Effects of Salt Stress on Photosynthetic Fluorescence Characteristics, Antioxidant System, and Osmoregulation of *Coreopsis tinctoria* Nutt.

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Abstract. *Coreopsis tinctoria* Nutt. (*C. tinctoria*) is used in composite tea material and has important medicinal functions. Soil salinization affects the growth and development of *C. tinctoria* in Xinjiang (China). Here, we discussed the changes in photosynthesis and physiological characteristics of *C. tinctoria* seedlings treated with different concentrations of NaCl [0 (CK), 50, 100, 150, 200, and 250 mmol·L⁻¹] for 12, 24, and 72 hours. The results showed that the net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (T_r), and stomatal inhibition rate (L_s) decreased significantly with increasing concentrations of NaCl. Salt stress promoted the accumulation of peroxidase (POD), catalase (CAT), soluble sugar, soluble protein, and free proline (Pro). A highly significant positive correlation was found between L_s and F_v/F_m ; L_s and F_v/F_o ; soluble sugar and CAT; soluble sugar and soluble protein. *C. tinctoria* was most sensitive to the concentrations of 150 to 250 mmol·L⁻¹ NaCl, and its salt stress tolerance was increased by reducing photosynthetic fluorescence parameters, improving the antioxidant enzyme system, and regulating osmotic substances.

Soil salinization has become a global eco-environmental problem (Amelie and Barbara, 2020). Saline-alkali soil accounts for approximately one-quarter of the total land area in China, which seriously restricts the sustainable development of agriculture. With irrational irrigation of arable land and other factors, the area of secondary salinized soil increases each year, and a large area of saline soil resources urgently needs to be developed (Ajay, 2015; Qiao et al., 2015; Zhang et al., 2018). Saline-alkali soil is well known for its wide distribution and salt types in Xinjiang, China (Lu et al., 2013). Screening and cultivating economically important plants with local salt-tolerant characteristics is an effective way to use saline-alkali land. Therefore, it is necessary to study the salt

tolerance mechanisms of plants and find methods to improve their salt tolerance (Hu et al., 2018; Zhang et al., 2017). *Coreopsis tinctoria* Nutt. is an annual herb of the genus *Coreopsis* in Compositae (Guo et al., 2015). This herb is an essential economic crop in Xinjiang (China). *C. tinctoria* is rich in natural active substances. It is a traditional health food with the effects of lowering blood sugar, blood pressure, and blood lipids and has broad market prospects (Zeng and He, 2019). *C. tinctoria* is grown widely in Xinjiang and northern China and is an important resource that can be further developed. At present, in studies of salt stress in plants, the effects of salt stress on the photosynthetic characteristics, antioxidant system, and osmotic regulation of multiple vegetative organs of various crops have been studied. Salt stress can inhibit photosynthesis in plants (Han et al., 2014). High salt stress can reduce g_s , transpiration, intercellular CO₂ concentrations, the activity of the PSII reaction center, and P_n of plants (Wang et al., 2017b). Other studies have shown that with increasing salt concentrations, plant P_n first increases and subsequently decreases or continues to increase (Li et al., 2015). Plants produce a large amount of reactive oxygen under salt stress, which causes peroxidation stress in plants. Improving or balancing the activity of antioxidant enzymes [e.g., superoxide dismutase (SOD), POD and CAT] can effectively reduce the pressure of peroxide stress (Wang et al., 2017a). With increasing salt concentration, many organic osmoregulation substances, including Pro,

soluble sugar, and soluble protein, accumulate to maintain the normal metabolism of plants, such as sugar beet (Huang et al., 2019), Volkamer lemon (Khalid et al., 2020a, 2021b), citru (Hussain et al., 2015), North American pea pear (Zhao et al., 2019), black wolfberry (Yang et al., 2019), and other crops. Research on the salt tolerance of plants has shown differences in the physiological response and photosynthetic fluorescence of different plants under salt stress. Previous studies on seed germination have found that the suitable range of NaCl salt tolerance for the germination of *C. tinctoria* bicolor seeds is from 0% to 1.3%, and there have been few studies on the membrane permeability, physiological characteristics, and photosynthesis of *C. tinctoria* under different concentrations of NaCl (Guo et al., 2013; Yeergen et al., 2014). However, the photosynthesis, antioxidase system, and osmotic regulators might be the physiological mechanism of *C. tinctoria* to the regulation of salt tolerance. In this study, seedlings of *C. tinctoria* were used as experimental materials to reveal the relationship between salt stress and photosynthetic characteristics, antioxidant enzymes, and osmosis to provide a reference for the study of salt tolerance in *C. tinctoria*.

Materials and Methods

Experimental materials and treatments.

The experiment was performed in the Vegetable Physiology Laboratory and Plant Factory Engineering Technology Research Center of Forestry and Horticulture, College of Xinjiang Agricultural University. The seeds of *C. tinctoria* were collected from Hotan, China (lat. 37°37'17.00" N, long. 78°16'58.80" E; altitude, 2196 m). Before being sown, the seeds of *C. tinctoria* were soaked in warm water at 30 °C for 8 to 12 h to fully absorb water and expand. Seeds of uniform size with no damage were selected for disinfection and cultured with distilled water in KRG-250A light incubator (temperature 25 ± 3 °C, humidity 60% to 70%, light 8 h a day). After 15 d, the seedlings were cultured with one-quarter to one-half concentration of Hoagland nutrient solution (the composition was shown in Supplemental Table 1) (25 ± 3 °C/20 ± 2 °C, humidity 60% to 70%, day/night 8/16 h, 600–1000 lx). During this period, the nutrient solution was changed every 5 d, and oxygen pumps supplied oxygen regularly. Then, seedlings with four to six pairs of leaves were treated with various concentrations of NaCl (50, 100, 150, 200, and 250 mmol·L⁻¹), the CK was treated with 0 mmol·L⁻¹ NaCl, and photosynthetic and physiological indexes of the leaves (the penultimate third pairs of leaves) were measured at 12, 24, and 72 h after NaCl treatment. Each treatment was repeated three times, and each treatment was repeated for 30 seedlings.

Photosynthetic indexes. The photosynthetic indexes were determined by an LI-6400 photosynthesis system (Li-COR Company, Lincoln, NE) at 12, 24, and 72 h after NaCl was applied at different concentrations. The leaf P_n , g_s , T_r , and intercellular CO₂ concentration (C_i) were measured by inverted

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H.J. and Y.Q. conceived and designed the experiments. H.J. and Z.L. carried out the experiments and analyzed the data. H.J. wrote the paper. Y.Q. and X.J. revised the manuscript.

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clover with consistent growth. A built-in light source was used, the constant light intensity was $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, the temperature was $25 \pm 1^\circ\text{C}$, and the CO_2 concentration was $400 \mu\text{mol}\cdot\text{mol}^{-1}$. Leaf water use efficiency (WUE) and stomatal limit value (L_s) were calculated as follows: $\text{WUE} = P_n/T_r$, $L_s = 1 - C_i/C_a$ (C_i is the concentration of intercellular CO_2 and C_a is the concentration of atmospheric CO_2) (Li et al., 2020).

Chlorophyll fluorescence parameters. The chlorophyll fluorescence parameters were measured by an FMS2 modulated fluorimeter (Hansatech Company, Norfolk, UK) at 12, 24, and 72 h after treatment with different concentrations of NaCl, and each leaf was repeated three times. After dark treatment for 30 min, the initial fluorescence (F_o), maximum fluorescence (F_m), variable fluorescence ($F_v = F_m - F_o$), maximum quantum efficiency of PSII (F_v/F_m), and potential activity ($F_v/F_o = F_m - F_o/F_o$) were measured. After activation for 30 min under artificial light, minimum fluorescence in light-adapted leaves (F_o'), maximum fluorescence (F_m'), and steady-state fluorescence (F_s) caused by photosynthesis and the photochemical quenching coefficient $qP = (F_m' - F_s)/(F_m' - F_o')$ were measured. The actual photochemical efficiency of PS II was determined as follows: $(\Phi \text{ PS II}) = (F_m' - F_s)/F_m'$ (Yan et al., 2016).

Antioxidant enzymes and osmoregulation substances. SOD, POD, CAT, and ascorbate peroxidase (APX) were determined by colorimetric methods using an Elabsience plant kit, following the provided instructions (Wuhan Ilerite Biotechnology Co., Ltd., Wuhan, China). Malondialdehyde (MDA) was determined by the thiobarbituric acid colorimetric method (TBA). The free proline (Pro) was determined by the indane trione staining method, soluble sugar content was determined by anthrone colorimetry, and soluble protein content was determined by the Coomassie Brilliant Blue Gmur 250 colorimetric method (Li, 2000).

Statistical analysis. Single-factor analysis of variance was carried out using SPSS 22.0, and the mean values were separated using the least significant difference test at $P = 0.05$. All figures were drawn using Origin 2018. The R language performance analytics package and OmicShare (<https://www.omicshare.com/tools/>) cloud platform were used to analyze the correlations among photosynthesis, chlorophyll fluorescence parameters, and physiological indexes.

Results

Effects of salt stress on gas exchange parameters and WUE in leaves of *C. tinctoria* seedlings. With increasing NaCl concentrations, P_n , g_s , and T_r decreased gradually at all

three time points, but the range of the decrease was different. The P_n under the 50 to 250 $\text{mmol}\cdot\text{L}^{-1}$ NaCl treatments reached 41%, 26.85%, 18.9%, 12.38%, and 3.76%, respectively, compared with the CK. The T_r under the 50 to 250 $\text{mmol}\cdot\text{L}^{-1}$ NaCl treatments reached 79.9%, 81.2%, 83.29%, 86.16%, and 93.47% of the CK at 72 h. The g_s under each NaCl treatment decreased by 62.5%, 84.58%, 83.33%, 92.92% and 91.67%, respectively, compared with that of the CK. The change of C_i was obvious at different concentrations. At 24 and 72 h, C_i of each NaCl treatment was lower than or trends to CK level. However, the changes in L_s and WUE were different at the three time points. With increasing NaCl concentrations, the L_s of most treatments increased at 12 and 24 h, but decreased gradually at 72 h. At 72 h, the L_s of 250 $\text{mmol}\cdot\text{L}^{-1}$ NaCl treatment was the lowest, reaching 25.77% of the CK level. The WUE increased gradually in 12 and 24 h with the increase of NaCl concentrations. At 72 h, 100 to 250 $\text{mmol}\cdot\text{L}^{-1}$ NaCl treatments decreased significantly, and the 250 $\text{mmol}\cdot\text{L}^{-1}$ NaCl treatment had the largest change range, reaching the lowest value at 57.2% of the CK level (Fig. 1).

Effects of salt stress on chlorophyll fluorescence characteristics in leaves of *C. tinctoria* seedlings. At the same treatment time, the fluorescence characteristics of leaves treated

Fig. 1. Effects of salt stress on gas exchange parameters and water use efficiency (WUE) in leaves of *Coreopsis tinctoria* seedlings. T_r = transpiration rate; C_i = CO_2 concentration; Cond = stomatal conductance; P_n = photosynthetic rate; L_s = stomatal inhibition rate.

Table 1. Effects of salt stress on chlorophyll fluorescence characteristics of leaves of *C. tinctoria* seedlings. F_o = initial fluorescence; F_v/F_m = potential maximum light energy conversion efficiency; F_v/F_o = potential maximum light energy activity; Φ PS II = actual photochemical efficiency of PS; qP = photochemical fluorescence quenching coefficient. Lowercase letters indicate the level of vertical difference ($P < 0.05$).

Index	Time (h)	NaCl mmol·L ⁻¹					
		CK	50	100	150	200	250
F_o	12	136.51 ± 0.50 e	146.33 ± 1.53 d	165.67 ± 5.03 c	161.67 ± 4.73 c	208.33 ± 5.03 a	199.33 ± 5.03 b
	24	135.65 ± 0.8 d	129.00 ± 5.29 d	135.67 ± 0.58 d	146.00 ± 5.29 c	154.67 ± 1.53 b	180.00 ± 7.00 a
	72	137 ± 1.00 c	135.67 ± 4.0 4c	136.00 ± 1.00 c	137.67 ± 2.52 c	142.67 ± 2.08 b	157.33 ± 3.06 a
F_v/F_m	12	0.87 ± 0.02 a	0.81 ± 0.02 a	0.83 ± 0.02 a	0.82 ± 0.02 a	0.81 ± 0.02 a	0.75 ± 0.03 b
	24	0.86 ± 0.02 a	0.86 ± 0.02 a	0.86 ± 0.01 a	0.84 ± 0.03 a	0.86 ± 0.01 a	0.86 ± 0.01 a
	72	0.87 ± 0.01 a	0.86 ± 0.00 ab	0.85 ± 0.01 bc	0.84 ± 0.01 c	0.82 ± 0.01 d	0.80 ± 0.02 e
F_v/F_o	12	6.50 ± 0.02 a	5.05 ± 0.15 b	4.99 ± 0.47 b	3.87 ± 0.49 cd	4.64 ± 0.70 bc	3.53 ± 0.10 d
	24	6.58 ± 0.16 a	6.66 ± 0.29 a	6.32 ± 0.13 ab	5.95 ± 0.38 b	5.98 ± 0.12 b	4.93 ± 0.22 c
	72	6.56 ± 0.19 a	6.41 ± 0.21 ab	6.22 ± 0.04 b	5.89 ± 0.09 c	5.53 ± 0.1 d	4.79 ± 0.11 e
qP	12	1.01 ± 0.02 a	0.92 ± 0.09 a	0.95 ± 0.04 a	0.97 ± 0.13 a	0.93 ± 0.10 a	1.01 ± 0.04 a
	24	1.00 ± 0.01 a	0.99 ± 0.03 a	0.98 ± 0.02 a	0.98 ± 0.02 a	0.99 ± 0.03 a	0.99 ± 0.01 a
	72	1.03 ± 0.01 a	0.99 ± 0.00 c	1.01 ± 0.01 b	0.99 ± 0.01 c	0.98 ± 0.01 c	0.98 ± 0.00 c
Φ PSII	12	0.81 ± 0.05 a	0.82 ± 0.08 a	0.80 ± 0.03 a	0.76 ± 0.06 a	0.81 ± 0.01 a	0.82 ± 0.04 a
	24	0.80 ± 0.04 a	0.81 ± 0.03 a	0.81 ± 0.01 a	0.82 ± 0.02 a	0.81 ± 0.02 a	0.81 ± 0.00 a
	72	0.82 ± 0.01 a	0.82 ± 0.01 a	0.81 ± 0.00 a	0.80 ± 0.00 a	0.80 ± 0.00 a	0.77 ± 0.02 b

with different NaCl concentrations were different. The F_o of the leaves of *C. tinctoria* showed an upward trend with increasing NaCl concentration. The increase of each treatment was the lowest at 72 h. There was no significant difference between F_v/F_m and qP at 12 and 24 h, but these values decreased with the increase in NaCl concentrations at 72 h, especially under the 200 to 250 mmol·L⁻¹ NaCl treatments. The F_v/F_o treatment at each time point showed a decreasing trend with increasing NaCl concentration. There was no significant difference in Φ PSII among the treatments. Φ PSII was the lowest under the 250 mmol·L⁻¹ treatment, which was 6.10% lower than that of the CK at 72 h (Table 1).

Correlation analysis of photosynthetic fluorescence indexes of *C. tinctoria* seedlings under salt stress. There were significant or highly significant differences in the correlation between photosynthetic indexes and chlorophyll fluorescence parameters of *C. tinctoria* under salt stress. Among these values, L_s was positively correlated with F_v/F_m ($r = 0.89$, $P < 0.01$), F_v/F_o ($r = 0.94$, $P < 0.01$), qP and P_n ($r = 0.88$, $P < 0.01$), and T_r and g_s ($r = 0.81$, $P < 0.01$); F_o was negatively correlated with P_n , g_s , L_s , T_r , and WUE, especially with L_s ($r = -0.81$, $P < 0.01$); C_i was not significantly associated with chlorophyll fluorescence (Fig. 2).

Effects of salt stress on antioxidant enzymes in *C. tinctoria* seedlings. Simultaneously, the changing trends of the SOD, POD, CAT, and APX contents under the various NaCl concentration treatments were different. With increasing NaCl concentrations, the change in the SOD content had no obvious pattern at the three time points, but the SOD content of the 50 and 100 mmol·L⁻¹ NaCl treatments was significantly higher than that of the other treatments at the same time. Similarly, The POD content of increased first and then decreased with the increase in the NaCl concentrations at each of the three time points, and the 150 mmol·L⁻¹ NaCl treatment group had significantly higher POD content than the other treatment groups at 24 and 72 h. There was no significant difference

in the CAT content among the other treatments except for the 50 mmol·L⁻¹ NaCl treatment at 12 h. At 24 and 72 h, with the increase in NaCl concentration, the CAT content under each treatment first increased and then decreased, and the CAT content under the 150 mmol·L⁻¹ NaCl treatment was significantly higher than that under the other treatments. The APX content under the 50 to 200 mmol·L⁻¹ NaCl treatment was significantly higher than that of the CK at 12 h. At 24 h, there was no significant difference between the APX content among the CK and all treatment groups. At 72 h, the APX content of all treatments tended to or lower than that of CK, and the content of 250 mmol·L⁻¹ NaCl treatment was the lowest (Fig. 3).

Effects of salt stress on MDA and osmoregulation substances of *C. tinctoria* seedlings. At the same sampling time, the MDA content increased with increasing NaCl concentration; in particular, the increase in the MDA content under each treatment was the highest at 72 h. The changes under the 50 to 200 mmol·L⁻¹ NaCl treatments were relatively stable, and the change under the 250 mmol·L⁻¹ NaCl treatment was significantly

higher than that of the other treatments. At 72 h, the MDA content of the 250 mmol·L⁻¹ NaCl treatment group peaked, which was ≈ 2.16 times higher than that of the CK. The changing trend of Pro content was consistent at 12 and 24 h. With increasing NaCl concentrations, the Pro content first increased, decreased under the 150 mmol·L⁻¹ NaCl treatment, and then increased significantly with the 200 to 250 mmol·L⁻¹ NaCl treatments. However, the content of Pro first increased and then decreased at 72 h and reached a maximum in the 150 mmol·L⁻¹ NaCl treatment group, which was ≈ 1.78 times that of CK. At the same time, the content of soluble sugar accumulated with the increase of NaCl concentration, and the soluble sugar content of 150 to 250 mmol·L⁻¹ NaCl treatments increased significantly. At 72 h, the soluble sugar content of 150 to 200 mmol·L⁻¹ NaCl treatments reached the maximum value, which was 58.95% and 57.28% higher than that of the CK, respectively. The content of soluble protein increased under all treatments at the three times, and the increase was the highest at 72 h. At the same sampling time, there were significant differences in soluble protein contents among different NaCl concentration treatments. The 150 mmol·L⁻¹ NaCl treatment group showed significantly higher soluble protein contents than other treatment groups, reaching the maximum at 72 h, which was 1.53 times higher than that of the CK (Fig. 4).

Correlation analysis of antioxidant enzymes and osmoregulation substances of *C. tinctoria* seedlings in response to salt stress. There was a positive correlation between most physiological indexes. Soluble sugar, CAT, and soluble protein showed a significant positive correlation ($r = 0.84$, $P < 0.01$; $r = 0.80$, $P < 0.01$). APX and Pro and MDA showed a significant negative correlation ($r = -0.51$, $P < 0.01$) (Fig. 5).

Discussion

It is commonly believed that under salt stress, stomatal and nonstomatal limitations

Fig. 2. Correlation analysis of photosynthetic fluorescence indexes of *C. tinctoria* seedlings under salt stress. ns represents nonsignificant; * and ** represent the significance of $P < 0.05$ and $P < 0.01$, respectively.

Fig. 3. Effect of salt stress on antioxidant enzymes of *C. tinctoria* seedlings. SOD = superoxide dismutase; POD = peroxidase; CAT = catalase; APX = ascorbate peroxidase.

are the main factors that reduce P_n . Stomatal limitations were manifested as decreases in g_s and C_i with an increase in L_s . In contrast, if the photosynthetic capacity of mesophyll cells significantly decreased, C_i may increase or not change even when g_s is low. At this time, the L_s value decreased, and nonstomatal limitation became the main factor in photosynthetic reduction. Stomata are the ordinary channels of CO_2 and H_2O in and out of leaves during photosynthesis and transpiration. They are essential factors in controlling the water-use level of leaves. The size of g_s has certain restrictions on P_n and T_r and then affects WUE (Luo et al., 2019; Su et al., 2019). The effect of the decrease in g_s on photosynthesis was less than that on transpiration. T_r had a strong dependence on stomata, and part of stomatal closure was beneficial to WUE (Wang, 2013). The results showed that under short-term salt stress, P_n , g_s , and T_r in the leaves of *C. tinctoria* showing a significant downward trend with the increase of NaCl concentrations. The C_i of most salt stress treatments were lower than that of the CK at 12, 24, and 72 h. At 12 and 24 h, the L_s of most salt stress treatments were higher than that of the CK. At 72 h, the L_s of 50 to 100 mmol·L⁻¹ NaCl treatments resulted in no significant difference from the CK, and the 150 to 250 mmol·L⁻¹ NaCl

treatments resulted in significantly lower levels than did the CK. This finding could explain why the photosynthesis of the seedlings of *C. tinctoria* was considerably inhibited, with stomatal limitations being prevalent at low concentrations (50 to 100 mmol·L⁻¹ NaCl) and nonstomatal limitations being prevalent at medium and high salt concentrations (150 to 250 mmol·L⁻¹ NaCl). The two mechanisms changed with the salt concentration. The WUE of 200 to 250 mmol·L⁻¹ NaCl treatments were significantly higher than that of the 50 to 150 mmol·L⁻¹ NaCl treatments at 12 and 24 h. The increase in WUE at high salt concentrations was due to the decrease in P_n and increase in L_s . The partial closure of stomata made the decrease in T_r more significant than the P_n decrease, thereby improving WUE. At 72 h, with the increase in NaCl concentration, WUE first increased and then decreased. The WUE under the 200 to 250 mmol·L⁻¹ NaCl treatments were significantly lower than that under the CK, which may have been due to the decrease in P_n and L_s . The reduction in the high concentration of P_n was mainly caused by nonstomatal factors, and the decrease rate of P_n was lower than that of T_r , which led to the decrease in WUE.

Under salt stress, the photosynthetic rate of plants decreases, and the absorption,

transmission and transformation of light energy by plants are inevitably affected, particularly causing a decline in photochemical activity (Wang, 2013). F_v/F_m is the maximum quantum yield of PSII, which reflects the potential maximum photosynthetic capacity of plants. $\Phi PSII$ reflects the actual primary light capture efficiency of PSII reaction center (Hu et al., 2020). At 72 h, this study showed that F_v/F_m decreased significantly under the 200–250 mmol·L⁻¹ NaCl treatments, and $\Phi PSII$ was the lowest in 250 mmol·L⁻¹ NaCl treatment. It indicated that light inhibition occurred in the leaves of *C. tinctoria* and showed that with the deepening of salt stress, the actual light energy capture efficiency of the PSII reaction center decreased gradually. Compared with the CK, qP decreased significantly, which was the lowest in 200 to 250 mmol·L⁻¹ NaCl treatments. It is suggested that the photosynthetic activity of *C. tinctoria* decreased under high salt stress, and qP is the fluorescence quenching caused by photosynthesis, which might be related to the decrease of photosynthetic efficiency. The correlation analysis also showed that L_s was positively associated with F_v/F_m and F_v/F_o , and qP was positively correlated with P_n and T_r , which indicated that the intensity of photosynthesis was closely related to chlorophyll fluorescence.

Fig. 4. Effects of salt stress on malondialdehyde (MDA) and osmoregulation substances of *C. tinctoria* seedlings.

The reactive oxygen species produced by photosynthetic organs under salt stress aggravated membrane lipid peroxidation, increased membrane lipid peroxide product MDA, and decreased photosynthetic capacity of leaves (Zhu et al., 2015). To avoid damage caused by reactive oxygen species, chloroplasts form a complete antioxidant system (Dong et al., 2018). SOD, POD, CAT, and APX play essential roles in the enzymatic system of plants (Guan et al., 2015). They reduce the damage of reactive oxygen species to the cell membrane, reduce membrane peroxidation, and stabilize membrane permeability by scavenging superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide. Therefore, the scavenging ability of the antioxidant system for reactive oxygen species is a key factor used to measure the resistance of plants to stress (Meng et al., 2019). In this study, the activities of POD and CAT first increased and then decreased with increasing salt concentration, but both were higher than those in the CK and reached their highest levels under the 150 mmol·L⁻¹ NaCl treatment. The APX content decreased, and activity was inhibited. The activities of POD and CAT increased accordingly to eliminate the accumulation of reactive oxygen species to achieve a new dynamic equilibrium. The MDA contents of the leaves of *C. tinctoria* under NaCl treatment was higher than that of the CK at three

sampling times. The MDA content of the 250 mmol·L⁻¹ NaCl treatment was the highest, being 2.16 times that of the CK. This result indicated that with increasing NaCl concentration, the leaf cells of *C. tinctoria* were gradually affected by membrane lipid peroxidation, and the degree of damage to the cell membrane structure was deepened. This finding was consistent with the research results of Liu et al. (2013), Ma et al. (2018), and Li et al. (2019) on *Salicornia bigelovii*, *Xanthoceras sorbifolia*, and *Narcissus tazetta* var. *chinensis*. The content of MDA in leaves of *C. tinctoria* seedlings reached the maximum in 250 mmol·L⁻¹ NaCl treatment, indicating that despite the existence of antioxidant system mechanism, salt stress could still cause membrane lipid peroxidation in seedling leaves.

Photosynthesis is the basis of plant metabolism, photosynthetic capacity is inhibited under salt stress, and plant physiological metabolism is also affected. Pro, soluble sugar, and soluble protein are important organic osmoregulation substances of plants that play an important role in participating in various physiological and biochemical metabolism processes (Wang, 2013). The increase in osmotic regulating substances content is one of the self-defense responses of plants under salt stress. The increase in proline content in cells can maintain the swelling and pressure

of cells and protect the enzyme and membrane system from toxicity. Salt stress can enhance the synthesis and metabolism of proteins in cells and trigger protein synthesis to reduce the osmotic potential of cells (Sun et al., 2017). In this study, the content of Pro, soluble sugar, and soluble protein increased under different NaCl concentrations at three sampling times, especially for the 150 to 250 mmol·L⁻¹ NaCl treatments, which was consistent with Yao et al. (2015), Adams et al. (1992), and Gao et al. (2017). It indicated that the leaves of *C. tinctoria* were most sensitive to high-salt stress and were protected, to a certain degree, against salt stress injury by accumulating proline, soluble sugar, and soluble protein.

Conclusion

With increasing NaCl concentration, P_n , g_s , T_r , and L_s in the leaves of *C. tinctoria* showed a significant downward trend. High concentrations of salt stress (200 to 250 mmol·L⁻¹ NaCl) significantly inhibited F_v/F_o and F_v/F_m in the leaves of *C. tinctoria*. The activities of the antioxidant enzymes POD and CAT were significantly promoted, and organic compounds, including soluble sugar, soluble protein, and Pro, accumulated significantly under salt stress. The results reveal the strategy by which *C. tinctoria* adapts to salt

Fig. 5. Correlation analysis of antioxidant enzymes and osmoregulation substances of *Coreopsis tinctoria* seedlings in response to salt stress. MDA = malondialdehyde; Pro = free proline; SS = soluble sugar; SP = soluble protein; SOD = superoxide dismutase; POD = peroxidase; CAT = catalase; APX = ascorbate peroxidase.

stress and provide a theoretical reference for further study of the salt tolerance of *C. tinctoria*.

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Supplemental Table 1. The composition of Hoagland solution.

Material	Chemical formula	Dosage (mg/L)
Calcium nitrate tetrahydrate	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	945
Potassium nitrate	KNO_3	607
Ammonium phosphate	$(\text{NH}_4)_2\text{HPO}_4$	115
Magnesium sulfate heptahydrate	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	493
Ethylenediaminetetraacetic acid disodium salt	EDTA-Na ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8$)	20–40
Orthoboric acid	H_3BO_3	2.86
Manganese sulphate	$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	2.13
Zinc sulfate	$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.22
Copper sulfate	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.08
Ammonium molybdate	$\text{H}_8\text{MoN}_2\text{O}_4$	0.02