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THE RESPONSES OF ANTIOXIDANT ENZYMES OF GENE EXPRESSION AND ACTIVITY TO SALINITY IN *ILEX VERTICILLATA*

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Abstract

Ilex verticillata is a deciduous shrub of great ornamental and commercial value owing to the red fruits that hang on its branches in winter. However, little is known about its salinity adaptability and mechanisms. Here, we investigated the mechanism of antioxidant gene expression and enzyme activity responses to salinity in *Ilex verticillata* under 50-100 mM NaCl treatments. Our results showed *I. verticillata* cutting seedlings were slightly affected under 50 mM treatment. However, 100mM NaCl inhibited the cutting seedlings growth, damaged chloroplasts and cell structures, and led to $O_2^{\cdot-}$ and H_2O_2 over-accumulated, whereas it was observed that the leaves fell off and new buds grew at NaCl treated for 20 days. In contrast, peroxidase (POD), catalase (CAT), and glutathione reductase (GR) activities, and AsA (ascorbic acid), GSH (glutathione) contents were significantly enhanced. Therefore, *I. verticillata* cutting seedlings could tolerate stress as high as 100mM NaCl by wilting young leaves to preserve buds. There were dose effects and time effects in enzyme activities and gene expressions. The enzyme activities (SOD, CAT and APX) and transcript levels (*IvCu-ZnSOD*, *IvCAT* and *IvAPX*) of cutting seedlings were asynchronous under 100mM NaCl treated for 20 days. This may be due to the synthesis and activation of enzymes lagging behind gene expression. This work would provide a reference for the cultivation of new varieties, and the breeding of elite genetic resources for salt tolerance. This is the first detailed report on salinity tolerance in *I. verticillata*.

Key words: *Ilex verticillata*; Salinity; Antioxidant enzymes; Gene expressions; Responses

Introduction

Soil salinity affects plant growth and yield as one of the most abiotic stress factors (Cai *et al*., 2025; Mariyam *et al*., 2023; Abdallah *et al*., 2024; Feng *et al.,* 2025). The arid regions, coastal fringes, and estuaries are sources of saline soils which are dominated by Na^+ ions above 40 mM NaCl (Munns & Tester, 2008; El Yamani *et al*., 2024). Drought and extensive irrigation also cause soil salinization and more than one billion hectares in the world are affected by salinity. Among them, 412 million hectares (20% of the world's irrigated arable land) is considered saline and alkaline (Montesinos *et al*., 2024; Du *et al*., 2023). In China, about 9.9×10^7 hectares and nearly 10.3% of the total land area is saline soil, and 4.88% of the total irrigated arable land (Ji *et al*., 2022; Gao *et al*., 2022). Therefore, it is very important and necessary for investigating the response to salt stress of plant and its adaptive mechanisms to salt stress.

Salinity could first cause osmotic stress, then led to ion toxicities and nutritional imbalance, and lastly result in various negative consequences such as growth retardation, pigments degradation, photosynthetic capacity decrease, ions uptake, and translocation restriction (Zhang *et al*., 2024; Mulet *et al*., 2023; El-Beltagi *et al*., 2024; Appiah *et al*., 2024). Salinity could further induce oxidative stress, a secondary stress, due to over synthesis reactive oxygen species (ROS), RNS (reactive nitrogen species), and reactive carbonyl species (RCS) (Sultana *et al*., 2022; Lu *et al*., 2023). Reactive oxygen species in plant mainly include superoxide ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) (Chen *et al*., 2018; Feng *et al*., 2022; Shahzad *et al*., 2024). Appropriate dose of ROS could play a signaling message role, whereas over high dose of ROS lead to oxidative stress, for example, it attacks cell membrane lipids, nucleic acids and proteins (Sultana *et al*., 2022; Mishra *et al*., 2023; Wang *et al.*, 2023).

However, plants could evolve a defensive strategy to respond to salinity stressful conditions, such as salt-induced, soluble sugars, proline and betaine (compatible osmolytes), and inorganic ions, etc. (Mansour & Ali, 2017; Kang *et al*., 2024; Shahzad *et al*., 2024). In addition, the plant also generates efficient antioxidant defense systems to scavenge over accumulation ROS. Superoxide dismutase (SOD, EC1.15.1.1), catalase (CAT, EC1.11.1.6), peroxidase (POD, EC1.11.1.7), glutathione reductase (GR, EC1.6.4.2), and ascorbate peroxidase (APX, EC1.11.1.1) are important antioxidant enzymes, and ascorbic acid (AsA), reduced glutathione (GSH) are antioxidant substances. These substances are key players in the antioxidant scavenging systems (Lu *et al*., 2023; Talaat *et al*., 2023; Garcia-Locascio *et al*., 2024; Noctor 2025).

The gene expressions of antioxidant enzymes at the transcriptome level could change plant tolerance to salinity stress (Qi *et al*., 2023). Cu/Zn-SOD (one of SOD isoenzymes) is located in chloroplasts, mitochondria, and chloroplasts and cytoplasm, respectively, and it is the most important SOD isoenzyme in the SOD superfamily (Xie *et al*., 2018; Qi *et al*., 2024). The gene expression of *Cu/ZnSOD* in *Corbicula fluminea* was sensitive to Cd^{2+}, Cu^{2+}, and Pb^{2+} ions contamination in water (Xie *et al*., 2018). Overexpression of the *APX1* gene from *Arabidopsis thaliana* enhances salinity tolerance in *Brassica juncea* (Saxena *et al*., 2020). Upregulation of *GpCAT1* gene expression in *Gymnocarpos przewalskii* increased the hydrophilicity of the *GpCAT1*

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protein, and enhanced its affinity for H_2O_2 (Qi *et al*., 2023). The transcript levels of *CsCAT* genes in cucumber showed differences under different abiotic stresses, and the levels of *CsCAT1* and *CsCAT2* were moderately decreased for salt and drought (Hu *et al*., 2016).

I. verticillata L. (common winterberry, America holly, black alder) is a deciduous shrub and dioecism found in wetland areas throughout eastern North America in the United States and southeast Canada (Gargiullo & Stiles, 1991; Lu *et al*., 2024). The red fruits (rarely yellow) ripen in September and maintained until late January. This species posses an important ornamental and commercial value, owing to its red fruits enjoyed in winter. Therefore the cutting branches are used for holidays such as Christmas and New Year's Day, and Spring Festival. In recent years, some deciduous holly species have been imported from the North America to China, and rapid propagation, drought and cold tolerance in the imported *I. verticillata* species were researched (Ma *et al*., 2020; Liu *et al*., 2021; Xie *et al*., 2023; Qin *et al*., 2023; Li *et al*., 2024). Currently, salt resistance has been reported about other plant of Ilex family, such as Ammopiptanthus (evergreen Ilex, local species, and are not of the same genus with *I. verticillata* (deciduous)) showed tolerance to low salt concentrations (<0.7%) (Li *et al*., 2008), while moderate salt (>80mmol.L^{-1}) significantly inhibited seedling growth (Zhang *et al*., 2014). However, little information is useable on *I. verticillata* (new imported species) of physiological and biochemical responses to salinity stress and adaptation.

The purpose of the present work was to elucidate the changes in antioxidant enzymes and gene expressions, antioxidant metabolites, and adaptation mechanism in *I. verticillata* seedlings when exposed to two concentrations of NaCl. This work would provide a reference for the introduction and cultivation of new *I. verticillata* species, and also provide elite genetic resources for salt tolerance plants in saline-alkali soil.

Material and Method

Culture conditions and plant treatments: At present, new varieties of Ilex deciduous are mainly propagated by cuttings and plant tissue culture technology. Therefore, two-year-old cutting seedlings used as experimental materials of this work. The two-year-old cutting seedlings of *I. verticillata* ('Oosterwijk') (approximately 50cm high) (from Nanjing QingzhuGuo Horticulture Co., LTD, China) were transferred individually into 15cm diameter plastic pots (cutting seedling/pot) containing nutrient soil, garden soil, and perlite (V=2:1:1), and cultured in a controlled chamber at 25 ± 3°C under a 14/10h, with 160μmol m^{-2} s^{-1} light intensity and 70–80% relative humidity (RH). The cutting seedlings were watered with the half-strength Hoagland's nutrient solution (Hoagland & Arnon, 1950) every 3 days and renewed once a week. The cutting seedlings were trained for four months and new shoots of 8 cm length were used for the experiment. After 4 months of cultivation, the seedlings were irrigated with NaCl solution (dissolved in the half-strength Hoagland's solution) including 0, 50, and 100mM treatments. A tray was placed at the bottom of the pot to collect the leaked salt solution. Cutting seedling per pot (one seedling/pot) was watered with 200mL NaCl solution every 4 days. Each experimental unit consisted of 10 cutting seedlings and completed with three replicates (total 30 cutting seedlings per treatment). After 10 or 20 days of treatment, leaves (the 3rd to 5th leaf from the top of the branch) were sampled and stored at -80°C, used for the active substance and transmission electron microscopy (TEM) analysis. After determination of the fresh weight (FW), the dry weight (DW) was obtained when leaves was dried to constant weight at 60°C.

Cutting seedling recovering: In order to verify the recovery ability of the seedlings, after treated with 50 and 100mM NaCl for 20 days, these cutting seedlings were then transferred to salt–free media after desalination for a further 20 days to recover the culture.

Chlorophyll content: Leaves cutting into about 0.2cm^2 were submerged in a mixed solution including acetone: ethanol: water (4.5:4.5:1) overnight. Chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid (Car) contents were measured following Chen *et al*., (2014).

REC, MDA, H_2O_2, and $O_2^{\cdot-}$ analyses: The REC (relative electrolytic leakage), malondialdehyde (MDA), and H_2O_2 content were analyzed (Kohnhehshahri & Demir, 2021). The MDA absorbance was determined at 532 and 600nm. H_2O_2 was extracted from leaves in cold acetone (−20°C) and centrifuged at 10,000 ×g at 4°C for 15min. Titanium sulfate (0.1 %) and ammonium hydroxide were added to the supernatant and centrifuged at 6,000 ×g at 4°C for 10 min. The precipitate (washed twice with cold acetone) dissolved in 2mol/L H_2SO_4 for absorbance measurement absorbance at 415 nm. The content of $O_2^{\cdot-}$ was analyzed following the method Vighi *et al*., (2017) and Feng *et al*., (2022).

Histochemical staining: The histochemical staining of H_2O_2 or $O_2^{\cdot-}$ production refers to the method of Negi *et al*., (2016). The leaves were infiltrated with DAB (3, 3-diaminobenzidine) or NBT (nitro blue tetrazolium) solutions at 25°C for 1h in the dark, respectively. Subsequently, the leaves were immersed in 95% ethanol for 6 hours to remove chlorophyll. DAB would give a reddish brown color, which was used to detect the presence of H_2O_2, whereas NBT would react with $O_2^{\cdot-}$ to give a dark blue insoluble compound.

Antioxidant enzyme activity analyses: Leaf tissue was macerated and homogenized in extraction buffer containing 100mM PBS (phosphate buffer sodium, pH 7.0), 0.1mM EDTA, and 10mM ascorbic acid. The extracts were then centrifuged at 10,000×g for 15 min at 4°C. The activities of SOD, CAT, measured as described by Panda *et al*., (2017). The SOD activity was determined by method of nitro blue tetrazolium (NBT) at 570nm. The activity of CAT was determined by ultraviolet absorption method at 240nm. The determination of POD activity was referred to the method of Chen *et al*., (2014). The APX and GR activity were determined according to Martinez-Ispizua *et al*., (2022), Kohnhehshahri & Demir (2021) method, respectively.

AsA and GSH content analyses: The AsA abundance was measured following Martínez-Ispizua *et al*., (2022). The GSH was modified according to Sanjida *et al*., (2022) in a reaction mixture containing supernatant, 100mM PBS (pH7.0), and 3mM DTNB (5, 5'-dithiobis - (2-nitrobenzoic acid)). The absorbance was determined using a spectrophotometer at 412nm.

RT-qPCR: Using an RNAprep Pure Plant Plus Kit (Tiangen, Beijing, China) extracted total RNA from the *Ilex verticillata* leaves, and using an Prime Script RT reagent Kit (TAKARA, Beijing, China) performed reverse transcription with appropriate primers. The RT-qPCR was carried using the Step One Real-Time PCR Detection System (ThermoFisher, Waltham, MA, USA). Primer sequences for *CuSOD*, *CAT*, *APX*, and GR GSL designed with Snapgene primer soft (Biotech LLC, America) are showed in Table 1. *GAPDH* gene was as an internal reference gene. The relative gene expressions were analyzed using the $2^{\Delta\Delta}$CT method (Livak & Schmittgen, 2001).

Transmission electron microscopy: The leaf ultrastructure was assayed according to Chen *et al*., (2018). Then fixed, and dehydrated, samples were embedded in Epon 812 resin, then were sectioned with a LKB-V ultramicrotome into about 70 nm sections. After staining the thin sections with lead citrate and uranyl acetate, the cell structure was photographed using a JEM-1400 transmission electron microscope (TEM) (JEOL Ltd operated at 75 kV).

Statistical analysis

All data were processed with ANOVA (one-way analysis of variance) of SPSS 20.0

Software (IBM, Armonk, NY). The Figures used by Prime8.0 and Origin 21.0 software. Duncan's multiple comparisons were used to determine significant differences at $p<0.05$, 0.01.

Results

Plantlet responses to NaCl treatment: The morphological responses to NaCl treatment in *I. verticillata* cutting seedlings under salt treatment for 20 days are shown in Fig. 1. The control leaves showed bright green and growing well (Fig. 1A-C). The lower dose of NaCl treatment (50mM) showed a slight effect on growth (Fig.1D-F), whereas the higher dose (100mM NaCl) treatment reduced cutting seedling growth (Fig.1G-H). The leaf margin and blade surface become red and dry in small and tender leaves under 50mM NaCl treatment (Fig. 1D-E). However, the leaves on the lower part of the branch had withered margins and brown spots (Fig.1G) and some tender leaves on the upper part of the branch were dried and curled at the edge in the 100mM NaCl treatment (Fig. 1H). Furthermore, new shoot length increments in 100 mM NaCl treatment were significantly lower (36.1% and 41.9%) than those in the control and 50mM NaCl treatments, respectively, after 20 days of treatment (Table 2). Two NaCl treatments both increased dry weight (DW) and decreased water content (WC) of leaves, whereas no significant changes in fresh leaf weight were observed compared to the control (Table 2). Interestingly, the injured cutting seedlings treated with 100mM NaCl for 20 days could recover when transferred to a NaCl-free medium (desalination) and cultured for a further 20 days. The new light and axillary buds appeared and resumed growth (Fig. 1I).

Effect of salinity on photosynthetic pigments: No significant changes in Chl a, Chlb, total Chl (a+b), and Caroline levels were observed between the 50 mM NaCl treatment and the control, whereas the Chl-a and total Chl contents in leaves decreased by 21.7% and 20.4% in 100 mM NaCl treatment on day 20, respectively, compared to control (Table 3). In contrast, the Chl a/b ratio increased significantly in 50 and 100 mM NaCl treatments, but the total Chl/Carotene ratio did not significantly change between the NaCl treatments and control (Table 3).

Table 1. Primer sequences in qPCR analysis.

Gene ID	Forward Primer	Reverse Primer
IvCuZnSOD	CCAGGCCTACATGGGTTCCA	AATCACCCGCATGTCGCTCT
IvCAT	TCGTGAGCGGATCCCAGAAC	GGCTCGCAGGAAATCAGCAC
IvAPX	CTCCCAAAGAAGGGCGCCTA	GAGCCCTTCCCAATGTGTGC
IvGR	ACACGTGGCATCGAAGGTCT	CCACAGTGCTCCCACCTTCA
IvGAPDH	CATCCCTTAGCACCTTCCAAC	ATCCTCACGTCAATGCTTCTC

Table 2. Effect of NaCl on growth indexes in *I. verticillata* cutting seedling leaves (20 day).

NaCl (mM)	New shoot length increments (cm)	Fresh weight of single leaf (mg)	Dry weight of single leaf (mg)	Water content (%)
0	3.25 ± 0.38a	114.33 ± 5.31a	32.00 ± 4.89b	72.15 ± 3.00a
50	3.58 ± 0.31a	110.67 ± 6.94a	45.33 ± 4.49a	58.62 ± 6.72ab
100	2.08 ± 0.29b	108.00 ± 5.71a	52.67 ± 5.31a	50.84 ± 7.52b

Values represent means. Different letters in the same row denote significant differences ($p<0.05$)

Table 3. Effect of NaCl on pigments contents in *I. verticillata* seedling leaves (20 day).

NaCl (mM)	Chl a ($mg.g^{-1}FW$)	Chl b ($mg.g^{-1}FW$)	Total Chl ($mg.g^{-1}FW$)	Caroline ($mg.g^{-1}FW$)	Chl a/b	Total Chl/Car
0	0.97 ± 0.14a	0.32 ± 0.06a	1.30 ± 0.19a	0.36 ± 0.06a	2.99 ± 0.42b	3.58 ± 0.20ab
50	1.05 ± 0.17a	0.30 ± 0.03a	1.36 ± 0.19a	0.36 ± 0.05a	3.47 ± 0.38a	3.72 ± 0.05a
100	0.76 ± 0.11b	0.24 ± 0.07a	1.00 ± 0.17b	0.30 ± 0.05a	3.23 ± 0.45a	3.34 ± 0.21b

Values represent means. Different letters in the same row denote significant differences ($p<0.05$)

Fig. 1. Morphology changes of *Ilex verticillata* cutting seedlings with NaCl treatments (A-C contrast; D-F: 50mM NaCl for 20 days; G-H:100mM NaCl for 20 days; I: Remove salt and restore 20 days after treatment with 100mM NaCl for 20 days).

Effect of salinity on leaf ultrastructure: Mesophyll cells and chloroplasts under TEM (transmission electron microscopy) revealed several changes in the size of the treated plants (Fig. 2). Chloroplasts from control leaves were close to the edge of cell, and ovalized with a typical arrangement of thylakoids and intact stacks of grana. TEM observations also showed that the organelles of the mesophyll cells were normal with many starch granules in chloroplasts, and a clear and dense cytoplasm in the control condition (Fig. 2A, B, C).

The ultrastructure of mesophyll cells in leaves of *I. verticillata* cutting seedlings showed slight changes after treated with low salinity (50 mM NaCl) for 20 days. The cells were slightly swollen, with an organized thylakoid membrane system and clearly differentiated, larger spaces in grana, fewer plastoglobuli, and more and larger starch granules than the control (Fig. 2D, E, and F).

Drastic changes were observed under higher saline conditions (100mM NaCl) for 20 days. For example, the distribution of chloroplasts was disorganized and irregularly arranged in the center of the cell rather than close to the cell membrane, few cells had starch granules, and thylakoid lamella was reduced compared to the control and 50mM NaCl treatment (Fig. 2 G, I). Furthermore, some cells with fragmented or cotton-like cytoplasm and severe plasmolysis were also observed in 100mM NaCl treatment (Fig. 2G, H, I). This illustrated *I. verticillata* cutting seedlings were damaged to some extent under 100mM NaCl treatment.

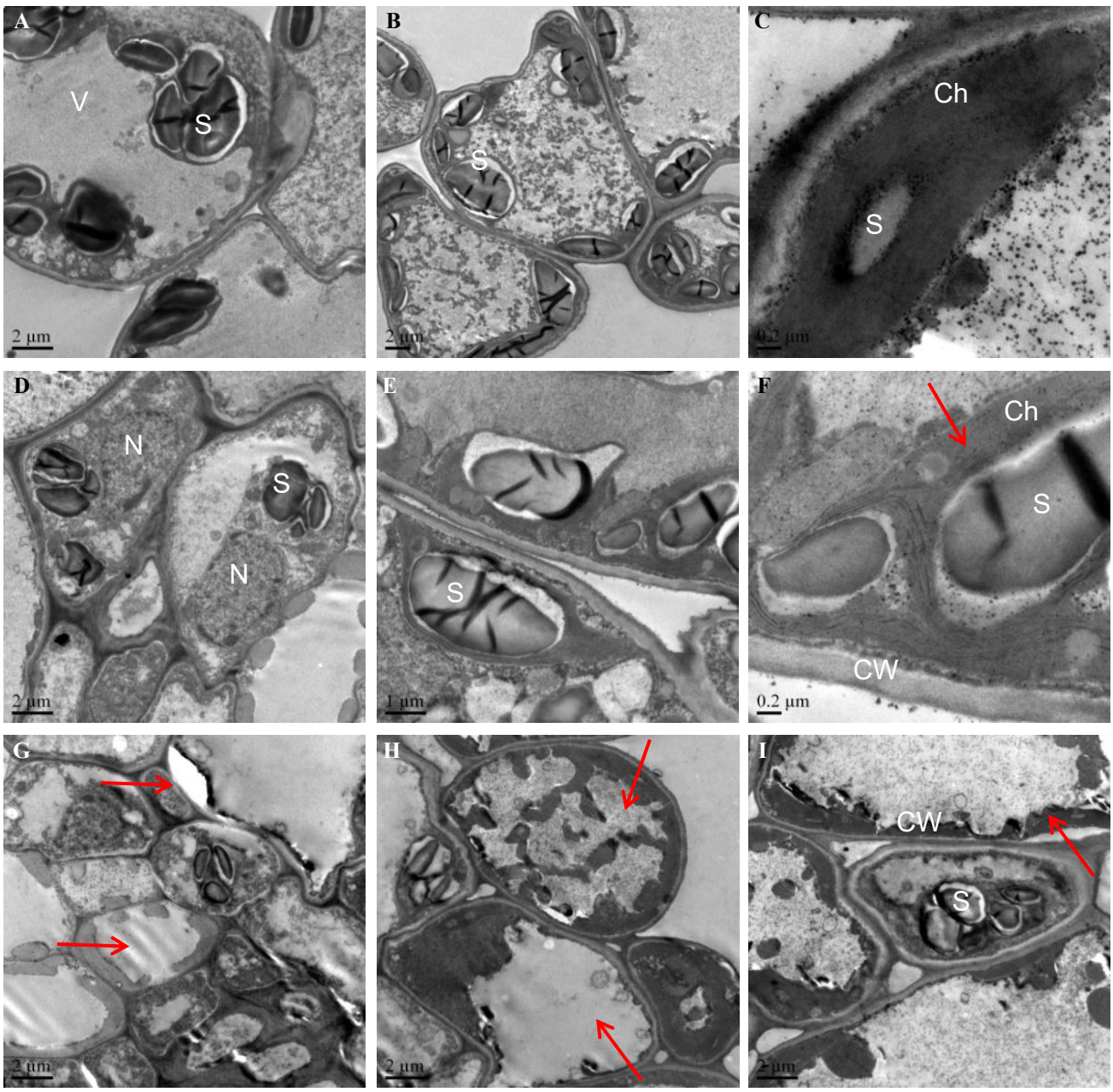

Fig. 2. Changes of cell ultrastructure in *I. verticillata* L. under salinity stress. (A-C: Control, D-F: 50mM NaCl treatment, G-I: 100mM NaCl treatment; N: Nucleus; S: Starch grain; CW: Cell wall; Ch: Chloroplast; V: Vacuole

Changes of REC, H_2O_2, MDA, and $O_2^{\cdot-}$: The REC of *I. verticillata* seedlings was not significantly changed in 50mM NaCl treatment for 10~20 days compared with the control. However, it increased sharply by 28.9% on day 10 or 84.9% on day 20 at 100mM NaCl treatment compared to the control (Fig.3 A, $p<0.05$).

Compared to the control, changes in H_2O_2 levels were more closely related to the duration of salt treatment time, with no significant statistical changes found on day10 under two NaCl treatments. However, after 20 days of exposure to 50mM and 100mM NaCl, the H_2O_2 content increased by 22.5% and 72.3 %, respectively. However, no significant statistical changes were found under 50mM NaCl treatment ($p>0.05$) (Fig. 3B).

After 10 days of salt stress, $O_2^{\cdot-}$ content in leaves increased with increasing the NaCl dose and duration time (Fig. 3C). After 20 days of salt stress, the content of $O_2^{\cdot-}$ was significantly enhanced in the two NaCl treatments and increased by 17.9% in 50 mM NaCl, and 74.8% in 100 NaCl treatment compared to the control (Fig. 3C).

The NaCl treatment also resulted in a significant increase in MDA contents at two doses. The MDA content was not significantly different at 50mM NaCl, while increased by 14.8% at 100mM NaCl compared to the control treated for 10 days. Moreover, the MDA content also increased by 13.8% and 41.3% at 50 and 100mM NaCl treated for 20 days, respectively (Fig. 3D).

Histochemical staining: Figure 4 presents the histochemical detections of H_2O_2 or $O_2^{\cdot-}$ with DAB and NBT staining, respectively. Leaves produced more brown staining by DAB with increasing NaCl dose, especially at 100mM NaCl treatment (Fig. 4B-C). Similarly, leaves showed more dark blue by NBT staining at 100mM than at 50mM NaCl and the control (Fig. 4D-F). These results indicated higher accumulation of H_2O_2 and $O_2^{\cdot-}$ in the 100mM NaCl treatment.

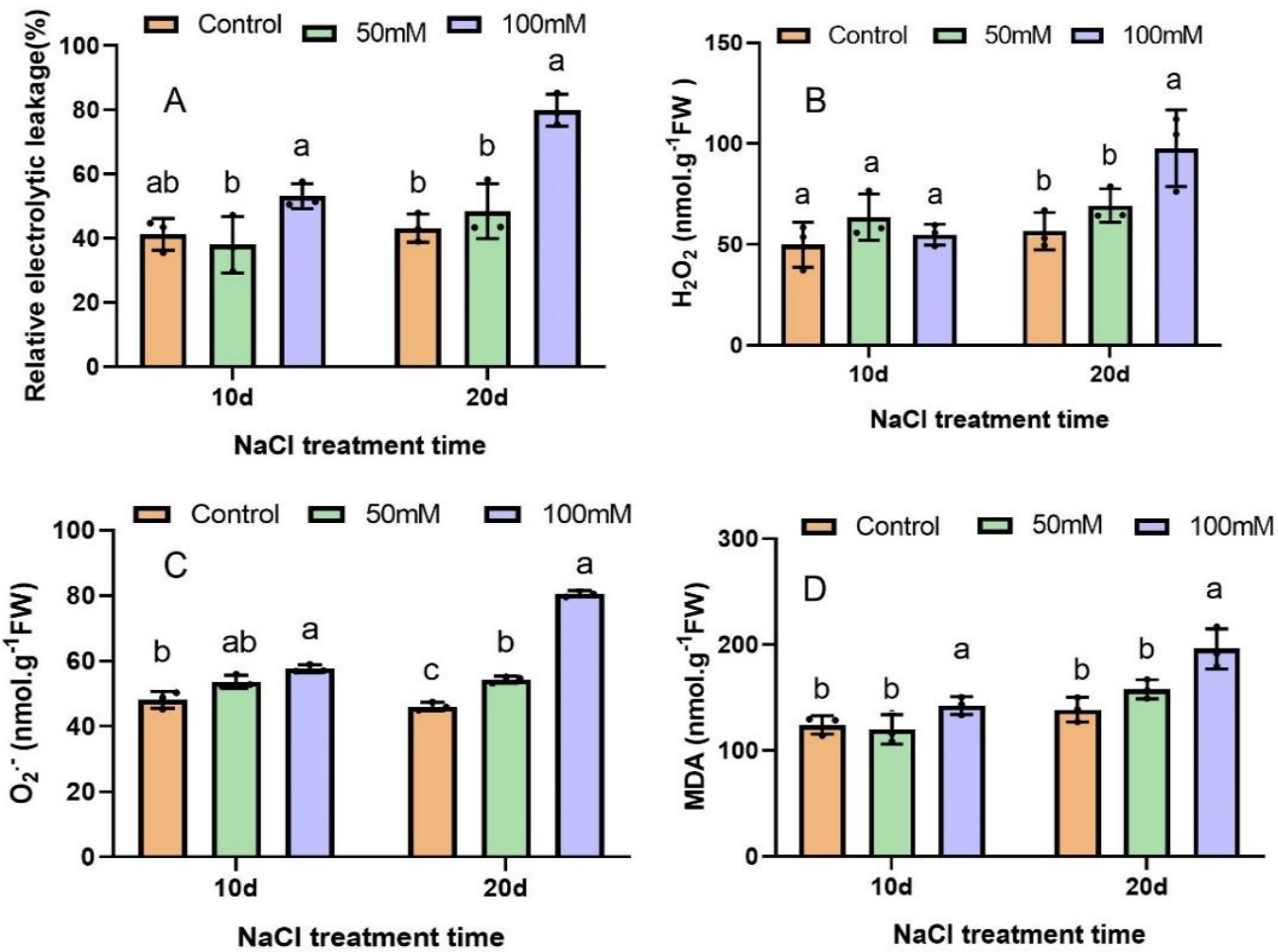

Fig. 3. Changes in REC, H_2O_2, $O_2^{\cdot-}$and MDA content in *I. verticillata* caused by different concentrations of NaCl. REC content (A); H_2O_2 content (B); $O_2^{\cdot-}$ content (C); MDA content (D). Values are means ± standard deviation. Means without a common letter are significantly different at $p<0.05$.

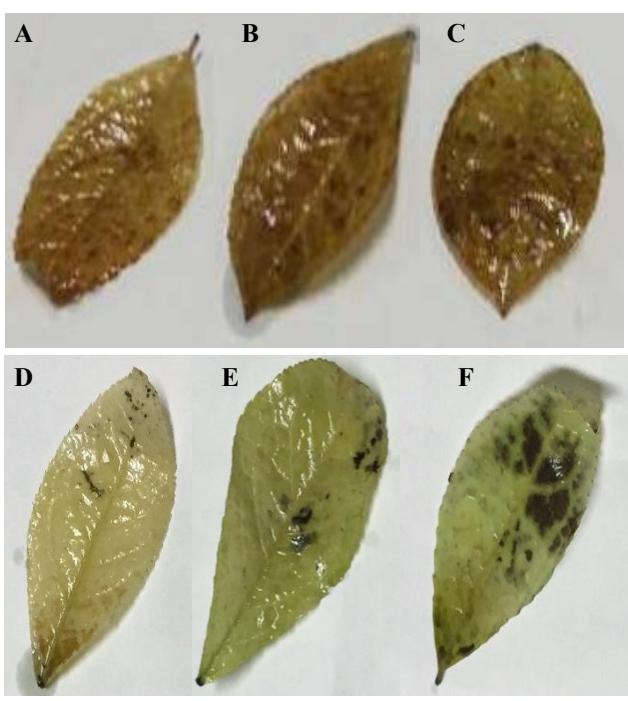

Fig. 4. Histochemical analysis of (A-C) H_2O_2 by DAB staining and (D-E) $O_2^{\cdot-}$ by NBT staining in the *I. verticillata* exposed to salinity stress or control. A-C: 0, 50, 100mM NaCl treatment; D-F: 0, 50, 100mM NaCl treatment.

Effect of antioxidant enzymes: Two NaCl treatments had different effects on antioxidant enzyme activities in *I. verticillata*. When treated NaCl for 20 days, the SOD activities were significantly decreased at 100mM but increased at 50mM. For example, SOD activity reduced by 8.0% at 100mM treatment for 20 days, whereas it increased by 25.1% at 50mM treatment for 20 days, respectively, compared to the control (Fig. 5A). The leaves of *I. verticillata* showed a significant increase in POD activity of 37.1% on day 10 in the 100mM treatment and 34.2% on day 20 in the 50mM treatment. However, the POD activity was reduced by 11.9% in the 100mM treatment compared to the 50mM NaCl treatment for 20 days (Fig. 5 B). Salinity treatment also significantly stimulated the CAT activity, and the 50 and 100mM NaCl treatments showed increased in CAT activity of 57.0% and 95.7% on day 10, and by 158.7% and 36.9% on day 20, respectively, compared to the control. In contrast, the CAT activity in the 100mM treatment was 47.1% lower than in the 50mM treatment (Fig. 5C).

APX activity varied with the dose and duration time of NaCl treatment. It increased by 79.0% at 100mM NaCl on day 10 and by 93.5% at 50mM NaCl on day 20 (Fig. 5D). Both NaCl treatments promoted GR activity which increased by 112.6% and 28.0% on day 10, and 44.1% and 64.8% on day 20 in the 50mM and 100mM NaCl treatments respectively, compared to the control. The highest activity of GR was observed in the 50mM NaCl treatment on day10 (Fig. 5E).

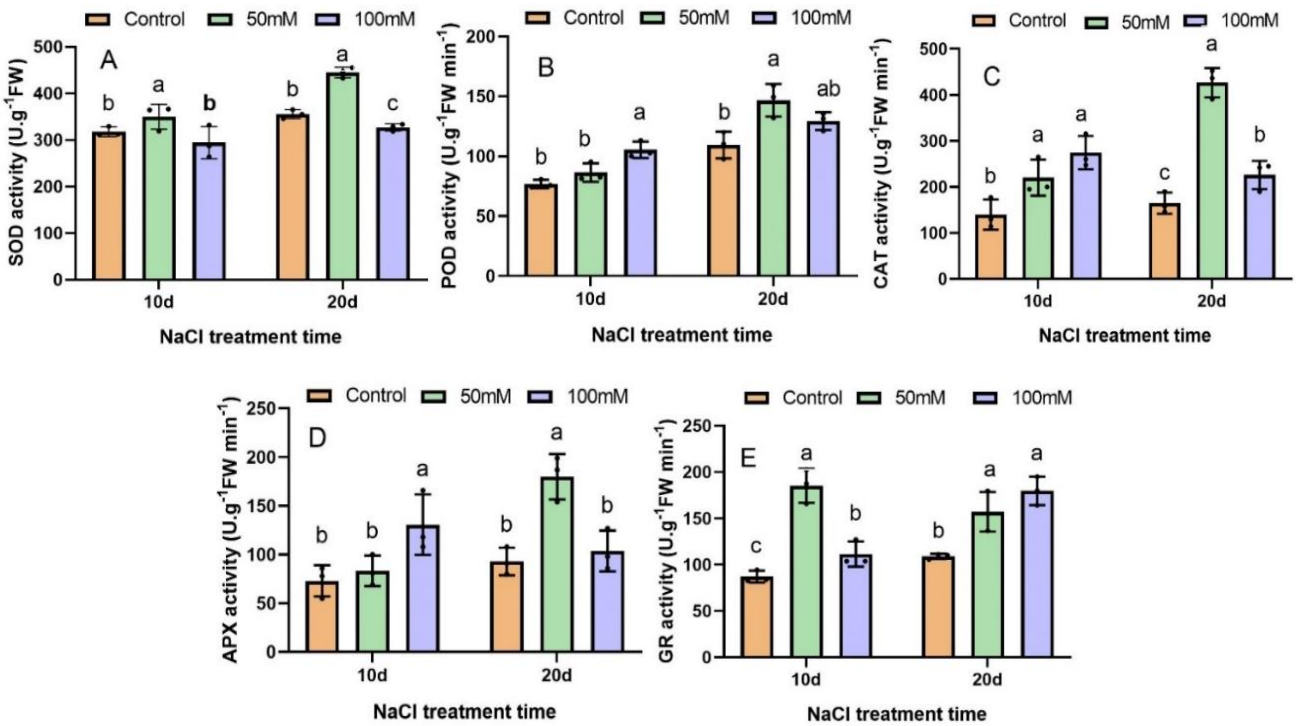

Fig. 5. Effect of NaCl treatments on SOD (A), POD (B), CAT (C), APX (D) and GR (E) activities in *I. verticillata.* Values are means ± standard deviation. Means without a common letter are significantly different at $p<0.05$.

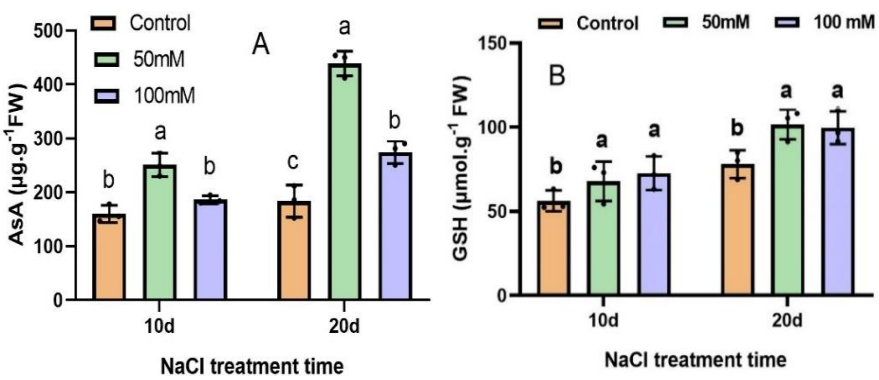

Fig. 6. Changes in AsA (A) and GSH (B) content in *I. verticillata* under different concentrations of NaCl. Values are means±standard deviation. Means without a common letter are significantly different at $p<0.05$.

Changes of antioxidants content: A substantial accumulation of AsA was observed which increased by 56.9% in the 50mM NaCl treatment on day 10, and by 138.9% and 49.3% in the 50 and 100mM treatments on day 20 compared to the control, respectively. The AsA content was higher in the 50mM treatment than in the 100mM treatment (Fig. 6A). In contrast, an increase in GSH levels was observed in *I. verticillata*, and increasing by 27.7% and 30.3% on day 10, and 20.6% and 29.2% on day 20 under the 50 and 100mM NaCl treatments, respectively, compared to the control. However, no significant changes were observed in the 50mM treatment, compared to 100mM treatment (Fig. 6B).

Relative gene expressions of antioxidant enzymes: The expressions of genes involved in the antioxidant response (*IvCu-ZnSOD*, *IvCAT*, *IvAPX* and *IvGR*) were measured by qRT-PCR to investigate the molecular regulation of Ilex cutting seedlings under NaCl treatments. NaCl treatments significantly upregulated the gene relative expression of the *IvCu-ZnSOD*, the degree of upregulation was higher in 50mM treatment (326% and 339%) than in 100mM treatment (113% and 162%) compared to the control) during the treatment (Fig. 7 A, C). In 10-day NaCl treatment, relative expression of the *IvCAT* gene was significantly upregulated with increasing NaCl dose, whereas the gene relative expression was downregulated by 14.1% in the 100mM treatment and upregulated by 51.4% in the 50mM treatment on day 20 (Fig. 7B, D). The change in *IvAPX* gene expression was similar to that of the *IvCAT* gene, and was significantly upregulated in the 50mM NaCl treatment during the treatment period (20 days), whereas the gene expression was upregulated on day 10 and downregulated on day 10 under the 100mM treatment (Fig. 7E, G). The relative expression of the *IvGR* gene was all significantly up-regulated in the two NaCl treatments during the treatment period, only the level on day 10 was lower than on day 20 in the 100 mM treatment. In general, the gene expression of these four enzymes was consistent with the change in enzyme activity (Fig.7 F, H).

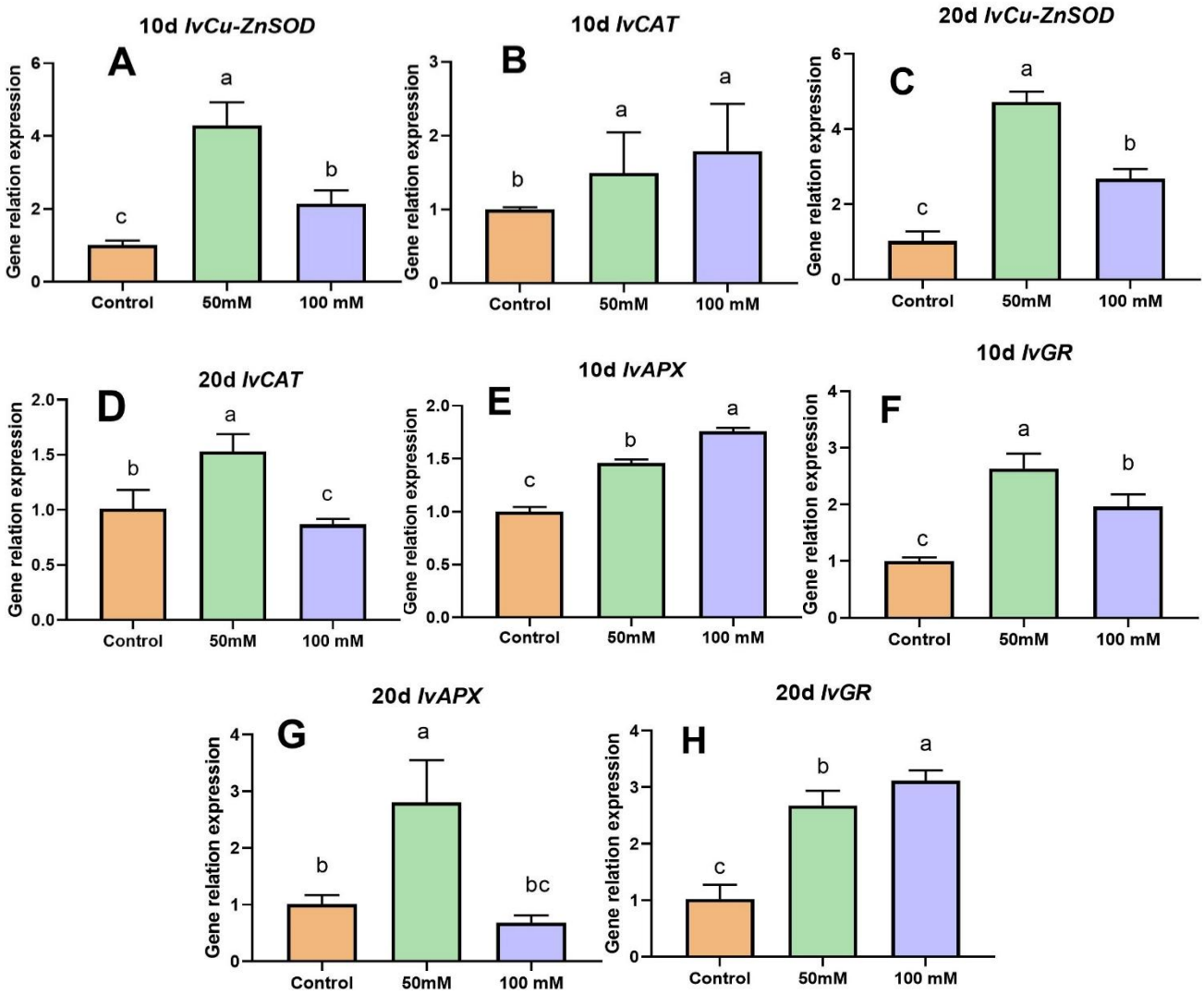

Fig. 7. The relative *gene* expressions of *IvCu-ZnSOD*, *IvCAT*, *IvAPX*, *IvGR* in *Ilex verticillata* seedlings under NaCl treatments. Values are means ± standard deviation. Bars not sharing a common letter are significantly different at $p<0.05$.

Principal component analysis and person correlations: The principal Component Analysis (PCA) was shown in Fig. 8A to gain the parameter relationships between control and NaCl treatments for 20 days in *Ilex verticillata*. The first principal component (PC1) and the second component (PC2) accounted for 46.9% and 40.4% of the variation, respectively, with 87.3% cumulative variation in both (PC1 and PC2) components. The activities of SOD, CAT, and APX and their relation gene expressions (*IvSOD*, *IvAPX*, and *IvCAT*), Chlt, POD and AsA were mainly in the first quadrant, while the indicators REC, H_2O_2, $O_2^{\cdot-}$, MDA, and GR, GSH, and GR gene expression (*IvGR*) were mainly in the fourth quadrant. Only WC was in the third quadrant. From Pearson's correlation analysis (Fig. 8B), the significant ($p\leq0.05$) and highly significant ($p\leq0.01$ or 0.001) positive correlations were observed between the activities of SOD, CAT, APX and their related gene expressions, and antioxidants such AsA and GSH. While WC (water content) exhibited a negative correlation with POD, GSH, GR, REC, H_2O_2, $O_2^{\cdot-}$, and MDA.

Cluster analysis of parameters under different salinity shows that most indicators except WC and Chlt were more significantly upregulated in the 50 mM NaCl treatment compared to the control. Whereas SOD, POD, CAT, APX, AsA, *IvCAT, IvAPX*, and Chlt were more significantly downregulated under 100mM NaCl treatment, whereas REC, H_2O_2, $O_2^{\cdot-}$, and MDA were significantly upregulated compared to 50 mM NaCl treatment for 20days (Fig. 8C).

Discussion

Ilex verticillata as an imported new variety has important ornamental and commercial value. An in-depth investigation of the salt adaptation mechanism in *I. verticillata* is of great significance and urgency in order to expand their range of cultivation. In the present study, the cutting seedlings at higher dose (100mM NaCl) showed some symptoms of salt injury, such as wilted margins and brown spots appeared in some leaves, and new shoot growth and water content decreased compared to the control and 50mM NaCl treatments. Although dry leaf margins and reddening of some new leaves appeared in the 50mM NaCl treatment, the changes of in new shoot growth and water content were not significantly different from the control (Table 2). The reduction in growth and biomass under salinity has also been observed in other plants, such as *Medicago sativa*, neem (*Azadirachta indica*), and tobacco, and these reductions are caused by the ion imbalance,

osmotic stress, and oxidative stress **(Pecetti** *et al*., 2024; Akram *et al*., 2024; Wu *et al*., 2024). In addition, when cultivated for a further 20 days after salt desalination, new shoots and axillary buds were able to regrow (Fig. 1I). Previous research showed that seed germination and seedling growth in Ammopiptanthus (the same as belonging to the Taxodiaceae family) were significantly inhibited at moderate salt (>80mM) (Zhang *et al*., 2014). Salinity thresholds were found to be 70.7, 58.7 and 50mM (6.5, 5.4 and 4.6 d Sm^{-1}) for *Hibiscus* ***syriacus*****,** *Physocarpus opulifolius*, and *Spiraea japonica*, respectively (Chen *et al.*, 2019). However, *Lycium nodosum* could be tolerant of 10‰ (171mM) NaCl via irrigation (Tezara, *et al.*, 2003) and *Amorpha fruticosa* seeds could germinate at 200mM NaCl when the pericarp was removed 2(Gao *et al.*, 2025). Compared to above landscape shrubs, *I. verticillata* cutting seedlings could tolerate stress up to 100mM NaCl through wilting young leaf margins to preserve buds. When NaCl was removed, the cutting seedlings could recover growth. This indicated that *I. verticillata* belong to a moderately salt-tolerant plant group, and may serve as an important genetic variety for the improvement of salt tolerance plants.

Chlorophyll is the major photosynthetic pigment and chloroplasts are the most sensitive organelles to salinity (Jia *et al*., 2020). In the present work, the levels of Chl a and total Chl t in *I. verticillata* cutting seedlings decreased under 100mM NaCl treatment, while the levels of pigments were no significantly different under 50mM NaCl treatment, indicating that high dose of NaCl affected photosynthetic pigment synthesis. Many researchers also reported that chlorophyll and carotenoid levels in plants decreased with increasing salt dose in a saline environment (El-Beltagi *et al*., 2024; Akram *et al*., 2024; Wu *et al*., 2024). In addition, chloroplasts showed some damage with disorganized and irregularly arranged under TEM observations, and plasmolysis was also observed in some cells under 100mM NaCl treatment (Fig. 2G-I). These salt injury symptoms were also observed in tobacco and grapevine seedlings under 60 mg/L KCl and 150mM NaCl treatments (Haider *et al*., 2019; Wu *et al*., 2024).

The changes in pigments and chloroplast structure under salinity stress could be due to degradation of pigment molecules, leading to slow synthesis in plant cells (Verma *et al*., 2018; Ji *et al*., 2022; El-Beltagi *et al*., 2024). Pigment degradation is associated with oxidative stress induced by ROS (reactive oxygen species) accumulation under salt stress, and ROS could cause chlorophyll degradation and membrane lipid peroxidation in plants (Todea *et al*., 2020; Lu *et al*., 2023b). In the present work, the levels of ROS ($O_2^{\cdot-}$ and H_2O_2), MDA, and REC were also significantly increased under the 100mM NaCl treatment (Fig. 2, Fig. 3). Moreover, the changes of these parameters were negatively correlated with the changes of pigment (Fig. 8). This indicates that 100mM NaCl induced oxidative stress and caused membrane disintegration in *I. verticillata* seedlings. Previous reports have also found that the ROS accumulate and MDA levels increase under salt stress, and higher levels of ROS and MDA (a biomarker of lipid peroxidation) might promote the chloroplast structural damage and chlorophyll degradation (Hannachi *et al*., 2018; Haider *et al*., 2019; Fatima *et al*., 2024).

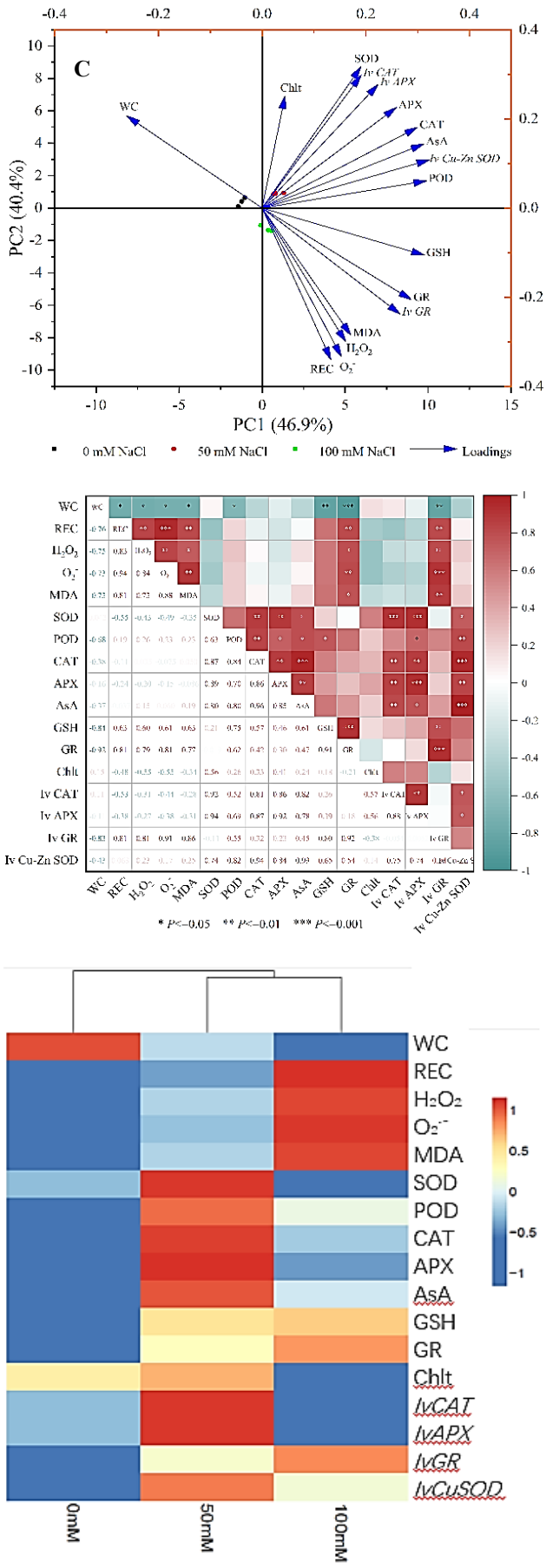

Fig. 8. The principal component analysis (PCA), pearson's correlation analysis and cluster analysis between parameters and NaCl treatments for 20 days.

The deleterious effects of ROS in cells are determined by the capacity of the antioxidant scavenging system in plants. Enzymatic or non-enzymatic antioxidants are essential for maintaining the cellular homeostasis between ROS scavenging and production. Antioxidant enzymes such as SOD, POD, CAT, APX, and GR play crucial roles in scavenging ROS generated during bio/abiotic stress (Feng *et al*., 2022; Chen *et al*., 2018; El-Beltagi *et al*., 2024). In the current findings, both the SOD activity and the gene expression of *IvCu-ZnSOD* in *I. verticillata* significantly increased under 50mM NaCl-treated, while the decrease of SOD activity and increase of *IvCu-ZnSOD* were found in the 100mM NaCl treatment compared to the control. This result indicated that a temporary *IvCu-ZnSOD* upregulation (transcript level) would not immediately enhance the protein translation level. Therefore, SOD was not sufficient to decompose the higher accumulation of $O_2^{\cdot-}$ caused by higher salt dose (100mM NaCl) in *I. verticillata*. Similar results were found for the halophyte *Salvadora persica,* walnut rootstock types and *Populus euphratica* (Rangani *et al*., 2016; Ji *et al*., 2022; Feng et al., 2022).

The activities of POD, CAT, APX, and GR were all higher under 100mM NaCl treatment than the control (except for APX on day 20, and the level was no significant different compared to control). This suggested that these protective enzymes are triggered to scavenge excess H_2O_2, thereby contributing to mitigate the adverse effects caused by 100mM NaCl stress. In the H_2O_2 scavenging system, CAT converts H_2O_2 to O_2 and H_2O, and APX scavenges H_2O_2 to H_2O via AsH-GSH using AsA as an electron donor (Feng *et al*., 2022). Our results are in agreement with the previous research on salinity stress (Haider et al., 2019; Li *et al*., 2023; Shahzad *et al*., 2024).

However, this inconsistency between enzyme activity and their transcript levels were also found in *IvCAT*, *IvAPX and IvGR* gene expression levels. The expression of the *IvGR* gene was significantly up-regulated and consistent with the changes in enzyme activity, whereas the expressions of *IvCAT* and *IvAPX* were downregulated. These results were also found in *Gymnocarpos przewalskii* under NaCl stress (Mhadhbi *et al*., 2011; Qi *et al*., 2023). This inconsistency might be due to the fact that the synthesis and activation of enzyme activity lag behind gene expression. Enzyme activation is more influenced by the level of ROS or RNS (reactive nitrogen species) in the environment, whereas genes have a spatiotemporal specific and tissue-specific expression in plants (Qi *et al*., 2023). The up-regulated gene expression could synthesise more antioxidant enzyme proteins, and which in turn could limit more ROS generation, and increase oxidation resistance.

In addition, AsA and GSH are two major non-enzymatic antioxidants and could stabilize cell membrane proteins in redox reactions (Shahzad *et al*., 2024). In our study, the levels of AsA and GSH in *I. verticillata* were increased under two NaCl treatments compared to the control, which could be attributed to the reduction of DHA (oxidized ascorbate) and GSSG (oxidized glutathione) to AsA and GSH. Many previous reports also indicated the accumulation of AsA and GSH in plants under salinity stress (Panda *et al*., 2017; Feng *et al*., 2022). This indicated that APX and GR combined AsA and GSH could maintain the balance of AsA-GSH R/O (reduction/oxidation) state to decompose H_2O_2 in plant cells (Feng *et al*., 2025). Therefore, AsA and GSH play crucial roles in the AsA-GSH cycle and cold be synergistic protective enzymes that work together to maintain the H_2O_2 at appropriate levels.

Conclusion

The growth of *I. verticillata* cutting seedlings was slightly affected under 50mM NaCl treatment. The cell structure and chloroplasts were normal, the accumulation of ROS ($O_2^{\cdot-}$ and H_2O_2) was less, the antioxidant enzymes activities (SOD, POD, CAT, APX, and GR) were increased and the expressions of *IvCu/ZnSOD*, *IvCAT*, *IvAPX*, and *IvGR* were enhanced under 50mM NaCl treatment. This indicates *I. verticillata* seedlings could grow normally under 50 mM NaCl conditions.

Under 100mM NaCl treatment, the growth of *I. verticillata* cutting seedlings was inhibited, and showed some symptoms of salt injury, chloroplasts and cell structures were damaged, $O_2^{\cdot-}$ and H_2O_2 were over-accumulated, POD, CAT, APX and GR, as well as AsA and GSH were synergistic to scavenge excess hydrogen peroxide. Therefore, *I. verticillata* seedlings could tolerate low to 100mM NaCl stress by wilting young leaf margins to preserve buds and belong to the moderately salt-tolerant plant group

Dose and time effects were observed in enzyme activities and gene expressions. The enzyme activities (SOD, CAT and APX) and transcript levels (*IvCu-ZnSOD*, *IvCAT* and *IvAPX*) of cutting seedlings were found to be asynchronous under 100mM NaCl treated for 20 days. This may be due to the synthesis and activation of enzymes lagging behind gene expression. This is the first detailed report on salinity tolerance in *Ilex verticillata*.

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References

Abdallah, M.B., K. Methenni, W. Taamalli and N.B. Youssef. 2024. Post-stress recovery from drought and salinity in olive plants is an active process associated to physiological and metabolic changes. *Acta Physiol. Plant.*, 46: 120. https://doi.org/10.1007/s11738-024-03748-3.

Akram, M., Z. Sajid, A.B.U. Farooq, I. Ahmad, A. Jamal, H. Rizwana, B.M. Almunqedhi and D. Ronga. 2024. Characterization of physiological and biochemical attributes of neem (*Azadirachta indica* A. Juss) under salinity stress. *Horticulturae*, 10: 702. https://doi.org/ 10.3390/ horticulturae10070702.

Appiah, C.S.O., S.A. Poku and K.O. Ayeh. 2024. Comparative morphological and physiological responses of three cowpea (*Vigna unguiculata* L. Walp.) cultivars to induced water, salinity and combined water and salinity stresses. *Pak. J. Bot*., 56(5): 1679-1686.

Cai, L.Y., Y.F. Shen, R.T. Jiang, J.W. Wang, S.Z. Ma, M.Q. Wu and P.M. He. 2025. Betacyanin accumulation mediates photosynthetic protection in *Suaeda salsa* (L.) Pall. under salt stress. *Planta*, 261: 100.

Chen, J.H., Y.X. Wang, A. Paudel and Y.P. Sun. 2019. Comparing the salt tolerance of three landscape plants using a Near-continuous Gradient Dosing System. *Hort-Technol.*, 29(5): 611-618.

Chen, Y., B.L. Yuan, Z.H. Wei, X. Chen, Y.Q. Chen and N.F. Qiu. 2018. The ion homeostasis and ROS scavenging responses in 'NL895' poplar plantlet organs under in vitro salinity stress. *In vitro Cell. Develop. Biol. Plant*, 54: 318-331.

Chen, Y., F.Z. Lin, H. Yang, L. Yue, F. Hu, J.L. Wang, Y.Y. Luo and F. L. Cao. 2014. Effect of varying NaCl doses on flavonoid production in suspension cells of *Ginkgo biloba*: relationship to chlorophyll fluorescence, ion homeostasis, antioxidant system and ultrastructure. *Acta Physiol. Plant*, 36: 3173-3187.

Du, Y., X. Liu, L. Zhang and W. Zhou. 2023. Drip irrigation in agricultural saline-alkali land controls soil salinity and improves crop yield: Evidence from a global meta-analysis. *Sci. Total Environ.*, 880: 163226.

El-Beltagi, H.S., M.F. El-Nady, M.I. Al-Daej, K.A. El-Naqma, A.A. Rezk, M.M. El-Afry, W.F. Shehata, A.M. Ismail, W.F. Eltonoby, and M.M.S. Metwaly. 2024. Exogenous application of manganese and arginine alleviates the adverse effects of salinity stress on pea (*Pisum sativum* L.). *Cogent Food Agri.*, 10(1): 2389445.

El-Yamani, M. and M.D.P. Cordovilla. 2024. Tolerance Mechanisms of Olive Tree (*Olea europaea*) under Saline Conditions. *Plants*, 13: 2094. https://doi.org/10.3390/plants13152094.

Fatima, A., S. Umbreen, S. Sadia, M. Waheed, F. Arshad, M.R. Malik, A. Hashem, A. Kumar and E.F.A. Allah. 2024. Mitigation of salinity-induced adverse effects through exogenous application of gibberellic acid in turnip (*Brassica rapa* L.). *Cogent Food Agri.*, 10(1): 2392042.

Feng, K., J.L. Lu, Y. Chen, Y.Y. Luo, Y.C. Hu, X.X. Li, S.Z. Zhong and L. Cheng. 2022. The coordinated alterations in antioxidative enzymes, *PeCu/ZnSOD* and *PeAPX2* expression facilitated in vitro *Populus euphratica* resistance to salinity stress. *Plant Cell, Tiss. Org. Cult.,* 150: 399-416.

Feng, T.T., J.X. Zhuge, Y. Chen, J.J. Di, L. Yue and Z.H. Wei. 2025. Apoplastic proteome and antioxidant system responses to *In vitro* salinity stress in'NL895' poplar plantlets *In Vitro Cell. Develop. Biol. Plant*, https://doi.org/10.1007/s11627-025-10589-3.

Gao, Y., H. Zou, B. Wang and F. Yuan. 2022. Progress and applications of plant growth promoting bacteria in salt tolerance of crops. *Int. J. Mol. Sci.*, 23(13): 7036.

Gao, Z., Y. Mu, F. Chen, L. He, X. Li, J. Li, K. Ding, Y. Cu, C. Mu and A. Rasheed. 2025. Effects of pericarp removal and different germination conditions on seed germination and saline-alkali tolerance of *Amorpha fruticosa*. *Turk. J. Bot*., 49(1): 40-51.

García-Locascio, E., E.I. Valenzuela and P. Cervantes-Aviles. 2024. Impact of seed priming with selenium nanoparticles on germination and seedlings growth of tomato. *Sci. Rep.*, 14: 6726.

Gargiullo, M.B. and E.W. Stiles. 1991. Chemical and nutritional differences between two bird-dispersed fruits: *Ilex opaca* and *Ilex verticillata*. *J. Chem. Ecol.*, 17(6): 1091-1103.

Haider, M.S., J. Sudisha, T. Pervaiz, Y.X. Zhao, N. Khan and J.G. Fang. 2019. Physiological and transcriptional variations inducing complex adaptive mechanisms in grapevine by salt stress. *Environ. Exp. Bot.*, 162: 455-467.

Hannachi, S. and L.M.C. Van. 2018. Salt stress affects germination, seedling growth and physiological responses differentially in eggplant cultivars (*Solanum melongena* L.). *Sci. Hort.*, 228: 56-65.

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. *California Agri. Exp. Station Circ*., 1: 347.

https:// doi. org/ 10. 3389/ fpls. 2016. 00050.

Hu, L., Y. Yang, L. Jiang and S. Liu. 2016. The catalase gene family in cucumber: Genome-wide identification and organization. *Gen. Mol. Biol.*, 39: 408-415.

Ji, X., J. Tang, W. Fan, B. Li, Y. Bai, J. He, D. Pei and J. Zhang. 2022. Phenotypic differences and physiological responses of salt resistance of walnut with four rootstock types. *Plants*, 11: 1557.

Jia, K., C.Y. Yan, H.Z. Yan and J. Gao. 2020. Physiological responses of turnip (*Brassica rapa* L. subsp. *rapa*) seedlings to salt stress. *Hortscience*, 55(10): 1567-1574.

Kang, J., X.L. Wang, S.J. Yan, H. Guo and Y.N. Cui. 2024. Comparative physiological and gene expression analyses provide insights into ion transports and osmotic adjustment of sweet sorghum under salt stress. *Agronomy*, 14: 1849.

Kohnhehshahri, S. and Y. Demir. 2021. Chitosan against to boron toxicity in maize. *Acta Scientiarum Polonorum Hortorum Cultus*, 20(3): 3-13.

Li, J.N., Q. Liu, P.P. Wang and W. Pan. 2008. The Response of *Ammopiptanthus mongolicus* seedlings to salt stress under hydroponics. *Bull. Bot. Res.*, 28(3): 342-346. (in Chinese).

Li, S. 2023. Novel insight into functions of ascorbate peroxidase in higher plants: More than a simple antioxidant enzyme. *Redox Biol.,* 64: 102789.

Li, X.X., S.Z. Zhong, X. Chen, J.J. Di and Y. Chen. 2024. The Stress responses and comparison of drought tolerance in four varieties of *Ilex verticillata* under drought stress. *J. Northeast Forest. Uni.*, 52(5): 34-41. (Chinese).

Liu, R., J.Y. Zhang, C.Q. Ge, L.L. Xie, Y. Chen, C. Zhang and X. Chen. 2021. Establishing an efficient regeneration protocol in vitro for rapid micro-propagation of two new varieties of *Ilex verticillata* L. *Bull. Bot. Res.*, 41(02): 221-231.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods*, 25: 402-408.

Lu, C., L. Li, X. Liu, M. Chen, S. Wan and G. Li. 2023b. Salt stress inhibits photosynthesis and destroys chloroplast structure by downregulating chloroplast related genes in *Robinia pseudoacacia* seedling. *Plants*, 12(6): 1283.

Lu, H.X., P. Zhou, F. Li, Y.J. Wang, J.Y. Gu, Y. Wang, S.B. Sun, M. Zhang and X.W. Wang. 2024. *Trichoderma guizhouense* NJAU4742 augments morpho-physiological responses, nutrient availability and photosynthetic efficacy of ornamental *Ilex verticillata. Tree Physiol.*, 44: tpae033.

Lu, J.L., Y.C. Hu, Y. Chen, R. Liu, J.J. Di, T.T. Feng and F.L. Cao. 2023. Hydrogen sulfide promoted cell differentiation, antioxidant ability, and flavonoids accumulation in *Ginkgo biloba* L. suspension cells. *Plant Cell Tiss. Org. Cult.*, 156: 36.

Ma, J.J., B. Zhao, Y. Chen, X.C. Ling, J. Yu and X. Chen. 2020. Physiological responses of seedlings of four *Ilex verticillata* varieties to low temperature stress and a comparison of their cold resistance. *J. Nanjing Forest. Uni. (Natural Sciences Edition*, 44(05): 34-40. (Chinese).

Mansour, M.M.F. and E.F. Ali. 2017. Evaluation of proline functions in saline conditions, *Phytochemistry*, 140: 52-68.

Mariyam, S., R. Bhardwaj, N.A. Khan, S.V. Sahi and C.S. Seth. 2023. Review on nitric oxide at the forefront of rapid systemic signaling in mitigation of salinity stress in plants: Crosstalk with calcium and hydrogen peroxide. *Plant Sci.*, 336: 111835.

Martínez-Ispizua, E., Á. Calatayud, J.I. Marsa, C. Cannata, F. Basile, A. Abdelkhalik, S. Soler, J.V. Valcárcel and M.R. Martínez-Cuenca. 2022. The nutritional quality potential of microgreens, baby leaves, and adult lettuce: An underexploited nutraceutical source. *Foods*, 11(3): 423.

Mhadhbi, H., V. Fotopoulos, P.V. Mylona, M. Jebara, M.E. Aouani and N.P. Alexios. 2011. Antioxidant gene–enzyme responses in *Medicago truncatula* genotypes with different degree of sensitivity to salinity. *Physiol. Plant.*, 141: 201-214.

Mishra, N., C. Jiang, L. Chen, A. Paul, A. Chatterjee and G.X. Shen. 2023. Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms. *Front. Plant Sci.*, 14: 1110622.

Montesinos, C., P. Benito, R. Porcel, J. Bellon, M. Gonzalez-Guzman, V. Arbona, L. Yenush and J.M. Mulet. 2024. Field evaluation and characterization of a novel biostimulant for broccoli (*Brassica oleracea* var. *italica*) cultivation under drought and salt stress which increases antioxidant, glucosinolate and phytohormone content. *Sci. Hort.*, 338: 113584.

Mulet, J.M., R. Porcel and L. Yenush. 2023. Modulation of potassium transport to increase abiotic stress tolerance in plants. *J. Exp. Bot.*, 74: 5989-6005.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol.*, 59: 651-681.

Negi, N.P., D. Shrivastava, S. Shekhar, V. Sharma and N.B. Sarin. 2016.Simultaneous overexpression of CuZnSOD and Capx from *Arachis hypogaea* leads to salinity stress tolerance in tobacco. *In Vitro Cell. Develop. Biol. Plant*, 52: 484-491.

Noctor, G. 2025. Hydrogen peroxide, ascorbate, and glutathione: building the Foyer–Halliwell–Asada pathway. *Planta*, 261: 132.

Panda, A., J. Rangani, A. Kumari and A.K. Parida. 2017. Efficient regulation of arsenic translocation to shoot tissue and modulation of phytochelatin levels and antioxidative defense system confers salinity and arsenic tolerance in the Halophyte *Suaeda maritima*. *Environ. Exp. Bot.*, 143: 149-171.

Pecetti, L., S. Tlahig, M. Confalonieri, M.V. Cornacchione, T. Hayek, S.P. Angueira and P. Annicchiarico. 2024. A comparison of procedures for evaluating and selecting alfalfa landrace germplasm for tolerance to salinity. *Crop Sci.*, 64: 2143-2157.

Qi, J., Y. Luo, H. Huang, S. Lu, F. Zhao, Z. Deng and Y. Qiu. 2023. Molecular mechanism of response and adaptation of antioxidant enzyme system to salt stress in leaves of *Gymnocarpos przewalskii*. *Plants*, 12: 3370.

Qin, S.N., S.Y. Fu, Y. Yang, Q.M. Sun, J.Q. Wang, Y.L. Dong, X.Y. Gu, T. Wang, X.T. Xie, X.R. Mo, H.J. Jiang, Y.X. Yu, J. Yan, J.F. Chu, B.S. Zheng and Y. He. 2023. Comparative microscopic, transcriptome and IAA content analyses reveal the stem growth variations in two cultivars *Ilex verticillata*. *Plants*, 12(10): 1941.

Rangani, J., A.K. Parida, A. Panda and A. Kumari. 2016. Coordinated changesin antioxidative enzymes protect the photosynthetic machinery from salinity induced oxidative damage and confer salt tolerance in an extreme halophyte *Salvadora persica* L. *Front. Plant Sci.*, 7:50.

Saxena, S.C., P. Salvi, N.U. Kamble, P.K. Joshi, M. Majee and S. Arora. 2020. Ectopic overexpression of cytosolic ascorbate peroxidase gene (APX1) improves salinity stress tolerance in *Brassica juncea* by strengthening antioxidative defense mechanism. *Acta Physiol. Plant.,* 42: 45. https://doi.org/10.1007/s11738-020-3032-5.

Shahzad, R., P.W. Harlina, S.U. Khan, S. Koerniati, B.R. Hastilestari, R.A. Ningrum, R. Wahab, I. Djalovic and P.V.V. Prasad. 2024. Iron oxide nanoparticles alleviate salt-alkaline stress and improve growth by modulating antioxidant defense system in cherry tomato. *J. Plant Int.*, 19(1): 2375508.

Sultana, M.S., S. Yamamoto, M.S. Biswas, C. Sakurai, H. Isoai and J. Mano. 2022. Histidine-containing dipeptides mitigate salt stress in plants by scavenging reactive carbonyl species. *J. Agri. Food Chem.*, 70(36): 11169-11178.

Talaat, N.B., A.A. Mostafa and S.N. Abd El-Rahman. 2023. A novel plant growth–promoting agent mitigates salt toxicity in barley (*Hordeum vulgare* L.) by activating photosynthetic, antioxidant defense, and methylglyoxal detoxification machineries. *J. Soil Sci. Plant Nutr.*, 23(1): 3 08-324.

Tezara, W., D. Martínev, E. Rengifo and A. Herrera. 2003. Photosynthetic responses of the tropical spiny shrub *Lycium nodosum* (Solanaceae) to drought, soil salinity and saline spray. *Ann. Bot.,* 92(6): 757-765.

Todea, I.M., S. González-Orenga, M. Boscaiu, M. Plazas, A.F. Sestras, J. Prohens, O. Vicente and R.E. Sestras. 2020. Responses to water deficit and salt stress in *Silver Fir* (*Abies alba* Mill.) seedlings. *Forests*, 11(4): 395.

Verma, S.S., R.S. Verma, S.K. Verma, A.L. Yadav and A.K. Verma. 2018. Impact of salt stress on plant establishment, chlorophyll and total free amino acid content of ber (*Zizyphus mauritiana* Lamk.) cultivars. *J. Pharmacog. Phytochem.*, 7(2): 556-559.

Vighi, L., L.C. Benitez, M.N. Amaral, G.P. Moraes, P.A. Auler, G.S. Rodrigues, S. Deuner, L.C. Maia and E.J.B. Braga. 2017. Functional characterization of the antioxidant enzymes in rice plants exposed to salinity stress. *Biol. Plant.*, 61(3): 540-550.

Wang, W.C., J.Y. Pang, F.H. Zhang, L.P. Sun, L. Yang, T.D. Fu and K.H.M. Siddique. 2023. Transcriptome analysis reveals key molecular pathways in response to alkaline salt stress in canola (*Brassica napus* L.) roots. *J. Plant Growth Regul.*, 42(5): 3111-3127.

Wu, Y.J., J.L. Lu, D.X. Wang, Y.Y. Li, Z.Y. Feng and X.F. Ye. 2024. Physiological and transcriptomic profiles of tobacco seedling leaves in response to high chloride accumulation. *J. Plant Interact.*, 19(1): 2355122.

Xie, X.T., Y.J. Gu, W. L. Wang, F. Abbas, S.N. Qin, S.Y. Fu, J.Q. Mei, J.Y. Wang, D.X. Ma, G.C. Wen, Y. Yang, A. Sharma, X.F. Wang, D.L. Yan, B.S. Zheng, Y. He and H.W. Yuan. 2023. Exogenous spermidine improved drought tolerance in *Ilex verticillata* seedlings. *Front. Plant Sci.*, 14. https://doi.org/10.3389/fpls.2023.1065208.

Xie, Y.H., H.B. Chen, S.Y. Zheng, X.L. Zhang and S.N. Mu. 2018. Molecular characterization of cu/Zn SOD gene in Asian clam *Corbicula fluminea* and mRNA expression and enzymatic activity modulation induced by metals. *Gene*, 663: 189-195.

Zhang, J., D.Z. Zhang, Y. Ma and Y.Y. Li. 2014. Effect of NaCl simulation of salt stress on seed germination and seedling growth of *Ammopiptanthus mongolicus*. *The North Hort.*, 14: 65-72.

Zheng, W.W., Y.T. Tian, H.L. Shi, M.M. Chen, S.B. Hong, K. Xu, J.H. Cheng and Y.X. Zang. 2023. Exogenous 5-aminolevulinic acid promotes plant growth and salinity tolerance of grape rootstocks in coastal areas. *Hort. Environ. Biotech.*, 64: 179-191.