EFFECTS OF EXOGENOUS $CaCl_2$ ON THE PHOTOSYNTHETIC FUNCTION AND ACTIVE OXYGEN METABOLISM OF *SALIX VIMINALIS* LEAVES UNDER PB STRESS

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Abstract. In order to provide some basic data for revealing the mechanism of exogenous $CaCl_2$ on improving drought resistance of *Salix viminalis* seedlings. The experimental results showed that: Spraying exogenous of different $CaCl_2$ concentrations significantly alleviated the damage degree of *S. viminalis* seedlings leaves caused by heavy metal stress, and the effect of 30 $\mu mol \cdot L^{-1}$ $CaCl_2$ was the most significant. Spraying exogenous $CaCl_2$ could regulate stomatal limitation of *S. viminalis* seedlings leaves under heavy metal stress, which was beneficial to water holding capacity of *S. viminalis* seedlings leaves under heavy metal stress, and enhanced photosynthetic carbon assimilation capacity of leaves under heavy metal stress; Spraying exogenous $CaCl_2$ can reduce the energy pressure of PSII reaction center by increasing the non-photochemical quenching (NPQ) of leaves of *S. viminalis* seedlings, alleviate the photoinhibition of PSII, and promote the electron transfer process, especially on the receptor side of PSII; Spraying exogenous $CaCl_2$ effectively reduced the production of reactive oxygen species in leaves of *S. viminalis* seedlings, as well as the degree of membrane peroxidation, which was also one of the important reasons for alleviating the inhibition of photosynthetic capacity.
Keywords: *heavy metals, S. viminalis, PSII, reactive oxygen, antioxidants*

Introduction

Heavy metal stress is an important limiting factor in agricultural production, and the yield of crops caused by heavy metal stress alone exceeds the sum of all pathogens. Lead (Pb) is one of the most harmful heavy metals in the environment. In recent years, with the rapid development of economy, mining, agriculture and recycling of waste metals have significantly increased the concentration of Pb in the soil, and the damage dealt by Pb pollution to the environment has gradually intensified, and the impact on human health has become a hot topic (Soffianian et al., 2014; Sauliutė and Svecevičius, 2015). The majority of Pb enters the human body through the soil-plant-human pathway and causes harm. To a certain extent, Pb in the soil will have a series of adverse effects on plant physiological metabolism, such as the increase of cell membrane permeability, the accumulation of reactive oxygen species, and the aggravation of membrane lipid peroxidation, and even lead to plant death in severe cases (Shu et al., 2012; Ogbomida et al., 2018).

Plant photosynthesis is one of the most sensitive processes to heavy metals. Persistent heavy metal stress will cause irreversible damage to plant photosynthetic apparatus (Zhang et al., 2018a), such as inhibition of photosynthetic pigment degradation (Albert et al., 2011; Guadagno et al., 2017; Chen et al., 2018; Zhang et al., 2018), photosynthetic phosphorylation and electron transport. Oxidative stress caused by oxidative stress in plant cell membrane caused by oxidative stress (Gill et al., 2010; Wang et al., 2017). Plant photosystem II (PSII) is one of the sensitive parts to heavy metal stress. A series of

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photosynthetic physiological processes such as light energy absorption, water photolysis and electron transfer are closely related to PSII (Allahverdiyeva et al., 2013; Chen et al., 2017). To ensure the stability of photosynthetic function, especially PSII function, of plant leaves under heavy metal stress plays an important role in maintaining the normal growth of plants and improving the stress resistance of plants.

Calcium is not only an essential mineral nutrient element for plants, but also a second messenger for intracellular physiological and biochemical reactions. Therefore, the stress resistance of plants can be improved by stabilizing cell wall and cell membrane structures and inducing the expression of specific genes (Ryan and Kochian, 1993; Larkindale and Huang, 2004). At present, there are a lot of reports about $CaCl_2$ can protect the normal physiology of PSII and increase the content of antioxidant enzymes in plant leaves under stress. *S. viminalis* is a *Salix* plant of *Salix family*. *S. viminalis* has the advantages of rapid growth, high biomass and strong ability to accumulate heavy metals is widely used in phytoremediation and biomass energy development in heavy metal contaminated soil areas, and has certain ecological and economic value (Zhai et al., 2016). Therefore, improving the resistance of *S. viminalis* seedlings to heavy metal stress is the key to ensure the survival and growth of seedlings after transplanting. However, the regulation of exogenous $CaCl_2$ on physiological function of *S. viminalis* seedlings under heavy metal stress, especially the regulation mechanism of PSII function, was less studied. In this experiment, the effects of spraying different concentrations of exogenous $CaCl_2$ on PSII function of *S. viminalis* leaves under heavy metal stress were studied, in order to provide some basic data for improving the drought resistance ability of *S. viminalis.*

Materials and methods

Test materials and treatment

The experiment was conducted in the laboratory of soil science of Jilin Agricultural University (Changchun, Jilin Province, China) from March to June 2019. The annual seedlings were raised by cutting. The culture substrate was fully mixed with peat soil and quartz sand; the ratio was 2:1 (V / V). It was cultured in an artificial climate box with temperature of 25/23 °C (light / dark), light intensity of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, photoperiod of 12/12 h (light/dark), and relative humidity of about 75%. After the seedlings had long functional leaves, 1/2 of Hoagland nutrient solution was irrigated once a week. When the seedlings grow to six leaves and one heart, they are transplanted. Before transplanting, the seedlings are irrigated once to make the relative water content of the soil basically reach saturation. The seedlings are transplanted into a cultivation bowl with a diameter of 12 cm and a height of 15 cm, and one plant is planted in each pot.

After transplanting, the seedlings with the same growth were selected as the experimental seedlings. The treatment group was sprayed with $CaCl_2$ solution with concentration of 15 and 30 $\mu mol \cdot L^{-1}$ (PH=7), and the control group was sprayed with water (CK). The foliar spray was carried out at 4:00 p.m., and the front and back sides were evenly sprayed until the solution on the leaves formed fine mist like uniform droplets ready to drop, after the water on the leaf surface evaporates naturally and the $CaCl_2$ solution is completely absorbed, repeat spraying 1 time with the same amount each time. 10 plants in each treatment were repeated. After spraying $CaCl_2$, the water on the surface of leaves is naturally evaporated. In order to fully absorb the spraying $CaCl_2$ on the leaf surface, after the $CaCl_2$ solution on the leaf surface evaporates, spray once more, and the usage and dosage are the same as the first time. The content of Pb in the test

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matrix was set at 200 mg·kg^{-1}. On the 15th day after treatment, the physiological indexes were determined after the results showed obvious differences among different treatments.

Determination parameters and methods

The growth parameters were determined: Vernier caliper was used to measure plant height and leaf area was measured by leaf area meter.

Photosynthetic gas exchange parameters were measured: A CIRAS-2 portable photosynthesis system (PPsystem Company, UK) was used to determine the net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) of the second fully expanded functional leaf of the penultimate leaf of *S. viminalis* seedlings was selected. The CO_2 concentration was fixed at 400 μl·L^{-1} in a CO_2 cylinder. The light intensity PFD was set to 1000 μmol·m^{-2}·s^{-1} with the light source built in the instrument. The net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r) and intercellular CO_2 concentration (C_i) of *S. viminalis* leaves under different treatments were measured. Repeat 5 times.

Chlorophyll fluorescence parameters were measured: The electron transfer rate (ETR) and non-photochemical quenching (NPQ), the maximum photochemical efficiency (F_v/F_m) and the actual photochemical efficiency (Φ_{PSII}) of PSII reaction center under light acclimation were measured by FMS-2 (Hansatch company, UK) for 5 times.

The chlorophyll fluorescence kinetic curve and its parameters were determined: After 30 min dark adaptation, the OJIP curve of leaves after dark adaptation was measured by handy pea (Hansatech compan, UK). According to Strasser et al.'s method (1995), the OJIP curves were standardized by $V_{O\text{-}P}=(F_t\text{-}F_o)/(F_m\text{-}F_o)$ and $V_{O\text{-}J}=(F_t\text{-}F_o)/(F_J\text{-}F_o)$ respectively. The relative variable fluorescence (V_J and V_K) of J point at 2 ms and K point at 0.3 ms were obtained, respectively. The differences between the standardized $V_{O\text{-}P}$ and $V_{O\text{-}J}$ curves of different treatments and the control were calculated, expressed as $V_{O\text{-}P}$ and $V_{O\text{-}J}$, respectively. The measured OJIP curve was analyzed by JIP test. The maximum photochemical efficiency (F_v/F_m) of PSII and the photosynthetic performance index (PI_{ABS}) based on absorbed light energy were measured.

Determination of ROS metabolism and other physiological indices: The rate of production of $O_2^{\bullet}$ was measured using the method of Zhang et al. (2007), The malondialdehyde (MDA) content was determined using the thiobarbituric acid method. The conductivity was measured by DDS-11C. Relative conductivity was used to express the electrolyte leakage rate. The content of superoxide dismutase activity (SOD) was determined using the NBT method. The activity unit (U) was 50% of the enzyme that inhibited the photochemical reduction of NBT in 1 ml of reaction solution in 1 h. The activity of ascorbic acid peroxidase (APX) was determined as described by Shen et al. (1996). An activity unit (U) is defined as the amount of enzyme that catalyzes 1 μmol ascorbic acid oxidation in one minute. Each index was measured five times.

Data and analysis

Excel and SPSS software (Version. 22) were used to conduct statistical analyses on the measured data. The data in the figure was denoted as mean ± standard deviation (SE). One-way ANOVA and least significant difference (LSD) were used to compare the differences among different data groups.

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Results and analysis

Effects of exogenous $CaCl_2$ on leaf growth of S. viminalis seedlings under Pb Stress

Under Pb stress, the leaf growth of *S. viminalis* seedlings changed significantly, and the increase of leaf area of *S. viminalis* seedlings without spraying exogenous $CaCl_2$ was significantly delayed (*Fig. 1*). Compared with CK, the plant height of *S. viminalis* seedlings was decreased by 20.36% ($P < 0.01$), reaching a very significant difference level. However, spraying different concentrations of exogenous $CaCl_2$ significantly alleviated the reduction of leaf area of *S. viminalis* seedlings under heavy metal stress, of which 15 and 30 $\mu mol \cdot L^{-1}$ were sprayed under heavy metal stress. The leaf area of *S. viminalis* seedlings treated with 30 $\mu mol \cdot L^{-1}$ exogenous $CaCl_2$ was 26.51% ($P<0.05$) and 37.21% ($P<0.05$), respectively.

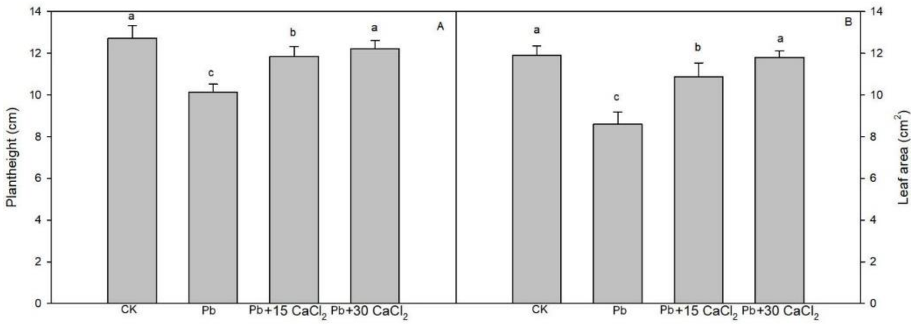

Figure 1. *Effects of $CaCl_2$ on leaf growth of S. viminalis seedlings under Pb Stress. CK: CK + 0 $CaCl_2$ contents; +$CaCl_2$: CK + different amounts of $CaCl_2$ contents. Note: Data in the figure are the mean ± SD; values followed by different lowercase letters indicate a significant difference ($p<0.05$)*

Effects of exogenous $CaCl_2$ on Photosynthetic gas exchange parameters of S. viminalis seedlings under Pb Stress

Compared with CK, P_n, G_s and T_r of *S. viminalis* seedlings leaves under Pb stress were significantly decreased, but spraying different concentrations of exogenous $CaCl_2$ significantly alleviated the reduction range of P_n, G_s and T_r. Except that there was no significant difference between spraying different concentrations of exogenous $CaCl_2$ and spraying $CaCl_2$ under heavy metal stress, spraying 15 and 30 $\mu mol \cdot L^{-1}$ in leaves of *S. viminalis* seedlings leaves Under $CaCl_2$ Treatment, P_n and G_s in leaves of *S. viminalis* seedlings were significantly increased compared with those without $CaCl_2$ treatment. It can be seen from *Fig. 2D* that spraying different concentrations of exogenous $CaCl_2$ also significantly alleviated the C_i reduction of *S. viminalis* seedlings under heavy metal stress, but there was no significant difference between the C_i of *S. viminalis* seedling leaves treated with 30 $\mu mol \cdot L^{-1}$ exogenous $CaCl_2$ and that without spraying exogenous $CaCl_2$.

Effects of exogenous $CaCl_2$ on F_v/F_m, Φ_{PSII}, ETR and NPQ of S. viminalis seedlings under Pb stress

Under Pb stress, F_v/F_m, Φ_{PSII}, and ETR of *S. viminalis* seedlings decreased significantly, while NPQ increased significantly (*Fig. 3*). However, spraying different concentrations of $CaCl_2$ significantly alleviated the decrease of F_v/F_m, Φ_{PSII} and ETR of *S. viminalis* seedlings leaves, especially under heavy metal stress, when spraying

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30 μmol·L^{-1} exogenous $CaCl_2$, the Φ_{PSII} of *S. viminalis* seedlings leaves increased by 73.24% (P<0.01). There was no significant difference in F_v/F_m, Φ_{PSII} and ETR between different concentrations of $CaCl_2$. Spraying different concentrations of $CaCl_2$ increased NPQ of *S. viminalis* seedlings leaves under heavy metal stress, but there was no significant difference among different concentrations of $CaCl_2$. However, NPQ of *S. viminalis* seedlings under 30 μmol·L^{-1} $CaCl_2$ treatment was significantly higher than that under Pb stress.

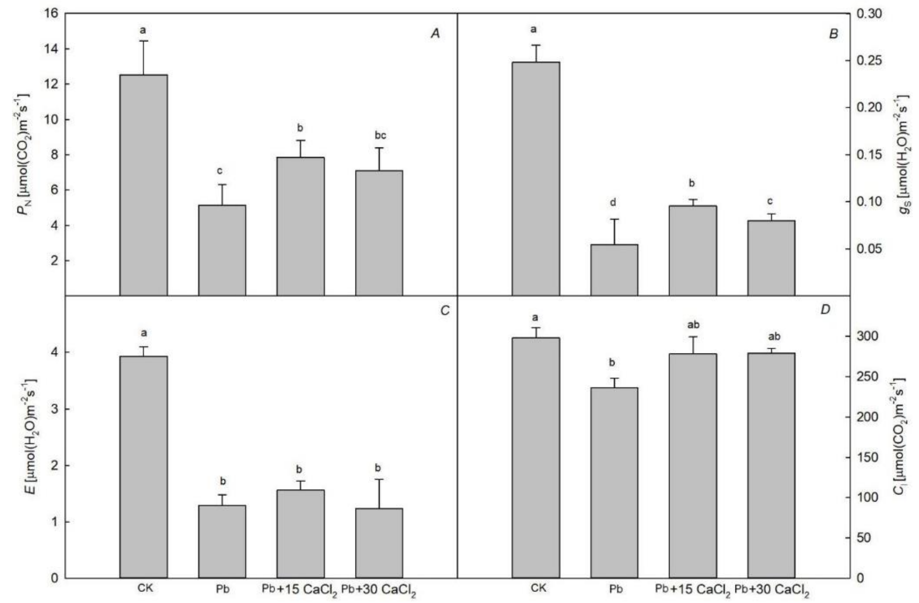

Figure 2. *Effects of different concentrations of exogenous $CaCl_2$ on net photosynthetic rate (a), stomatal conductance (b), transpiration rate (c) and intercellular CO_2 concentration (d) of S. viminalis seedlings under Pb Stress. CK: CK + 0 $CaCl_2$ contents; +$CaCl_2$: CK + different amounts of $CaCl_2$ contents. Note: Data in the figure are the mean ± SD values followed by different lowercase letters indicate a significant difference (p<0.05)*

Effects of exogenous $CaCl_2$ on OJIP curve of S. viminalis seedlings under Pb stress

Compared with CK, the relative fluorescence intensity of O point and J point had no significant change under Pb stress, while the relative fluorescence intensity of I and P decreased significantly, especially that of P point (*Fig. 4*). However, spraying different concentrations of exogenous $CaCl_2$ could significantly reduce the change range of OJIP curve of *S. viminalis* seedlings under heavy metal stress.

Effects of exogenous $CaCl_2$ on standardized O-P curve, V_J and V_I of S. viminalis seedlings under heavy metal stress

Compared with CK, the relative variable fluorescence (V_J) of each point on the $V_{O\text{-}P}$ curve of *S. viminalis* seedlings leaves under Pb stress increased significantly with the relative variable fluorescence V_J of J point at 2 ms (*Fig. 5*). the increase range of V_J in leaves of *S. viminalis* seedlings treated with different concentrations of exogenous $CaCl_2$ was significantly less than that of no $CaCl_2$ treatment. At 0.3 ms, the change range of K point relative to variable fluorescence V_K was small, and it was not significantly affected by spraying $CaCl_2$.

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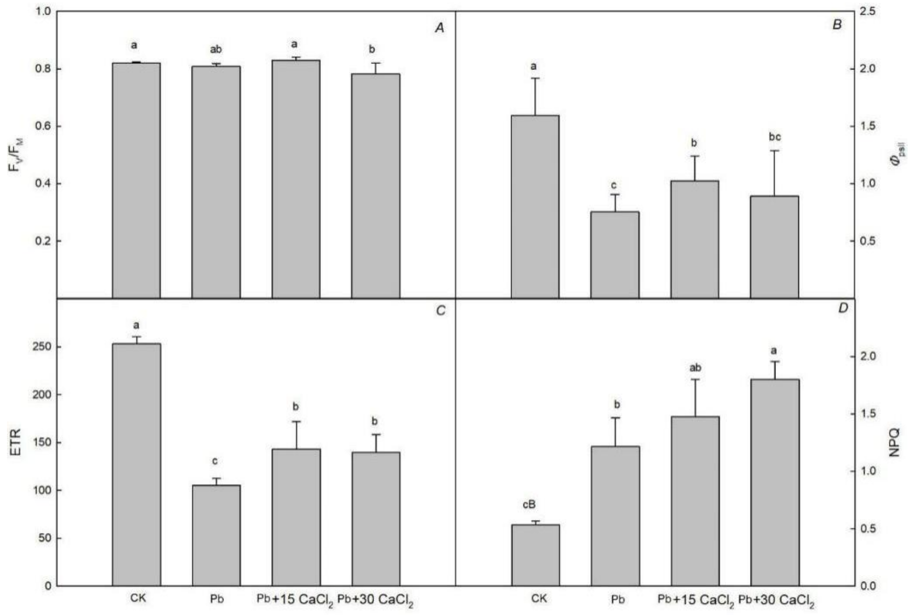

***Figure 3.** Effects of different concentrations of exogenous $CaCl_2$ on the maximum photochemical efficiency (F_v/F_m) and the actual photochemical efficiency (Φ_{PSII}) of PSII reaction center electron transfer rate (ETR) and non-photochemical quenching (NPQ) in leaves of S. viminalis seedlings under Pb Stress. CK: CK + 0 $CaCl_2$ contents; +$CaCl_2$: CK + different amounts of $CaCl_2$ contents. Note: Data in the figure are the mean ± SD values followed by different lowercase letters indicate a significant difference ($p<0.05$)*

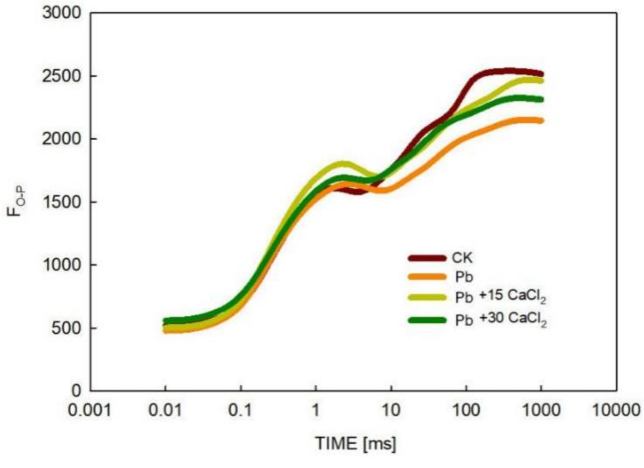

***Figure 4.** Effects of different concentrations of exogenous $CaCl_2$ on OJIP curve of S. viminalis seedlings under Pb Stress. CK: CK + 0 $CaCl_2$ contents; +$CaCl_2$: CK + different amounts of $CaCl_2$ contents*

The results of quantitative analysis of V_J and V_K showed that the V_J of *S. viminalis* seedling leaves under Pb stress increased by 24.63% ($P<0.05$), while the V_J of leaves of *S. viminalis* seedlings treated with 15 and 30 μmol·L^{-1} $CaCl_2$ increased by 21.77% ($P<0.05$) and 18.44% ($P<0.05$), respectively. Under Pb stress, the V_K of *S. viminalis* seedlings leaves had no significant change compared with CK, and spraying different concentrations of $CaCl_2$ did not significantly change the V_K of *S. viminalis* seedlings leaves.

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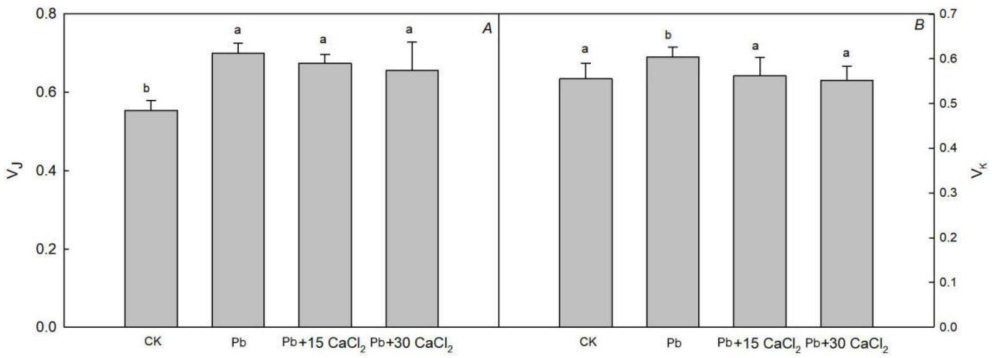

Figure 5. *Effects of different concentrations of exogenous $CaCl_2$ on V_J (a) and V_K (b) in leaves of S. viminalis seedlings under Pb Stress. CK: CK + 0 $CaCl_2$ contents; +$CaCl_2$: CK + different amounts of $CaCl_2$ contents. Note: Data in the figure are the mean ± SD; values followed by different lowercase letters indicate a significant difference (p<0.05)*

Effects of exogenous $CaCl_2$ on reactive oxygen species and membrane peroxidation of S. viminalis seedlings under Pb Stress

Compared with CK, the results showed that the treatment of 30 $\mu mol \cdot L^{-1}$ exogenous $CaCl_2$ had the most obvious effect, and the $O_2^{\bullet -}$ production rate and H_2O_2 content decreased by 25.83% ($P<0.05$) and 32.22% ($P<0.05$), respectively, which resulted in the decrease of MDA content and electrolyte leakage rate of membrane lipid peroxidation by 21.43% ($P<0.05$) and 25.14% ($P<0.05$), respectively (*Fig. 6*).

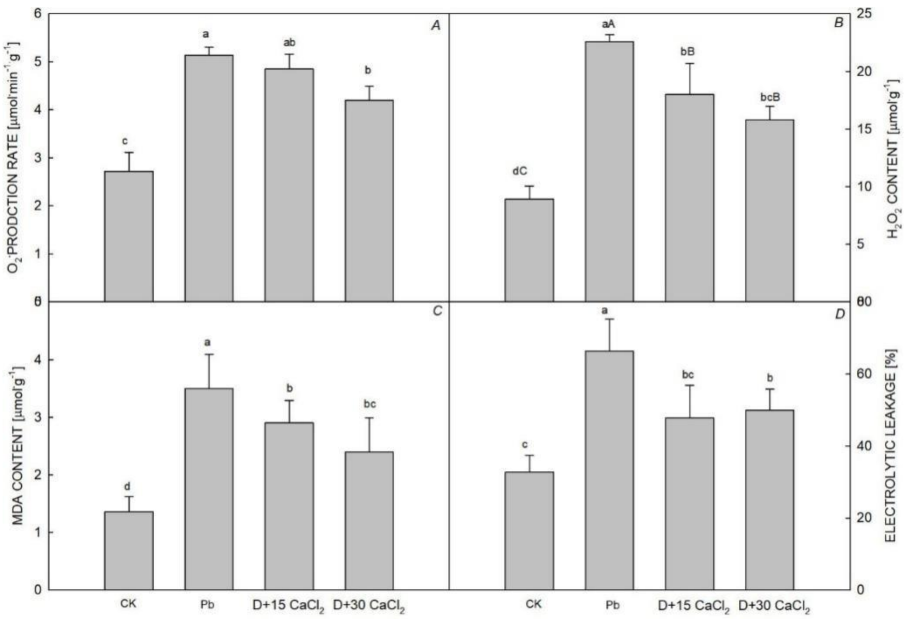

Figure 6. *Effects of different concentrations of exogenous $CaCl_2$ on $O_2^{\bullet -}$ production rate (a), H_2O_2 Content (b), MDA content (c) and electrolyte leakage rate (d) in leaves of S. viminalis seedlings under Pb Stress. CK: CK + 0 $CaCl_2$ contents; +$CaCl_2$: CK + different amounts of $CaCl_2$ contents. Note: Data in the figure are the mean ± SD; values followed by different lowercase letters indicate a significant difference (p<0.05)*

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Discussion

Pb is a kind of non-essential element for plant growth, which has different effects on different plants when it enters into plants. Many studies have shown that low concentrations of Pb can stimulate plant growth, moderate concentrations of Pb can cause plant metabolic disorders and inhibit growth, while heavy concentrations of Pb can cause plant death (Del et al., 1999a,b; Dong et al., 2008), $CaCl_2$ could alleviate the growth inhibition of heavy metals on plants (Saradhi et al., 2001; Li et al., 2011; Zhang et al., 2014). In this study, Pb significantly reduced the growth rate of plant height and leaf area; Application of $CaCl_2$ after Pb stress could reduce the inhibition of Pb on leaf area and plant height growth of *S. viminalis*.

Under Pb stress, the photosynthetic carbon assimilation capacity of *S. viminalis* seedlings leaves was limited, which was mainly manifested in the decrease of P_n, along with the decrease of G_s, T_r and C_i. According to Farquhar's photosynthetic stomatal factor analysis theory (Farquhar et al., 2003), the reason for the decrease of photosynthetic carbon assimilation capacity of *S. viminalis* seedlings under heavy metal stress was directly related to the decrease of stomatal conductance Although the decrease of water loss can effectively prevent the loss of water, it also directly reduces the supply of carbon assimilation material (CO_2)), which limits its carbon assimilation capacity. In this experiment, spraying different concentrations of exogenous $CaCl_2$ increased P_n in different degrees, which was similar to the change of G_s, and accompanied by the increase of C_i, indicating that spraying exogenous $CaCl_2$ could improve the photosynthetic capacity of *S. viminalis* seedlings by improving stomatal limitation.

Under Pb stress, the dark response of plant leaves was inhibited, and the accumulation of assimilative capacity (ATP and NADPH) would feedback inhibit the light response process, resulting in the excess of electrons in the photosynthetic electron transport chain (Li et al., 2000; Liu et al., 2006). In addition, the decrease of PSII activity also inhibited the process of light energy absorption and electron transfer. In this experiment, although F_v/F_m, ETR and, Φ_{PSII} of *S. viminalis* seedlings were significantly decreased under Pb stress. F_v/F_m and, Φ_{PSII} were important indexes reflecting the photochemical activity of PSII, and the sensitivity of Φ_{PSII} was significantly higher than that of F_v/F_m (Kalaji et al., 2016). Therefore, the results showed that the photochemical activity of PSII in leaves of *S. viminalis* seedlings was inhibited under heavy metal stress, and the process of PSII electron transport was hindered. However, the decrease of PSII photochemical activity was alleviated by spraying different concentrations of exogenous $CaCl_2$, especially 30 $\mu mol \cdot L^{-1}$. The results showed that exogenous $CaCl_2$ could alleviate the photoinhibition of *S. viminalis* seedlings under heavy metal stress and promote electron transfer. NPQ was positively correlated with heat dissipation dependent on xanthophyll cycle. Under Pb stress, NPQ in leaves of *S. viminalis* seedlings increased significantly, which means that the excess excitation energy in PSII could be dissipated by increasing NPQ under Pb stress, so as to reduce the pressure of PSII reaction center. The NPQ of *S. viminalis* seedlings leaves treated with different concentrations of exogenous $CaCl_2$ increased to varying degrees. Ivanov et al. (1995) found that $CaCl_2$ treatment enhanced the xanthophyll cycle in barley seedlings, which enhanced the opening degree of photosystem II reaction center and promoted the utilization rate of light energy. Therefore, the reason why spraying exogenous $CaCl_2$ to alleviate the decrease of PSII in leaves of *S. viminalis* seedlings under heavy metal stress may be related to the mechanism of energy dissipation dependent on xanthophyll cycle induced by $CaCl_2$.

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Under stress conditions, the blocking sites of photosynthetic electron transfer often occur on the electron donor side and receptor side of PSII reaction center, especially the transfer of Q_A to Q_B is the main inhibition site (Zhang et al., 2018a). The increase of J point relative to variable fluorescence V_J at 2 ms on the normalized O-P curve indicated that the electron transfer from Q_A to Q_B was blocked in the photosynthetic electron transport chain (Zhang et al., 2017; Zhang, 2018b). The increase of relative variable fluorescence V_K at 0.3 ms on the normalized O-J curve was considered as a specific marker of damage to OEC activity of PSII electron donor side oxygen complex (Zhang et al., 2012, 2016). In this experiment, the V_J of *S. viminalis* seedlings leaves increased significantly under Pb stress, but the V_K did not change significantly, which indicated that the reason for the decrease of PSII photosynthetic electron transfer rate under Pb stress mainly occurred in PSII receptor side. However, the change of V_K was not only affected by the donor side injury of PSII, but also by the receptor side injury of PSII. When the injury degree of the recipient side was greater than that of the donor side, the V_K did not increase significantly (Xu et al., 2018; Zhang et al., 2018c). Therefore, in this study, the effect of heavy metal stress on the donor side of PSII may be due to the insensitivity of OEC to Pb stress, or it may be due to the excessive damage on the receptor side. However, spraying different concentrations of exogenous $CaCl_2$ significantly alleviated the increase of V_J, but the effect on V_K was not significant, which indicated that exogenous $CaCl_2$ could promote the electron transfer from Q_A to Q_B in PSII receptor side of *S. viminalis* seedlings under Pb stress.

Pb usually leads to excessive reduction of photosynthetic electron transport chain in plants, and produces a large number of ROS in chloroplasts and mitochondria (Ramachandra et al., 2004; Asada, 2009; Ahmed et al., 2009). Excessive ROS breaks the redox balance in plants, causes membrane peroxidation, damages membrane system, and causes oxidative damage to cell components and structures (Jiang and Zhang, 2004; Gill et al., 2010). Under normal conditions, the production and elimination of intracellular free radicals are in a dynamic equilibrium state. When the concentration of exogenous Pb^{2+} reaches a certain level, this dynamic balance will be broken, resulting in membrane lipid peroxidation and osmotic stress (Amhed et al., 2009; Li and Li, 2011). The results also showed that under Pb stress, the relative permeability of cell membrane increased, the production rate of $O_2^{\bullet-}$ and the content of MDA increased, which caused lipid peroxidation in the inner membrane of *S. viminalis*. Under Pb stress, $O_2^{\bullet-}$ production rate and H_2O_2 content were significantly increased, MDA content and electrolyte leakage rate were also significantly increased, which indicated that excessive ROS caused membrane system peroxidation and increased membrane permeability. However, spraying different concentrations of exogenous $CaCl_2$ significantly alleviated ROS production and membrane lipid peroxidation of *S. viminalis* seedlings, especially the treatment of 30 $\mu mol \cdot L^{-1}$ $CaCl_2$ was the most effective, which was consistent with the change of photosynthetic parameters of *S. viminalis* seedlings. Spraying exogenous $CaCl_2$ could alleviate ROS production by enhancing photosynthetic capacity and reducing PSII damage degree of *S. viminalis* seedlings, and the decrease of ROS production could also alleviate the photoinhibition of *S. viminalis* seedlings leaves under heavy metal stress.

Conclusion

The photosynthetic capacity of *S. viminalis* seedlings was decreased under Pb stress, mainly due to stomatal factors, and also related to the decrease of PSII photochemical

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activity and the damage of membrane system caused by ROS. Under Pb stress, spraying different concentrations (15 and 30 μmol·L^{-1}) of exogenous $CaCl_2$ promoted the photosynthetic capacity of *S. viminalis* seedlings, especially 30 μmol·L^{-1} $CaCl_2$. The main reason is that exogenous $CaCl_2$ can effectively reduce the energy pressure of PSII reaction center in leaves of *S. viminalis* seedlings under Pb stress by inducing the increase of NPQ, thus alleviating the photoinhibition degree of PSII under Pb stress, promoting the electron transfer rate, especially the electron transfer ability of PSII receptor side. Under Pb stress, spraying exogenous $CaCl_2$ could also effectively reduce ROS production in leaves of *S. viminalis* seedlings and alleviate the degree of membrane lipid peroxidation. Therefore, the inhibition of ROS production by spraying $CaCl_2$ under heavy metal stress is not only related to the enhancement of photosynthetic capacity, but also to the increase of enzymatic or non-enzymatic related ROS scavenging system induced by $CaCl_2$, but this needs further study.

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