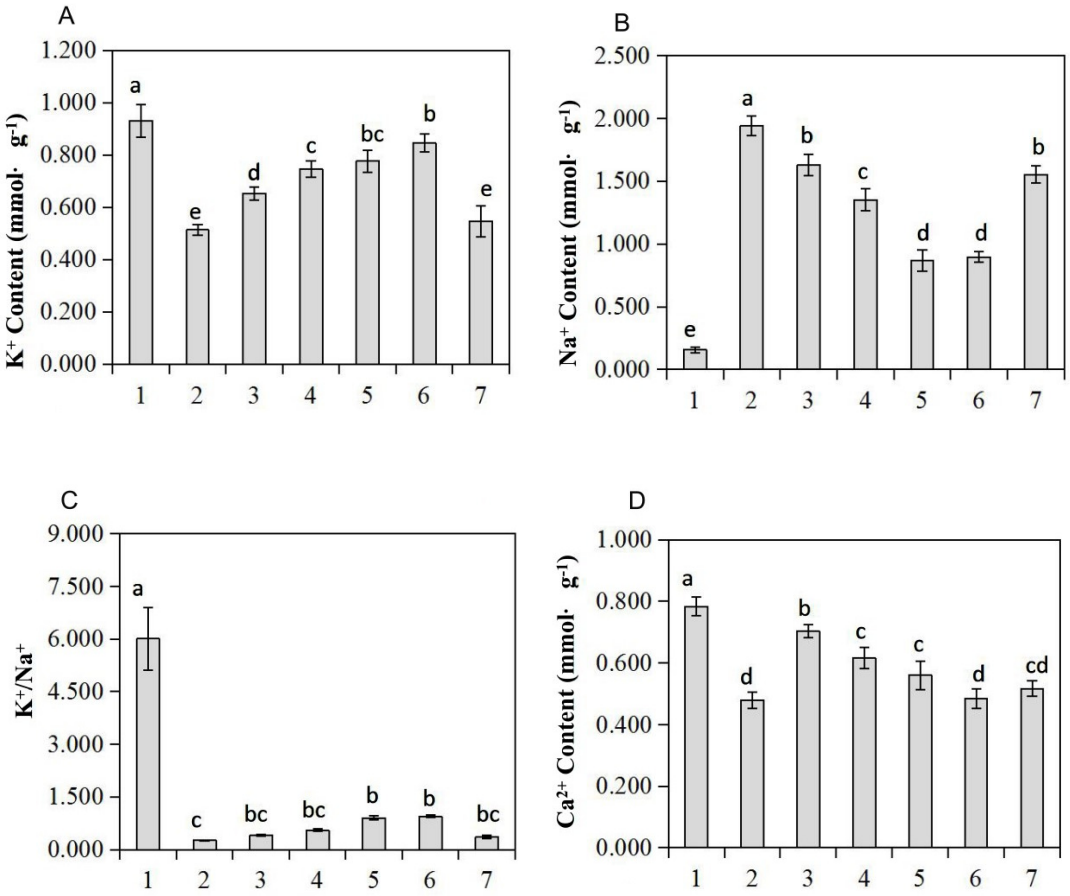
The photochemical quenching coefficient (qP) reflects the share of light energy absorbed by PSII natural pigments for photochemical electron transfer. The qP increases with increasing electron transfer activity of

PSII. For 'Zheyou-18', with the increase of Spd concentration, the qP first increases and then decreases (Figure 2 C). The highest qP, 0.138, achieved when treatment with 160 mg L^{-1} Spd under NaCl stress, where the qP decreases significantly compared to the control group.

Non-Photochemical Quenching (NPQ) reflects the part of light energy absorbed by the PSII antenna pigment and dissipated in the form of heat for not used for photochemical electron transfer. The increase in non-photochemical energy dissipation helps in dissipating excess excitation energy and preventing photosynthesis from excess excitation energy. For 'Zheyou-18', with the increase of Spd concentration, NPQ first decreases and then increases (Figure 2 D). The highest NPQ, 0.885, achieved when treatment with 80 mg L^{-1} Spd under NaCl stress, where the NPQ increases significantly compared to the control group.

The content of sodium, potassium, and calcium in Brassica napus seedlings under salt stress

NaCl stress significantly reduced the K^+ content in rapeseed seedlings ($p < 0.05$) (Figure 3 A). However, the K^+ content increased with exogenous application of Spd (≤ 160 mg·L^{-1}) compared with the only NaCl treated plants. Therefore, K^+ content in plants increased remarkably with the increase of Spd concentration under salt stress. But when the concentration of Spd reached 200 mg·L^{-1}, the content of K^+ decreased sharply. Content of Na^+ increased due to NaCl stress and application of Spd declined its accumulation significantly (Figure 3 B). Relative to the control, Na^+ increased by 12 times. However, the application of five concentrations of Spd reduced the Na^+ by 16.1%, 30.4%, 55.3%, 53.8%, and 19.9% over the only NaCl treated plants, respectively. Salt stress also could result in a significant reduction in the content of Ca^{2+} ($p < 0.05$), and 40-120 mg·L^{-1} concentrations of Spd significantly increased the calcium content in plants, except 160 and 200 mg·L^{-1} Spd treatments (Figure 3 D).



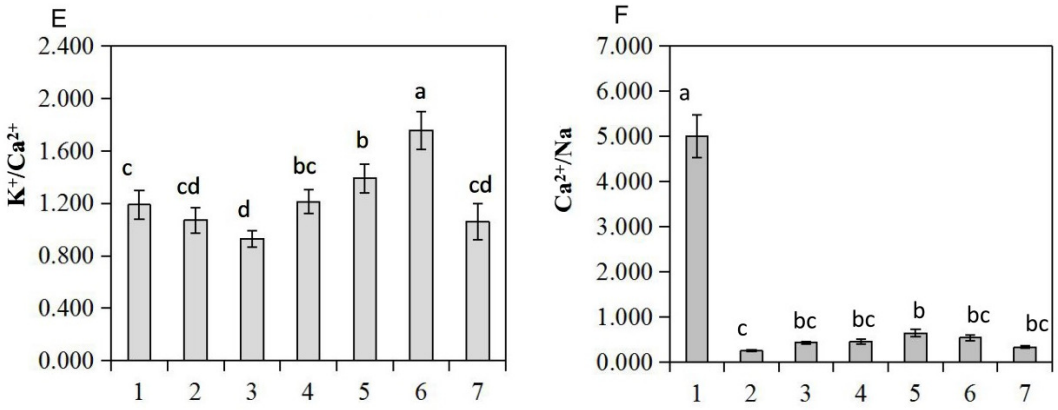


Figure 3. Effects of exogenous spermidine on the content of sodium, potassium and calcium, and the rotios of these inorganic metal ions in rapeseed seedlings under NaCl stress. A, potassium content; B, sodium content; C, the ratio of potassium and sodium; D, calcium content; E, the ratio of potassium and calcium, and F, the ratio of calcium and sodium

1: 0 mg·L^{-1} Spd / 0 mM NaCl; 2: 0 mg·L^{-1} Spd / 200 mM NaCl; 3: 40 mg·L^{-1} Spd / 200 mM NaCl; 4: 80 mg·L^{-1} Spd / 200 mM NaCl; 5: 120 mg·L^{-1} Spd / 200 mM NaCl; 6: 160 mg·L^{-1} Spd /200 mM NaCl; 7: 200 mg·L^{-1} Spd / 200 mM NaCl. Values are means ± standard error (n=6). Bars followed by the same letter do not differ statistically at $p < 0.05$ (Duncan Multiple Range Test)

Relative to the changes in Na^+, K^+ and Ca^{2+} contents, the ratio of $[K^+]/[Na^+]$ and $[Ca^{2+}]/[Na^+]$ were also increased in the seedlings by the treatment of Spd comparing with the same sections of NaCl treated plants (Figure 3 C, F). Moreover, there was no significant difference between the treatment of salt stress and the control in the ratio of $[K^+]/[Ca^{2+}]$, no increase with the application of low concentrations of Spd (40-80 mg·L^{-1}). The 120 and 160 mg·L^{-1} Spd treatments could significantly increased the ratio of $[K^+]/[Ca^{2+}]$ ($p < 0.05$) compared with the control and the only NaCl treated plants (Figure 3 E).

The MDA and reactive oxygen species production in Brassica napus seedlings under NaCl stress

NaCl stress increased oxidative parameters, such as H_2O_2 content, $O_2^{\cdot-}$, MDA content, and relative conductivity (Figure 4 A, B, C, and D). Relative to the control, NaCl treatment increased H_2O_2 content (59.09%), $O_2^{\cdot-}$ content (104.27%), MDA content (113.59%), and relative conductivity (128.17%). However, the application of exogenous Spd decreased these parameters remarkably with the maximal decline of 52.83%, 29.64%, 40.77%, and 34.61% in the content of H_2O_2, $O_2^{\cdot-}$, MDA, and relative conductivity respectively over the NaCl treated plants. Furthermore, multiple comparative analyses showed that the application of 160 mg·L^{-1} Spd significantly reduced the indexes, without H_2O_2, compared with the other Spd concentrations. Suggesting that 160 mg·L^{-1} Spd could markedly alleviate NaCl-induced damage effects of membrane and reactive oxygen species.

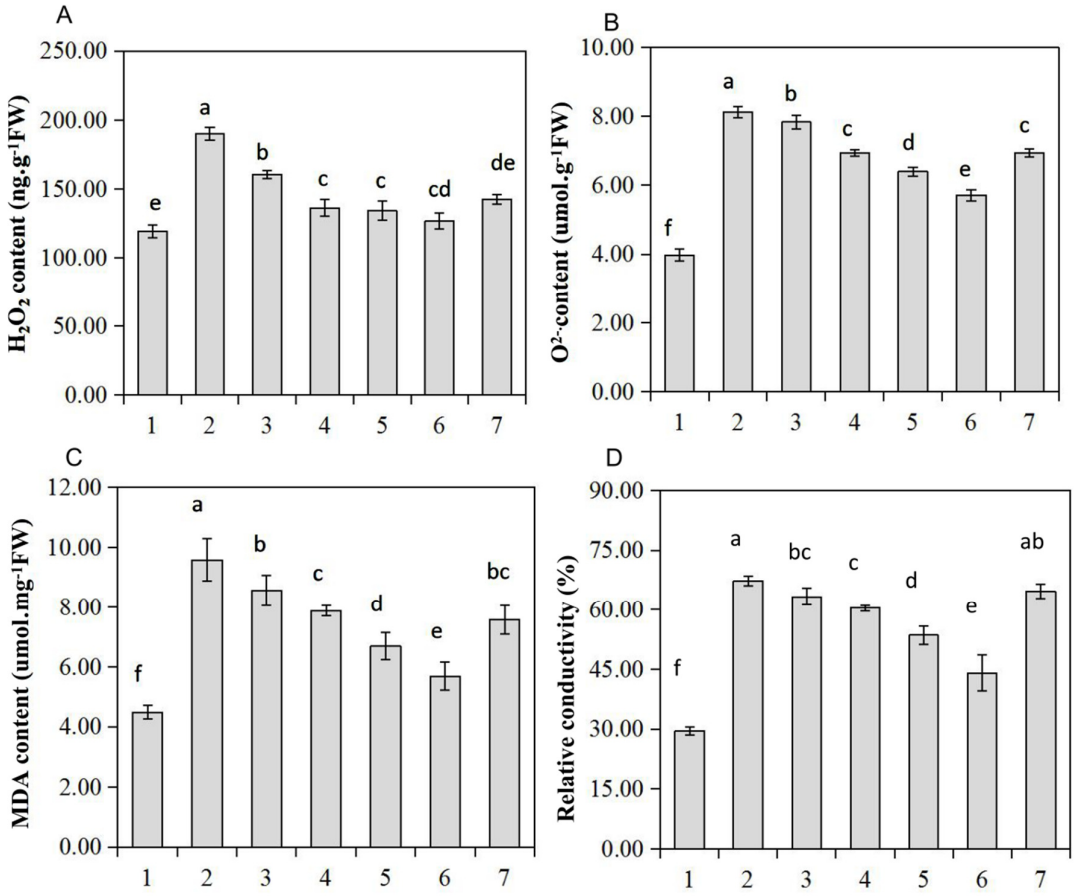


Figure 4. Effects of exogenous spermidine on membrane damage and reactive oxygen species production in rapeseed seedlings under NaCl stress. A, H_2O_2 content; B, $O_2^{\cdot-}$ content; C, MDA content, and D, relative conductivity

1: 0 mg·L^{-1} Spd / 0 mM NaCl; 2: 0 mg·L^{-1} Spd / 200mM NaCl; 3: 40 mg·L^{-1} Spd / 200 mM NaCl; 4: 80 mg·L^{-1} Spd / 200 mM NaCl; 5: 120 mg·L^{-1} Spd / 200 mM NaCl; 6: 160 mg·L^{-1} Spd /200 mM NaCl; 7: 200 mg·L^{-1} Spd / 200 mM NaCl. Values are means ± standard error (n = 6). Bars followed by the same letter do not differ statistically at $p < 0.05$ (Duncan Multiple Range Test)

Effect of exogenous Spd on activities of antioxidant enzymes, including SOD, POD, CAT, and APX, under salt stress in *Brassica napus* seedlings were examined. As shown in figure 5, there was a steep rise between the salt treatment and the control in activities of antioxidant enzymes, indicating the induction of the defense system under salt stress. Stressed plants treated with exogenous Spd application showed the highest activities of SOD, CAT, POD, and APX compared to the control samples, especially, 48.33% for SOD, 36.99% for POD, 20.8% for CAT, and 68.77% for APX (Figure 5). In addition, 160 mg·L^{-1} Spd addition combined with 200 mM NaCl treatment dramatically increased POD and APX activities (Figure 5, B and D). Statistical analysis revealed that Spd significantly affected the activities of the two enzymes under salt stress ($p < 0.05$) compared with the other treatments. Therefore, the prohibitive effect of Spd on activities of antioxidant enzymes under salt stress should be specially noted.

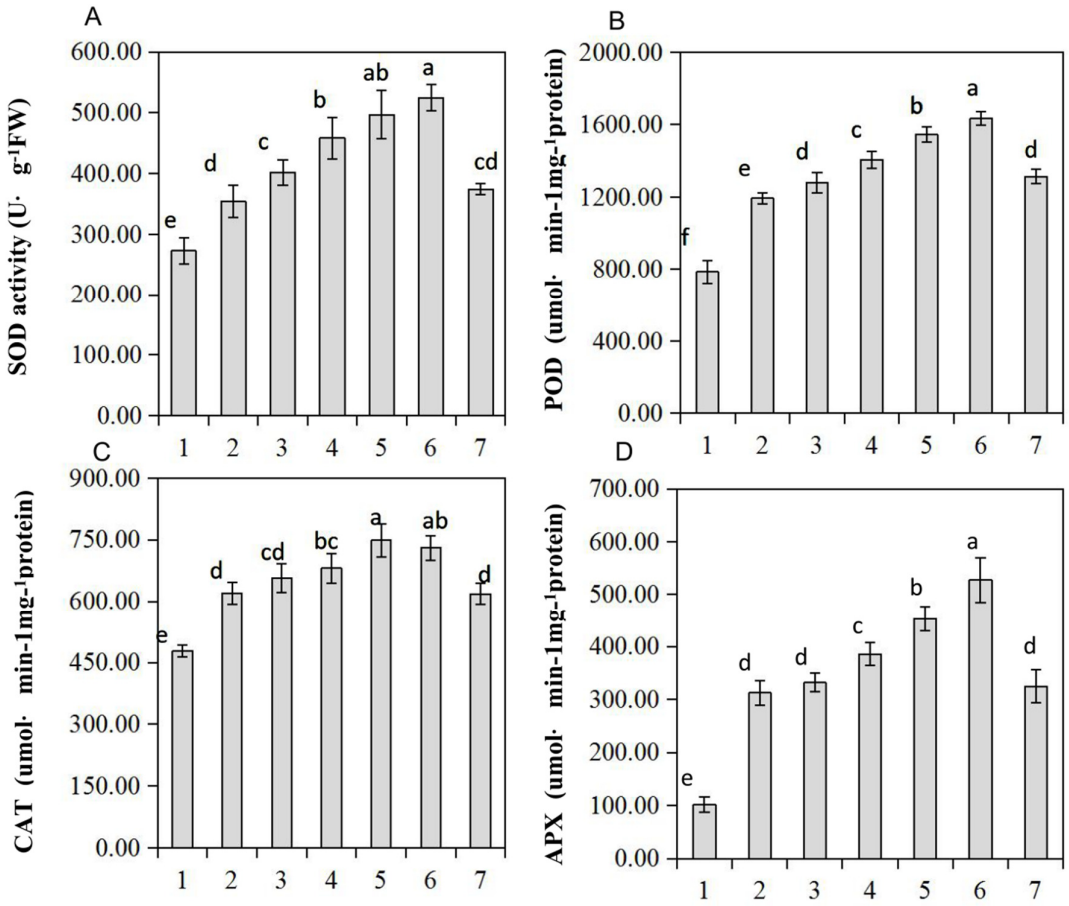


Figure 5. Effects of exogenous spermidine on antioxidant enzyme activities of rapeseed seedlings under NaCl stress. A, SOD activity; B, POD activity; C, CAT acitivity and D, APX activity
1: 0 mg·L^{-1} Spd / 0 mM NaCl; 2: 0 mg·L^{-1} Spd / 200mM NaCl; 3: 40 mg·L^{-1} Spd / 200 mM NaCl; 4: 80 mg·L^{-1} Spd / 200 mM NaCl; 5: 120 mg·L^{-1} Spd / 200 mM NaCl; 6: 160 mg·L^{-1} Spd /200 mM NaCl; 7: 200 mg·L^{-1} Spd / 200 mM NaCl. Values are means ± standard error (n = 6). Bars followed by the same letter do not differ statistically at $p < 0.05$ (Duncan Multiple Range Test); SOD: Superoxide dismutase, POD: Peroxidase, CAT: Catalase, APX: Ascorbate peroxidase

Discussion

Effect of spermidine on rapeseed photosynthesis under salt stress

Salinity stress causes disruption of ion homeostasis, damage to macromolecules and cellular structures, and limited photosynthesis and growth of plants (Duan *et al*., 2008). Previous studies have shown that the main effect of salt stress on plant photosynthesis is stomatal restriction (Morales *et al*., 1992; Lu *et al*., 2003). Our study revealed that, under salt stress, the net photosynthetic rate, stomatal conductance, and intercellular CO_2 concentration of rapeseed leaves decreased. This study also indicated that the decrease of rapeseed stomatal conductance may lead to a decrease in intercellular CO_2 concentration, and in turn reduces the rate of rapeseed CO_2 assimilation. Under exogenous Spd application, the net photosynthetic rate, stomatal conductance, and intercellular CO_2 concentration of rape leaves increase significantly with the increase of Spd concentration. This indicates that the application of Spd can significantly alleviate the inhibition of salt stress on the photosynthesis of rapeseed leaves.

The light energy absorbed by chloroplasts under normal conditions is mainly consumed through three ways: photosynthetic electron transfer, chlorophyll fluorescence, and heat dissipation. Previous studies have shown that salt stress destroyed the photosynthetic machinery of chloroplasts, damaged the PSII reaction center, inhibited the original reaction of photosynthesis, hindered the process of photosynthetic electron transfer, and significantly reduced the original light energy conversion efficiency of PSII (Morant-Manceau *et al*., 2004; Athar *et al*., 2015; Shin *et al*., 2020). Therefore, chlorophyll fluorescence is an important way to detect and analyze the photosynthetic function of plants, providing various parameters for the photosystem and the electron transfer process study. It is an ideal indicator for studying the physiological status of plant photosynthesis, and the relationship between plants and adversity stress (Athar *et al*., 2015). In this study, the effect of salt stress on the chlorophyll fluorescence of rape is represented by the decrease of Fv/Fm, qP, ΦPSII, and the increase of NPQ. The decrease of Fv/Fm indicates that PSII was damaged and the plant was photo-inhibited to a certain extent. The decrease of qP indicates that the electron transfer from the oxidation side of PSII to the PSII reaction center was inhibited. The sharp decrease of ΦPSII indicates that ATP and NADPH are equivalent. The formation of force was hindered, the net photosynthetic rate of plants decreased, and the NPQ was increased. These results indicate that plants can consume excessive excitation energy and reduce damage to the stress environment by increasing heat dissipation through non-photochemical quenching and then protect themselves. Exogenous Spd treatment increased Fv/Fm, ΦPSII, qP and decreased NPQ values of rapeseed seedlings. This indicates that exogenous Spd can effectively alleviate the growth inhibition induced by salt stress. Especially, the light energy utilization rate of rapeseed was enhanced, the excess light energy was dissipated promptly, and then PSII Photochemical activity was improved lead to the smooth progress of photosynthesis. These results indicate that Spd can ameliorate and protect the integrity of PSII from salinity-induced damage, corroborating the findings of previous studies (Sheng, 2012).

Exogenous Spd alleviated the salt damage of rapeseed by adjusting ion balance

Salt stress leads to nutrient imbalance in plants, mainly due to the excessive accumulation of Na+. The accumulation causes ion poisoning and the deficiency of other mineral nutrients, such as K^+ and Ca^{2+}(Zhao, 2020; Zhu, 2003). The balance of Na^+/K^+ and Na^+/Ca^{2+} concentration in cells is the key to ensure normal physiological metabolism of plants under salt stress (Maathuis and Amtmann, 1999; Yang and Guo, 2018). K^+ plays an important role in regulating osmotic pressure and acid-base balance in plants, and high K^+ content in vacuoles maintains higher enzyme activity to resist salt stress (Zhang and Shi, 2013). Ca^{2+} plays an essential regulatory role in cells, especially under stress conditions, it can participate as signal substance response transduction of salt stress signals (Zeng *et al*., 2017). Plants reduce the intracellular Na+ concentration by efflux or compartmentalization of Na^+ and establish new ion homeostasis adapting to the high-salt environment (Yong *et al*., 2015; Yang and Guo, 2018). In the present study, salt stress significantly increased the accumulation of Na^+ and reduced the accumulation of K^+ and Ca^{2+} in the seedlings of rapeseed. However, the application of Spd exogenously (≤ 160 mg·L^{-1}) leads to increasing K^+ and Ca^{2+} and reducing Na+ in the seedlings of rapeseed. Under salt stress, K^+ content in plants increased remarkably with increasing Spd concentration. It should be noticed that the alleviation for ion balance in rapeseed is less productive when the Spd concentration higher than 200 mg L^{-1}, indicating that the effect depends on the applied concentration of Spd under salt damage in rapeseed.

Exogenous Spd improves salt tolerance of rapeseed by scavenging active oxygen

Reactive oxygen species (ROS) is a strong oxidant, which can cause cell plasma membrane damage, irreversible metabolic disorders, and cell death (Ashraf, 2004; Mittler *et al*., 2004). Besides acting as a toxic molecule, ROS is also a signalling molecule that regulates many important biological processes, such as growth, development, and response to abiotic and biotic stress (Purty *et al*., 2008; Miller *et al*., 2010). The active oxygen metabolism system was disordered without maintaining the original equilibrium state under salt stress (Zhang

et al., 2016). Subsequently, a large number of active oxygen accumulates with higher active oxygen scavenging system activity. Likewise, the antioxidant defence system works and controls the content of active oxygen. Then, the active oxygen exerts a positive effect, and the damage caused by active oxygen was prevented or reduced. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) are the main protective enzymes in the enzymatic defence system (Zhang *et al*., 2016). In this study, salinity stress significantly increased oxidative parameters, such as H_2O_2, O_2^-, MDA content, and relative conductivity. However, the H_2O_2, O_2^-, and MDA content decreased remarkably with exogenous application of Spd. The activities of antioxidant enzymes, including SOD, POD, CAT, and APX increased significantly with the application of exogenous Spd. The result indicates that exogenous application of Spd can reduce the level of active oxygen by increasing the activity of antioxidant enzymes and increase the salt tolerance of rapeseed.

Physiological system of enhancing salt tolerance of rapeseed by exogenous application of Spd

Based on our findings, we present a mechanistically integrated model (Figure 6) where salt stress triggers three fundamental perturbations in rapeseed: (1) osmotic imbalance causing cellular dehydration, stomatal restriction, and photosynthetic inhibition; (2) ionic dysregulation through Na^+-mediated suppression of K^+/Ca^{2+} homeostasis; and (3) oxidative damage from accumulated ROS. Our data demonstrate that exogenous spermidine (Spd) coordinates multi-level protection by simultaneously stabilizing cellular water status and CO_2 assimilation, restoring $Na^+/K^+/Ca^{2+}$ equilibrium via selective ion transport modulation, and activating the SOD-CAT-APX enzymatic antioxidant system. This is consistent with the findings of Alharbi *et al.*(2025). Although this framework successfully explains the observed physiological responses, we emphasize that the cellular signaling or gene regulatory mechanisms underlying Spd's modulatory functions remain to be elucidated.

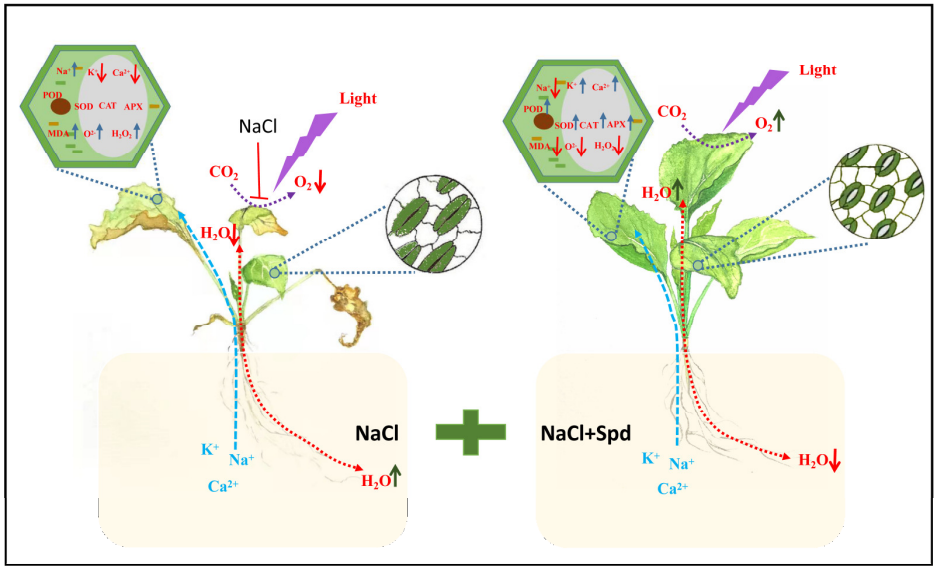


Figure 6. Schematic presentation of exogenous application of spermidine to improve salt tolerance in rapeseed

Conclusion

Exogenous spermidine (Spd) application improves salt tolerance in rapeseed under NaCl stress. The treatment with 160 mg·L^{-1} Spd alleviated growth inhibition, enhanced photosynthetic efficiency, and increased antioxidant enzyme activity. Additionally, Spd reduced Na+ absorption while promoting the uptake of K^+ and Ca^{2+}. These findings suggest that Spd can effectively mitigate salt stress in rapeseed by improving photosynthesis, ion balance, and reducing oxidative damage.

Authors' Contributions

Conceived and designed the experiments: TX, HW and CZ. Performed the experiments and analyzed the data: TX, HW and XW. Data acquisition: JC, YY and LL. Wrote the paper: TX. Contributed to writing the manuscript: HW and XD; Revised the manuscript: CZ and YD.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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