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REGULAR ARTICLE

# Promotive effects of 5-aminolevulinic acid on growth, photosynthetic gas exchange, chlorophyll, and antioxidative enzymes under salinity stress in *Prunnus persica* (L.) Batseh seedling

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## ABSTRACT

The physiological characteristics of peach (*Prunus persica* L. Batsch) seedlings under NaCl stress were investigated to ameliorate the effects of 5-aminolevulinic acid (ALA). Growth and physiological parameters of NaCl stressed peach seedlings treated with exogenous ALA were investigated; these parameters included shoot and root dry mass, leaf area, plant height, stem diameter, intercellular $CO_2$ concentration (*Ci*), stomatal conductance (*Gs*), net photosynthetic rate (*Pn*), transpiration rate (*Tr*), membrane permeability, and root vitality. The contents of chlorophyll, proline, soluble sugars, soluble proteins, malondialdehyde (MDA), as well as the activities of antioxidant enzymes were evaluated. Results showed that the physiological characteristics of peach seedlings under NaCl stress (100mM) were inhibited. Peach seedlings treated with 100mM NaCl and 200mg/l ALA for every growth parameters recovered. Moreover, peach seedlings treated with 200mg/l ALA exhibited improved effectively physiological characteristics. Treatment with exogenous ALA increased stem diameter, plant height, leaf area, shoot and root dry mass, net photosynthetic rate (*Pn*), transpiration rate (*Tr*), intercellular $CO_2$ concentration (*Ci*), stomatal conductance (*Gs*), and root vitality. The treatment also increased the contents of chlorophylls, soluble sugars, soluble proteins, and proline but decreased membrane permeability and MDA content. Furthermore, treatment with ALA significantly enhanced the activities of superoxide dismutase, peroxidase, and catalase. These findings indicate that exogenous ALA treatment could improve the growth and relieve the NaCl stress injury of peach seedlings by increasing photochemical efficiency, osmotic content, and antioxidant enzyme activity.

**Keywords:** 5-Aminolevulinic acid; Growth and physiological parameters; NaCl stress; *Prunus persica* (L.) Batsch

## INTRODUCTION

With expanding human activities in farming, in the past two decades has led to a gradual increase in ground water salinity, soil salinization has increased irreversibly (Rozema and Flowers, 2008). Environmental stresses, such as high salinity, adversely affect plant growth and productivity (Youssef and Awad, 2008; Van-Ha et al., 2014). The rate of photosynthesis in plants decreases because of ion toxicity, osmotic stress, and hyponutrition under salinity stress; as a result, plant growth is inhibited and senescence is accelerated (Chaves et al., 2009). Thus, plant growth and development are seriously affected.

Peach (*Prunus persica* L. Batsch 'Chunmei'), a plant native to China, is a member of the subgenus *Amygdalus* L., and family Rosaceae; this plant is a major economic fruit in temperate regions worldwide and has been bred and cultivated for more than 4,000 years (Verde et al., 2013). Peach is not only fragrant and sweet but also provides special flavor and abundant nutrients; the plant contains several kinds of amino acids and holds important economic value because of its applications in manufacturing drugs and active carbon materials as well as in oil extraction for industrial purposes (Dirlewanger et al., 2002). Peach grows better in fertile, loose, and well-drained soil or in slightly acidic sandy loam in temperate regions instead of alkaline soil or heavy clay soil. Salinity is one of the most serious threats to crop growth and yield of One-year-old of *Prunus persica* (L.) Batsch, salinity stress decreases the water potential in peach (Karakas et al., 2000; El-Khashab et al., 1997). As a result, the growth and development of

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peach trees are limited, and physiological and metabolic disorders arise as membrane permeability increases. Other processes also occur. For instance, photosynthesis is inhibited, respiration is altered because plant breathing is facilitated by low NaCl concentration and inhibited by high NaCl concentration, protein synthesis is reduced, protein decomposition is increased, and NaCl toxicity is induced by toxic substances accumulated from ion imbalance (You et al., 2015). Excessive amounts of NaCl in soil may cause growth stagnancy or death of peach seedlings (Karakas et al., 2000).

5-Aminolevulinic acid (ALA) is a key precursor compound for the biosynthesis of porphyrin derivatives (Wang et al., 2003). ALA is widely found in fungi, bacteria, animals, and plants, especially in non-protein amino acids in living cells. ALA is also involved in important plant physiological functions, including respiration, photosynthesis, and other metabolic activities (Zhang et al., 2015). ALA can improve not only plant photosynthesis, yield, coloring, and fruit quality but also NaCl tolerance. NaCl tolerance is enhanced by increasing the osmolyte content and the physiological activity of protective enzyme systems in plants (Al-Qurashi et al., 2011). Exogenous ALA can improve protective enzyme activity and leaf photosynthesis in various crops, such as *Cucumis sativus* L. (Zhen et al., 2012), *Solanum lycopersicum* (Zhang et al., 2015), *Helianthus annuus* L. (Akram et al., 2012), *Brassica campestris* L.(Naeem et al., 2010), *Brassica rapa* L. (Wang et al., 2005), *Solanum tuberosum* (Zhang et al., 2006), *Oryza sativa* L. (Nunkaew et al., 2014), and *Creeping bentgrass* (Yang et al., 2014), under salinity stress. As a result, the development and production of crops are facilitated, in addition, the inhibitory actions of salinity stress on plants are alleviated. However, the roles of ALA in peach seedlings exposed to salinity stress have yet to be reported. This study investigated the effects of ALA treatment on Growth, physiological, and biochemical parameters, such as stem diameter, plant height, leaf area, shoot and root dry mass, leaf photosynthesis, chlorophylls content, root vigor, membrane permeability, contents of malondialdehyde (MDA), soluble protein, soluble sugar and proline, and antioxidase activity, of peach seedlings exposed to NaCl stress. The regulatory mechanisms that relieve peach seedlings from NaCl stress were also explored. This study aimed to provide a way for the alleviation of NaCl stress problems in peach cultivation.

## MATERIALS AND METHODS

### Plant materials and treatments

*Prunnus persica* (L.) Batseh. 'Chunmei' seeds were sterilized in 0.5% mercury chloride solution for 10 min and 75% alcohol for 5 min to wipe out possible bacteria, fungi, and viruses before stratification treatment. The seeds were washed with distilled water for 5 min and dried for 40 min before use (Korkmaz, 2005). The seeds were then sown in polystyrene germination boxes (300 mm in length, 250 mm in diameter, and 400 mm in height) filled with sterilized substrate (perlite: soil: vermiculite = 1:1:5). The seedlings were grown in an illuminated incubator (light 16 h/day, 60%–70% relative humidity, eight lamps of 40W, and light intensity of 500 μmol $m^{-2}$ $s^{-1}$; GTOP-430D, Topper Instrument Co. Ltd., China) at day/night temperatures of 28 °C and 20 °C. At one- and two-leaf stages, plants of nearly the same sizes per pot were irrigated with nutrient solution (Zhenzhou Deminxin Agricultural Science & Technology Co. Ltd., China) twice a week.

After a two week acclimatization period, seedlings were separated into four groups. The experimental designs included control (sterile water) as well as treatments with NaCl (Chemical Reagent Co. Ltd., NanJing, China; 100mM NaCl containing 0.02% Tween-20), ALA (Cosmo Oil Co. Ltd., Japan; 200mg/l ALA contain 0.02% Tween-20), and NaCl and ALA (100mM NaCl and 200mg/l ALA containing 0.02% Tween-20). Each treatment was replicated four times. The seedlings were sprayed with an aqueous solution of 200mg/l ALA at the same time as root irrigation of 100ml NaCl per pot, and then directly used for assessment of photosynthetic parameters. Leaves and stems were sampled separately and stored in a cold container for measurement of related indicators.

### Determination of plant biomass

The seedlings were carefully placed in polystyrene germination boxes, washed with deionized water, and softly wiped with filter papers. Dry mass of shoot and root parts of each peach seedling were separately recorded 36h after hot air treatment at 50°C. Growth parameters, namely, plant height (cm), and diameter (cm), of each examined treatment plant were recorded (Ali et al., 2013). Leaf area ($cm^2$) can be calculated by means of planimetric techniques (Jonckheere et al., 2004). After leaf collection, a leaf can be horizontally fixed to a flat surface, its contour can be measured with a planimeter (Li-3000, Licor, NE, USA), and its area can be computed from this contour assessment.

### Determination and analysis of photosynthetic parameters

Seedling leaves of intercellular $CO_2$ concentration ($C_i$), stomatal conductance ($G_s$), photosynthetic rate ($P_n$), and transpiration rate($T_r$) were measured using at least four leaves per replicate through the open system of LI-6400XT apparatus (LI-COR-6400, Inc., USA). During measurement, the intact topmost completely expanded leaf after 50 min. The leaf temperature was adjusted to 18°C,

and the relative humidity was 50%–70% (Memon et al., 2009). Each measurement was replicated for three times.

### Measurement of chlorophylls contents

Chlorophyll a and b and total chlorophyll contents were determined according to the method (Liu et al., 2011). Leaf tissues (2g) were placed into test tubes, extracted with 30 ml of DMSO (Sigma–Aldrich), and incubated overnight at 70°C. Chlorophylls contents [mg/g FW (fresh weight)] were calculated at 665 and 649 nm by a spectrophotometer (UV-2800, Unico, GER).

### Contents of soluble sugars, soluble proteins, and proline

Tissue samples (5g) were ground and extracted with 6 ml of sulfosalicylic acid for 2h, filtered, and centrifuged at 4,000×g for 5min. The supernatant was subjected to sugar analyses by spectrophotometry at 630nm to determine soluble sugar content through sulfuric acid anthrone colorimetric method (Moustakas et al., 2011). Soluble sugar contents were expressed as percentage of leaf fresh weight (w/w, FW).

Soluble protein in fresh leaf tissues was extracted with boiling ethanol (Kosar et al., 2015). The samples were added to sulfosalicylic acid solutions containing 2.5g of ninhydrin and 15 ml of glacial acetic acid and analyzed using a UV spectrophotometer at 520nm for 90s.

Proline concentration was determined using a standard curve in accordance with the method Briefly, 5g of fresh leaves were ground before mixing with quartz sand and 7 ml of aqueous sulfosalicylic acid solution containing glacial acetic acid, sterile water, and phosphoric acid. The mixture was incubated in aboiling water bath for 30min and centrifuged at 12,000×g for 50s. The supernatant was determined through UV spectrophotometry at 546nm (Claussen, 2005).

### Measurement of membrane permeability, malondialdehyde contents, and root vitality

Malondialdehyde (MDA) content was determined and analyzed using the method. Plant fresh tissues (1g) from each treatment were extracted in 8 ml of 0.5% thiobarbituric acid (TBA), ground in a mortar, and added to 3 ml of 15% trichloroacetic acid (TCA). The homogenates were incubated in a water bath and centrifuged at 12,000rpm for 60s. The mixture was then incubated at 50°C for 2h, and MDA content was determined through UV spectrophotometry at 600 and 540nm (Naeem et al., 2011).

Fresh leaves were subjected to membrane permeability determinations in accordance with the method (Inal and Tarakcioglu, 2001). The leaves (10g) were placed in a Bunsen beaker containing distilled water. The reaction mixture was incubated at 40°C for 120 min. The mixture was tested for conductivity and surveyed with an electric conductometer (LA-EC20, HACH, USA). Electrolyte conductivity was measured at boiling (C1) and room temperatures (C2). The membrane permeability of fresh leaf tissues was calculated as Percent EC (C1/C2)×100 (Sperling et al., 2014).

Root vitality was assessed using by a modified 2, 3, 5-triphenyltetrazolium chloride (TTC) method in accordance with the procedures proposed (Gao et al., 2015). The roots were shredded, ground, weighed accurately (200mg), and placed in sterile test tubes. The seedlings were treated with 3 ml of 0.6% (w/v) TTC solution in 0.05M $Na_2HPO_4$-$K_2HPO_4$ buffer (pH 7.4) and placed in a water bath at 30°C for 24h. The samples were extracted in 4 mL of 95% ethanol at 85°C in a water bath for 5min. The supernatant was determined through UV spectrophotometry at 485nm (Li et al., 2008).

### Activities of antioxidative enzymes

Activities of total superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11), and catalase (CAT, EC 1.11.1.6) were determined in accordance with the method with some modifications (Pasquariello et al., 2015). SOD activity was assayed by inhibiting the photochemical reduction of nitroblue tetrazolium (NBT), followed by spectrophotometric reading at 560nm (Ding et al., 2016). One unit of SOD activity was defined as the content that causes inhibition of NBT photoreduction by 50%. The assay for POD activity was conducted through guaiacol reduction method. Absorbance was read at 470 nm through UV spectrophotometry (Gengmao et al., 2015). The solution was extracted in 60mM phosphate buffer solution containing 1% insoluble polyvinylpyrrolidone and 1.5% guaiacol and then placed in a thermostatic water bath at 35°C for 3min.The reaction mixture was added with 0.6% $H_2O_2$ and enzyme extract and then subjected to measurements after 5min. POD activity was calculated using 1μmol oxidized guaiacol $min^{-1}$ $g^{-1}$ at 25°C. APX activity was assessed after ascorbate oxidation, and spectrophotometry analysis was conducted at 290 nm for 20s. The mixture (6 mL) contained 0.2 ml of 1mM hydrogen peroxide, 1 ml of 0.2mM EDTA–$Na_2$, 1 ml of 0.5mM ascorbic acid, 0.5 ml of enzyme extract, 2 ml of 50mM phosphate(pH 7.0), and 1.3ml of sterile water (Reddy et al., 2015). CAT activity was determined using absorbance at 240 nm and assayed based on the decomposition of $H_2O_2$ buffer containing 0.2 ml of 2 mM EDTA–$Na_2$, 1 ml of 7 mM phosphate buffer, 0.4 ml of 20 mM $H_2O_2$, 1.2 ml of distilled water, and 0.2 ml of enzyme extract.

### Statistical analysis

Data from all experiments are mean values. Measurements were performed on three replicates ± standard errors (S.E.). The data in the articles were subjected to analysis of variance (ANOVA). Comparisons between means were evaluated by Duncan's new multiple range test, values at $P \leq 0.05$ were considered statistically significant (SPSS, USA).

## RESULTS

### Effect of ALA on biomass accumulation of peach seedling under NaCl stress

The effects of treatments of 200mg/l ALA and 100mM NaCl on plant growth parameters such diameter, height, shoot and root dry mass, and leaf area are given in Table 1. The diameter, height, shoot and root dry mass, and leaf area of peach seedlings treated with 100mM NaCl significantly decreased compared their respective controls; these parameters decreased by 18.3%, 18.9%, 14.0%, 9.7%, and 8.0%, respectively. The height as well as shoot and root dry mass of the plants treated with 200mg/l ALA significantly increased compared with those in the blank control. However, diameter and leaf area treated with 200mg/l ALA no significantly increased compared to the control. Treatment with 200mg/l ALA and 100mM NaCl enhanced NaCl-stress-induced biomass accumulation compared with treatment with 100mM NaCl, the height, diameter, and shoot and root dry mass of the plant increased by 16.0%, 17.3%, 14.6%, and 7.5%, respectively. However, the leaf area of peach seedlings was not significantly affected by ALA application under NaCl stress. Furthermore, the NaCl stress of seedlings treated with 200mg/l ALA was not significantly different from the control in height and shoot and root dry mass.

### Effect of ALA on the photosynthesis of peach seedlings under NaCl stress

Changes in photosynthetic parameters including intercellular CO2 concentration (*Ci*), photosynthetic rate (*Pn*), stomatal conductance (*Gs*), and transpiration rate (*Tr*) under different conditions are shown in Table 2. Treatment with 200mg/l ALA significantly increased the *Pn*, *Tr*, and *Gs* of the leaves of peach seedlings by 21.5%, 28.4%, and 36.2%, respectively. However, $C_i$ treated with 200mg/l ALA no significantly increased compared to the control. Table 2 shows that the $C_i$, *Pn*, *Tr*, and *Gs* are lower in peach leaves treated with 100mM NaCl compared with those in the control. The *Pn*, *Tr*, and $C_i$ of peach leaves treated with 200 mg/l ALA and 100mM NaCl are 19.7%, 18.4%, and 6.5% higher than those in peach leaves treated with 100 mM NaCl alone. Nonetheless, the *Gs* of peach seedlings treated with 200 mg/l ALA was not significantly different under NaCl stress.

### Effect of ALA on the membrane permeability, MDA, and root activity of peach seedlings under NaCl stress

The levels of membrane permeability and MDA decreased, indicating that the plant under study was resistant (Naeem et al., 2011). As shown in Table 3, the membrane permeability and MDA contents increased by 132.8% and 62.8%, respectively, in the leaves of peach seedlings treated with 100mM NaCl compared with those in samples treated without ALA and NaCl (control). Treatment with 200mg/l ALA and 100mM NaCl significantly decreased the membrane permeability and MDA content of peach compared with treatment with 100mM NaCl, the membrane permeability and MDA of the plant increased by 32.0% and 27.6%, respectively. The membrane permeability level significantly decreased by 35.5% under treatment with 200mg/l ALA compared with that of the control. Meanwhile, ALA treatment did not significantly decrease the MDA content in peach seedlings. Table 3 shows that root vitality was reduced in samples exposed to 100mM NaCl. Treatment with 200mg/l ALA conferred peach seedlings with ability to adjust under NaCl stress. The root vitality significantly increased by 200mg/l ALA treatment and eventually reached the maximum value. The root vitality of peach seedlings treated with 200mg/l ALA and 100mM NaCl significantly increased by 18.3% compared with that under NaCl stress alone.

### Effect of ALA on chlorophylls accumulation of peach seedlings under NaCl stress

The concentrations of chlorophyll a, chlorophyll b, and total chlorophyll in the leaves of peach seedlings significantly increased by 80.1%, 18.9%, and 59.4%, respectively, after application of 200mg/l ALA compared with those in the control (Table 4). Compared with the control, chlorophyll a, chlorophyll b, and total chlorophyll contents decreased by 27.2%, 17.1%, and 24.0%

**Table 1: Effects of ALA and NaCl treatments on diameter, leaf area, plant height, and shoot and root dry mass in peach seedling**

| Treatment | Diameter (cm) | Dry mass of shoot (g) | Dry mass of root (g) | Height (cm) | Leaf area (cm²) |
|---|---|---|---|---|---|
| 0mM NaCl+0mg/L ALA | 0.428±0.015a | 13.150±0.954b | 4.229±0.139b | 46.130±2.080b | 11.485±0.523a |
| 0mM NaCl+200mg/L ALA | 0.431±0.021a | 14.993±0.892a | 4.746±0.224a | 53.950±3.190a | 11.524±0.428a |
| 100mM NaCl+0mg/L ALA | 0.347±0.013c | 11.305±1.021c | 3.818±0.312c | 37.670±3.220c | 10.566±0.710b |
| 100mM NaCl+200mg/L ALA | 0.407±0.019b | 12.952±0.758b | 4.103±0.286b | 43.720±1.950b | 10.497±0.847b |

Each value represents the mean of three replicates of each treatment, different letters denote statistically significant differences by the Duncan multiple comparison tests ($P \leq 0.05$)

**Table 2: Effects of ALA and NaCl treatments on intercellular CO2concentration ($C_i$), stomatal conductance ($G_s$) net photosynthetic rate ($P_n$), and transpiration rate ($T_r$) in the leaves of peach seedling**

| Treatment | $C_i$ [mmol ($H_2O$ ) $m^{-2}$ $s^{-1}$] | $G_s$ [mmol ($H_2O$) $m^{-2}$ $s^{-1}$] | $P_n$ [µmol ($CO^2$) $m^{-2}s^{-1}$] | $T_r$ [mmol ($H_2O$) $m^{-2}s^{-1}$] |
|---|---|---|---|---|
| 0mM NaCl+0mg/L ALA | 324.140±17.640a | 0.461±0.016c | 11.452±1.502b | 7.256±0.490b |
| 0mM NaCl+200mg/L ALA | 329.270±23.750a | 0.628±0.020a | 13.918±1.164a | 9.320±0.517a |
| 100mM NaCl+0mg/L ALA | 285.510±25.620c | 0.524±0.018b | 9.455±1.003c | 6.079±0.521c |
| 100mM NaCl+200mg/L ALA | 304.160±21.720b | 0.521±0.023b | 11.321±0.878b | 7.198±0.714b |

Each value represents the mean of three replicates of each treatment, different letters denote statistically significant differences by the Duncan multiple comparison tests ($P \leq 0.05$)

**Table 3: Effects of ALA and NaCl treatments on root vitality, malondialdehyde (MDA) and membrane permeability contents in the leaves of peach seedling**

| Treatment | MDA (nmol $g^{-1}$ FW) | Membrane permeability (ms/cm) | Root vitality [mg/(g·h)] |
|---|---|---|---|
| 0mM NaCl+0mg/L ALA | 12.857±1.328c | 0.160±0.010c | 0.569±0.053b |
| 0mM NaCl+200mg/L ALA | 12.611±1.116c | 0.103±0.009d | 0.900±0.042a |
| 100mM NaCl+0mg/L ALA | 20.934±1.390a | 0.371±0.017a | 0.408±0.025d |
| 100mM NaCl+200mg/L ALA | 15.152±1.607b | 0.253±0.009b | 0.482±0.028c |

Each value represents the mean of three replicates of each treatment, different letters denote statistically significant differences by the Duncan multiple comparison tests ($P \leq 0.05$)

**Table 4: Effects of ALA and NaCl treatments on chlorophyll a, chlorophyll b, and total chlorophyll contents in the leaves of peach seedling**

| Treatment | Chlorophyll a (mg/g FW) | Chlorophyll b (mg/g FW) | Total chlorophyll (mg/g FW) |
|---|---|---|---|
| 0mM NaCl+0mg/L ALA | 0.926±0.041b | 0.475±0.018b | 1.401±0.105b |
| 0mM NaCl+200mg/L ALA | 1.668±0.056a | 0.565±0.033a | 2.233±0.117a |
| 100mM NaCl+0mg/L ALA | 0.674±0.027d | 0.394±0.03c | 1.068±0.054d |
| 100mM NaCl+200mg/L ALA | 0.859±0.019c | 0.482±0.017b | 1.341±0.102c |

Each value represents the mean of three replicates of each treatment, different letters denote statistically significant differences by the Duncan multiple comparison tests ($P \leq 0.05$)

respectively, in peach seedlings treated with 100 mM NaCl. NaCl stress obviously decreased the chlorophylls contents of peach seedling leaves. Treatment with 200 mg/l ALA and 100mM NaCl showed that chlorophyll a, chlorophyll b, and total chlorophyll contents increased by 27.4%, 22.3%, and 25.6%, respectively, compared with those in 100mM NaCl treatment. ALA treatment obviously increased the chlorophylls contents of peach seedling leaves under NaCl stress.

### Effect of ALA on proline, soluble proteins, and soluble sugars of peach seedlings under NaCl stress

The contents of proline, soluble proteins, and soluble sugars significantly increased in peach seedlings treated with 100 mM NaCl or 200mg/l ALA as sole treatment compared with those in the control (Table 5). Moreover, the contents of soluble sugars, soluble proteins, and proline increased in peach seedlings treated with 100mM NaCl and 200mg/l ALA by 104.9%, 54.0%, and 84.2%, respectively, compared with those in the control. Treatment with combined NaCl and ALA increased the contents of soluble sugars (44.4%), soluble proteins (22.3%), and proline (46.2%) compared with those treated with 100mM NaCl alone in the leaves of peach seedlings.

### Effect of ALA on the antioxidant enzyme activities of peach seedlings under NaCl stress

The activities of APX, CAT, POD, and SOD in peach seedlings treated with NaCl stress and ALA are shown in Table 6. The activities of APX, CAT, POD, and SOD in peach seedlings treated with 100mM NaCl increased by 82.5%, 72.8%, 63.4%, and 160.8%, respectively, compared with those in the control. ALA (200 mg/l) treatment enhanced POD and SOD activities by 26.8% and 39.0% compared with those in the control. However, CAT treated with 200 mg/l ALA no significantly increased compared to the control, in addition, the content of APX was decrease. The APX, CAT, POD, and SOD activities in NaCl-stressed peach seedlings treated with 200 mg/l ALA increased by 122.2%, 84.3%, 54.3%, and 142.1%, respectively, compared with those in the control. Compared with those in the 100mM NaCl, the activities of CAT and SOD in peach seedlings treated with 100 mM NaCl and 200 mg/l ALA reduced by 10.7% and 7.1%, respectively. Treatment with 100mM NaCl and 200 mg/l ALA significantly increased the activity of POD (36.0%) compared with those treated with 100mM NaCl alone in the leaves of peach seedlings.

**Table 5: Effects of ALA and NaCl treatments on proline, soluble protein, and soluble sugar contents in the leaves of peach seedling**

| Treatment | Proline (μg·g$^{-1}$FW) | Soluble protein (mg·g$^{-1}$FW) | Soluble sugar (μg·g$^{-1}$FW) |
|---|---|---|---|
| 0mM NaCl+0mg/L ALA | 37.422±2.151d | 7.311±0.381d | 10.821±0.638c |
| 0mM NaCl+200mg/L ALA | 51.041±4.310b | 9.576±0.837b | 16.568±1.317 b |
| 100mM NaCl+0mg/L ALA | 47.149±4.109c | 9.208±0.187c | 15.359±1.551 b |
| 100mM NaCl+200mg/L ALA | 68.932±3.163a | 11.262±0.731 a | 22.180±1.060 a |

Each value represents the mean of three replicates of each treatment, different letters denote statistically significant differences by the Duncan multiple comparison tests ($P \leq 0.05$)

**Table 6: Effects of ALA and NaCl treatments on ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (POD), and superoxide dismutase (SOD) activities in the leaves of peach seedling**

| Treatment | APX (mmol g$^{-1}$FW ) | CAT (mmol g$^{-1}$FW) | POD (mmol g$^{-1}$FW) | SOD (U g$^{-1}$FW) |
|---|---|---|---|---|
| 0mM NaCl+0mg/L ALA | 6.315±0.508b | 91.67±2.85c | 715.363±31.510d | 281.972±15.010d |
| 0mM NaCl+200mg/L ALA | 5.147±0.326c | 93.74±8.10c | 907.079±34.761c | 391.941±26.228c |
| 100mM NaCl+0mg/L ALA | 11.528±0.725a | 158.41±7.24a | 1168.897±62.168b | 735.493±45.722a |
| 100mM NaCl+200mg/L ALA | 11.639±0.613a | 141.44±6.52b | 1589.707±59.248a | 682.714±19.687b |

Each value represents the mean of four replicates of each treatment, different letters denote statistically significant differences by the Duncan multiple comparison tests ($P \leq 0.05$)

## DISCUSSION

Our current study indicated that NaCl stress severely inhibits the growth of peach seedlings and remarkably decreases the diameter, leaf area, plant height, and shoot and root dry mass. NaCl stress changes the thermodynamic equilibrium of water and ions. As a consequence, the water potential of the rhizosphere decreases and difficulties in root water uptake occur. Thus, the root absorption capability is reduced, and the supply of water from roots to shoots is decreased (Wang et al., 2012). Plant roots under NaCl stress are often the first to experience stress and produce corresponding physiological responses. Therefore, root and shoot growth were inhibited (Choi et al., 2014). As shown in Table 1, ALA treatment can alleviate salinity stress during the growth of peach seedlings, improve dry matter accumulation, promote plant growth, and relieve salinity stress damages. This increase in growth parameters are caused by ALA treatment, which improves daytime photosynthesis and reduces nighttime respiration (Zhang et al., 2015). ALA of 200mg/L treatment caused no significant of diameter and leaf area, this may be due to ALA has not started its mechanism of resistance under non-stress condition. These findings indicated that exogenous NaCl treatment significantly inhibited biomass accumulation of peach cultivar. Moreover, treatment with 200mg/l ALA can obviously alleviate damages caused by salinity stress in seedlings.

Under salinity stress, *Gs* and *Tr* decrease remarkably. This decrease causes a reduction in shoot transpiration. Thus, the leaf stomata of peach seedlings have closed. The $Na^+$ concentration from leaves may be limited by reducing the leaf transpiration rate because the amount of $Na^+$ transported to plants is in proportion to the leaf transpiration rate, with a relatively reduced absorption of toxic ions (Contreras-Cornejo et al., 2014). The decrease in the leaf transpiration rate of peach seedlings under short-term salinity stress is an adaptive response. This phenomenon belongs to a mechanism of non-halophytes to prevent $Na^+$ stress and to adapt to osmotic stress (Silva et al., 2015). Table 2 shows that the *Ci*, *Tr*, *Pn*, and *Gs* are lower in peach leaves treated with 100mM NaCl compared with those in the control. Salinity stress may interfere with the normal metabolism of plant tissues and slows down plant growth or causes death (Sgherri et al., 2000). This condition is usually accompanied by a decrease in the Pn and other physiological phenomena. The reduction in *Pn* of *Lycium barbarum* under salinity stress is mainly caused by stomatal constraints (Zheng et al., 2001). The application of 100mM NaCl can reduce the *Ci*, *Tr*, *Pn*, and *Gs*, while under 100mM NaCl stress, ALA application could also improve them (Table 2). Similarly, in our experiment the concentration of *Ci* has no significant in peach seedlings treated with ALA under normal condition. This may be due to ALA can plays a definite role in under NaCl stress, however it has not started its mechanism of resistance under the condition of normal growth. These results suggest that ALA can regulate the opening and closing of stomata. Therefore, ALA possibly improves *Pn* of peach seedlings under NaCl stress, and this role is likely related to the enhanced *Gs* and increased $C_i$.

Salinity stress may increase reactive oxygen species (ROS), such as superoxide radical, perhydrol, and hydroxyl radical, in plant cells. Salinity stress may also induce the peroxidation of membrane lipids and unsaturated fatty acids. This condition results in membrane lipid

peroxidation and excessive MDA accumulation. MDA is the final product of membrane lipid peroxidation; its content and membrane permeability are important indicators to evaluate membrane lipid peroxidation and plasma lemma damage (Salama and Mansour, 2015). Zhang et al. (2013) revealed that ALA treatment can increase the anti-lipid peroxidation of the leaves of *Cassia obtusifolia* L. seedlings exposed to salinity stress by reducing the MDA content in leaves. The NaCl stress treatment induced a significant increase in MDA content in peach seedlings (Table 3). This result is significantly higher than that in normally grown plants. Therefore, the plasma membrane of plant cells undergoes peroxidation and exhibits physiological disruption. Seedlings treated with 200mg/L ALA and 100mM NaCl showed significant reduces in MDA content, which suggest that ALA treatment could relieve NaCl-stress-induced inhibition of MDA content in peach seedlings. Therefore, membrane lipid peroxidation is alleviated and cell membrane stability is improved (Song et al., 2011). ALA of 200mg/L treatment caused no significant of MDA content, this may be due to ALA has not started its mechanism of resistance under the condition of normal growth. Root vitality under high NaCl conditions were the worst, which became fragile and thinner and also showed strong inhibition of branching and growth. The change in physiological indicators of plant may be influenced by soil environment conditions (Li et al., 2008; Gao et al., 2015). 100mM NaCl treatment caused reduction of root vitality. The highest root vitality was observed in the 200mg/L ALA treatment, treatment with the 100mM NaCl and 200mg/L ALA caused a obvious increase in root vitality (Table 3). These results suggest that ALA treatment could relieve NaCl-stress-induced inhibition of root vitality in peach seedlings.

Chlorophyll concentration was significantly decreased by 100mM NaCl treatment (Table 4). Similarly, Zhang et al. (2013) also reported that chlorophyll content was decreased by NaCl stress in *Cassia obtusifolia* L, Salinity stress undermines cell membrane permeability and causes the leakage of numerous chlorophylls molecules and reduces chlorophylls content (Mehta et al., 2010). Chlorophylls are essential for photosynthesis. Chlorophylls complete the transmission, distribution, and conversion of absorbed light energy and ensures normal photosynthesis. Chloroplast is a NaCl-sensitive organelle mainly involved in photosynthesis. Thus, the chlorophylls content in leaves directly determines leaf photosynthesis and acts as an important indicator to evaluate the NaCl tolerance of plants. Chlorophyllase is an enzyme that functions in chlorophylls catabolism (Moradi et al., 2007). As shown in Table 4, Chlorophyll a, Chlorophyll b, and total chlorophyll contents in peach seedlings exposed to NaCl stress decreases. After exogenous ALA was applied, the chlorophylls content in peach leaves is higher than the control level. At the same time, the Chlorophyll a, Chlorophyll b, and Total chlorophyll contents content in peach seedlings exposed to salinity stress returns to the control level. ALA is a precursor of chlorophyll biosynthesis, the application of exogenous ALA may promote chlorophyll biosynthesis (Schlicke et al., 2014). The research demonstrated that ALA treatment may promote the generation of sufficient Chl*a*, which is favorable for the conversion of Chl*a* into Chl*b*. Chl*b* is considered a photosynthetic pigment involved in plant resistance against stress. Chl*b* may also prevent chlorophyll decomposition (Liu et al., 2015; Hui et al., 2015). These results showed that 200mg/L ALA application increased the chlorophylls content of peach seedlings under NaCl stress.

Soluble sugars are an important protective substance that confers plants with resistance to NaCl stress, improved protoplasm protection, and protection against protein colloid aggregation and non-deformation under NaCl stress. Accumulation of soluble proteins and proline in plants could reduce the osmotic potential, which is contributed in the cytoplasm by an important osmotic protective substance (Arndt et al., 2001). Plants exposed to stress absorb inorganic ions in cells, synthesize, and accumulate soluble protein, soluble sugar, proline, and small organic molecules (Qados, 2011). Soluble sugar and soluble protein are osmoprotectants; they are also sources of carbon skeletons and energy for the synthesis of other organic solutes. When their contents increase under salinity stress, their cytosolic concentration increases, cellular water potential decreases, and water absorption enhances (Kasuga et al., 1999). Our results demonstrated that ALA can improve soluble sugar, soluble protein and proline contents under NaCl stress (Table 5). Soluble protein in plants exposed to stress may also induce the formation of new stress-tolerant proteins, such as salinity stress-related proteins, drought-related proteins, detoxifying enzymes, and water channel proteins. These products can enhance the stress resistance of plants (Aghaei and Komatsu, 2013). Proline may regulate cytoplasmic osmotic pressure to protect the membrane and other enzyme structures, maintain the metabolic activities of many important enzymes in cells, and relieve stress when plants suffer from NaCl damages (Nunkaew et al., 2014). The proline content increases under salinity stress. Hence, the absorption and accumulation of ions in crops are affected, their cytosolic concentration is reduced, and cellular water potential is increased. ALA may increase cellular fluid concentration under salinity stress, reduce cellular water potential, and enhance water absorption in cells by increasing proline content. Our results also show that spraying ALA onto the leaves enhances the growth of peach seedlings exposed to salinity stress to a certain extent. The contents of soluble

protein, soluble sugar, and proline are increased. Therefore, the role of ALA in relieving peach seedlings from salinity stress is related to its functions in the osmoregulation of plants and inhibitory effects on membrane lipid peroxidation.

Salinity stress may also promote the establishment of plant defense systems. Antioxidase is another protective enzyme in plant defense systems. Superoxide dismutase may catalyze the disproportionation reaction in vivo and thus transforms superoxide radical into $O_2$ and $H_2O_2$. As a result, plants are protected against damage by reactive oxygen under salinity stress (Lee and Lee, 2000). Nishihara et al. (2003) found that exogenous ALA treatment in spinach can reduce superoxide radical accumulation, enhance antioxidase activity in plants. In this experiment, the antioxidase activity in the leaves of peach seedlings is increased at 100mM NaCl. An increase in the antioxidase activity may maintain the metabolic balance of ROS in peach seedlings, protect the integrity of membrane structure, and avoid damage induced by NaCl. The regulatory mechanism involves ferroheme-containing POD as a prosthetic group and ALA as a precursor of porphyrin heme biosynthesis. ALA treatment promotes the synthesis of ferroheme and increases the activity of POD as a prosthetic group; thus, resistance to oxidative stress is enhanced (Hunter et al., 2005). After 200mg/L ALA was applied, the CAT, SOD, and POD activities in the leaves of peach seedlings are improved under NaCl stress (Table 6). The CAT and APX activities play an important role in scavenging $H_2O_2$ to form $H_2O$ and $O_2$, in protecting a plant against oxidative stress. However, there were no significant differences in APX activity between NaCl treatment and ALA and NaCl treatment conditions. The CAT activity in peach seedlings was similar between the ALA treatment and control as there were no significant differences. This suggests that the CAT activity is affected without significantly under ALA treatment alone. In addition, after 200mg/L ALA was applied, the APX activity had no obvious change under NaCl stress in the current study. This effect enhanced the influence of reactive oxygen removal on the plant. In the present study, exogenous ALA treatment may enhance the NaCl tolerance by increasing the POD activity and reducing CAT and SOD activity in peach seedlings and ensure the normal growth of peach seedlings under NaCl stress. Thus, this study provides a new basis for feasibility studies on enhancing plant resistance to stress by applying exogenous substances.

The results suggest that the growth parameters of exogenous ALA treatment are significantly increased than those under NaCl stress. Two main pathways are speculated: (1) ALA treatment may induce a high antioxidase activity in the leaves of peach seedlings under salinity stress. Thus, the abilities of scavenging ROS from tissues and cells are enhanced and damages from membrane lipid peroxidation are reduced (Zhang et al., 2006; Naeem et al., 2011). (2) The contents of osmolytes, such as chlorophylls, proline, soluble sugar, and soluble protein, in the leaves of peach seedlings under salinity stress are reduced after ALA was administered. Osmoregulation in cells increases. Hence, the leakage of electrolytes through the membrane is reduced and the integrity of the membrane is maintained (Youssef and Awad, 2008; Yang et al., 2014).

## CONCLUSIONS

Exogenous ALA treatment may enhance the stress resistance of peach seedlings under NaCl stress and exhibit good performance in terms of the physiological parameters. ALA treatment can also enhance the antioxidase activity and chlorophylls content in peach seedlings, inhibit the accumulation of lipid peroxidation products, enhance membrane permeability, maintain the integrity of membrane systems, improve osmoregulation, and alleviate salinity stress damages on peach seedlings. ALA treatment can also increase the net *Pn* in peach leaves and promote the photosynthesis, accumulation of photosynthetic products, and plant growth. ALA treatment also relieves peach seedlings from NaCl stress. As a consequence, the stress resistance of crops is enhanced. Thus, ALA treatment is essential for agricultural culture and production.

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### Authors' contributions

J.B. Ye conducted the experiment, analyzed the results, and drafted the manuscript. Q.W. Chen sampled collection and data analysis. T.T. Tao analyzed the data. G.Y. Wang drafted the manuscript. F. Xu designed the overall experiment and improved the manuscript. All authors read and approved the final manuscript.

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