

Increase of nitrogen to promote growth of poplar seedlings and enhance photosynthesis under NaCl stress

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Abstract The solution culture method was used to study the effect of increasing nitrogen on the growth and photosynthesis of poplar seedlings under 100 mmol L⁻¹ NaCl stress. Increase in nitrogen reduced stomatal limitation of leaves under NaCl stress, improved utilization of CO₂ by mesophyll cells, enhanced photosynthetic carbon assimilation capacity, significantly alleviated saline damage of NaCl, and promoted the accumulation of aboveground and root biomass. Increased nitrogen enhanced photochemical

efficiency (Φ_{PSII}) and electron transport rates, relieved the reduction of maximum photochemical efficiency (F_v/F_m) under NaCl, and reduced the degree of photoinhibition caused by NaCl stress. Increased nitrogen applications reduced the proportion of energy dissipating in the form of ineffective heat energy and hence a greater proportion of light energy absorbed by leaves was allocated to photochemical reactions. Under treatment with increased nitrogen, the synergistic effect of heat dissipation and the xanthophyll cycle in the leaves effectively protected photosynthetic PSII and enhanced light energy utilization of leaves under NaCl stress. The increased nitrogen promoted photosynthetic electron supply and transport ability under NaCl stress evident in enhanced functioning of the oxygen-evolving complex on the electron donor side of PS II. It increased the ability of the receptor pool to accept electrons on the PSII electron acceptor side and improved the stability of thylakoid membranes under NaCl stress.

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Therefore, increasing nitrogen applications under NaCl stress can promote poplar growth by improving the efficiency of light energy utilization.

Keywords Poplar · Nitrogen · NaCl stress · Photosynthetic characteristics

Introduction

About 20% of the world's arable land has been salinized which limits the production of plants (Zhu 2001; Bhatnagar et al. 2008). Nitrogen is a necessary macronutrient required for growth and development and an important component of plant proteins and nucleic acids (Mi et al. 2007). However, soil salinization limits nitrogen mineralization and reduces the availability of nitrogen (Calderon et al. 2000; Wang et al. 2004). Nitrogen deficiency is an important limiting factor on the growth of plants. Rational application of nitrogen fertilizer can help promote plant growth and restore vegetation in vulnerable ecological areas and improve plants resistance under stress (Yuan et al. 2009). The application of nitrogen fertilizer not only promotes plant growth, it ameliorates the saline-alkaline soil environment (Yuan et al. 2009), improves photosynthetic and protein synthesis abilities (Wang et al. 2015), and plays an important role in enhancing plant resistance (Pompeiano et al. 2014; Skeffington and Jeffrey 1988). For example, increasing nitrogen to improve resistance to abiotic stressors of plants such as Indian mustard (*Brassica juncea* (L.) Czern.; Nathawat et al. 2007), barley (*Hordeum* L.; Ali et al. 2001), and rape (*Brassica campestris* L.; Yang et al. 2012), has been confirmed.

Photosynthesis is the foundation of plant production and plants continue to grow only by maintaining a relatively high photosynthetic capacity in adverse environments. Increased nitrogen promotes nitrogen metabolism and enhances photosynthetic carbon metabolism (Li and Gao 2007). Chloroplasts contain up to 75% of the nitrogen in plant leaves, and nitrogen metabolism is closely related to both the light dependent and light independent reactions of photosynthesis (Shangguan et al. 2000). Except for driving photochemical reactions, a large portion of the photosynthetic electron flow in photosynthesis is consumed in nitrogen metabolism. Therefore, there is a relationship of interdependence and competition between nitrogen metabolism and photosynthetic carbon metabolism. In adverse conditions, carbon metabolism is inhibited and the light energy absorbed by leaves cannot be fully used for electron transfer; the result is an excess of light energy. Nitrogen metabolism can then consume part of this energy and reduce the damage caused by excess light energy, and

hence protect the photosynthetic apparatus (Foyer et al. 2001).

Some salt ions in the soil are indispensable nutrient elements for normal growth (Wang et al. 2001), but excess salt causes osmotic stress and ion toxicity as well as interferes with nutrient ion equilibrium affecting plant growth and physiological functions (Munns et al. 2006; Yan et al. 2006; Ali et al. 2017). Salt stress affects chlorophyll synthesis and photosynthetic capability of plants (Gong et al. 2013; Dąbrowski et al. 2016), often leading to the reduction of PSII(Photosystem II)reaction center activity, inhibition of electron transport, limitation of carbon assimilation capacity and may even lead to peroxidation or dissociation of thylakoid membranes (Mitsuya et al. 2000; Takahashi et al. 2017).

In long-term evolution, plants generate a series of salt-tolerant mechanisms such as the salt exclusion effect, the accumulation of osmotic adjustment substance, and/or the start-up of anti-oxidation systems in the root systems (Song et al. 2006; Askari et al. 2006; Zhao et al. 2016). Plant root systems control the passing in and out of Na^+ using ion channels (such as a voltage-dependent non-selective cation channel NSCC, and the glutamate activation channel GLR) or transporters (such as the Na^+/H^+ antiporter and high affinity K^+ transporter HKT), as well as adapting to salt stress by adjusting intracellular ion equilibrium via an ion compartmentation effect (Wu et al. 2012; Cheng et al. 2009; Apse and Blumwald 2007). The adaptation mechanisms of different root systems to salt stress are obviously different. Poplar is a common species for afforestation and greening in northern China (Song et al. 2013). It is a pioneer species for vegetation restoration projects in degraded ecosystems. In areas with high salt and alkali contents, soil nitrogen deficiency has been an important limiting factor for plant restoration (Yuan et al. 2009; Wang et al. 2004). However, there are few studies on the effects of nitrogen application on the growth and photosynthesis of poplar under NaCl or sodium chloride stress. The solution culture method was adopted to study the effects of increasing nitrogen on the growth and photosynthesis of poplar seedlings under NaCl stress. In addition, we investigated the effects on photosynthetic electron transport and energy allocation of poplar leaves under NaCl stress. This study also aimed to clarify the relationship between nitrogen metabolism and photosynthetic carbon metabolism in leaves under increasing nitrogen treatments. This will provide theoretical grounds for rational nitrogen management for vegetation restoration in vulnerable ecological areas dominated by saline and alkaline soils.

Materials and methods

Materials

This work was conducted at the plant physiological laboratory of the Northeast Forestry University, Harbin, between March and June of 2014. Black poplar hybrid (*Populus simonii* × *P. nigra*) seeds were sown at the beginning of March in vermiculite. They were regularly irrigated with 1/2 Hoagland nutrient solution and the water content was maintained at 80%. Water content was determined using the TZS-1K instrument (TOP, China). When seedlings had five leaves, they were carefully removed from the vermiculite, the roots washed, and seedlings fixed on a black foam board with punched holes using a sponge. The foam board was then floated in an opaque box filled with 1/2 Hoagland solution for hydroponics. Each hydroponic box was filled with a 10-L nutrient solution with two poplar seedlings. The seedlings were continuously ventilated by an electric air pump and the nutrient solution replaced every 3 days. The treated seedlings were incubated under greenhouse culture lamps at temperatures of $25\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$, light intensity of $400\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$, photoperiods of 12 h/12 h (light/dark), and a relative humidity of 75%. After 15 days of hydroponics, the experiment was conducted when new white roots emerged on the existing seedling roots.

Experimental design

The 18 seedlings under relatively consistent growth conditions were randomly assigned to three treatment groups with six seedlings in each. The NaCl stress treatment was initiated by adding NaCl to the 1/2 Hoagland solution and the NaCl concentration was allowed to reach 100 mmol L^{-1} . The increased nitrogen treatment (T2) started by adding Ca (NO_3)₂ into the 1/2 Hoagland solution containing 100 mmol L^{-1} of NaCl. (The NaCl concentration was according to Wang et al. 2004), which made the concentration of NO_3^- -N reach 17.5 mmol L^{-1} (NO_3^- -N in a 1/2 Hoagland solution was 7.5 mmol L^{-1}) (Xu et al. 2017). Hydroponics in a 1/2 Hoagland solution was used as the control (CK). To eliminate the effect of introducing Ca^{2+} in the T2 treatment, an appropriate amount of CaCl_2 was added in the CK and NaCl stress treatments to make the Ca^{2+} concentration consistent with the T2 treatment. After 20 days of incubation, the indices were determined when the phenotypic differences between treatments became obvious.

Methods

Leaf photosynthesis measurements

For each treatment, three pieces of the last third or fourth fully expanded leaf from the bottom of the plant were used to determine the net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i). The Li-6400 photosynthesis system was utilized to maintain a CO_2 concentration of $450\text{ }\mu\text{L L}^{-1}$, light intensity of $800\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$, environment temperature of $23\text{ }^{\circ}\text{C}$, and a relative humidity of 75%.

Determination of the fluorescence index of chlorophyll

A 0.5 h dark adaptation treatment was conducted on poplar seedling leaves with dark adaptation clamps. Using a portable pulse modulated fluorometer FMS-2 (Hansatch Co., UK) and following the method of Hu et al. (2007), initial fluorescence (F_o), maximal fluorescence (F_m), variable fluorescence (F_v), the maximum fluorescence under light adaptation (F'_m), minimal fluorescence under light adaptation (F'_o), and steady-state fluorescence (F_s) were determined. The maximal photochemical efficiency of PSII (F_v/F_m), actual photochemical efficiency (Φ_{PSII}), electron transport rate (ETR), and excess light energy, $(1 - q_p)/\text{NPQ}$, were calculated as follows:

$$\Phi_{\text{PSII}} = (F_m/F_s)/F'_m \quad (1)$$

$$\text{ETR} = 0.5 \times 0.85 \times \Phi_{\text{PSII}} \times \text{PFD} \quad (2)$$

$$q_p = (F'_m - F_s)/(F'_m - F'_o) \quad (3)$$

$$\text{NPQ} = (F_s/F'_m) - (F_s/F_m) \quad (4)$$

where PFD is the light intensity ($\mu\text{mol m}^{-2}\text{ s}^{-1}$). Each sample was replicated three times. The direction of the light energy absorbed by the PSII reaction center was determined (Hendrickson et al. 2004; Zhou et al. 2007), i.e., the quantum yield used for the photochemical reaction (Y_{PSII}), quantum yield depending on the proton gradient on both sides of the thylakoid membrane and xanthophyll cycle (Y_{NPQ}), basic fluorescence quantum yield and quantum yield of heat dissipation ($Y_{\text{f,D}}$), and quantum yield of heat dissipation in inactivated the PSII reaction center (Y_{NF}). The calculation of parameters are as follows:

$$\Phi_{\text{PSII}} = [1 - (F_s/F'_m)] \cdot [(F_v/F_m)/(F_v/F_m)] \quad (5)$$

$$\Phi_{\text{NPQ}} = [(F_s/F'_m) - (F_s/F_m)] \cdot [(F_v/F_m)/(F_v/F_{mM})] \quad (6)$$

$$\Phi_{\text{f,D}} = (F_s/F_m) \cdot [(F_v/F_m)/(F_v/F_{mM})] \quad (7)$$

$$\Phi_{\text{NF}} = 1 - [(F_v/F_m)/(F_v/F_{mM})] \quad (8)$$

where the sum of parameters equals one, namely $\Phi_{\text{NF}} + \Phi_{\text{PSII}} + \Phi_{\text{NPQ}} + \Phi_{\text{I,D}} = 1 \cdot F_v/F_{\text{mM}}$ is the maximal photochemical efficiency without photoinhibition. Each treatment was measured three times.

Determination of the chlorophyll fluorescence kinetic curve

The second fully-expanded leaf from the top of the seedlings was selected for measurement. The OJIP curve of the leaves after a 30-min dark adaptation treatment was measured using the Mini modulated chlorophyll fluorometer (FluorPen FP 100 max, Czech). The measurement of each treatment was repeated five times. The OJIP curve was induced by a pulsed red light of $3000 \mu\text{mol m}^{-2} \text{s}^{-1}$. The relative fluorescence intensity of the OJIP curve at points O, J, I, and P (F_o , F_J , F_I , and F_m , respectively) corresponded to the times of 0, 2, 30, and 1000 ms, while the points of L and K (F_L and F_K , respectively) corresponded to the times of 0.15 and 0.30 ms, respectively. To analyze the changes of relative variable fluorescence at points J, I, L, and K (V_J , V_I , V_L , and V_K , respectively), the curves of O–P, O–J, and O–K were normalized based on the method of Strasser et al. (1995). Three replications were measured for each treatment.

Determination of growth parameters

Growth parameters of all treated poplar seedlings were measured after the photosynthetic and chlorophyll fluorescence parameters were determined. After measuring height and root length for each seedling, the root surface was dried with absorbent papers and the leaves, stems, and roots of seedlings in each treatment were separately placed in aluminum boxes at a fixed temperature of $105 \text{ }^\circ\text{C}$ for 30 min and then dried at $60 \text{ }^\circ\text{C}$ for 30 h. The quality was measured until it was constant, and then leaf, stem, and root biomass were obtained. Each measurement was repeated six times.

Statistical analysis

Excel and SPSS (22.0) software were used for the statistical analysis. The data in figures and tables are the average of three replicates \pm standard deviation (SE). The differences between different groups were compared using a one-way analysis of variance (ANOVA) and the least significant difference (LSD) method.

Results

Effects of increased nitrogen on growth of poplar seedlings under NaCl stress

NaCl significantly inhibited the growth of poplar seedlings. Compared with the CK, there was no significant change in plant heights but root lengths significantly decreased by 37.8% ($P < 0.05$) (Fig. 1). In addition, the biomass of each part of poplar seedling leaves under NaCl stress was significantly lower than that of the CK. However, an increase of nitrogen significantly promoted the growth of seedlings under NaCl stress. The difference in heights and root lengths between NaCl + N and NaCl treatments was not significant but the biomass of roots, stems, and leaves increased by 48.1% ($P < 0.05$) and 37.5% ($P < 0.05$), respectively, compared with NaCl treatment.

Effects of increased nitrogen on photosynthetic gas exchange parameters of leaves under NaCl stress

Compared with the CK, P_n , T_r , and G_s of leaves decreased by 31.0% ($P < 0.05$), 40.4% ($P < 0.05$), and 36.4% ($P < 0.05$) under NaCl stress, respectively, but C_i significantly increased by 24.6% ($P < 0.05$; Fig. 2). Increased nitrogen increased the size of stomatal openings of leaves under NaCl stress, and higher stomatal conductance promoted gas exchange in the leaves; P_n and T_r of leaves also significantly increased and the utilization of CO_2 by the mesophyll cells was enhanced, which showed that the C_i of leaves in the NaCl + N treatment decreased by 9.1% compared with the NaCl treatment ($P > 0.05$).

Effects of increased nitrogen fertilizer on chlorophyll fluorescence of leaves under NaCl stress

NaCl significantly reduced F_v/F_m , Φ_{PSII} , and ETR of poplar leaves and $(1 - q_p)/NPQ$ increased 2.4 times compared with the CK (Fig. 3). Greater nitrogen levels increased the electron transport rate and photochemical efficiency of seedlings under NaCl stress, $(1 - q_p)/NPQ$ significantly decreased, photoinhibition was reduced, among which the F_v/F_m , Φ_{PSII} , and ETR of leaves in the NaCl + N treatment increased by 3.8% ($P < 0.05$), 96.1% ($P > 0.05$) and 98.2% ($P > 0.05$) respectively, compared with NaCl stress treatment.

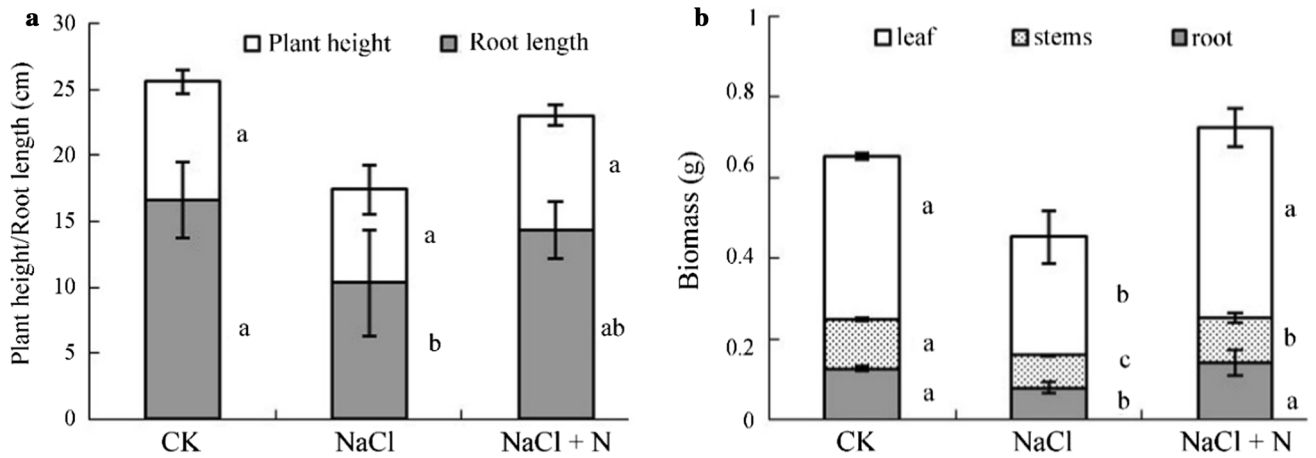


Fig. 1 Effects of increased nitrogen fertilizer on growth characteristics of poplar seedlings under NaCl stress. Each point is the mean of six separate observations. SE was plotted for clarity. Results are means. SE; n = 6

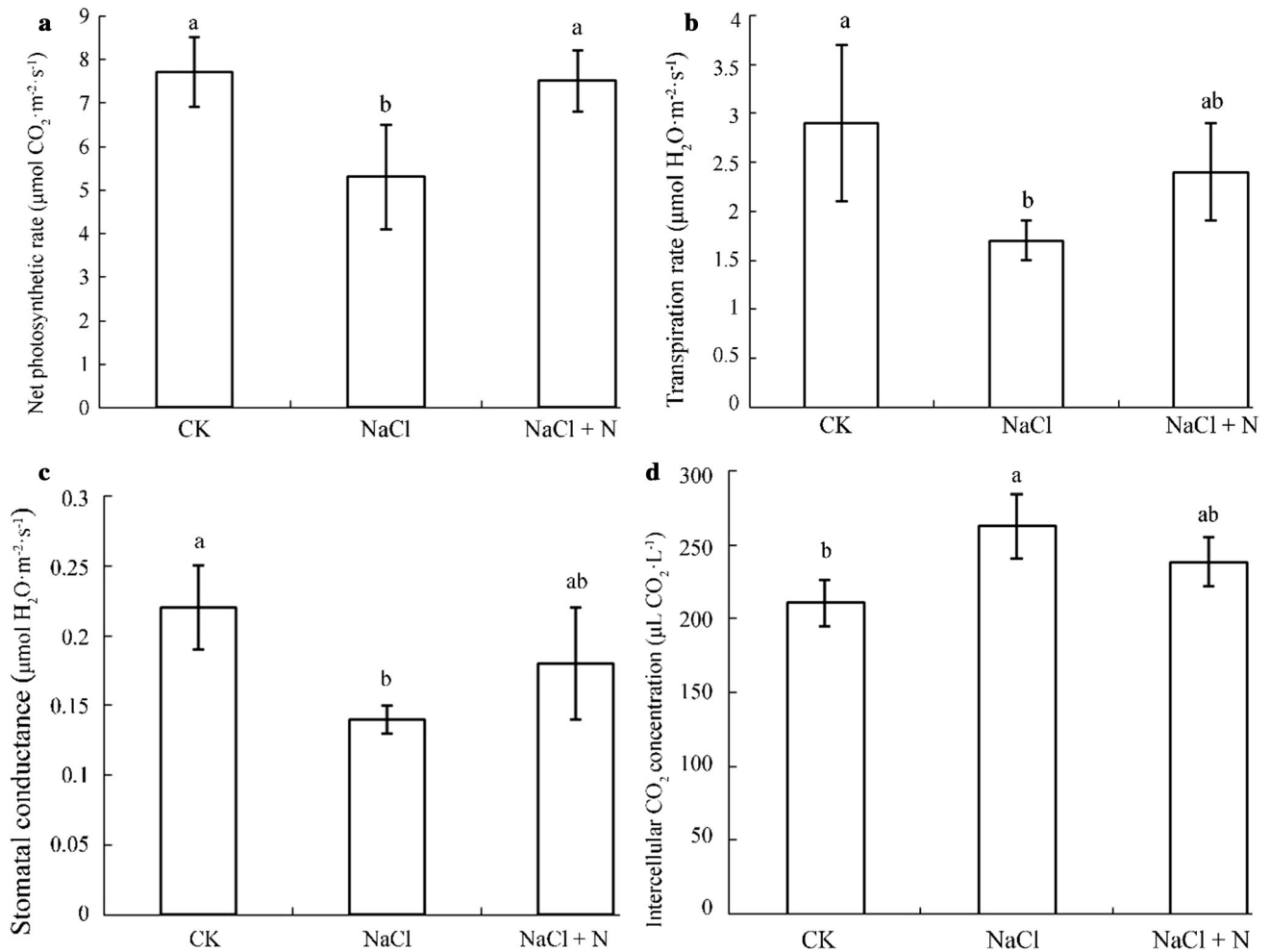


Fig. 2 Effects of increased nitrogen fertilizer on photosynthetic gas exchange parameters of poplar seedling leaves under NaCl stress. Each point is the mean of six separate observations. SE was plotted for clarity. Results are means. SE; n = 6

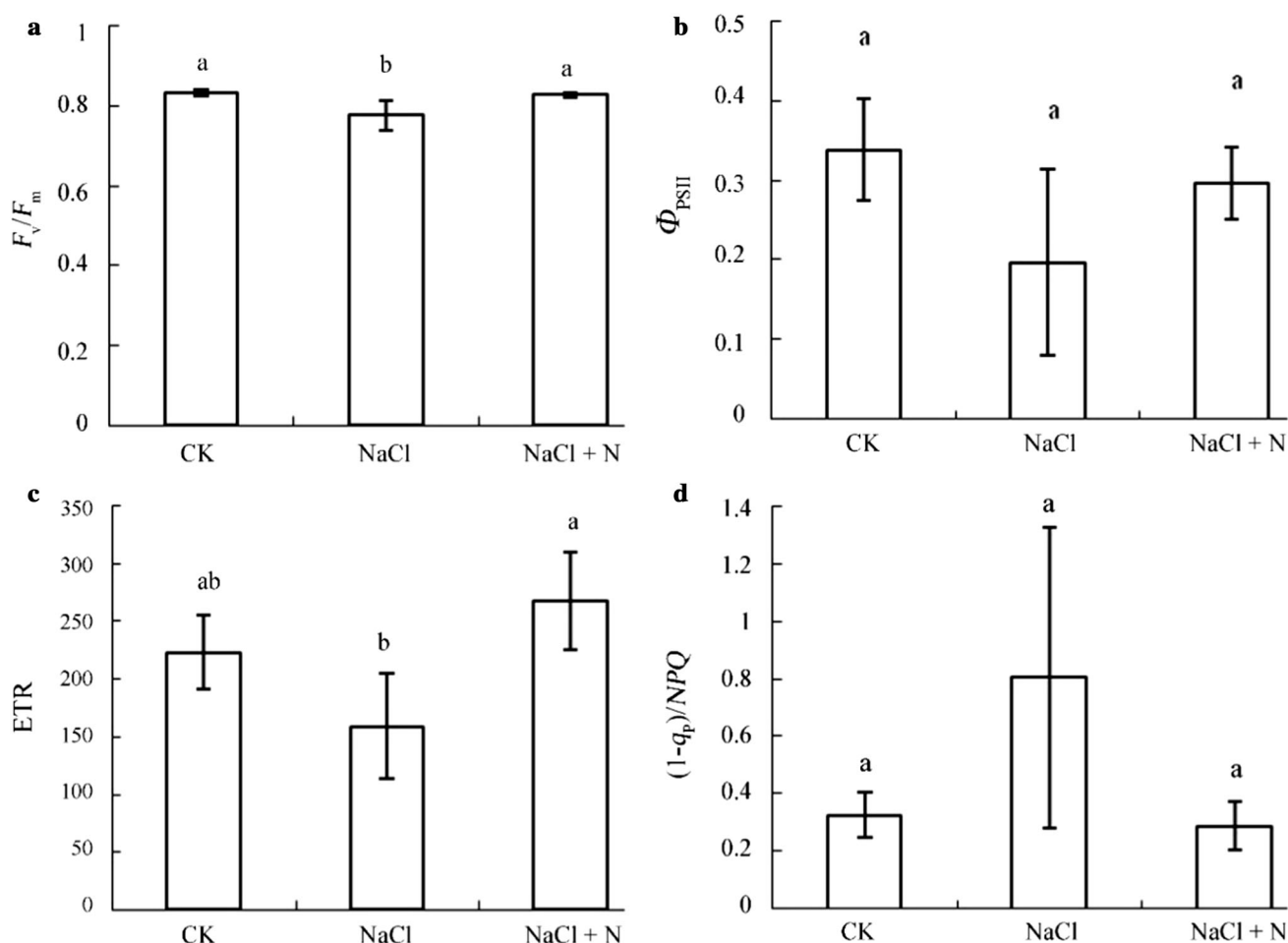


Fig. 3 Effects of increased nitrogen fertilizer on chlorophyll fluorescence parameters of poplar seedling leaves under NaCl stress. Each point is the mean of six separate observations. SE was plotted for clarity. Results are means

Effects of increasing nitrogen fertilizer on light energy parameters in the PSII reaction center of leaves under NaCl stress

The Y_{PSII} and Y_{NPQ} of leaves under NaCl stress noticeably decreased, while the $Y_{f,D}$ and Y_{NF} increased, particularly Y_{NF} , which increased 3.5 times (Fig. 4). Increased nitrogen increased the quantum yield for the photochemical reaction absorbed by the PSII reaction center. The Y_{PSII} of leaves in the NaCl + N treatment increased by 108.9% compared with the NaCl treatment, while the $Y_{f,D}$ and Y_{NF} declined by 44.9 and 83.5%, respectively.

Effects of increased nitrogen on the standardized O–P curve of leaves under NaCl stress

The relative fluorescence intensity of point O was defined as zero while points P, J, and K were defined as 1. After the OJIP curves of leaves in different treatments were normalized, the relative variable fluorescence (V_J and V_I) of

points J and I on the standardized O–P curve increased more than those of the controls (CK) under NaCl stress (Fig. 5). The increasing rate of V_J was obviously greater than V_I . The relative variable fluorescence (V_K and V_L) of points K and L on the standardized O–J and O–K curves also showed an increasing trend compared with CK (Fig. 5). However, increased nitrogen significantly alleviated the increase of relative variable fluorescence at each characteristic point of leaves under NaCl stress.

Effects of increased nitrogen fertilizer on V_J , V_I , V_K , and V_L of leaves under NaCl stress

Quantitative analysis of changes in V_J , V_I , V_K , and V_L of poplar seedling leaves under NaCl stress showed that they increased by 20.7% ($P < 0.05$), 22.6% ($P < 0.05$), 20.7% ($P < 0.05$) and 22.6% ($P < 0.05$) compared with the CK, respectively (Fig. 6). However, increased nitrogen fertilizer alleviated the increasing rate of relative variable fluorescence of each characteristic point of leaves under NaCl

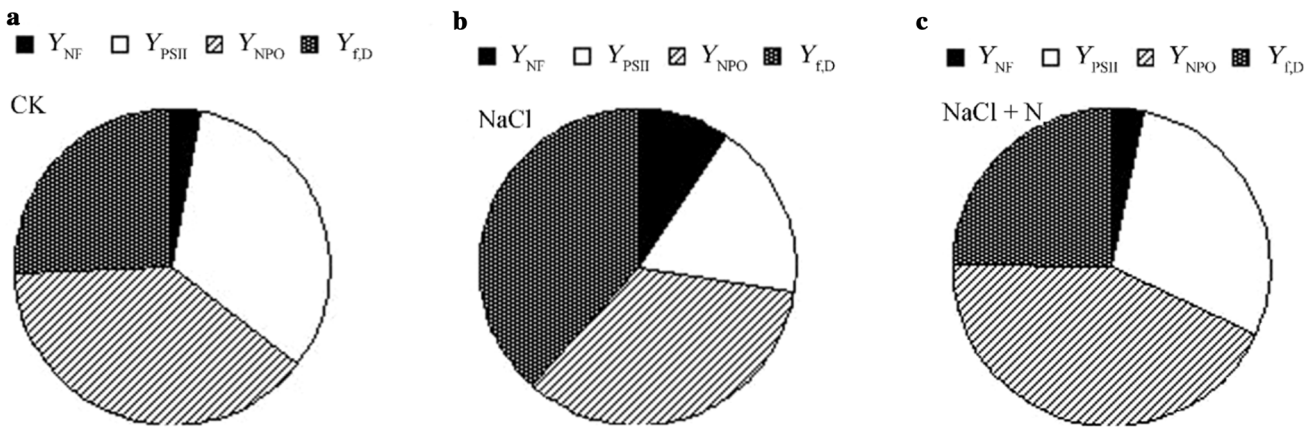


Fig. 4 Effects of increased nitrogen on light energy allocation parameters in the PSII reaction center of leaves under NaCl stress

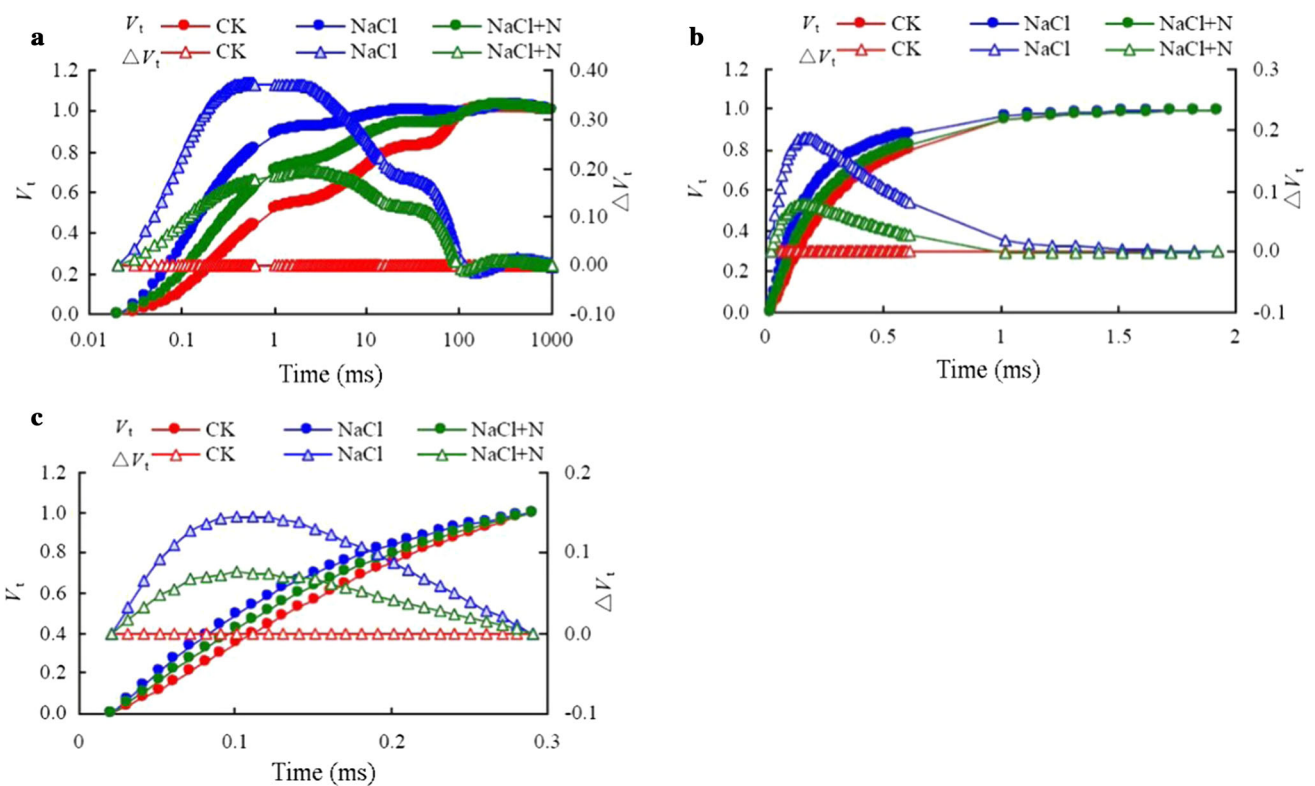


Fig. 5 Effects of increased nitrogen on the standardized O-P, O-J, and O-K curves of poplar seedling leaves under NaCl stress

stress, but the differences of each parameter between NaCl + N and NaCl treatments were insignificant.

Discussion

The damage of NaCl to plants not only included ion toxicity but the secondary action of saline ions also resulted in the inhibition of photosynthesis. NaCl reduced the stomatal conductance of poplar seedling leaves, and blocked the photosynthetic gas exchange which reduced P_n and T_r .

However, the C_i showed an increasing trend, indicating that the decrease of P_n caused by NaCl was the result of the combined effects of stomatal and non-stomatal factors. In an adverse environment, due to the enhancement of starch hydrolysis and the accumulation of carbohydrates in mesophyll cells and a slowdown of output of photosynthetic products, photosynthesis was inhibited (Huppe and Turpin 1994). In this study, NaCl inhibited the photosynthetic carbon assimilation capacity of seedlings but increased nitrogen significantly increased photosynthetic capacity under NaCl stress. Since NO_3^- -N uses organic carbon

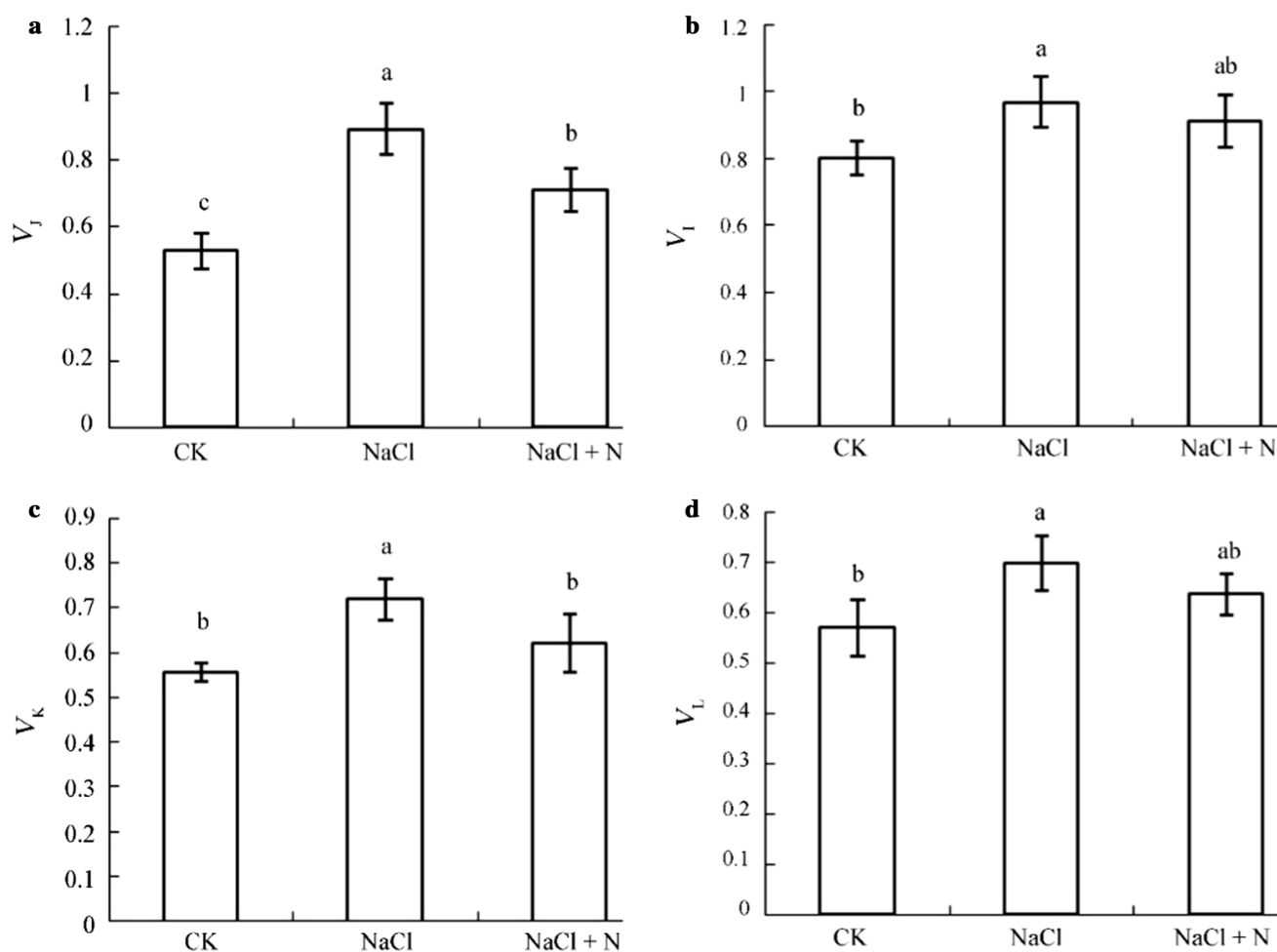


Fig. 6 Effects of increased nitrogen fertilizer on V_j , V_i , V_k , and V_l of leaves under NaCl stress

formed by photosynthetic carbon assimilation (Huppe and Turpin 1994), and the light reduction of NO_3^- requires carbohydrates provided by photosynthesis. In NO_3^- reduction, a large amount of substances and energy were consumed and therefore there was a competitive relationship with carbon metabolism (Chen and Li 1984). Under NaCl stress, increased nitrogen may accelerate the transport and output of photosynthates and promote normal photosynthetic functioning in poplar seedling leaves.

With high levels of NaCl, the photochemical capacity of leaves declined and electron transport was blocked. This not only induced an insufficient assimilation power supply, it also accumulated an excessive number of electrons in the electron transport chain. If excessive electrons are not removed in time, they will attack the active oxygen species of the monovalent oxygen molecules in the thylakoid membrane, thus inhibiting photosynthesis (Reddy et al. 2004; Yamori et al. 2010; Zhang et al. 2017). This study demonstrated that under NaCl stress, the *ETR* (electron transport rate) of poplar seedlings decreased, excess light energy $(1 - q_p)/NPQ$ accumulated in leaves, and the *Fv*/*Fm*

(maximum photochemical efficiency of PS II) declined, indicating that photoinhibition occurred in leaves under NaCl stress. Research has found that during nitrogen metabolism, the reduction of nitrite (NO_2^-) to ammonium had a similar effect to NADP^+ (Nicotinamide adenine dinucleotide phosphate) which also uses the electrons transported by reduced ferredoxin (Fd_{red}) (Robinson 1986). The energy allocated to nitrogen metabolism from Fd_{red} can reach 20–25% and can be as high as 55% in certain plants (Tian et al. 2010). In this study, increased nitrogen increased the electron transport rate of leaves under NaCl stress and reduced the extent of photoinhibition. This may be that, under NaCl stress, greater nitrogen increased the receptive ability of photosynthetic electrons in the electron transport chain, reduced excess electrons, and promoted the normal functioning of electrons in the chain. As the light-independent reaction of leaves was inhibited under NaCl (Fig. 2), the ability to use assimilation power (ATP and NADPH) produced by photoreaction declined, resulting in an excess of assimilation power that not only produces feedback inhibition on the transport of photosynthetic

electrons but is the main reason for the production of excess excitation energy in plant cells. Yang et al. (2012) found that NO_3^- will compete with CO_2 for assimilation power in the reduction process, although the NO_3^- reduction process cannot directly use the NADPH produced by the light-dependent reaction, but rather the NADPH produced through the path of the “malic acid—oxaloacetic acid shuttle pathway” or “phosphoglycerate-dihydroxyacetone phosphate shuttle pathway”, the dicarboxylate shuttle was converted into the NADH specifically required by nitrate reductase, which can be used indirectly (Klepper et al. 1971). Therefore, under NaCl stress, increased nitrogen partially allocated the assimilation power of poplar leaves to the nitrogen metabolism process, increased the utilization ability of assimilation power, and reduced the potential pressure generated by excess excitation energy. The degree of photoinhibition of poplar seedlings under NaCl stress was thereby reduced and normal photosynthetic functioning and growth were promoted.

NaCl significantly changed the light energy allocation parameters absorbed by the PS II reaction center of poplar leaves; the Y_{PSII} (the quantum yield of the photochemical reaction) decreased significantly and the Y_{NF} increased noticeably, indicating that NaCl inhibited the activity of the PS II reaction center. To reduce the pressure on the center, the proportion of light energy absorbed by poplar leaves for non-photochemical reactions increased. The light energy allocation parameter $Y_{\text{f,D}}$, includes two parts: quantum yield of fluorescence and quantum yield of thermal dissipation. Because the proportion of fluorescence emitted by plants is very small (Maxwell and Johnson 2000), $Y_{\text{f,D}}$ mainly reflects the proportion of heat dissipated in light energy allocation during the non-radiative energy dissipation. This study shows that the $Y_{\text{f,D}}$ (quantum yield of heat dissipation) of poplar leaves under NaCl stress increased while the Y_{NPQ} (quantum yield depending on the proton gradient on both sides of the thylakoid membrane and xanthophyll cycle) slightly decreased. Other studies have demonstrated that NPQ has a positive correlation with heat dissipation dependent on the xanthophyll cycle (Goss et al. 1998; Li et al. 2000). The results of this study indicate that poplar seedlings under NaCl stress reduce the generation of excess excitation energy by increasing the proportion of heat dissipation, while the mechanism of photosynthetic protection based on the xanthophyll cycle is limited. Increased nitrogen increased the proportion of light energy absorbed by leaves used for photochemical reactions under NaCl stress. This would increase the photosynthetic electron transport and maintain the supply of power required for carbon assimilation and thus improve light energy utilization. Increased nitrogen also reduced the $Y_{\text{f,D}}$ while Y_{NPQ} increased, indicating that increased nitrogen reduced

the proportion of dissipation in the form of ineffective heat. The synergistic effect of heat dissipation and the xanthophyll cycle protect the normal physiological functioning of PS II so that the proportion of the inactive reaction center decreased under NaCl, providing a guarantee of normal functioning of photosynthesis.

To understand how increased nitrogen helped alleviate the extent of damage to the PS II reaction center in leaves under NaCl stress, OJIP curves were standardized by O–P, O–J, and O–K to specifically analyze the changes of V_J , V_L , V_K , and V_L . The increase of V_J is a sign of blocked transport from Q_A to Q_B (Govindjee 1995; Zhang et al. 2016). The appearance of point I was related to the transport process from Q_A^- to Q_B (Strasser et al. 1995). The increase of V_K was related to the damage of an oxygen-evolving complex (OEC) and the increase of V_L was an important sign of thylakoid membrane dissociation (Strasser 1997). Under NaCl stress, V_J , V_I , V_K , and V_L of poplar seedling leaves increased compared with the controls (CK), indicating that the stress not only inhibited the normal water splitting function of OEC on the PS II donor side but also reduced electron transport on the receptor side. In addition, NaCl resulted in changes to the thylakoid membranes of poplar leaves which might be related to the blocked electron transmission caused by the leakage of electrons in the electron transport chain, the leakage electron attacked the free oxygen molecules, which could produce superoxide anion and reactive oxygen. The increase of those reactive oxygen molecules led to the peroxidation of thylakoid membrane. (Reddy et al. 2004). Increased nitrogen reduced the variable fluorescence of each point in leaves, suggesting that nitrogen can promote photosynthetic electron transport of poplar leaves under stress, and play an important role in protecting the oxygen-evolving complex and alleviating peroxidation damage of thylakoid membranes.

Conclusions

Under NaCl stress, photosynthesis declined; the photochemical activity of the PS II reaction center decreased and the electron donor and receptor side of PS II were affected to different degrees. Increased nitrogen alleviated saline damage to poplar seedlings under NaCl stress, improved photosynthesis, reduced stomatal limitations, and increased CO_2 utilization by mesophyll cells. In addition, under NaCl stress, increased nitrogen enhanced the functioning of the oxygen-evolving complex on the PS II electron donor side and the ability to accept electrons on the receptor side. Nitrogen increased the stability of the thylakoid membrane structure and alleviated the degree of damage to the PS II reaction center of poplar leaves.

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