



Full Length Article

Salinity Induced Cross Tolerance to Drought in Cotton Seedlings is Associated with Reduced Lipid Peroxidation and Photoprotection through Activation of Antioxidant System

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Abstract

To evaluate the effects of soil salinity on the drought tolerance of cotton, the influence of NaCl on the biomass, photosynthesis and lipid peroxidation in the leaves of cotton seedlings were studied in pot-culture under individual drought (D), salinity (S), combined drought and salinity stress (D+S), and control. The results showed that D or S reduced the shoot biomass in cotton seedlings, but compared to D, D+S caused relatively higher biomass ($p < 0.01$). The leaf relative water content and net photosynthetic rate of D+S were significantly higher than D ($p < 0.05$). Membrane lipid peroxidation and the damage to the photosynthetic apparatus evaluated by net photosynthetic rate, intercellular CO₂ concentration, and chlorophyll fluorescence were more severe in D than in D+S. The activities of antioxidant enzymes, SOD, POD, and CAT, increased due to diverse stresses. Particularly under the severe treatment D, the effect on antioxidant enzyme activities was distinct, while the presence of salinity stress could relatively relieve this notable affect induced by individual drought stress. This suggests that, compared to the cotton seedlings under D, the seedlings subjected to D+S could maintain higher leaf water content, alleviate membrane lipid peroxidation, improve photosynthetic properties and increase biomass accumulation under drought conditions. © 2019 Friends Science Publishers

Keywords: Salinity stress; Drought; Photosynthesis; Lipid peroxidation; Plant growth

Introduction

Drought and salinity stress are the most common and crucial environmental stress factors that affect the normal growth of crop plants and induce decreased production (Aroca *et al.*, 2012; Sun *et al.*, 2015; Forni *et al.*, 2017). Drought stress causes an imbalance in plant water relation, which leads to metabolic disorders, growth inhibition and lower photosynthetic efficiency (Farooq *et al.*, 2009; Anjum *et al.*, 2011; Singh *et al.*, 2012; Nahar *et al.*, 2016). Salinity stress can lead to ion imbalance, hyperosmotic stress and secondary oxidative damage (Shaheen *et al.*, 2013; Acosta-Motos *et al.*, 2017), which all affect the normal growth of plants. In arid and semi-arid regions, even though the irrigation could help to increase productivity, the excessive evaporation and some ill-conceived agricultural practices often lead to soil salinization (Forni *et al.*, 2017). When the Na⁺ concentration increases in the surrounding environment, the high extracellular level of Na⁺ (relative to the cytosol) and inner negative membrane potential establish a steep thermodynamic gradient for Na⁺ influx (Niu *et al.*, 1995). Therefore, under drought conditions, more Na⁺ will be absorbed and accumulate in plants. In general, the combined effects of salinity and drought on yield are more detrimental

than the effects of each individual stress, as observed in potato (Levy *et al.*, 2013) and wheat (Yousfi *et al.*, 2012). However, certain Na⁺ accumulation is known to have a beneficial effect on the growth, development, yield and quality of some plants (Harmer and Benne, 1945; Truog *et al.*, 1953). Slama *et al.* (2007) suggested that the Na⁺ intake enables plants to accumulate more osmotica with low energy consumption, and this is more economical and efficient than synthesis and accumulation of organic osmolytes. Ma *et al.* (2012) found that soil salinity helps to lower the osmotic potential of plant tissue and enhance the driving force for water absorption under drought conditions. Some desert plants can exploit the accumulation of Na⁺ as an effective strategy to adapt to the arid environment (Wang *et al.*, 2004). In this sense, a moderate amount of NaCl in soil may effectively alleviate the adverse effects of drought. Currently, the studies on the role of Na⁺ accumulation in plant adaptation to drought have mainly focused on halophytes and xerophytes (Wang *et al.*, 2004; Mori *et al.*, 2011; Tan *et al.*, 2013), but with little research on other crop plants.

Cotton (*Gossypium hirsutum* L.) is the most important fiber crop in the world and is believed to have some tolerance to salinity (Richards, 1954; Meloni *et al.*, 2003). In this study, we performed an initial analysis to determine whether a

moderate amount of NaCl in soil can alleviate damage of drought stress on the lipid peroxidation and photosynthesis of cotton seedlings by assessing photosynthetic performance, antioxidant levels and other physiological parameters under individual drought stress, salinity stress and combined drought and salinity stress. This study will enrich the cognizance on the role of Na⁺ in the adaptation of plants to drought stress.

Materials and Methods

Experimental Material and Growth Conditions

Experiments were performed in the greenhouse from April to June, 2016. The test material was cotton (*Gossypium hirsutum* L., variety: Xinnongkang 13). Cotton seeds were sown in pots (internal diameter 38.5 cm × height 34.0 cm) filled with 26.5 kg of haplic luvisols (brown soil), with timely watering. A total of 20 pots were planted in this experiment. After germination, cotton seedlings were thinned to 6 seedlings per pot and to 3 seedlings per pot at 4 leaf stage. The soil had a bulk density of 0.95 g cm⁻³, a field water capacity of 47.8%, a pH of 7.2, an electrical conductivity of 0.78 mS cm⁻¹, and available N, P, K of 73.36, 32.63 and 50.25 mg kg⁻¹. The potted seedlings were placed in a greenhouse with a long-day photoperiod (16 h light/8 h dark). During the experiment, the minimum and maximum temperatures inside the greenhouse were 19.2°C and 35.5°C, respectively. The maximum photosynthetic photon flux density (PAR) on sunny days was about 1450 μmol m⁻²s⁻¹ outside the greenhouse, and about 960 μmol m⁻²s⁻¹ inside. Relative humidity ranged from 40.2 to 78.6%.

Experimental Treatments

Cotton seedlings were subjected to stress treatment at a height of approximately 15 cm (50 days old). There were four different treatments: control, individual salinity stress, individual drought stress, and combined drought and salinity stress (subsequently abbreviated as CK, S, D and D+S, respectively), and each treatment of 5 pots of cotton seedlings (5 replications). More concretely, at first, the CK and D cotton seedlings were irrigated fully with water alone (10 L deionized water was added to each pot). The S and D+S cotton seedlings were irrigated fully with 100 mmol L⁻¹ NaCl solution (10 L solution was added to each pot). The electrical conductivity of the soil under the different treatments are represented in Table 1. Thereafter, CK and S groups were watered in a timely manner, keeping 75–80% relative soil water content. The D and D+S groups were no longer watered, achieving natural water consumption and leading to progressive drought stress. On the 0, 5th, 10th, 15th, 20th, 25th and 30th day after treatment (the following day after full irrigation is as the first day after treatment), the photosynthetic parameters, antioxidant enzyme activity, malondialdehyde (MDA) content and relative leaf water content were determined.

Table 1: The electrical conductivity (EC) of the soil under the different treatments (mS cm⁻¹)

Treatment	CK	S	D	D+S
0 d after treatment	0.78±0.03a	1.23±0.03bc	0.78±0.03a	1.23±0.03bc
30 d after treatment	0.80±0.03a	1.26±0.03c	0.76±0.05a	1.23±0.03bc

CK: control; S: individual salinity stress; D: individual drought stress; D+S: combined drought and salinity stress. Values are means ± SD (n = 5). Different letters indicate significant differences among treatments at the 0.05 level

On the 30th day after treatment, at which point the leaves of the plantlets treated with progressive drought stress showed permanent wilting and shoot biomass was determined.

Measurement Method

Photosynthesis: Net photosynthetic rates (P_n) and intercellular CO₂ concentration were measured on cotton leaves on days 0–30 of the experiment under greenhouse conditions, once every 5 days. Measurements were made using a TPS-2 portable photosynthesis system (PP system, USA) between 8:30 and 11:00 am. The youngest fully expanded leaf of each plantlet was used as the measured object and at least five leaves from each treatment were measured to minimize measuring error. Light was controlled with light-emitting diodes, and light levels were set at a photosynthetic photon flux density of 950 μmol m⁻²s⁻¹. The temperature, relative humidity and reference CO₂ concentration were not regulated.

Chlorophyll fluorescence Parameters

Chlorophyll fluorescence was measured on the youngest fully expanded leaf using a Handy PEA chlorophyll fluorometer (Hansatech Ltd., UK). The leaves were dark-adapted for at least 15 min in leaf-clips before measurements were performed. The minimal fluorescence intensity (F_0) and maximum quantum yield of open photosystem II (PSII) (F_v/F_m) were measured between 8:30 and 10:00 am. Fifteen leaves were measured per treatment.

Antioxidant Enzymes Activity

Antioxidant enzymes were extracted according to Zhu *et al.* (2010). Fresh leaves were homogenized in 5 mL phosphate buffer (100 mmol L⁻¹, pH 7.8) and centrifuged at 10,000×g for 20 min at 4°C. The supernatant was used for superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) assays from 5 replications per treatment.

SOD activity was measured based on the ability of SOD to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide radicals that were generated photochemically (Zhu *et al.*, 2010). One unit of SOD was defined as the amount of enzyme required to inhibit the reduction rate of NBT by 50%.

CAT activity was measured by the disappearance of H_2O_2 (Rao *et al.*, 1996). The change in absorbance at 240 nm was monitored for 3 min, and 1 μ mol H_2O_2 destroyed per minute was defined as one unit of CAT.

POD activity was determined using guaiacol oxidation (Fu and Huang, 2001). One unit of POD was defined as an absorbance change of 0.01 per minute.

Malondialdehyde (MDA) Content

MDA was measured via the thiobarbituric acid (TBA) reaction, as described by Zhu *et al.* (2010). Five replications were performed for per treatment.

Leaf Relative Water Contents (Leaf RWC)

Five leaves per treatment were weighed immediately after harvest to obtain the fresh weight (FW). Next, the leaves were soaked in water for 18 h at 4°C, in the dark, and the turgid weight (TW) was determined. The leaves were then oven-dried at 70°C for 8 h, and the dry weight (DW) was obtained. Leaf RWC was estimated using the following formula: $RWC (\%) = (FW - DW) / (TW - DW) \times 100$ (Souza *et al.*, 2013).

Shoot Biomass

Five individual plantlets (not including underground parts) from each treatment were harvested at the 30th day after treatment. Dry weight was obtained from oven-dried samples after drying the plant material at 70°C for 72 h.

Statistical Analysis

The experimental data were subjected to one-way analysis of variance (ANOVA). Means were compared by Duncan's test.

Results

Photosynthetic Performance

A significant decrease was observed in the net photosynthetic rate (P_n) in cotton leaves under individual drought and/or salinity stress (Fig. 1a). The P_n of the D leaves started to decline rapidly at the 10th day (no significant soil water deficit in the first 10 days after treatment), down to nearly 0 on the 30th day after treatment; the P_n of the S and D+S leaves decreased soon after treatment (adverse effect from the presence of salinity stress), but their P_n were higher than the D leaves from the 15th day after treatment, and the differences increased as exposure time increased (Fig. 1a).

The results indicated that the C_i of the D leaves declined in the first 20 days after treatment and thereafter it raised robustly, but the C_i of the S and D+S leaves changed slightly (Fig. 1b). The decrease in the P_n of the leaf of the D leaves may be due primarily to the stomatal factor (stomata closure or contraction resulting in insufficient carbon dioxide) in the

first 20 days after treatment. Thereafter, the decrease in P_n was due to non-stomatal factors *i.e.*, the decrease in the photosynthetic activity of mesophyll cells caused by damage to the photosynthetic apparatus. However, the decrease in the P_n of the S and D+S leaves were due mainly to stomatal factors rather than the effect of the photosynthetic apparatus over the entire experimental period. Thus, a moderate amount of NaCl in soil might alleviate damage caused by drought stress to the photosynthetic apparatus.

In this study, compared to the control (CK), the F_o of the D leaves rose rapidly on the first 10 treatment days and declined rapidly from the 20th day after treatment. And the ratio of F_v/F_m appeared a continuous rapid decline from the 10th day after treatment. However, the F_o and F_v/F_m of the S and D+S leaves only showed a slight rise and fall, respectively, from the 10th day after treatment (Fig. 2). This indicated that the PSII reaction center of the D leaves began to inactivate on the 10th day after treatment; until the day 20, the photosynthetic apparatus was severely damaged. In comparison, the PSII reaction center of the S and D+S leaves showed only mild inactivation but not damage.

Lipid Peroxidation and Antioxidant Enzymes Activities

In this study, MDA content of the CK cotton leaves showed no significant change during the entire process, while the D, S and D+S cotton leaves increased as stress time extended. Meanwhile, the MDA content of the D leaves was significantly higher than the S and D+S after 15 days of treatment (Fig. 3a), reaching almost two times of the level for the D+S on the 30th day after treatment.

The SOD activities of the CK leaves were relatively stable, while those of the D, S and D+S leaves increased due to stress. The increase in SOD activity was much higher in the D than for the S and D+S from the 15th to 20th day after treatment, and a sharp decline in SOD activity appeared on the 20th day after treatment D (Fig. 3b). POD activities of the D, S and D+S leaves increased as treatment time increased. POD activities of the D leaves increased rapidly after 15 days of treatment, and the levels were significantly higher than in the S and D+S leaves (Fig. 3c). The trends in CAT activities were similar to those of SOD (Fig. 3d).

Leaf Relative Water Content (RWC)

The RWC of the CK and S leaves was higher due to high soil relative water content, and the RWC of the S leaves was slightly lower than the CK due to salinity stress. The RWC of the D and D+S leaves sustained rapid decline resulting from progressive drought stress. However, the RWC of the D+S leaves was significantly higher than the D after 20 days. On the 30th day after treatment, the leaf RWC of the D fell below 50%, and the D+S remained above 60%. The higher leaf RWC is beneficial to maintain cell turgor pressure and metabolism; therefore, the D+S treatment is more conducive to cotton seedling growth than the D treatment under drought conditions (Fig. 4).

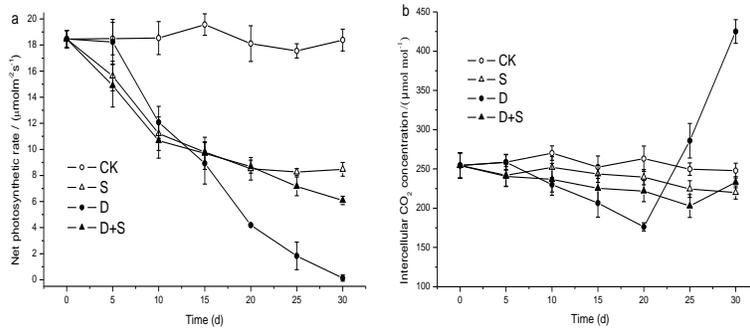


Fig. 1: Changes in net photosynthetic rate (a) and intercellular CO_2 concentration (b) in leaves of cotton seedlings with treatment time. CK: control; S: individual salinity stress; D: individual drought stress; D+S: combined drought and salinity stress. Values are means and vertical bars indicate SD (n = 5)

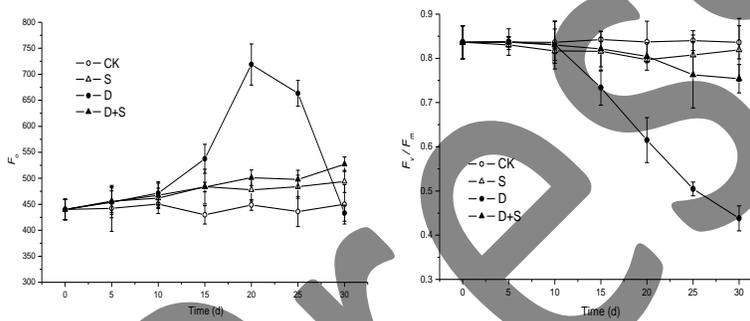


Fig. 2: Changes in chlorophyll fluorescence parameters F_o and F_v/F_m in cotton leaves with treatment time. CK: control; S: individual salinity stress; D: individual drought stress; D+S: combined drought and salinity stress. Values are means and vertical bars indicate SD (n = 5)

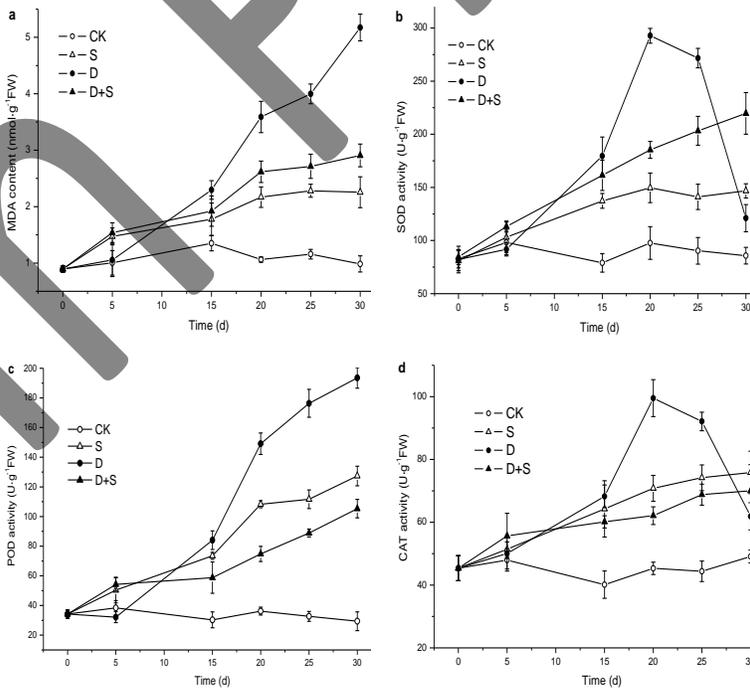


Fig. 3: Changes in MDA content and antioxidant enzyme activities with treatment time. CK: control; S: individual salinity stress; D: individual drought stress; D+S: combined drought and salinity stress. Values are means and vertical bars indicate SD (n=5)

Biomass

The shoot biomass of the S, D and D+S plants were significantly less compared to the control ($p < 0.01$), while the D+S was significantly higher than the D ($p < 0.01$). This indicated that both drought and salinity stress inhibited the growth of cotton seedlings. However, moderate NaCl in soil alleviated the inhibitory effect of drought stress on cotton growth (Fig. 5).

Discussion

Drought stress can cause water shortage in plant cells, which affects all aspects of plant metabolism and growth, including a decline in photosynthetic rate, aggravation in membrane lipid peroxidation and growth inhibition (Singh *et al.*, 2012; Li *et al.*, 2014; Nahar *et al.*, 2016). However, plants can decrease osmotic potential by accumulating organic and inorganic osmotic adjustment substances to enhance the power of water absorption and maintain cell turgor, plant metabolism and growth under drought conditions (Chen and Jiang, 2010; Blum, 2017). However, some researchers indicated that the certain concentrations of NaCl can enhance osmotic adjustment ability, increase tissue water content and improve growth in halophytes under osmotic stress (Slama *et al.*, 2007; Ma *et al.*, 2012). Our results showed that cotton seedlings with the D+S treatment (combined drought and salinity stress) showed higher leaf relative water contents and more biomass accumulation than the D treatment (Fig. 4 and 5). This indicated that moderate soil NaCl could maintain relatively high water content of cotton seedlings, which is very conducive to the plant growth under drought conditions.

Photosynthesis is the primary process affected by drought and/or salinity (Chaves *et al.*, 2009). Chlorophyll fluorescence is a rapid, non-invasive natural probe for monitoring the photosynthetic performance of leaves (Baker, 2008). This provides a sensitive examination of the effects of various stresses on the photosynthetic apparatus. F_o (initial/minimal fluorescence) is the level of fluorescence when the plastoquinone electron acceptor pool (Q_A) is fully oxidized (PSII centers open). An increase in F_o is characteristic of PSII inactivation (Zlatev and Yordanov, 2004), and a decrease in F_o can reflect damage to regulatory processes external to the reaction center of PSII and degradation of photosynthetic pigments (Li *et al.*, 2006). F_v/F_m reflects the maximum quantum efficiency of PSII (Kalaji *et al.*, 2012). A decrease in F_v/F_m indicates that the photosynthetic apparatus is damaged (Huang *et al.*, 2013). The experimental results showed that F_o experienced a rapid increase, followed by a rapid decrease, and F_v/F_m decreased continuously in the D cotton leaves. Conversely, the F_o and F_v/F_m of the D+S cotton leaves fluctuated little (Fig. 2). Under drought conditions, the D leaves suffered damage to the photosynthetic apparatus and the destruction of photosynthetic pigments; the D+S leaves had not.

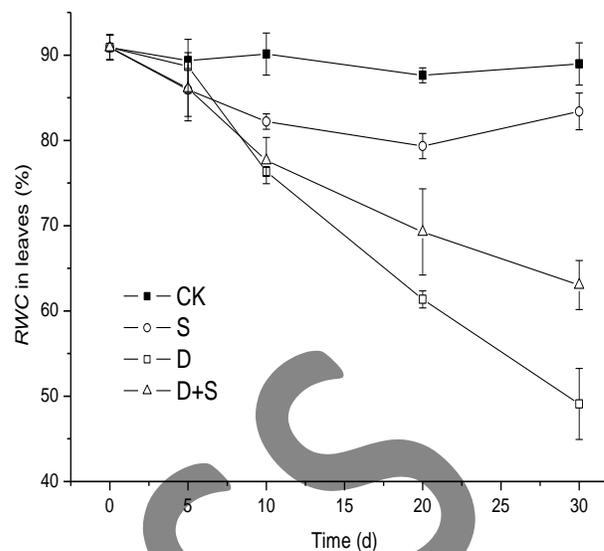


Fig. 4: Changes in RWC in cotton leaves under different treatments. CK: control; S: individual salinity stress; D: individual drought stress; D+S: combined drought and salinity stress. Values are means and vertical bars indicate SD (n = 5)

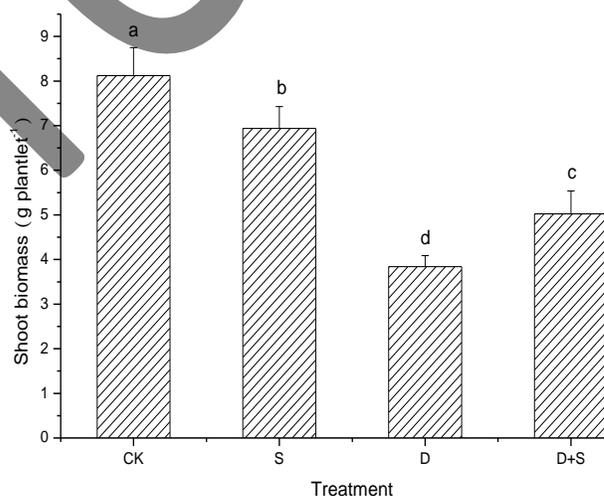


Fig. 5: The shoot biomass under different treatments. CK: control; S: individual salinity stress; D: individual drought stress; D+S: combined drought and salinity stress. Values are means and vertical bars on the top indicate SD (n=5). Different letters in data columns mean significant differences at the 0.01 level

Meanwhile, analysis of the gas exchange parameters also showed that a decrease in P_n of the D leaves was due to insufficient CO_2 resulted from stomatal factors in the first 20 days after treatment, followed by photosynthetic apparatus damage; however, a decrease in the P_n of the D+S leaves was always due to insufficient CO_2 . These results

indicated that moderate NaCl in soil might alleviate the damage caused by drought in the photosynthetic apparatus, and might be beneficial to maintain the photosynthesis of cotton leaves under drought conditions. This suggested that the drought stress might cause the PSII reaction center to inactivate and possibly injure the photosynthetic apparatus, but a moderate amount of NaCl in soil could mitigate the adverse effect.

As known, drought stress induces the production of active oxygen species, increases lipid peroxidation, causes damage to the cell membrane system and affects plant metabolism (Türkan *et al.*, 2005; Li *et al.*, 2014). SOD, POD and CAT are the main antioxidant enzymes preventing cellular damage. Many reports have underlined that the environmental stress resistance of plants is intimately related to the activity of antioxidant enzymes (Bowler *et al.*, 1992; Bor *et al.*, 2003; Gapinska *et al.*, 2008; Dwivedi *et al.*, 2016). Wang *et al.* (2012) reported that the activities of these enzymes in drought-stressed apple leaves were heightened at first and then fell over time; levels were always higher than values of the control plants. Similar results were reported in *Oryza sativa* L. (Sharma and Dubey, 2005), *Boehmeria nivea* L. (Huang *et al.*, 2013) and potato (Li *et al.*, 2017). Our experiments showed that the activities of antioxidant enzymes (SOD, CAT and POD) increased sharply in the D cotton leaves, with the peaks of SOD and CAT occurring at day 20 before a rapid decrease. Although the activities of these enzymes also increased in the D+S cotton leaves, the change was smaller and no reduction in SOD and CAT activities was observed. Meanwhile, the MDA contents which show the membrane lipid peroxidation and the degree of plant cell injury (Du *et al.*, 2014), in the D+S leaves was significantly lower than in the D (Fig. 3). Based previous research and analysis, it was suggested that the sharp rise in SOD, CAT and POD activities in the D cotton leaves resulted from a protective response induced by drought stress, and the following sharp decline in SOD and CAT activities was due to severe cellular damage caused by extreme drought stress. The D+S leaves were not harmed during the progressive drought. These above results indicate that a moderate amount of soil NaCl can alleviate adverse effects caused by drought stress on cotton seedlings by reducing membrane lipid peroxidation under drought conditions.

Conclusion

Based on the present study results, it can be inferred that under drought conditions, moderate soil NaCl can increase the water contents of cotton leaves, thereby alleviate the damages resulted from lipid peroxidation induced by drought stress to the cell membrane and photosynthetic apparatus and then mitigate adverse effects of drought stress on the cotton growth and biomass accumulation. This suggested that moderate soil salinity may improve the drought tolerance of cotton seedlings.

Acknowledgements

This work was funded by the National Natural Science Foundation of China [Grant number 31601994] and Youth Fund of Shandong Provincial Natural Science Foundation, China [Grant number ZR2016CQ11].

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(Received 12 February 2018; Accepted 25 September 2018)