EFFECTS OF EXOGENOUS ABSCISIC ACID ON SALT TOLERANCE OF WATERMELON SEEDLINGS UNDER NaCl STRESS

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Abstract. Effects of exogenous abscisic acid (ABA) treatment on growth, chlorophyll fluorescence parameters, and antioxidant enzyme activities of watermelon seedlings under NaCl stress were investigated in this study to explore physiological mechanism of ABA-induced salt tolerance response of watermelon. Results showed that NaCl stress significantly reduced the growth and photosynthetic efficiency of watermelon seedlings and affected activities of antioxidant enzymes. ABA treatment at a certain concentration significantly reduced salt injury index and increased fresh and dry weights of watermelon seedlings under NaCl stress. The maximum photosynthetic efficiency, actual photosynthetic efficiency, relative electron transport rate, and photochemical quenching of photosystem II of watermelon seedlings under NaCl stress increased first and then decreased with the increase of ABA concentration, while non-photochemical quenching, quantum yield of regulated energy dissipation, and quantum yield of non-regulated energy dissipation showed the opposite trend. ABA treatment increased activities of superoxide dismutase, catalase, and peroxidase in plants and reduced the content of malondialdehyde. Treatment with ABA concentrations of 1 and 5 mg·L^{-1} demonstrated an enhanced effect that can improve salt tolerance and effectively alleviate injury of watermelon seedlings under NaCl stress.

Keywords: *ABA, salt stress, salt tolerance, salt injury, photosynthesis*

Introduction

Soil salinization is a global problem that affects 20% of arable land and 33% of irrigated agricultural land worldwide and severely reduces crop yields (Wu et al., 2021). Watermelon (*Citrullus lanatus* (Thunb.) Matsum. et Nakai), a widely cultivated annual trailing herb from the gourd family, is used as a fruit for consumption and demonstrates high edibility and medicinal value. Secondary salinization of soil in facilities has occurred in recent years due to irrigation methods, fertilization, soil texture, groundwater level, and other factors (Huan et al., 2007). The secondary salinization of soil has seriously affected the yield and quality of watermelon and hindered the sustainable development of watermelon facility cultivation. Therefore, investigating physiological and biochemical changes of watermelon under salt stress as well as methods for improving the salt tolerance of plants is urgently necessary.

Abscisic acid (ABA) is a key plant hormone produced in response to abiotic stress conditions and an activator and regulator of abiotic stress resistance mechanism in plants (Hauser et al., 2017). Accumulation of ABA increases significantly under drought, cold, and salt stress (Jiang and Zhang, 2004). Drought resistance mechanism of *Camellia oleifera* was controlled by maintaining the growth of root system to obtain the required water, increasing contents of osmotic substances in leaves to maintain water holding capacity, and reducing water transpiration by increasing ABA and other hormone contents in leaves (He et al., 2020). Spraying ABA regulated the osmotic protection and antioxidant mechanisms of grape leaves and promoted metabolic response under water stress (Pontin et al., 2021). Exogenous ABA improved cold tolerance of wheat, and the ultrastructure of chloroplasts also changed evidently to maintain the photosynthetic activity (Venzhik et al., 2016). Alfalfa seedlings were stressed with alkaline solution after pretreatment with ABA. Compared with the control, ABA pretreatment significantly reduced leaf injury and increased fresh weight, water content, and survival rate of alfalfa seedlings under alkaline condition (Wei et al., 2021). A long-term study on salt tolerance induced by ABA revealed the mechanism of ABA regulation at the molecular level and demonstrated that salt stress symptoms of *Vicia faba* could be alleviated by continuously changing transcription pattern of key genes and improving photosynthesis (Sagervanshi et al., 2021). A study on the effect of ABA on *Toona sinensis* seedlings under salt stress showed that ABA participated in signal transduction under NaCl stress, promoted synthesis of related proteins, maintained integrity of cell membrane structure, alleviated osmotic and ion stresses caused by excessive salt in plants, and maintained water balance of plants (Yao et al., 2020). However, effects of ABA treatment on photosynthetic parameters and antioxidant enzyme activities of watermelon seedlings remain to be elucidated.

Watermelon was used as the research material in this work to explore effects of different concentrations of exogenous ABA treatment on the growth and physiological indicators of watermelon seedlings under NaCl stress and obtain the appropriate ABA concentration. This study aims to provide a theoretical basis for reasonably solving the problem of salt injury in watermelon production.

Materials and Methods

Test materials

Seeds of watermelon cultivar ‘Zaojia’ were provided by New Century Agricultural High-technology Development Center of Changji, Xinjiang, China. ABA was purchased from Beijing Solarbio Science & Technology Co., Ltd., China.

Sowing

Seeds were first disinfected with 1% methanal solution for 30 min and then rinsed several times with sterile distilled water. Seeds were then incubated at 30℃ for germination after soaking for 6 h. Peat, vermiculite, and perlite were mixed at a volume ratio of 2:1:1 and then placed into plastic pots (10 cm×10 cm). Seeds were germinated and then sown into a substrate. The plastic pots were placed in a controlled growth chamber under conditions of 28℃/22℃ (day/night), relative humidity of 75% ± 5%, and photoperiods of 12 h (PAR 200 $\mu mol\cdot m^{-2}\cdot s^{-1}$) after sowing.

Experimental treatments

Watermelon seedlings with emergence of two true leaves were sprayed with different concentrations of exogenous ABA. Seedlings were irrigated with 1/2 strength Hoagland’s nutrient solution containing 180 mM of NaCl (50 mL per plant) after 1 day and then every day onward. The optimal concentration of NaCl stress was determined to be 180 mM from a pre-experiment. Another treatment without NaCl stress and ABA spraying was set as the control. Thirty plants were used for each treatment. The six treatments are listed below.

Control: 0 mg·L^{-1} ABA + 0 mM NaCl
ABA0: 0 mg·L^{-1} ABA + 180 mM NaCl
ABA1: 1 mg·L^{-1} ABA + 180 mM NaCl
ABA5: 5 mg·L^{-1} ABA + 180 mM NaCl
ABA25: 25 mg·L^{-1} ABA + 180 mM NaCl
ABA50: 50 mg·L^{-1} ABA + 180 mM NaCl

Determination of indicators

Second leaves from the bottom of four randomly selected seedlings in each treatment were used for measurement after 6 days of NaCl stress. Chlorophyll fluorescence parameters, including maximum photosynthetic efficiency (Fv/Fm), actual photosynthetic efficiency (Y(II)), relative electron transport rate (rETR), photochemical quenching (qL), non-photochemical quenching (NPQ), quantum yield of regulated energy dissipation (Y(NPQ)), and quantum yield of non-regulated energy dissipation (Y(NO)) of photosystem II (PSII), were measured using a portable modulated chlorophyll fluorimeter (PAM-2500, Walz, Germany) after 30 min of dark adaptation.

Second leaves of randomly selected seedlings were harvested from each treatment after 6 days of NaCl stress. Superoxide dismutase (SOD) activity was measured by the method of Giannapoliti and Ries (1977); catalase (CAT) activity was determined according to Aebi’s assay (1984); peroxidase (POD) activity was measured using a procedure described by Li and Yi (2012); the content of malondialdehyde (MDA) was assayed as described by Hodges et al. (1999). Measurement of these indicators was performed in three biological replicates using two plants per replicate.

Symptoms of leaf injury in each treatment were investigated and recorded and the salt injury index was calculated according to Zhen et al. (2010) after 10 days of NaCl stress. Meanwhile, 10 plants were randomly selected from each treatment for fresh and dry weight measurements.

Statistical analysis

The experimental data were statistically analyzed using two-way ANOVA through the SPSS software, version 27 (IBM SPSS, Chicago, USA). Duncan multiple range test was used to compare differences among different treatments.

Results

Effects of exogenous ABA on salt injury index and fresh and dry weights of watermelon seedlings under NaCl stress

The investigation and comparison of the salt injury index of watermelon seedlings pretreated with exogenous ABA under NaCl stress (*Figure 1*) revealed that the certain concentrations (1 and 5 mg·L^{-1}) of ABA treatment alleviated symptoms of salt injury and significantly reduced the salt injury index, which decreased by 26.8% and 19.6% compared with that at a concentration of 0 mg·L^{-1}, whereas an excessive concentration led to the aggravation of the degree of injury (*Figure 2A*). Data in *Figure 2B* showed a significant decrease in fresh and dry weights of watermelon seedlings under NaCl stress compared with the control, indicating that seedling growth was evidently inhibited. However, the inhibition effect was alleviated with a certain concentration of ABA. The fresh weight of seedlings treated with 1 mg·L^{-1} of ABA was significantly higher than that of seedlings treated with 0, 25, and 50 mg·L^{-1} of ABA but showed no significant differences compared with that of seedlings treated with 5 mg·L^{-1} of ABA. ABA treatment at a concentration of 1 mg·L^{-1} was significantly higher than that of 0 and 50 mg·L^{-1} while no significant differences were observed between the treatment of 1, 5, and 25 mg·L^{-1} for the dry weight of seedlings under NaCl stress. Overall, fresh and dry weights of watermelon seedlings under NaCl stress increased first and then decreased with increasing ABA treatment concentration.

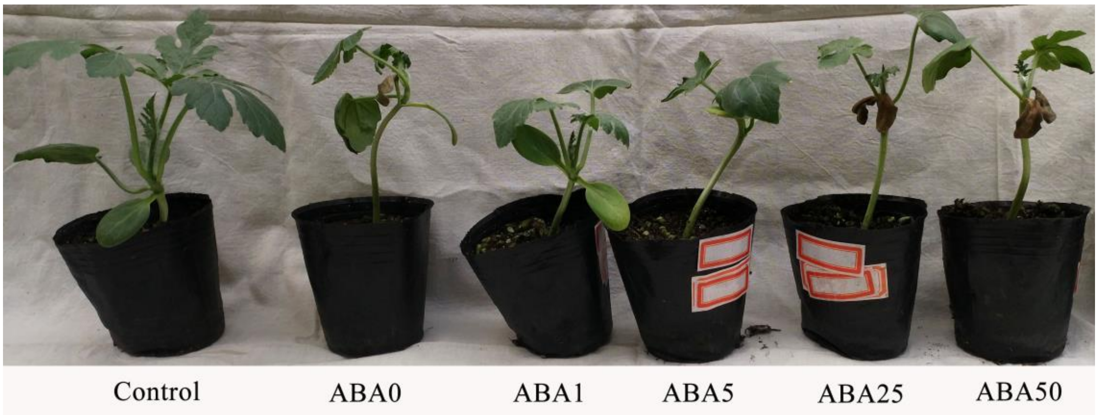

Figure 1. *Effect of ABA on watermelon seedling growth under NaCl stress. Control: seedlings grown in substrate under normal conditions; ABA0: seedlings grown in substrate with 180 mM NaCl; ABA1: seedlings pretreated with 1 mg·L^{-1} ABA, grown in substrate with 180 mM NaCl; ABA5: seedlings pretreated with 5 mg·L^{-1} ABA, grown in substrate with 180 mM NaCl; ABA25: seedlings pretreated with 25 mg·L^{-1} ABA, grown in substrate with 180 mM NaCl; ABA50: seedlings pretreated with 50 mg·L^{-1} ABA, grown in substrate with 180 mM NaCl. The photograph was taken after 10 days of NaCl stress*

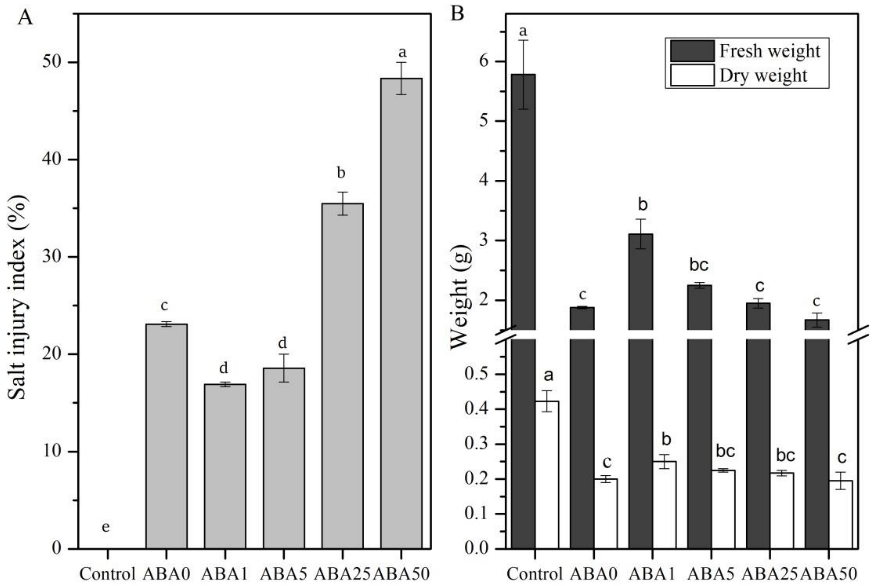

Figure 2. *Effects of exogenous ABA on salt injury index and fresh and dry weights of watermelon seedlings under NaCl stress. (A) Salt injury index; (B) Fresh weight and dry weight. Bars represent mean ± standard error of mean and those labeled with different lowercase letters are significantly different ($p < 0.05$)*

Effects of exogenous ABA on chlorophyll fluorescence parameters of watermelon seedlings under NaCl stress

As shown in *Figure 3,* significant differences existed in chlorophyll fluorescence parameters of watermelon seedlings due to NaCl stress and exogenous ABA treatments. *F*v/*F*m, Y(II), rETR, and qL of watermelon seedlings were significantly lower than those of the control while NPQ, Y(NPQ), and Y(NO) were significantly higher than those of the control under NaCl stress alone. *F*v/*F*m, Y(II), rETR, and qL values increased initially and then decreased with the increase of ABA concentration. *F*v/*F*m was higher when the exogenous ABA concentration was 1 mg·L^{-1}, while Y(II), rETR, and qL were higher when the exogenous ABA concentration was 25 mg·L^{-1}. NPQ, Y(NPQ), and Y(NO) first decreased and then increased with the increase of exogenous ABA concentration. Y(NO) of ABA treatments at low and medium concentrations was significantly lower than that of the non-ABA treatment.

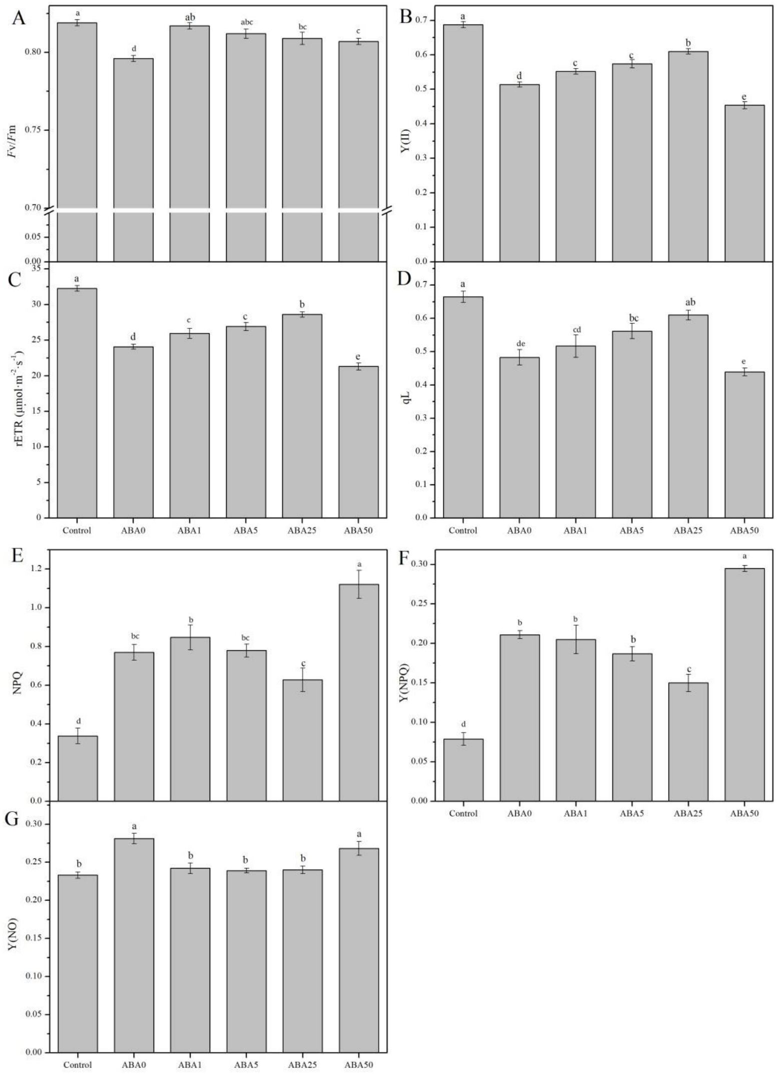

Figure 3. *Effect of exogenous ABA on chlorophyll fluorescence parameters of watermelon seedlings under NaCl stress. (A) Fv/Fm; (B) Y(II); (C) rETR; (D) qL; (E) NPQ; (F) Y(NPQ); (G) Y(NO). Bars represent mean ± standard error of mean and those labeled with different lowercase letters are significantly different ($p < 0.05$)*

Effects of exogenous ABA on antioxidant enzyme activities and MDA content of watermelon seedlings under NaCl stress

Figure 4 shows that NaCl stress and exogenous ABA treatment exerted significant effects on antioxidant enzyme activities and MDA content of watermelon seedlings. SOD activity, POD activity, and MDA content of watermelon seedlings were significantly higher than those of the control under NaCl stress. SOD, CAT, and POD activities first increased and then decreased with the increase of ABA treatment concentration. SOD and CAT activities when seedlings were treated with 5 mg·L^{-1} of ABA were significantly higher than those of seedlings treated with 0 mg·L^{-1} of ABA. POD activity was higher in 25 mg·L^{-1} of ABA treatment than that in 0 mg·L^{-1} of ABA treatment. The results showed that activities of antioxidant enzymes can improve via exogenous ABA. By contrast, the content of MDA decreased first and then increased with the increase of ABA treatment concentration and reached a low value when the ABA treatment concentration was 1 mg·L^{-1}, which indicated that a certain concentration of ABA treatment can reduce the MDA content.

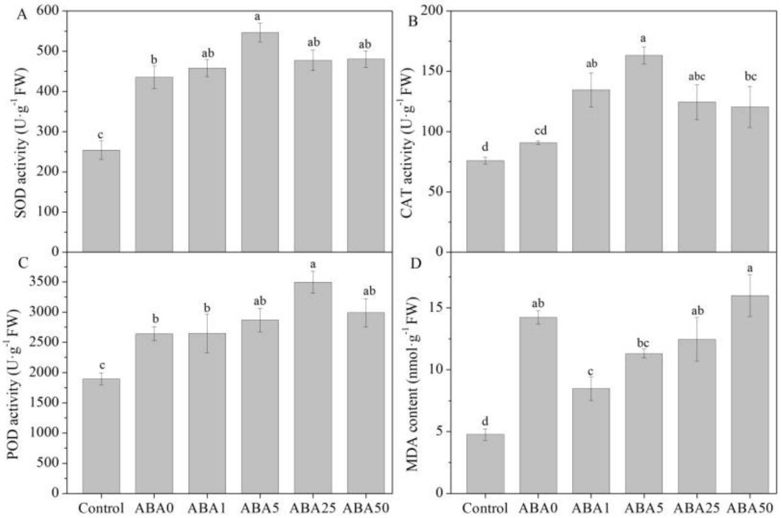

Figure 4. *Effects of exogenous ABA on antioxidant enzyme activities and MDA content of watermelon seedlings under NaCl stress. (A) SOD activity; (B) CAT activity; (C) POD activity; (D) MDA content. Bars represent mean ± standard error of mean and those labeled with different lowercase letters are significantly different ($p < 0.05$)*

Discussion

Salt stress is considered an obstruction of nonhalophytic plants that results in physiological drought of plants, reduces enzyme activity, changes membrane permeability, and affects the normal growth of plants (Hu et al., 2018). For example, germination of cottonseed was inhibited (Chen et al., 2021) and dry weight of tomato seedlings was significantly reduced under NaCl stress (Hu et al., 2021). Salt stress leads to the inhibition of absorption and transport of plant nutrients and results in the imbalance of mineral nutrients (Farissi et al., 2014). The low water potential in soil due to salt stress reduces not only the water potential (Flowers and Colmer, 2010) and stomatal conductance of leaves (Rahnama et al., 2010) but also the content of CO_2 entering stomata while inhibiting photosynthesis, thereby reducing carbon fixation and biomass accumulation (Munns, 2010; Setia et al., 2013). Watermelon seedlings in this experiment showed evident symptoms of salt injury and fresh and dry weights significantly reduced under NaCl stress. Low concentrations of ABA treatments reduced the salt injury index and increased fresh and dry weights of seedlings, thereby indicating enhanced growth compared with those under NaCl stress alone, which demonstrated that a certain concentration of ABA treatment can improve salt tolerance and alleviate the inhibition of salt stress on the growth of watermelon seedlings. However, an excessively high concentration of ABA exerted a negative effect on plant growth. This finding is similar to the results obtained in the experiment where ABA was applied to alleviate the toxicity of NaCl to grape plants. The weight, leaf number, leaf area, and bud dry weight of grape plants under NaCl stress were lower than those without NaCl stress. However, these indicators increased with an ABA treatment of 100 μM (Karimi et al., 2021). The study of physiological characteristics of Tartary buckwheat under salt stress demonstrated that applying an appropriate amount of ABA can improve seedling fresh weight and root activity (Lu et al., 2021). Similar results were also obtained for tomato (Martínez-Andújar et al., 2021; Xue et al., 2021).

Chlorophyll fluorescence kinetics is a fast, sensitive, and non-invasive approach often used to explore and evaluate the effect of environmental stress on the photosynthetic performance of plants (Krause and Weis, 1984; Baker, 2008). Each chlorophyll fluorescence parameter demonstrates a certain biological meaning. *F*v/*F*m and Y(II) represent the maximum and actual photosynthetic quantum yield of PSII, respectively; qL represents the proportion of light energy absorbed by PSII for photochemistry; rETR represents the relative electron transport rate; NPQ reflects the proportion of dissipation of excess light energy by heat emission; and Y(NPQ) and Y(NO) reflect the part of energy that is dissipated in the form of heat via the regulated mechanism and passively dissipated in the form of heat and fluorescence, respectively (Maxwell and Johnson, 2000; Klughammer and Schreiber, 2008; Kalaji et al., 2014). Values of *F*v/*F*m, Y(II), qL, and rETR of watermelon seedlings under NaCl stress were significantly lower than those of the control in this experiment. This finding suggested that PSII of leaves was damaged to a certain extent. However, the significant increase of chlorophyll fluorescence parameters after ABA treatment compared with those without ABA treatment suggested that a suitable concentration of ABA can enhance the photochemical efficiency of leaves. Small values of NPQ, Y(NPQ), and Y(NO) under a certain concentration of ABA treatment suggested low heat dissipation and high light energy conversion efficiency. These results indicated that ABA treatment can improve the photosynthetic efficiency to a certain extent and protect the photosynthesis of leaves, thereby alleviating the injury caused by excess light energy to photosynthetic apparatus of watermelon seedlings under NaCl stress.

SOD, CAT, and POD are key enzymes in the antioxidant defense system of scavenging reactive oxygen species in plants. The increase of activities of these enzymes indicates that the ability of scavenging reactive oxygen enhances and the membrane lipid peroxidation reduces to maintain the normal metabolism of cells (Moradi and Ismail, 2007). Activities of SOD, CAT, and POD of watermelon seedlings under NaCl stress increased and reflected the stress response of plants themselves in this study. The ABA treatment significantly increased the activities of SOD, CAT, and POD in watermelon seedlings under NaCl stress and effects increased first and then decreased with the increase of ABA concentration. MDA is the end product of membrane lipid peroxidation and its content is an important indicator of the degree of membrane lipid peroxidation directly related to the injury degree of cell membranes (Yang et al., 2012). In this experiment, MDA content in the leaves of watermelon seedlings was significantly elevated by NaCl stress compared with the control. This result suggested that NaCl stress caused injury to the cell membrane system. However, ABA protected the integrity of cell membranes to some extent by reducing membrane lipid peroxidation, inhibiting the accumulation of MDA, and thus effectively alleviating the injury of NaCl stress on watermelon seedlings.

Conclusions

ABA treatment within a certain concentration range can improve the salt tolerance in watermelon seedlings by increasing biomass of plants, exerting a beneficial effect on photosynthetic efficiency of leaves to a certain extent, enhancing activities of SOD, CAT, and POD, and reducing the content of MDA in plants, and thus effectively alleviate the injury of NaCl stress on watermelon seedlings. ABA treatment of 1 and 5 mg·L^{-1} exerted acceptable effects on alleviating the injury caused by NaCl stress; however, effects were very poor when the ABA concentration was increased to 50 mg·L^{-1}. This finding indicated that low ABA concentrations exert alleviating effects whereas high ABA concentrations exert negative effects. ABA has protective effect on plants depending on the treatment concentration.

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APPENDIX

Two-way ANOVA (related to Figure 2)

Factors	**P value (salt injury index)**	**P value (Fresh weight)**	**P value (dry weight)**
NaCl	0.000	0.000	0.000
ABA	0.000	0.077	0.556

Two-way ANOVA (related to Figure 3)

Factors	**P value (*F*v/*F*m)**	**P value (Y(II))**	**P value (rETR)**	**P value (qL)**	**P value (NPQ)**	**P value (Y(NPQ))**	**P value (Y(NO))**
NaCl	0.000	0.000	0.000	0.000	0.000	0.000	0.000
ABA	0.002	0.000	0.000	0.001	0.000	0.000	0.001

Two-way ANOVA (related to Figure 4)

Factors	**P value (SOD)**	**P value (CAT)**	**P value (POD)**	**P value (MDA)**
NaCl	0.002	0.388	0.042	0.000
ABA	0.114	0.036	0.117	0.012