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Effects of exogenous abscisic acid on the photosynthetic function and reactive oxygen species metabolism of tobacco leaves under drought stress

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Abstract

In this study, water solutions with different concentrations of abscisic acid (ABA) (10, 20, and 40 μmol L^{-1}) were sprayed on leaves of drought-stressed tobacco seedlings. The results showed that under drought stress, both water content and photosynthetic carbon assimilation ability were reduced, PSII activity was also inhibited. Drought stress also led to the outbreak of reactive oxygen species in tobacco leaves and increased the degree of membrane peroxidation. However, spraying exogenous ABA significantly alleviated the damage caused by drought stress on the leaves. The potential mechanisms by which drought resistance in tobacco is improved by spraying exogenous ABA include: (*1*) regulation of the stomatal limitation of leaves, which is beneficial to water retention ability and can enhance photosynthetic function; (*2*) reduction of the energy pressure of the PSII reaction center by increasing nonphotochemical quenching (NPQ); and (*3*) effective reduction of the production of reactive oxygen species in the leaves and reducing the degree of membrane peroxidation.

Additional key words: chlorophyll fluorescence; oxidative damage; photoinhibition.

Introduction

Drought stress is an important limiting factor in agricultural production. At present, in the context of global climate change, drought occurs frequently, leading to declines in crop yield and quality. Plant photosynthesis is a drought-sensitive process; in the initial stage of drought stress or under mild drought stress conditions, the inhibition of plant photosynthesis is usually a combined result of stomatal and nonstomatal factors (Xu *et al.* 2017). After stomata are closed, water loss is reduced, but stomatal closure also leads to a decline in intercellular CO$_2$ concentration (C_i) and photosynthetic rate (P_N). Prolonged drought stress causes irreversible damage to the photosynthetic apparatus of plants *via* the degradation of photosynthetic pigments (Nikolaeva *et al.* 2010) and the inhibition of photosynthetic phosphorylation and electron transport (Albert *et al.* 2011, Guadagno *et al.* 2017, Chen *et al.* 2018, Zhang *et al.* 2018a). Under drought stress, the content of reactive oxygen species (ROS) in plant cells sharply increases. In this case, an excessive amount of ROS breaks the redox balance in plants, resulting in membrane peroxidation and destruction of the membrane system, and eventually causes oxidative damage to the cellular components and structures (Ramachandra *et al.* 2004, Asada 2006, Gill and Tuteja 2010, Wang *et al.* 2017). Plant PSII is one of the most sensitive sites to drought stress and is closely associated with multiple photosynthesis-related physiological processes such as light energy absorption, water photolysis, and electron transfer (Allahverdiyeva

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Abbreviations: ABA – abscisic acid; C_i – intercellular CO$_2$ concentration; E – transpiration rate; ETR – electron transfer rate; F_J – chlorophyll fluorescence intensity at the point J (2 ms); F_M – chlorophyll fluorescence intensity at the point P (1,000 ms); F_O – chlorophyll fluorescence intensity at the point O (0.01 ms); $F_{O\text{-}P}$ – OJIP curve; F_V/F_M – PSII maximum photochemical efficiency; g_s – stomatal conductance; MDA – malondialdehyde; NPQ – nonphotochemical quenching; PI_{ABS} – absorption-based photosynthetic performance index; P_N – net photosynthetic rate; ROS – reactive oxygen species; RWC – relative water content; V_J – the relative variable fluorescence transient at point J (2 ms);V_K – the relative variable fluorescence transient at point K (0.3 ms); $V_{O\text{-}J}$ – OJIP curves were normalized as $V_{O\text{-}J} = (F_t – F_O)/(F_J – F_O)$; $V_{O\text{-}P}$ – OJIP curves were normalized as $V_{O\text{-}P} = (F_t – F_O)/(F_M – F_O)$.

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et al. 2013, Chen *et al.* 2017). To maintain normal growth and improve the stress resistance of plants, it is of vital significance to maintain normal photosynthesis in leaves under drought stress, especially the stability of PSII.

Abscisic acid (ABA) is an endogenous plant hormone that is involved in controlling plant growth, inhibiting seed germination, and promoting senescence (Guo *et al.* 2014, Hir *et al.* 2017). In addition, ABA also plays an important role in the stress responses to drought, high salinity, and low temperature, and acts as a signal to trigger *in vivo* adaptive regulation (*i.e.*, inducing gene expression) of plants following the initial abiotic stress response. Therefore, ABA is considered a plant 'stress hormone' (Ijaz *et al.* 2017, Li *et al.* 2017, Lv *et al.* 2017). Stress conditions can dramatically upregulate the ABA content in plants. The ABA synthesized in plant roots constitutes a direct source of the ABA in plant leaves. To prevent dehydration under drought and other stress conditions, plants close stomata through an ABA-based mechanism to improve water utilization (Cai *et al.* 2017, Jalakas *et al.* 2017, Krugman *et al.* 2010).

Exogenous ABA can significantly improve a plant's resistance to stress (Loik and Nobel 1993). Previous research has shown that 67% of the *Arabidopsis* genes that have altered expression during drought stress are also regulated by exogenous ABA, indicating a close link between ABA and drought resistance in *Arabidopsis* (Huang *et al.* 2008). ABA can maintain the normal physiological metabolic functions, including sugar metabolism and enzyme activities, and also control the process of leaf senescence of plants under drought stress (Ruan *et al.* 2012). Under drought stress conditions, exogenous ABA treatment can increase carbohydrate accumulation in wheat during the flowering stage and facilitate carbohydrate transport to the grain, significantly alleviating the stress effect of moderate drought on wheat and increasing the yield of dry land wheat (Nayyar and Kaushal 2002, Travaglia *et al.* 2007, 2010). For corn under drought stress during the flowering stage, spraying ABA can promote the synthesis of endogenous ABA, thus maintaining the relative water content and increasing the net photosynthetic rate of leaves under drought stress (Souza *et al.* 2013). Exogenous ABA can also enhance the water absorption capacity of plant roots by increasing osmotic regulation substances such as soluble sugar and proline (Li *et al.* 2010). Many studies have revealed that ABA can protect the physiological function of PSII (Saradhi *et al.* 2000) and increase the content of antioxidant enzymes in plant leaves (Jiang and Zhang 2004).

Tobacco is one of the main economic crops in Northeast China. However, in the process of transplanting tobacco seedlings into the field, they often encounter stressful conditions, such as soil and atmospheric drought, which affect their survival rate. Therefore, increasing the resistance of tobacco seedlings to drought stress is the key to ensuring the normal survival and growth of tobacco seedlings after transplanting. However, previously published studies have rarely explored how exogenous ABA affects the physiological function of tobacco seedlings under drought stress conditions, especially the regulatory mechanism of ABA on the function of PSII. In this study, by spraying different concentrations of exogenous ABA on leaves, we investigated how ABA affects the function of PSII in tobacco leaves under drought stress conditions. The purpose of this study was to obtain basic data that could be used to improve the drought resistance of tobacco.

Materials and methods

Test materials and treatment: The experiment was carried out in the Plant Physiology Laboratory of Northeast Forestry University in 2016. The tobacco cultivar used was 'Longjiang 911', a major variety popularized in Heilongjiang Province, China; the seeds were provided by the Heilongjiang Tobacco Research Institute. Plants were seeded in early March 2016, and the culture medium consisted of peat soil and quartz sand in a ratio of 2:1 (v/v). Plants were cultivated in an artificial climate chamber with a temperature of 25/23°C (light/dark), a PAR intensity of 400 μmol(photon) m^{-2} s^{-1}, a photoperiod of 12/12 h (light/dark), and a relative humidity of approximately 75%. Watering and seedling management were done following the common methods used in the region. After the seedlings grew to four leaves, they were watered once per week with 1/2 Hoagland nutrient solution. When the seedlings had six leaves, they were transplanted and irrigated once before transplanting, so that the relative water content of the soil was saturated. The seedlings were transplanted into a culture pan (one seedling per pan) with a diameter of 12 cm and a height of 15 cm. The culture medium was peat soil with good water retention.

Seedlings with the same growth characteristics were selected as the test subjects. At 16:00 h daily, leaves of the treatment group were sprayed with 0, 10, 20, and 40 μmol(ABA) L^{-1} solutions (referred to as D, D+ABA_{10}, D+ABA_{20}, and D+ABA_{40}); water was sprayed on the leaves of control group plants under normal-watered conditions (referred to as CK). A fine mist solution was sprayed both on the front and back of leaves until uniform, small liquid beads were about to drip from the leaf surface. For each treatment, ten seedlings were prepared. After spraying with ABA, we waited until the blade surface dried through natural evaporation. Then, to guarantee the full absorption of ABA sprayed on the blade surface, the same ABA solution was sprayed once again following the same method. To mimic drought stress, seedlings were not watered. After 7 d, soil moisture content declined from 85.6% before treatment to 38.7% after treatment. When the phenotypes of the treatment and control groups were significantly different, we measured a series of physiological parameters.

Photosynthetic gas-exchange parameters: The second fully expanded leaf from the top was selected from each treated tobacco seedling. The concentration of CO_2 was fixed at 400 μL L^{-1} by using a CO_2 cylinder. Using the built-in light, the PAR was set to 1,000 μmol(photon) m^{-2} s^{-1}. For each treatment, tobacco leaves were measured for the following parameters: net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i). In the measurement process, PAR of 1,000 μmol(photon) m^{-2} s^{-1} was used for treatment for 3–5 min; when photosynthetic gas-exchange parameters became stable, data were recorded. All parameters were measured five times.

The electron transfer rate (ETR) and nonphotochemical quenching (NPQ) of PSII reaction centers under light adaptation were determined using a portable pulse-modulated fluorometer *FMS-2* (*Hansatech*, UK). After 30 min of dark adaptation, the leaves of tobacco seedlings were measured using the *FMS-2* instrument with saturated pulsed light of 8,000 μmol(photon) m^{-2} s^{-1}, and the maximum fluorescence (F_M) was determined. In this procedure, the leaf adapts to the light for the first 30 s, and the light intensity was the same as the ambient light intensity before the measurement began [PAR of 1,000 μmol(photon) m^{-2} s^{-1}], then, saturated pulsed light was turned on for 8,000 μmol(photon) m^{-2} s^{-1} for 0.7 s. The maximum fluorescence (F_M') and steady-state fluorescence (F_s) under light-adapted conditions were measured. $ETR = 0.5 \times 0.85 \times (F_M' - F_s)/F_M' \times PAR$, $NPQ = (F_M - F_M')/F_M'$. For each treatment, ETR and NPQ were measured five times as described by Zhang *et al.* (2018b). Each measurement was repeated three times.

Chlorophyll (Chl) fluorescence and kinetics parameters: The second fully expanded leaf from the top of the tobacco seedlings was collected. After 30 min of dark adaptation, the OJIP curve of each dark-adapted leaf was determined using *Handy-PEA* (*Hansatech*, UK), and normalized using $V_{O\text{-}P} = (F_t - F_O)/(F_M - F_O)$ and $V_{O\text{-}J} = (F_t - F_O)/(F_J - F_O)$ according to the method proposed by Tsimilli-Michael and Strasser (2013); F_t represents the Chl fluorescence intensity at each time point. Subsequently, the relative variable fluorescence (V_J and V_K) at point J (2 ms) and V_J and V_K at point K (0.3 ms) were obtained, and the difference between the normalized $V_{O\text{-}P}$ and $V_{O\text{-}J}$ curves of the treated leaves and the control was calculated, as represented by $\Delta V_{O\text{-}P}$ and $\Delta V_{O\text{-}J}$, respectively. Two Chl fluorescence parameters, PSII maximum photochemical efficiency (F_V/F_M) and light energy absorption-based photosynthetic performance index (PI_{ABS}), were obtained through a JIP-test analysis on the OJIP curve. Each measurement was repeated three times.

The moisture content of leaves was determined by drying the leaves, and then the rate of yield of superoxide anions ($O_2^{\bullet-}$) was determined by the hydroxylamine hydrochloride method (Wang and Luo 1990). H_2O_2 content was determined by the titanium tetrachloride precipitation method (Lin *et al.* 1988). The malondialdehyde (MDA) content was determined using the thiobarbituric acid chromogenic method (Wang *et al.* 2003). The electrolyte leakage rate was measured using a conductivity meter *(DDS-11C*) and was represented by relative conductivity. Each indicator was measured three times.

Statistical analysis was performed using *Excel* and *SPSS* (*22.0*) software. The data in the figure are presented as the mean ± standard deviation (SE) from three replicates, and one-way analysis of variance (*ANOVA*) and the least significant difference (LSD) tests were used to compare the differences between different datasets.

Results

Plant morphology and leaf relative water content: Under drought stress, the leaves of tobacco seedlings showed obvious symptoms of water loss and wilting; older leaves were yellow (Fig. 1). However, spraying different concentrations of exogenous ABA alleviated the wilting of tobacco leaves. The moisture content of the tobacco leaves under drought stress was reduced by 18.8% compared with CK, which is an extremely significant ($P<0.01$) difference (Fig. 2). However, spraying different concentrations of exogenous ABA significantly alleviated the reduction of the moisture content of leaf under drought stress conditions when compared with the control. Specifically, after spraying leaves with 10, 20, and 40 μmol L^{-1} of exogenous ABA under drought stress, the water content increased by

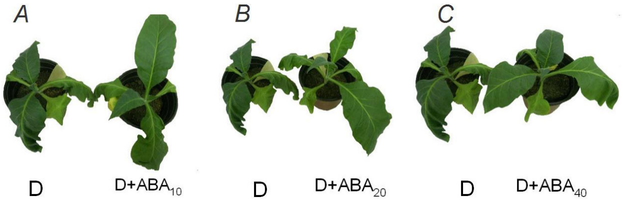

Fig. 1. Effects of different concentrations of exogenous abscisic acid (ABA) on the plant morphology of tobacco seedling leaves under drought stress. D is the drought treatment. $D+ABA_{10}$, $D+ABA_{20}$, and $D+ABA_{40}$ indicate 10, 20, and 40 μmol(ABA) L^{-1} treatments under drought stress, respectively.

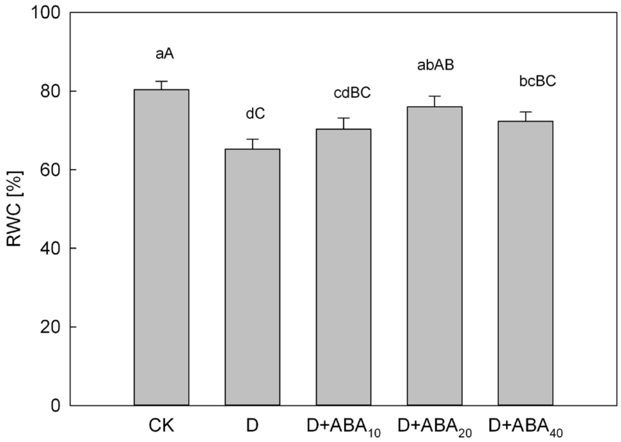

Fig. 2. Effects of different concentrations of exogenous abscisic acid (ABA) on the relative water content (RWC) of tobacco seedling leaves under drought stress. Significant differences were expressed by *different small letters* ($P<0.05$), and very significant differences were expressed by *different capital letters* ($P<0.01$). CK is the nonstressed control. D is the drought treatment. $D+ABA_{10}$, $D+ABA_{20}$, and $D+ABA_{40}$ indicate 10, 20, and 40 μmol(ABA) L^{-1} treatments under drought stress, respectively. Values are means ± SE ($n = 3$).

3.0, 11.3, , and 5.9%, respectively, when compared with the control.

Photosynthetic gas-exchange parameters: The values of P_N, g_s, and E of tobacco leaves were significantly reduced under drought stress when compared with the CK, but spraying different concentrations of exogenous ABA significantly alleviated such reductions (Fig. 3*A*–*C*). P_N and g_s showed significant differences after spraying with 10 and 20 µmol(ABA) L^{-1}, whereas no significant difference was observed for E. However, the 40 µmol(ABA) L^{-1} treatment did not cause a significant change in these variables. Spraying different concentrations of exogenous ABA also significantly alleviated the decrease of the C_i of tobacco leaves under drought stress, but the difference between 20 µmol(ABA) L^{-1} treatment and the CK was not significant (Fig. 3*D*).

ETR and NPQ: The ETR of tobacco seedling leaves decreased significantly under drought stress, while the NPQ increased significantly (Fig. 4). Notably, spraying different concentrations of ABA significantly alleviated the decrease of ETR in tobacco leaves, but the leaves treated by different concentrations of ABA did not show a significant difference in ETR. Treatment with different concentrations of ABA increased the NPQ of tobacco leaves under drought stress, but the difference between ABA treatments was not significant. After treatment with 20 or 40 µmol(ABA) L^{-1}, the NPQ was significantly higher than that of the control.

OJIP curve: Compared with the CK, the relative fluorescence intensity of leaves under drought stress showed no significant difference at point O or point J, while the intensity at points I and P showed a significant decrease, especially for point P (Fig. 5). However, treating leaves with different concentrations of exogenous ABA alleviated the changes of the OJIP curve of tobacco seedlings under drought stress.

Photochemical efficiency of PSII: The F_V/F_M of tobacco leaves under drought stress decreased slightly compared with CK, but the difference was not significant (Fig. 6*A*). Yet, PI_{ABS} decreased by 52.4% compared with CK and the difference was extremely significant (Fig. 6*B*). Beside from the 40 µmol(ABA) L^{-1} treatment, all the other treatments significantly alleviated the decrease in F_V/F_M and PI_{ABS} of leaves under drought stress. Notably, tobacco leaves treated with 20 µmol(ABA) L^{-1} showed a 73.2% higher PI_{ABS}.

Normalized O-P curve, V_J, and V_I: Among the points on the $V_{O\text{-}P}$ curve of tobacco leaves under drought stress, the relative variable fluorescence at point J (2 ms) increased

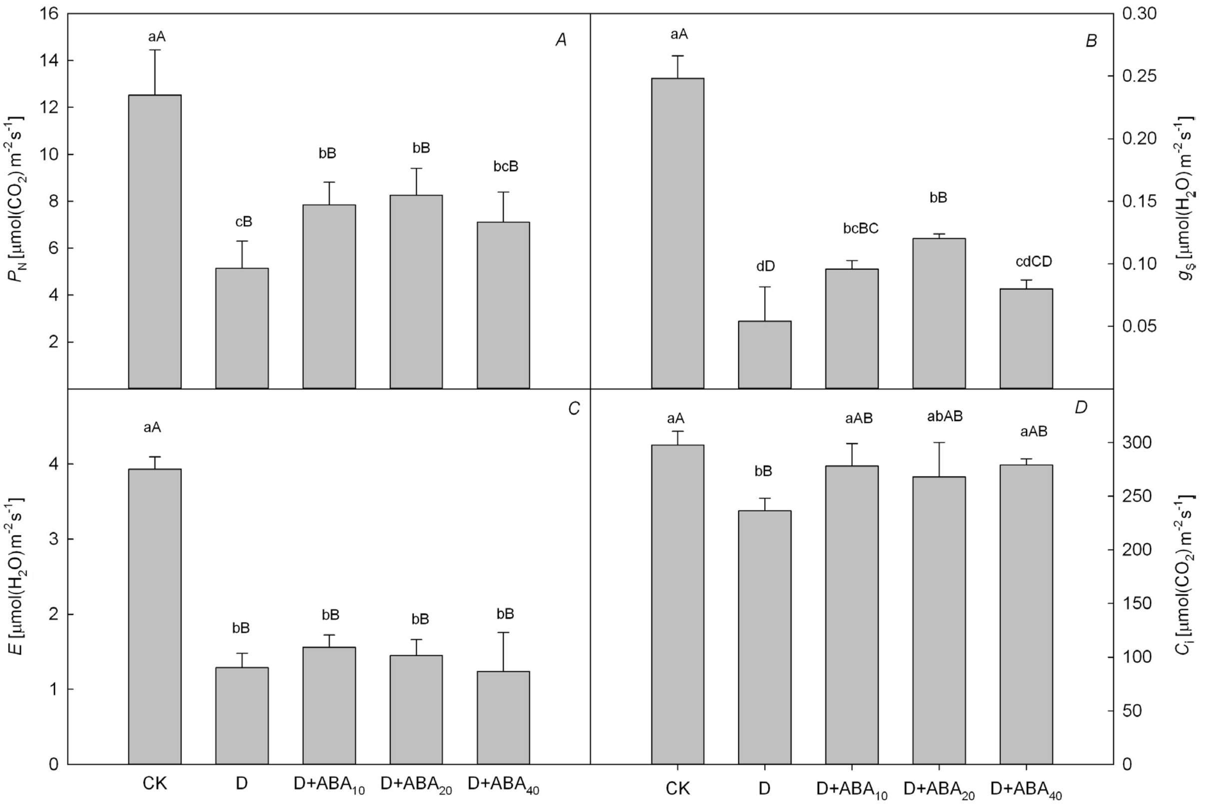

Fig. 3. Effects of different concentrations of exogenous abscisic acid (ABA) on the net photosynthetic rate (P_N) (*A*), stomatal conductance (g_s) (*B*), transpiration rate (*E*) (*C*), and intercellular CO_2 concentration (C_i) (*D*) in tobacco seedlings under drought stress. Significant differences were expressed by *different small letters* ($P<0.05$), and very significant differences were expressed by *different capital letters* ($P<0.01$). CK is the nonstressed control. D is the drought treatment. D+ABA_{10}, D+ABA_{20}, and D+ABA_{40} indicate 10, 20, and 40 µmol(ABA) L^{-1} treatment under drought stress, respectively. Values are means ± SE (n = 5).

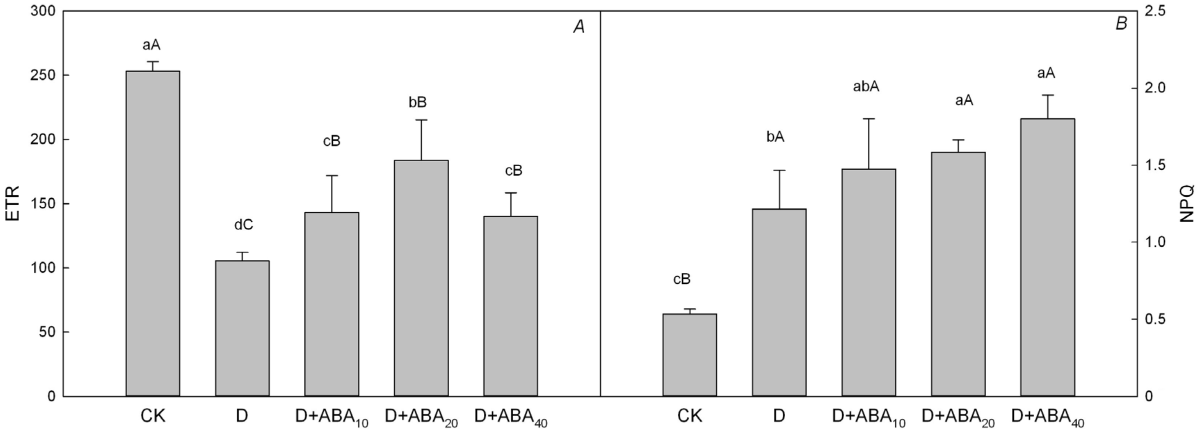

Fig. 4. Effects of different concentrations of exogenous abscisic acid (ABA) on the electron transfer rate (ETR) (*A*) and nonphotochemical quenching (NPQ) (*B*) of tobacco seedlings under drought stress. Significant differences were expressed by *different small letters* ($P<0.05$), and very significant differences were expressed by *different capital letters* ($P<0.01$). CK is the nonstressed control. D is the drought treatment. D+ABA$_{10}$, D+ABA$_{20}$, and D+ABA$_{40}$ indicate 10, 20, and 40 µmol(ABA) L^{-1} treatments under drought stress, respectively. Values are means ± SE ($n = 3$).

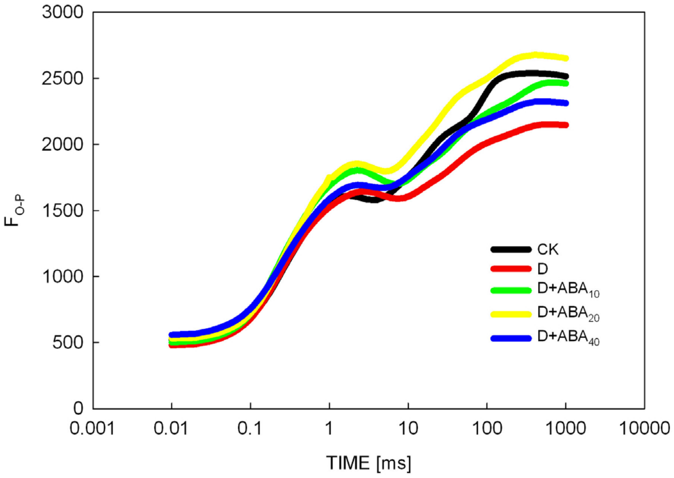

Fig. 5. Effects of exogenous abscisic acid (ABA) on the OJIP curve of tobacco seedlings leaves under drought stress. CK is the nonstressed control. D is the drought treatment. D+ABA$_{10}$, D+ABA$_{20}$, and D+ABA$_{40}$ indicate 10, 20, and 40 µmol·L^{-1}ABA treatments under drought stress, respectively.

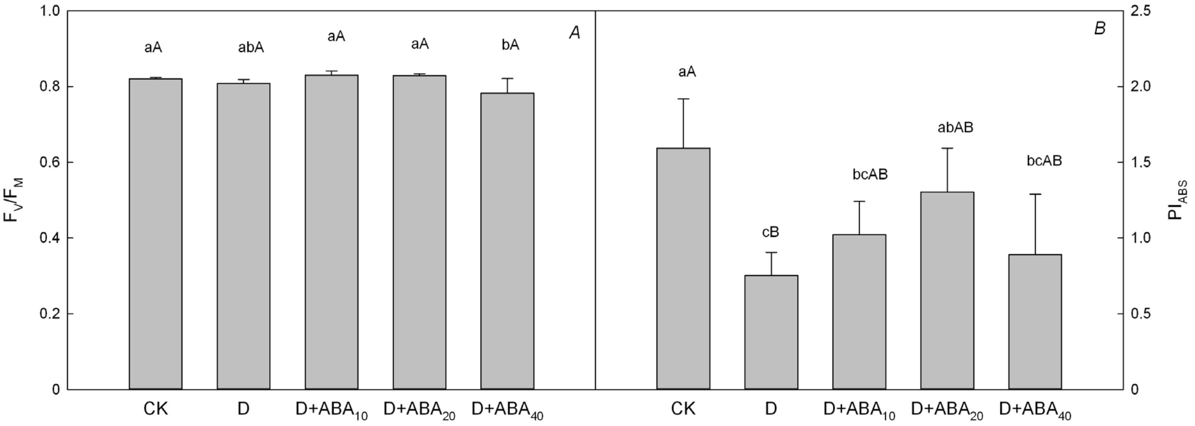

Fig. 6. Effects of exogenous abscisic acid (ABA) on the PSII maximum photochemical efficiency (F_V/F_M) (*A*), and absorption-based photosynthetic performance index (PI_{ABS}) (*B*) of tobacco seedlings leaves under drought stress. Significant differences were expressed by *different small letters* ($P<0.05$), and very significant differences were expressed by *different capital letters* ($P<0.01$). CK is the nonstressed control. D is the drought treatment. D+ABA$_{10}$, D+ABA$_{20}$, and D+ABA$_{40}$ indicate 10, 20, and 40 µmol(ABA) L^{-1} treatments under drought stress, respectively. Values are means ± SE ($n = 3$).

significantly when compared with the CK (Fig. 7*A*,*C*). By calculating the difference between the $V_{O\text{-}P}$ curve of the treatments and CK, it was clear that the V_J increase was always compromised by applying exogenous ABA. The variation of the variable fluorescence at point K (at 0.3 ms) was minor on the normalized O-J curve and was not significantly affected by treatment with ABA (Fig. 7*B*,*D*).

Quantitative analysis of V_J and V_K (Fig. 8*A* and 8*B*, respectively) revealed that the V_J of tobacco seedling leaves under drought stress increased by 26.4% ($P<0.01$) compared with the CK. Treatment with 20 and 40 μmol(ABA) L^{-1} increased the V_J by 14.1 and 18.4%, respectively. The V_K of tobacco seedling leaves under drought stress showed no significant change compared with the CK, and spraying different concentrations of ABA did not significantly alter the V_K of tobacco leaves.

Active oxygen and membrane peroxidation levels: Compared with the CK, the rate of $O_2^{\bullet-}$ production (Fig. 1S*A*, *supplement*), H_2O_2 content (Fig. 1S*B*), MDA content (Fig. 1S*C*), and electrolyte leakage rate (Fig. 1S*D*) of tobacco seedling leaves under drought stress presented similar increasing trends. However, spraying different concentrations of exogenous ABA under drought stress alleviated this increase. In particular, spraying with 20 μmol(ABA) L^{-1} showed the most dramatic effect, especially on the rate of $O_2^{\bullet-}$ production and H_2O_2 content, which decreased by 17.0 and 36.7%, respectively, in comparison with the CK. The changes further caused decreases in MDA content and electrolyte leakage rate (indicators of membrane peroxidation levels) by 37.1 and 35.4%, respectively.

Discussion

The ability of tobacco leaves to assimilate carbon from photosynthesis is restricted under drought stress, which is reflected by reduced P_N, g_s, E, and C_i values. According to the theory of photosynthetic stomatal factors proposed by Farquhar and Sharkey (2003), the decrease of the ability of tobacco seedlings to assimilate photosynthetic carbon under drought stress is directly related to the decrease of stomatal conductance. Although the decrease of stomatal conductance under drought stress can effectively prevent water loss, it also directly reduces the supply of CO_2, a source for carbon assimilation in tobacco leaves, thereby limiting their carbon assimilation capacity. Some studies have shown that plant stomatal closure under drought stress can induce roots to synthesize a large amount of ABA in response to drought stress. Under drought conditions, ABA can promote ion flux and lower the osmotic pressure of guard cells. Consequently, stomatal closure occurs,

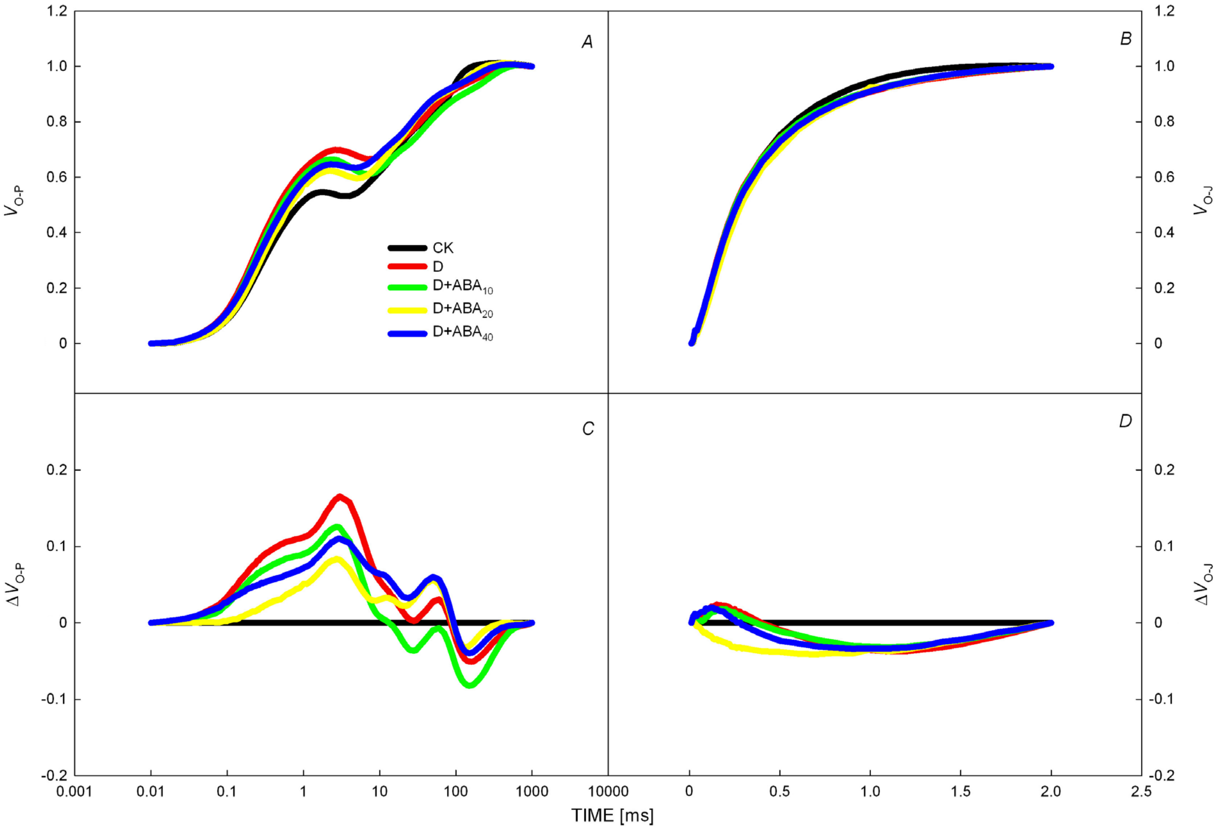

Fig. 7. Effects of exogenous abscisic acid (ABA) on the normalized O-P curve (*A*), normalized O-J curve (*B*), difference normalized O-P curve between ABA treatment and CK (*C*), difference normalized O-J curve between ABA treatment and CK (*D*). CK is the nonstressed control. D is the drought treatment. $D+ABA_{10}$, $D+ABA_{20}$, and $D+ABA_{40}$ indicate 10, 20, and 40 μmol·L^{-1}ABA treatments under drought stress, respectively.

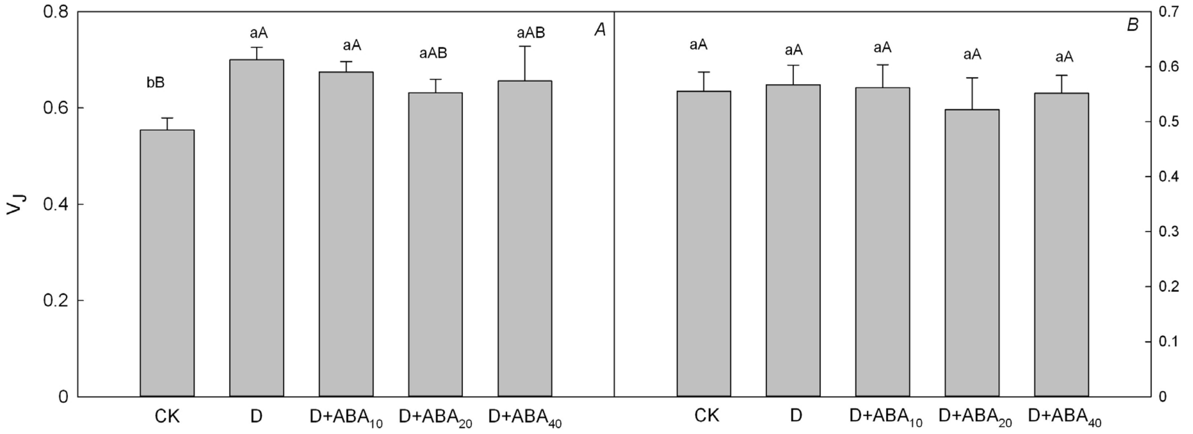

Fig. 8. Effects of exogenous abscisic acid (ABA) on the relative variable fluorescence transient at point J (V_J) (*A*) and the relative variable fluorescence transient at point K (V_K) (*B*) of tobacco seedlings leaves under drought stress. Significant differences were expressed by *different small letters* ($P<0.05$), and very significant differences were expressed by *different capital letters* ($P<0.01$). CK is the nonstressed control. D is the drought treatment. D+ABA$_{10}$, D+ABA$_{20}$, and D+ABA$_{40}$ indicate 10, 20, and 40 μmol·L^{-1}ABA treatments under drought stress, respectively. Values are means ± SE (*n* =3).

thereby reducing water loss and improving the water retention capacity of plants under drought conditions (Chen *et al.* 2006, Yu *et al.* 2016a,b). However, the closure of stomata also causes a decrease in the intercellular CO_2 concentration, resulting in a decline in photosynthetic capacity (Kano *et al.* 2011).

In this study, the P_N of seedling leaves treated with different concentrations of exogenous ABA increased to varying extents, which is similar to the change of g_s; these changes were accompanied by the increase of C_i, suggesting that the application of exogenous ABA could improve the photosynthetic capacity of tobacco leaves by alleviating stomatal closure. However, the effects of different concentrations of exogenous ABA on the *E* of tobacco seedlings were not significantly different, which played an important role in effectively reducing water loss in tobacco seedlings and increasing the water retention rate of leaves. The phenotype and leaf moisture content (Figs. 1, 2) also showed that spraying different concentrations of exogenous ABA could significantly alleviate the wilting of tobacco leaves under drought stress and increase their water content. Therefore, the decrease of photosynthetic carbon assimilation of tobacco seedlings under drought stress after treatment with ABA might be related to the high leaf moisture content induced by exogenous ABA. Souza *et al.* (2013) showed that under drought stress, spraying ABA on maize could promote endogenous ABA synthesis, maintain leaf relative water content, and increase leaf net photosynthetic rate. Studies have also found that exogenous ABA can induce the expression of the dehydrin gene (Wang *et al.* 2002, Boominathan *et al.* 2004), which is often related to plant stress resistance (Allagulova *et al.* 2003). Exogenous ABA can also enhance the water absorption capacity of plant roots by increasing osmotic regulation substances such as soluble sugar and proline (Li *et al.* 2010).

Under drought stress, the dark reaction is inhibited, and the accumulation of energy substances (ATP and NADPH) inhibits the photoreaction process through a feedback mechanism, resulting in excess electrons in the photosynthetic electron transport chain. In addition, the decrease of PSII activity also leads to the suppression of light energy absorption and an inhibited electron transfer process (Liu *et al.* 2006). In this study, although the F_V/F_M of tobacco leaves was not significantly changed under drought stress, significant decreases were observed in both ETR and PI_{ABS}. F_V/F_M and PI_{ABS} are important indicators of the photochemical activity of PSII, and PI_{ABS} is significantly more sensitive to change than F_V/F_M (Kalaji *et al.* 2014, Zhang *et al.* 2019a). Therefore, the results of this experiment showed that the photochemical activity of PSII in tobacco seedling leaves was inhibited under drought stress, and the PSII electron transport process was also compromised. However, the treatment of tobacco seedling leaves with different concentrations of exogenous ABA alleviated the decrease of PSII photochemical activity to varying extents, indicating that the application of exogenous ABA could alleviate the photoinhibition of tobacco seedling leaves under drought stress and promote electron transfer. In particular, 20 μmol(ABA)·L^{-1} had a more significant effect. Under adverse conditions, plant photosynthetic electron transport is often blocked on the electron donor and acceptor sides of the PSII reaction center; the transfer of Q_A to Q_B is the main inhibitory site (Zhang *et al.* 2019b). The increase in the relative variable fluorescence V_J at point J (2 ms) on the normalized O-P curve indicated that the electron transfer from Q_A to Q_B in the photosynthetic electron transport chain was blocked (Zhang *et al.* 2017, 2018c). The increase in the relative variable fluorescence V_K at point K (0.3 ms) on the normalized O-J curve was considered a specific marker of the damage to the activity of oxygen-evolving complex on the PSII electron donor side (Zhang *et al.* 2012, 2016). In this experiment, the V_J value of tobacco leaves increased significantly under drought stress, while the V_K did not change significantly, indicating that the decrease of PSII photosynthetic electron transport rate in leaves under drought stress mainly occurred on the PSII acceptor side. However, the change of V_K was affected by both the damage to the donor side of PSII and by damage to its acceptor side. When the degree of damage to the acceptor side was greater than the degree of damage to the donor side, V_K would no longer significantly increase (Xu *et al.* 2018, Zhang *et al.* 2018d). Therefore, in this study, the effect of PSII on the donor side under drought stress might have been caused by the insensitivity of the oxygen-evolving complex to drought stress or by excessive damage on the acceptor side. The application of different concentrations of exogenous ABA significantly attenuated the increase of V_J in tobacco seedling leaves; however, the effect on V_K was not significant, indicating that exogenous ABA could promote electron transfer from Q_A to Q_B on the PSII acceptor side of tobacco leaves under drought stress. The transfer of electrons from Q_A to Q_B was blocked mainly due to the synthesis and turnover of the D1 protein (Zhang *et al.* 2011), of which the synthesis is primarily regulated by *psbA* (Erinle *et al.* 2018). Some studies have found that exogenous ABA can promote the expression of *psbA* in wheat chloroplasts under drought stress (Wang *et al.* 2011). Thus, the promoting effect of ABA on the electron transport of the PSII acceptor side in tobacco leaves under drought stress might be explained by the protection of the D1 protein.

Studies have shown that antheraxanthin and zeaxanthin content have a significant positive correlation with energy dissipation (Eskling *et al*. 1997). According to Ruban and Horton (1999) and Frank *et al.* (1994), *in vitro* zeaxanthin can directly quench the excited state of Chl, which is an important mechanism that protects the photosynthetic apparatus from excessive light energy damage (Demmig-Adams and Adams 1996, Pieters *et al.* 2003). NPQ is positively correlated with the heat dissipation dependent on the xanthophyll cycle (Li *et al.* 2000). Under drought stress, the NPQ of tobacco seedlings increases significantly, suggesting that plants under drought stress can dissipate excess excitation energy in the PSII by increasing NPQ, thereby reducing the pressure on the PSII reaction center. Under drought conditions, the NPQ of tobacco seedlings treated with exogenous ABA increased to varying extents compared with the tobacco not treated with ABA. ABA in higher plant cells is mainly synthesized by the degradation of zeaxanthin, which is opposite to the direction from xanthophylls to zeaxanthin when the xanthophyll cycle occurs (Zeevaart and Creelman 1988, Milborrow 2001). These two processes, ABA synthesis and the formation of zeaxanthin in the xanthophyll cycle, share the same precursor, violaxanthin. The two processes have a competitive relationship, so an increased ABA content may inhibit the conversion of violaxanthin to ABA through a feedback mechanism, promoting the conversion from violaxanthin to zeaxanthin and enhancing the xanthophyll cycle (Mayaba *et al.* 2001). Ivanov *et al.* (1995) found that treating barley seedlings with ABA could enhance the process of xanthophyll cycle, thereby strengthening the openness of the PSII reaction center and promoting the utilization of light energy. Accordingly, spraying tobacco seedlings with exogenous ABA under drought stress relieved the stress on PSII, which may be because of an ABA-induced energy dissipation mechanism in the xanthophyll cycle.

Drought conditions typically lead to excessive reduction of plant photosynthetic electron transport chains, and the production of large amounts of ROS in chloroplasts and mitochondria (Ahmed *et al.* 2009). Excess ROS negatively impact the redox balance in plants, causing peroxidation of phospholipids, resulting in destruction of the membrane system, and causing oxidative damage to cellular components and structures (Gill and Tuteja 2010). Under drought stress conditions, the $O_2^{\bullet-}$ production rate and H_2O_2 content in tobacco seedling leaves significantly increased, which was accompanied by the increase of the rate of electrolyte leakage and MDA content, suggesting that excess ROS caused the peroxidation of the membrane system and increased membrane permeability. However, treatment with different concentrations of exogenous ABA significantly alleviated the degree of peroxidation and the ROS content. Treatment with 20 μmol(ABA)$\cdot$L^{-1} had the most significant effect, consistent with the data on other photosynthetic parameters. Spraying exogenous ABA could reduce ROS yield by enhancing the photosynthetic capacity of tobacco leaves and reducing the degree of damage to PSII. A lower ROS content would also alleviate the photoinhibition of tobacco seedling leaves under drought stress. Studies have found that spraying exogenous ABA affects ROS scavenging under drought stress (Duan *et al.* 2013). For example, Jiang and Zhang (2001) showed that ABA could protect against oxidative stress by increasing the activity of ROS scavenging enzymes in maize leaves. Li *et al.* (2011) showed that spraying exogenous ABA on the leaves of *Astragalus* under drought stress could significantly increase the activity of catalase. In addition, studies have shown that ABA treatment can increase ascorbic acid content in tobacco BY-2 suspension cells (Bueno *et al.* 1998). Therefore, spraying ABA reduces ROS in leaves of tobacco seedlings and is not only related to the enhancement of photosynthetic capacity, but also associated with ABA-induced enzymatic or nonenzymatic function enhancements of the ROS scavenging system. However, this needs further study.

Conclusion: The photosynthetic capacity of tobacco seedling leaves decreased under drought stress mainly due to restriction by stomatal factors, the decrease of the photochemical activity of PSII, and the damage of the membrane system caused by ROS. Spraying different concentrations of exogenous ABA (10, 20, and 40 μmol L^{-1}) promoted the photosynthetic capacity of tobacco seedlings to varying extents under drought stress, and the 20 μmol$\cdot$L^{-1} of ABA treatment had the most significant effect. The main mechanism of this effect might be that the application of exogenous ABA effectively alleviated the stress on the PSII reaction center in tobacco seedling leaves under drought stress *via* inducing the increase of NPQ, thus alleviating the PSII photoinhibition and promoting the electron transfer rate. The electron transfer rate of the PSII acceptor side was promoted. Spraying exogenous ABA also effectively reduced ROS yield in tobacco seedlings leaves and alleviated the peroxidation of membrane lipids.

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