Na\(^+\) accumulation alleviates drought stress induced photosynthesis inhibition of PSII and PSI in leaves of *Medicago sativa*

Zhang Huihu\(^{a,b}\), Huo Yuzhe\(^b\), Guo Kaiwen\(^b\), Xu Zisong\(^b\), Shiqi Liu\(^b\), Qiao Wang\(^b\), Xuecong Wang\(^b\), Xu Nan\(^c\), Yining Wu\(^c\) and Sun Guangyu\(^a\)

\(^a\)Key Laboratory of Saline-alkali Vegetation Ecology Restoration, Ministry of Education, College of Life Sciences, Northeast Forestry University, Harbin, People’s Republic of China; \(^b\)College of resources and environment, Northeast Agricultural University, Harbin, People’s Republic of China; \(^c\)Natural Resources and Ecology Institute, Heilongjiang Sciences Academy, Harbin, People’s Republic of China

**ABSTRACT**

To probe the mechanism of action of NaCl in regulating drought adaptation in *Medicago sativa*, we pretreated *M. sativa* with different concentrations (0, 50, 100 and 200 mM) of NaCl, then studied the effects of NaCl pretreatment on water content, chlorophyll content, photosynthetic function, and membrane peroxidation of *M. sativa* plants subjected to drought stress. Although pretreatment with different concentrations of NaCl significantly increased the Na\(^+\) content in the aboveground of *M. sativa*, it had no significant effect on the activity of PSII and PSI. However, different concentrations of NaCl pretreatment significantly increased the water content of the aboveground of *M. sativa* under drought stress, alleviated the degree of photoinhibition of PSII and PSI, and maintained the flow and stability of donor and acceptor electrons in PSII. NaCl pretreatment promotes the increase of non-photochemical quenching (NPQ), which relieves oxidative damage in the leaves of *M. sativa* under drought stress, playing an important role in maintaining the activity of PSII and PSI. In conclusion, pretreatment with a certain concentration of NaCl can help *M. sativa* leaves maintain relatively high-water content and alleviate the photosynthesis inhibition of PSII and PSI under drought stress, thereby improving the drought resistance of *M. sativa*.

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Abiotic stresses such as drought and salt are the main environmental factors that affect plant growth, development and lead to crop yield reduction (Aroca et al. 2012). Of these various abiotic stresses, drought leads to the greatest loss in crop yield and drought-related losses exceed the losses caused by other stresses combined. Due to global warming, the global risk of drought will continue to increase (Gosling and Arnell 2016). In arid and semi-arid regions, evaporation far exceeds precipitation, resulting in a gradient of increasing salt concentration in the surface soil, creating an environment of dual stress from both drought and increased soil salt. Soil salinization not only leads to ion toxicity, but also decreases the water potential of the soil solution, generating osmotic stress that makes it difficult for plants to absorb water (Shaheen et al. 2013). Although high concentration of Na\(^+\) can damage plants (Parida and Das 2005), some studies show that low concentrations of salt have little effect on the growth and physiological functions of plants and can even promote the growth of a variety of plants (He et al. 2019).

One of the important ways by which plants adapt to drought stress is through osmotic regulation, which increases intracellular solute concentration through metabolic activities, reduces their osmotic potential and water potential, and maintains water absorption from the external environment to maintain cellular turgidity (Chen and Jiang 2010). Molecules used by plants for osmotic adjustment mainly include soluble sugar (Hong et al. 2000), proline (Sperdouli and Moustakas 2015), polyamine (Ruiz 2007; Evelin et al. 2009), betaine (Ashraf and Foolad 2007), and other organic compounds. In addition, inorganic substances also play an important role in osmotic balance. Under adverse conditions, plants can reduce their osmotic potential by absorbing salt ions to improve the ability of roots to absorb water. This indicates that the absorption and accumulation of salt may enhance the ability of plants to resist drought (Bai et al. 2012; Tan et al. 2013; Xie et al. 2015). K\(^+\) is an important component of osmotic balance in plants (Ahanger and Agarwal 2017; Zahoor et al. 2017) and its effects are especially pronounced under drought stress (Hosseini et al. 2016). Aside from K\(^+\), Na\(^+\) can also play a similar role as K\(^+\) to some extent when it comes to osmotic balance (Erel et al. 2014). Although excess Na\(^+\) is considered as a toxic ion, when adapting to salt stress, in addition to the salt-repelling effect of roots (Wang et al. 2006) and the salt secretion of salt glands (Maheshi and Larkin 2017), Na\(^+\) can be partitioned into vacuoles (Apse and Blumwald 2007), and the partitioned Na\(^+\) can be used for osmotic adjustment to reduce water potential and increase drought resistance (Kang et al. 2015; Cui et al. 2019). Relatively less energy is required for plants to use Na\(^+\) for osmotic regulation when compared with the energy required for the synthesis and accumulation of organic molecules, making regulation using Na\(^+\) more economical and efficient (Inès et al. 2007; Chen et al.)
et al. (2012) have found that a suitable concentration of NaCl can reduce the water potential of plants and improve their water status. In addition, other studies have found that mannitol can reduce the water potential of plants and improve their drought resistance (Zhao et al. 2019a). The ability to maintain photosynthesis under drought stress plays an important role in alleviating the yield and quality reduction in crop plants faced with drought. Drought conditions lead plants to significantly reduce the stomatal conductance of leaves to reduce water loss (Yi et al. 2016), and photosynthetic capacity is often also reduced with decreased stomatal conductance (Zhang et al. 2010). PSII and PSI are the main photosynthetic systems that are inhibited under adverse conditions (Zhang et al. 2020a, 2020b), and drought often leads to a decrease in the photochemical activity of PSII and PSI, resulting in the blockage of electron transport and the inhibition of energy production (ATP and NADPH) and carbon capture (Zhang et al. 2019b). Inhibition of photosynthesis under drought stress also results in excess production of reactive oxygen species (ROS), leading to increased levels of membrane peroxidation (Forni et al. 2017). But plants can alleviate photosynthesis inhibition under drought stress by enhancing the rapid turnover of D1 protein (Yang et al. 2014), CEF around PSI (Huang et al. 2018; Zhang et al. 2020c), state transitions in the photosystems (Wollman 2001; Depege et al. 2003), photorespiration (Hu et al. 2015; Messant et al. 2018), and xanthophyll cycle (Pieters et al. 2003; Ruban et al. 2010), and initiating the antioxidant system (Nie et al. 2007; Chao and Kao 2010). Because of this, maintaining an effective photosynthesis inhibition defense mechanism plays an important role in alleviating photosynthesis inhibition in plants under stress. Some studies have found that an appropriate concentration of NaCl can improve the hydration state and photosynthetic performance of plants by regulating their osmotic potential, thereby maintaining a higher plant growth rate (Ma et al. 2012; Liu et al. 2017). Accumulated Na+ in leaves can also enhance the photosynthetic function of plants and alleviate photosynthetic inhibition (Wakeel et al. 2011; Yue et al. 2012) by increasing ROS metabolism-related enzyme activities in plants (Lu 2003) and by improving stomatal movement (Yan et al. 2015). *Medicago sativa* is a perennial leguminous plant with high yield, high quality and is tolerant of frequent harvest. It has strong resistance to adverse conditions such as poor soil, high salinity, and drought, and has a pivotal place in livestock production and ecological management (Zhang et al. 2017). However, *M. sativa* has a strong, deep root system and requires a large amount of water. Drought stress often leads to a decline in its yield and quality (Hund et al. 2009). In China, *M. sativa* is often planted in arid and semi-arid areas that are non-ideal for agricultural use. In these areas, due to the large amount of surface evaporation, the soil is also affected by salinization in addition to severe water shortage. Does the salt in the soil aggravate or alleviate the drought resistance of alfalfa? Can *M. sativa* perform the necessary osmotic regulation brought on by drought stress through the accumulation of inorganic Na? Can salt pretreatment relieve photosynthesis inhibition of *M. sativa* caused by drought stress? If so, what type of regulation is used to produce this effect? In order to further explore the above questions, this study evaluated the effect of pretreatment with different concentrations of NaCl on ion and water status, PSII and PSI activity, and membrane peroxidation in *M. sativa* leaves under persistent drought stress to reveal the mechanism by which salt in soil affects plant drought resistance under persistent drought conditions.

1. Materials and methods

1.1. Experimental materials and treatment

The experiment was carried out in the Soil Science Laboratory of Northeast Agricultural University in 2019. The tested plant species was *Medicago sativa* CV. Zhaodong, and the seeds were provided by the Crop Research Institute of Heilongjiang Academy of Land Reclamation Sciences. Mature, full-bodied, and similarly sized seeds were place in a cultivation dish for germination. After the germ grew to approximately 0.5 cm, germinated seeds with relatively uniform growth were selected and grown in a 1:1:1 volume mixture of peat, perlite and vermiculite. Two plants were planted per container and the seed was covered with approximately 1 cm of soil. The culture was carried out in an incubator with a temperature of 25°C, an illumination intensity of 400 μmol-m⁻²-s⁻¹, and a photoperiod of 12/12 h (light/dark).

After the seedlings were grown for 30 days, the experiment was carried out. Pretreatment using different concentrations of NaCl was performed as follows: 2 L of 50, 100 and 200 mM NaCl solution were placed in 100 cm × 80 cm trays and the seedling culture vessels were immersed in a tray with nutrient solution for NaCl pretreatment. The control (CK) plants were soaked in distilled water and all samples were soaked for 24 h to absorb sufficient NaCl in the culture medium. Each treatment was performed in 3 trays, each tray contained 10 culture vessels. The different culture vessels were removed from the salt solution and watering was ceased to simulate drought conditions (denoted as drought 0 d). On drought days 0, 3, 6, and 9, various data were collected.

1.2. Assay parameters and methods

Determination of chlorophyll fluorescence induction curve (OJIP curve) and 820 nm optical reflection curve (MR820).

Fully developed leaves on *M. sativa* plants from different treatment groups were selected on the 0th, 3rd, 6th and 9th day of drought. After dark adaptation for 30 min, the M-PEA (Hansatch, UK) was used to determine the OJIP curve and the MR820 curve of each dark-adapted leaf. Five replicates were performed for each treatment. The curve was induced using far-red light of 3000 μmol-m⁻²-s⁻¹ and chlorophyll fluorescence signal was recorded within 1 s. The points O, J, I, and P on the OJIP curve represent the relative fluorescence intensities at 0.01, 2, 30, and 1000 ms, respectively, and are expressed as \( F_o \), \( F_h \), \( F_i \) and \( F_m \), respectively. The PSII...
maximum photochemical efficiency \((F_o/F_m)\) was calculated and used to characterize the photochemical activity of \(M. \ sativa\) leaf PSII, where \(F_o/F_m = (F_m - F_o)/F_m\). PSII activity is reflected by the relative drop in MR820 curve signal, i.e. \(ΔI/I_o\), where \(I_o\) is the maximum value of the reflected signal on the MR820 curve, and \(ΔI\) is the difference between the maximum and minimum values of the reflected signal on the MR820 curve (Zhang et al. 2018a).

For the specific analysis of the changes in the relative variable fluorescence \(V_j\) and \(V_k\) at points \(J\) (2 ms) and \(K\) (0.3 ms) on the OJIP curve, the OJIP curve was normalized to \(O-P\) and \(O-J\) according to the formula \(V_{O,P} = (F_i - F_o)/(F_m - F_o)\) and \(V_{O,J} = (F_i - F_o)/(F_m - F_o)\) to obtain the \(V_{O,P}\) and \(V_{O,J}\) curves (Zhang et al. 2016), where \(F_i\) is the relative fluorescence intensity at each time point on the OJIP curve. \(V_j\) was obtained from the \(V_{O,P}\) curve and \(V_k\) was obtained from the \(V_{O,J}\) curve, and the \(V_{O,P}\) and \(V_{O,J}\) curves of the different treatment groups were compared against those of the CK group, and the differences were expressed as \(ΔV_{O,P}\) and \(ΔV_{O,J}\) respectively.

**Determination of non-photochemical quenching (NPQ).** \(M. \ sativa\) leaves were dark-adapted for 30 min using dark-adaptation clamps. The initial fluorescence \(F_o\) and maximum fluorescence \(F_m\) of leaves from different NaCl treatment groups were determined using the FMS-2 (Hansatech, UK) instrument with saturated pulsed light of 8000 \(\mu\)mol-m\(^{-2}\)-s\(^{-1}\). Then, the light intensity of 1000 \(\mu\)mol-m\(^{-2}\)-s\(^{-1}\) was irradiated for 4.2 min with the instrument’s light source, and the maximum fluorescence \(F'_m\), minimum fluorescence \(F'_o\) and steady-state fluorescence \(F_s\) under light adaptation were measured, the photochemical quenching coefficient \(q_p\) and non-photochemical quenching (NPQ) were obtained, \((F'_m - F'_o)/(F'_m - F'_o)\cdot\text{NPQ} = (F_m - F_o)/(F_m - F_o)\) and the excess light energy \(1 - q_p)/\text{NPQ}\) was calculated. Each value was measured in triplicate and followed the method published by Zhang et al. (2020d).

**Determination of plant phenotype, biomass and physiological indicators.** \(M. \ sativa\) plants were photographed using a digital camera after different days of drought stress to observe their phenotypic characteristics. The content of malondialdehyde (MDA) was determined using the thiobarbituric acid colorimetric method (Wang et al. 2003) and was performed in triplicate. The seedlings were pulled from the culture medium, the roots were washed, and the fresh weight of the aboveground and the underground were taken after the entire plant was weighed. The aboveground and underground were dried in an oven at 80 °C until constant weight to obtain aboveground and underground biomass. Leaf water content was calculated as follows, leaf water content = (aboveground fresh weight - aboveground dry weight)/aboveground dry weight. The dried sample was pulverized and passed through a 40-mesh sieve. The mixture was dehydrated with concentrated \(H_2SO_4\)-\(H_2O_2\) and K and Na contents were determined by flame photometry (Bao 2005).

### 1.3. Data processing

Excel and SPSS (22.0) were used to analyze the measured data. All data were the mean ± standard error (SE) of three repetitions, and the differences among different treatments were compared by one-way ANOVA and LSD.

### 2. Results and analysis

#### 2.1. Plant phenotype and biomass

**Figure 1(A)** shows that on day 0 of drought stress, pretreatment with 50 and 100 mM NaCl had little effect on the morphology of \(M. \ sativa\) plants when compared with CK, but the plants pretreated with 200 mM NaCl showed some drooping of the middle and lower leaves. On the 3rd day of drought stress, the morphological differences were minor in the different treatment groups. On the 6th and 9th days of drought, the degree of wilting in the CK plants was markedly greater than that of the pretreated plants. Our observations show that pretreatment with 50, 100, or 200 mM NaCl can alleviate the drought damage symptoms in \(M. \ sativa\) plants and help to maintain relatively high water content in \(M. \ sativa\) leaves. The effect of pretreatment with 50 and 100 mM NaCl is greater than pretreatment using 200 mM NaCl.

**Figure 1(B,C)** show that the effects of NaCl pretreatment at different concentrations on the 0th and 3rd day of drought were not significant for the aboveground and underground biomass of \(M. \ sativa\), but on the 6th and 9th day of drought, although pretreatment resulted in no significant difference between the underground biomass when compared to the CK group, the aboveground biomass was higher than CK to different degrees, and this was especially pronounced on the 9th day of drought, when the aboveground biomass of the pretreated plants showed significantly higher biomass than that of CK.

Note: in the figure, 0, 50, 100 and 200 indicate NaCl pretreatment concentration (mM). Different uppercase letters indicate significant differences after different numbers of drought days \((P < 0.05)\). Different lowercase letters indicate significant differences between different concentrations of NaCl treatment with the same number of drought days \((P < 0.05)\). The same applies below.

#### 2.2. Leaf water content and leaf chlorophyll content

**Figure 2(A,B)** show that the water content of the leaves decreased slightly after pretreatment with different concentrations of NaCl (day 0 of drought), while the SPAD values increased to various extents, but did not show significant difference compared with CK. With increasing numbers of drought days, the leaf water content and SPAD value of \(M. \ sativa\) showed a decreasing trend. For the entire duration of drought stress, the difference in SPAD between leaves pre-treated with different concentrations of NaCl was not significant, but after 6 and 9 days of drought, the water content of \(M. \ sativa\) treated with different concentrations of NaCl was significantly higher than that of CK, especially in the 50 mM NaCl pretreatment group, where the effect was most pronounced.

#### 2.3. Aboveground K and Na content

**Figure 3** shows that on day 0 of drought, with increasing concentration of NaCl pretreatment, the content of Na in the aboveground of \(M. \ sativa\) increased significantly, while the content of K decreased, but the difference in K in the aboveground of \(M. \ sativa\) was only significant between the 200 mM pretreatment group and the CK group. With
increasing number of drought days, the content of Na in the aboveground of *M. sativa* increased, while the content of K showed a decreasing trend. After different numbers of drought days, the Na content in the aboveground of *M. sativa* was significantly higher after pretreatment with different concentrations of NaCl when compared to CK, but the difference in K content in the aboveground is not significant.

### 2.4. OJIP and MR820 curve and PSII and PSI photochemical activity

Figure 4(A) shows that pretreatment with different concentrations of NaCl has little effect on the relative fluorescence intensity $F_o$ and $F_m$ at points O and P on the OJIP curve of *M. sativa* leaves, but with the increase of salt concentration, the relative fluorescence intensity of point J on the...
**2.5. Normalized O-P and O-J curves and relative variable fluorescence at points J and K on the curve**

Figure 5(A) shows that at the 0th day of drought, with increasing NaCl concentration in the pretreatment solution, the relative variable fluorescence $V_I$ of the point J at 2 ms on the $V_{O-P}$ curve shows an increasing trend. On the 3rd (Figure 5(B)), the difference in $V_I$ became smaller between the $V_{O-P}$ curves of pretreated $\textit{M. sativa}$ leaves and CK. On the 6th and 9th day of drought (Figure 5(C,D)), $V_I$ on the $V_{O-P}$ curve of $\textit{M. sativa}$ leaves decreased to some extent in the leaves pretreated with different concentrations of NaCl when compared with CK.

Figure 5(E) shows that, on the 0th day of drought, the relative variable fluorescence $V_K$ at the point K at 0.3 ms on the $V_{O-J}$ curve of the 50 and 100 mM NaCl pretreated $\textit{M. sativa}$ leaves did not change significantly, while the $V_K$ of the 200 mM NaCl pretreated $\textit{M. sativa}$ increased significantly. On the 3rd and 6th day of drought (Figure 5(F,G)), the difference of $V_K$ in pretreated leaves compared to CK leaves was smaller, and did not show significant differences when compared with day 0 of drought. On the 9th day of drought, the $V_K$ of the leaves increased significantly compared with days 0, 3 and 6 of drought, and the $V_K$ of the leaves treated with different concentrations of NaCl was lower than that of CK (Figure 5(H)).

Quantitative analysis of $V_I$ changes are shown in Figure 5(I). The $V_I$ of leaves pretreated with different concentrations of NaCl on drought days 0 and 3 showed different degrees of increase compared with CK, but the differences were not significant. On the 6th day of drought, the $V_I$ of leaves treated with 50 mM NaCl and that of CK were significantly different, but the $V_I$ of 50, 100 and 200 mM NaCl pretreated $\textit{M. sativa}$ leaves were lower than that of CK by 26.55% ($P < 0.05$), 22.13% ($P < 0.05$), and 19.26% ($P > 0.05$), respectively.

Figure 5(J) shows that there is no significant change in $V_K$ of $\textit{M. sativa}$ leaves within 0–6 d of drought, and there is no significant difference between pretreatment with different concentrations of NaCl and CK. However, on the 9th day of drought, $\textit{M. sativa}$ leaves $V_K$ was significantly increased compared with the 0th, 3rd and 6th day of drought, and the $V_K$ of the leaves pretreated with different concentrations of NaCl was significantly lower than that of CK. The $V_K$ of 200 mM NaCl pretreated leaves was higher than that of leaves pretreated with 50 and 100 mM NaCl by 15.38% ($P < 0.05$) and 13.66% ($P > 0.05$), respectively.

**2.6. NPQ and (1-q_p)/NPQ**

Figure 6(A) shows that on day 0 of drought, the NPQ of $\textit{M. sativa}$ leaves increased with the increase of NaCl concentration in the pretreatment solution. With the increase of drought days, the NPQ of $\textit{M. sativa}$ leaves increased at first and then decreased once reaching a maximum value on the 3rd day of drought. On the 9th day of drought, the NPQ of $\textit{M. sativa}$ leaves decreased significantly. During the duration of the drought, the NPQ of NaCl pretreated $\textit{M. sativa}$ leaves were higher than CK. Figure 6(B) shows that there was no significant change in leaf (1-q_p)/NPQ of the $\textit{M. sativa}$ on the 0th and 3rd day of drought, and the difference of the leaves (1-q_p)/NPQ between the different concentrations of NaCl showed no significant differences when compared with CK. However, on the 6th day of drought, the (1-q_p)/NPQ of the leaves began to increase, and the increase was significant on the 9th day of drought when compared with day 0. The (1-q_p)/NPQ of the NaCl pretreated groups on the 6th and 9th day were significantly lower than CK.

**2.7. MDA content**

Figure 7 shows that pretreatment with different concentrations of NaCl does not affect the MDA content of $\textit{M. sativa}$ leaves after 0 d drought conditions. When 3 d drought was compared with 0 d, the MDA content of $\textit{M. sativa}$ leaves did not increase significantly, and pretreatment with different concentrations of NaCl did not result in any significant differences from CK. However, after 6 and 9 d of drought, the MDA content of $\textit{M. sativa}$ leaves increased significantly, but the MDA content of leaves treated with 50 and 100 mM NaCl pretreatment was significantly lower than that of CK. The difference in MDA content between CK and leaves treated with 200 mM NaCl was not significant.

**3. Discussion**

Under drought stress, the roots of plants have difficulty absorbing water, which affects water balance in plants and can even cause plant death. Plants have developed a series of adaptations during their evolutionary process, such as closing the stomata to prevent water loss (Zhang et al. 2019a) and increasing the permeability of regulatory organic or inorganic molecules to promote water absorption (Ashraf and Foolad 2007; Cicatelli et al. 2010; Sperdouli and Moustakas 2015; Ahanger and Agarwal 2017). In terms of
osmotic regulation, plants can reduce cell osmotic potential by actively absorbing K⁺ or Na⁺ and maintaining cell turgidity, and this requires less energy than synthesizing organic compounds to regulate osmotic potential (Cui et al. 2019). Some studies suggest that the role of Na⁺ as an osmotic regulator in plants under drought stress is still controversial. For example, Martínez et al. (2005) believe that Na⁺ has a rather weak osmotic regulation effect in Atriplex halimus.
L. However, numerous studies have shown that Na$^+$ can play a role in osmotic regulation under drought stress (Wang et al. 2004; Gattward et al. 2012; Ma et al. 2012; Erel et al. 2014). In our experiment, different concentrations of NaCl pretreatment increased the Na$^+$ content in the aboveground of *M. sativa* and this had an obvious concentration-dependent effect. Although the K$^+$ content in the aboveground of the *M. sativa* plant decreased with the increase of NaCl concentration in the pretreatment, the difference between pretreatment at different concentrations was not significant. With increased drought duration, the Na$^+$ content in the aboveground part of the *M. sativa* increased.

**Figure 5.** Effects of different concentrations of NaCl pretreatment on $V_{o-p}$ curve on the 0th (A), 3rd (B), 6th (C) and 9th (D) day of drought, $V_{o-j}$ curve on the 0th (E), 3rd (F), 6th (G) and 9th (H) day of drought and $V_i$ (I), $V_o$ (J) of *M. sativa* under drought stress.
that a suitable concentration of NaCl can improve the water status of *Reaumuria soongorica* and of the succulent xerophyte *Zygophyllum xanthoxylum* under drought stress, and improve their photosynthetic capacity. In addition, through our study on the effects of electron transport on the donor side and the acceptor side of PSII in the leaves of *M. sativa*, we found that although the *V_{f}\text{ and }V_{k}\text{ of the leaves did not change significantly after 6 d of drought, the } V_{f}\text{ and }V_{k}\text{ of the leaves were significant increased after 9 d of drought, but the } V_{f}\text{ and }V_{k}\text{ of }M.\text{ sativa leaves pretreated with different concentrations of NaCl are lower than those of }CK.\text{ The increase of } V_{f}\text{ can reflect the accumulation of } Q_{A}\text{, that is, the electron transfer from } Q_{A}\text{ to }Q_{B}\text{ in the photosynthetic electron transport chain is blocked. The increase in } V_{k}\text{ is considered to be a specific marker of damage to the oxygen evolving complex (OEC) activity of the PSI electron donor side (Zhang et al. 2012). Therefore, it can be shown that under severe drought stress, both the donor side and the receptor side of PSII are significantly affected, PSI non-cyclic electrons transfer was blocked, but different concentrations of NaCl pretreatment could increase the stability of electron transport on the donor and acceptor sides of PSI.}

The enhancement of non-photochemical quenching (NPQ) plays a strong role in dissipating excess energy in PSI and in alleviating PSI photosynthesis inhibition (Porcel et al. 2015; Da et al. 2018). Adverse conditions such as salt stress often lead to an increase in NPQ to reduce excess excitation energy in PSI (Xu et al. 2018; Zhang et al. 2020). A similar conclusion was obtained in this experiment. The NPQ of the pretreated leaves increased to different degrees, and trended with increasing concentrations of NaCl in the pretreatment solution. The increased NPQ observed in NaCl pretreated samples could be related to (1) increased thermal dissipation conferring a better resistance to photodestruction or (2) increased photooxidation due to possibly destabilized photosystems. While, the second hypothesis is unlikely, considering the other data herein reported (Figure 4), indicating that different concentrations of NaCl pretreatment can activate NPQ-dependent energy dissipation mechanism in *M. sativa* leaves and prevent the accumulation of excess light energy (1-qP)/NPQ. With prolonged drought time, the NPQ of *M. sativa* leaves first increased and then decreased, especially on the 9th day of drought, the NPQ in plants pretreated with different concentrations of NaCl was less reduced when compared to the 6th day of drought. Studies have found that if excess excitation energy fails to pass through the NPQ pathway and other electron pools in time, PSI photosynthesis inhibition will gradually increase and the additional accumulated ROS can destroy...
the photosynthetic system (Asada 1999). Under severe stress, plant leaf NPQ will often decrease (Zhang et al. 2018b). Therefore, under severe drought stress, significant photosynthesis inhibition of PSII and PSI in M. sativa leaves was observed, and the reason for the increase of MDA content was mainly related to the destruction of the NPQ-dependent energy dissipation mechanism. However, under drought stress, the NPQ in pretreated leaves was significantly higher than that of CK, especially on the 6th and 9th day of drought, the decrease of NPQ in the pretreated leaves was significantly lower than that in CK leaves, so that the accumulation of (1-qP)/NPQ is also significantly lower than in CK. Therefore, under severe drought stress, M. sativa leaves pretreated with NaCl can continue to dissipate excess energy via the NPQ-dependent energy dissipation mechanism and alleviate the degree of photosynthesis inhibition of PSII and PSI and the degree of membrane peroxidation in leaves of M. sativa during long-term drought stress.

The mechanism by which NaCl pretreatment enhances the drought resistance of M. sativa and alleviates the photosynthesis inhibition of PSII and PSI in M. sativa leaves is summarized in Figure 8.

4. Conclusion
Pretreatment with less than 200 mM NaCl has little effect on the growth and photosynthesis of M. sativa. However, the Na⁺ accumulation in M. sativa leaves increased significantly upon pretreatment with different concentrations of NaCl, which played an important role in increasing the water content and in alleviating leaf wilting under increased drought stress. Under drought stress, the activity of PSII and PSI decreased, the electron transport chain was blocked, and photosynthesis inhibition occurred. However, the photosynthesis inhibition of PSII and PSI in the leaves of M. sativa was significantly alleviated by different concentrations of NaCl pretreatment. We found that using 50 mM NaCl pretreatment is better than 100 and 200 mM NaCl in relieving the damage to M. sativa under drought stress, indicating that although M. sativa can increase its drought resistance by accumulating Na⁺, there are concentration limits and excessive NaCl concentration may aggravate the damage of drought stress.

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Notes on contributors
Zhang Huihui is an Associate Professor in Northeast Forestry University. His research interests lie in the area of Plant physiology and molecular biology.
Huo Yuze is a Postgraduate Student in Northeast Agricultural University. His research interests lie in the area of Plant physiology.
Guo Kaiwen is a Postgraduate Student in Northeast Agricultural University. His research interests lie in the area of agricultural resources and environment.
Xu Zisong is a Postgraduate Student in Northeast Agricultural University. His research interests lie in the area of agricultural resources and environment.
Shiqi Liu is an Undergraduates Student in Northeast Agricultural University. His research interests lie in the area of agricultural resources and environment.
Qiao Wang is an Undergraduates Student in Northeast Agricultural University. Her research interests lie in the area of agricultural resources and environment.
Xuecong Wang is an Undergraduates Student in Northeast Agricultural University. Her research interests lie in the area of agricultural resources and environment.
Xu Nan is an Assistant researcher Assistant researcher in Heilongjiang Sciences Academy. His research interests lie in the area of Plant physiology.
Yining Wu is an Assistant researcher Assistant researcher in Heilongjiang Sciences Academy. His research interests lie in the area of soil microbes and plant physiology.
Sun Guangyu is a Professor in Northeast Forestry University. His research interests lie in the area of Plant physiology and molecular biology.

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