RESEARCH ARTICLE

Roles of salicylic acid in selenium-enhanced salt tolerance in tomato plants

Hong Wu · Shuya Fan · Haijun Gong · Jia Guo

Abstract

Aims Selenium (Se) has been reported to mitigate the harmful effects of salt stress on plants; however, the internal mechanisms remain unknown. Here, the effects of Se supplementation on tomato plants under salt stress were investigated.

Methods The biomass, relative electrical conductivity (REC), relative water content (RWC), photosynthetic parameter, inorganic ion contents, malondialdehyde (MDA), soluble sugar and proline contents, as well as the regulation of plant hormones of Se application in tomato plants were investigated after exposure to Se and salt stress treatments.

Results Exogenous Se application improved photosynthesis and the water use efficiency (WUE) of tomato plants under salt stress, thereby promoting the growth of tomato plants under salt stress. Se supplementation also maintained the K^+ and Na^+ homeostasis, reduced the REC, decreased MDA, H_2O_2 and $O_2^{\bullet-}$ contents, and mitigated the oxidative damage caused by salt stress. In addition, exogenous Se increased the salicylic acid (SA) content in tomato leaves and roots via up-regulating the PAL or ICS pathways involved in SA biosynthesis. After pretreatment with the SA inhibitor 1-aminobenzotriazole, the photosynthetic efficiency of tomato plants decreased, plant growth was weakened, and the REC was increased, indicating that the alleviating role of Se on salt stress was abolished.

Conclusions Our results clarified the roles of Se and its regulation mechanisms in plant salt stress tolerance and the critical involvement of SA in this process. The study of Se in plant abiotic stress tolerance will give a more theoretical foundation for using exogenous Se in agricultural production to enhance crop growth and yield under adversity stresses.

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Abbreviations

ABT	1-Aminobenzotriazole
DAB	Diaminobenzidine
H_2O_2	Hydrogen peroxide
ICS	Isochorismate synthase
MDA	Malondialdehyde
NBT	Nitroblue tetrazolium
$O_2^{\bullet-}$	Superoxide anion
PAL1	Phenylalanine ammonia-lyase 1
qRT-PCR	Quantitative real-time PCR

ROS   Reactive oxygen species
SA   Salicylic acid
SABP2   Salicylic acid-binding protein 2
SAMT   Carboxymethyltransferase
Se   Selenium
WUE   Water use efficiency

Introduction

Soil salinity is a major abiotic stress that severely restricts plant growth and crop yield (Soltabayeva et al. 2021; Shrivastava and Kumar 2015), which has been exacerbated by poor irrigation practices, rising population, and industrial pollution (Yu et al. 2020). Currently, 20% of the world's arable land and 50% of the irrigated areas are significantly affected by extreme salt stress (Wichern et al. 2020). Salt stress leads to ionic toxicity and osmotic and oxidative stress, which affect many plant physiological and biochemical processes, including photosynthesis, energy metabolism, and water and nutrient uptake (Yang et al. 2020). Therefore, plants have developed strategies to survive in highly saline environments, such as ion homeostasis and compartmentalization, biosynthesis of osmoprotectants, activation of antioxidant compounds, and regulation of several stress-responsive genes (Gupta and Huang 2014). However, applying exogenous substances (melatonin, silicon, brassinosteroids, polyamine, and selenium (Se), etc.) may be a promising strategy to improve plant salt tolerance effectively, saving time and effort (Rady et al. 2020).

Se is an essential trace element required by humans and animals (Gupta and Gupta 2017). Adequate daily Se supplementation is essential for many physiological functions in the human body, such as preventing cardiovascular diseases and type 2 diabetes, regulating immunity, and reducing cancer risk (Rayman 2012). Since plants are the primary source of dietary Se, studies on Se compounds in plants are essential (Zhu et al. 2018). Selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}) are the main forms of Se available to plants (Gupta and Gupta 2017). Although there is no direct evidence that Se is essential for higher plants, recent studies have shown that low concentrations of Se could influence plant growth and physiological processes, such as enhancing photosynthesis (Borbély et al. 2021) and root and shoot growth (Diao et al.

2014), promoting germination and nitrogen assimilation, increasing starch accumulation, and delaying senescence (Hajiboland et al. 2019). Other studies have reported that Se could mitigate the adverse impacts on plants of different environmental stresses, e.g., salt, drought, high temperature, and heavy metals (Elkelish et al. 2019; Rady et al. 2020). Jiang et al. (2017) reported that Se enhanced the plant growth index of maize under salt stress. Other studies have shown that Se could protect plants against severe stresses by improving the antioxidant capacity, increasing the photosynthetic indices, and promoting secondary metabolism (Elkelish et al. 2019; Zsiros et al. 2019). However, much research remains to be done to explore the impacts of exogenous Se supplementation on plants in response to salinity.

Previous studies have shown that phytohormones played essential roles in Se-enhanced tolerance of abiotic stresses, such as abscisic acid (Zahedi et al. 2019), auxin (Jia et al. 2018), jasmonic acid, and ethylene (Tamaoki et al. 2008). However, it is unknown whether salicylic acid (SA) is involved in the Se-induced salt tolerance of plants. SA is an endogenous plant hormone that is crucial in regulating physiological and developmental processes in plants, including growth, maturity, senescence, photosynthesis, and transpiration (Koo et al. 2020). SA also serves as a signaling molecule, triggering allergic reactions and inducing stress resistance of plants to salt, drought, heat, heat, cold, and heavy metals (La et al. 2019; Wassie et al. 2020). Khalvandi et al. (2021) found that 0.5 mM SA treatment could improve photosynthetic performance, maintain membrane permeability, induce stress-response-related proteins, and increase antioxidant enzyme activity, effectively alleviating the negative impacts caused by drought stress on winter wheat growth. Ardebili et al. (2014) found that a combination of Se and SA mitigated the effects of salt stress in soybean more successfully than either Se or SA alone. Although Se or SA can improve salt stress tolerance, their interactions during salt stress are unknown. Therefore, the underlying mechanisms of Se-enhanced plant tolerance under salt stress, and the impact of plant hormones like SA, should be investigated.

Cultivated tomato (*Solanum lycopersicum* L.), one of the most popular vegetable fruit crops in the world, has moderate sensitivity to soil salinity (Fan et al. 2021). Sustainable approaches are needed to

enhance the growth and production of tomato plants under salinity. Previous studies reported that Se treatment promoted seed germination and plant growth of salt-stressed tomato plants and improved the nutritional quality of tomato fruit (Zhu et al. 2018). However, the precise mechanism through which exogenous Se improves the salt stress resistance of tomatoes remains unclear. This study investigated the Se involvement in plant regulatory mechanisms under salt stress by analyzing the photosynthesis, plant growth indicators, chloroplast antioxidant systems, and plant hormone regulation following Se application. Our findings will clarify the mechanisms underlying Se's ability to enhance salt resistance in tomato plants and provide theoretical support for using Se fertilizer for agricultural production in high-salinity areas.

Materials and methods

Plant materials and treatment

The experimental materials were tomato (*Solanum lycopersicum* L.) cultivar "Ailsa Craig". Plump and uniform seeds were sterilized by soaking in deionized water for 15 min at 55 °C, then germinated for 2 days on wet filter paper in a growth chamber without light at 28℃. The germinated seeds were planted in the medium in the greenhouse with a day/night temperature of 25℃/18℃. At the three-leaf stage, the seedlings were moved to the half-strength modified Hoagland solution (Gou et al. 2020a), which was aerated for 10 min every 3 h. The culture solution pH was adjusted to 6.2 and changed every 3 days. Five days later, the seedlings were subjected to Se or salt stress treatment by adding 25 μM sodium selenite (Na_2SeO_3) or 150 mM sodium chloride (NaCl) to the nutrient solution. Among the different Se doses tested, supplementation with 25 μM Se had the most noticeable effect in relieving salt stress damage in our early pre-experiment (data not shown). These experiments include four different treatments: (1) CK: no Se and no NaCl; (2) Se: 25 μM Na_2SeO_3; (3) NaCl: 150 mM NaCl; (4) NaCl + Se: 150 mM NaCl + 25 μM Na_2SeO_3. After 15 days of salt stress, the fully expanded leaves and roots of tomato seedlings were wrapped in tin foil and preserved at -80℃ after being frozen in liquid nitrogen.

The biomass, root morphological traits, relative electrical conductivity (REC), and relative water content (RWC)

A straightedge and a vernier caliper were used to measure the plant height and stem diameter of six randomly picked plants. The tomato seedlings were divided into above-ground and roots, and their fresh weight (FW) was assessed. Then the plants were dried at 105 °C for 15 min, stayed at 75 °C for 1 d, and weighed to determine the plant dry weight (DW). Total root length, root surface area, root volume and average root diameter were investigated by a dual lens system (V700. EPSON, Japan).

The REC of tomato leaves was measured as follows: the fully expanded third leaves were taken and cut into pieces. Weigh 0.1 g sample and put it into a centrifuge tube containing 20 ml of deionized water, shaken for 2 h and its electrical conductivity was measured (EC1). The sample was then boiled for 10 min, cooled and measured for electrical conductivity (EC2). The REC was calculated with the formula: REC (%) = EC1/EC2*100. The RWC of leaves was measured as described by Fan et al. (2022).

Measurement of photosynthetic pigments

The contents of chlorophyll and carotenoid were determined in fresh weight (FW) of leaves by sample homogenization with 95% (v/v) ethanol. The absorbance was measured at 665 nm for chlorophyll a, 649 nm for chlorophyll b, and 470 nm for carotenoids using an ultraviolet spectrophotometer (UV-2450, Shimadzu, Japan). Chlorophyll and carotenoid contents were calculated as described by Arnon (1949).

Measurement of photosynthetic parameters and chlorophyll fluorescence parameters

The second fully expanded leaves of tomato plants were used to determine the photosynthetic parameters using the Li-6800 portable photosynthesis system (LI-COR, Lincoln, USA). The formula: WUE = Pn/Tr was used to calculate the instantaneous water use efficiency (WUE). The efficiency of PSII (Fv/Fm) and quantum yield of PSII (ΦPSII) were determined in fully expanded intact leaves using a PAM chlorophyll fluorometer (Heinz Walz, GmbH, Effeltrich, Germany).

Determination of Se, K, Na, and Cl contents

Plant tissues were dried for 3 days under 80 °C in an oven and ground into powder. 0.2 g sample was weighted and placed in a digestion tube containing 5 mL extracting solution (nitric acid: perchloric acid=4:1). After incubation overnight, the solution was first digested at 120 °C in the metal bath for 2 h, and then warmed up to 170 °C. The solution was heated until the remaining volume is about 2 mL, cooled, and diluted to 30 mL. 5 mL of the solution was taken out, added the same volume of 6 mol/L hydrochloric acid, reaction at 100 °C for 30 min. After making the blank control and Se standard curve, an atomic fluorescence spectrometer (LC-AFS-8530, China) was applied to determine the Se content. To measure the content of K^+ and Na^+, the oven-dried plant shoots and roots were digested in 5 mL HNO_3–H_2O_2 solution and heated at 250 °C. The concentration of K^+ and Na^+ was determined by flame spectrophotometry (M410, Sherwood, Britain). The Cl^- content was determined with silver nitrate titration as described by Zhang et al. (2011).

The malondialdehyde (MDA), soluble sugar and proline content

The MDA content was measured using thiobarbituric acid reaction according to Gou et al. (2020a). According to Fan et al. (2022), the soluble sugars were determined with the sulphate ketone colorimetry method.

The proline was measured as described by Shi et al. (2016). 0.1 g of sample was added with 3 mL of 3% sulfosalicylic acid, incubated for 10 min in a boiling water bath, then cooled down and centrifuged at 6,000 g for 10 min. After that, 1 ml of the supernatant was collected and added with 4 mL extraction buffer (distilled water: glacial acetic acid: acidic ninhydrin=1:1:2). The mixture solution was boiled for 1 h and cooled down. After that, 2 mL of toluene was added and stratification was carried out for 2 h. After full extraction, the red toluene phase was taken for colorimetric analysis at 520 nm. The proline content was calculated based on a standard curve.

Quantification and visualization of reactive oxygen species (ROS)

The hydrogen peroxide (H_2O_2) content was assayed with a fluorescent dye 2,7-dichlorofluorescin diacetate as described by Han et al. (2020). The superoxide anion ($O2^{•-}$) content was determined using the sulphanilic acid and α-naphthylamine solution as described by Shi et al. (2014). The visualization of H_2O_2 and $O^{2•-}$ accumulation was performed with diaminobenzidine (DAB) and nitroblue tetrazolium (NBT) solution as reported by Zhu et al. (2020).

Antioxidant enzymes activity assays

The weight of 0.3 g of frozen material was ground into homogenate with 4 mL ice-cold sodium phosphate buffer (100 mM, pH 6.8). The homogenate was centrifuged under 12,000 g at 4 °C for 20 min, and the supernatant was collected to determine the antioxidant enzymes activity (Gou et al. 2020b).

Plant hormone content

The weight of the 0.1 g of frozen material was ground into powder with liquid nitrogen, then mixed thoroughly with 2 mL of the extraction buffer (isopropanol: sterile water: HCl=2:1:0.002) and incubated at 4℃ overnight. The supernatant was obtained by centrifuging the solution at 7000 g for 12 min at 4 °C. The supernatant was collected, added with 2 mL of dichloromethane and shaked in an oscillation incubator at 4 °C for 1 h, and centrifuged at 7000 rpm for 10 min at 4 °C. Then, 2 mL of the lower fraction was taken, blew dry with nitrogen, added with 100 μL methanol and filtered through a 0.22 μm microporous membrane. The liquid chromatography (QTRAP5500, AB, USA) was applied to determine the content of various phytohormones.

Analysis of gene expression

Total RNA was isolated and purified using Trizol Reagent (Omega, Norcross, USA), and first-strand cDNA was generated with HiScript® II Q RT SuperMix for qPCR kit (Vazyme, Nanjing, China). The gene expression analysis was performed on a QuantStudio®5 Real-Time PCR System (ABI, Carlsbad, CA) using SYBR Premix Ex Taq^{TM} kit (Takara). The specific primers were listed in Table S1. The GAPDH (GenBank: NM_001321306) gene was selected as internal control.

SA biosynthesis inhibitor treatment

Two days in advance, the four-leaf stage seedings were pre-sprayed with a salicylic acid biosynthesis inhibitor ABT (1-aminobenzotriazole, Yang et al. 2018), followed by the addition of Se and NaCl. The experiment included four treatments: (1) CK; (2) CK + 100 μM ABT; (3) 150 mM NaCl; (4) 150 mM NaCl + 100 μM ABT; (5) 150 mM NaCl + 25 μM Se; and (6) 150 mM NaCl + 25 μM Se + 100 μM ABT. The PH of culture solution was adjusted to 6.2 and changed every 3 days.

Statistical analysis

The data was analyzed using SPSS 19.0 software (IBM, Armonk, NY). Data are means ± SD (n = 3) calculated with three biological replicate samples. One-way analysis of variance (ANOVA) and Duncan's multiple range test or t-test in Excel (2010) were performed for comparison of significant differences between treatments (P < 0.05). All the experiments were repeated at least three times.

Results

Effects of Se on plant growth parameters under salt stress

In order to investigate the function of Se on plant growth and development under salt stress, tomato seedlings were applied with 150 mM NaCl and exogenous Se to evaluate the salt resistance of various treatment groups (Fig. 1, Table 1). Under normal conditions, Se supplementation significantly increased plant height, but did not change the stem diameter or the fresh and dry weights of tomato shoots and roots (Table 1). Compared with the controls, salt stress treatment for 15 d significantly reduced plant height and stem diameter. Compared with the seedlings without Se treatment, the plant heights and stem diameters of Se-treated tomato seedlings significantly increased by 17.7 and 23.0%, respectively, and the fresh weight of shoots by 13.9%, under salt stress treatment (Table 1). The shoot dry weights were also higher in the Se-treated tomato seedlings under salt stress, although these findings were not significant (Table 1). Moreover, the tomato seedlings without Se

treatment showed obvious wilting and petiole drooping, whereas the Se-treated plants did not exhibit apparent symptoms of salt damage (Fig. 1A). In addition, under normal and salt stress conditions, Se treatment significantly promoted tomato root growth (Fig. 1B, Table 1). Under normal conditions, the total root surface area, total root volume, and average root diameter of Se-treated tomato plants increased by 22.3, 43.5, and 58.6%, respectively, compared to control plants without Se addition (Table 1). Under salt stress, Se treatment increased the total root length, total root volume, and average root diameter of tomato plants by 29.1, 39.0, and 28.6%, respectively, compared to plants without Se treatment (Table 1).

The RWC of tomato seedlings from different treatment groups was tested (Fig. 1C). We also investigated the REC, which is a key parameter of stress damage (Fig. 1D). Exogenous Se treatment did not change the RWC and REC of tomato plants compared to the control plants under non-stress conditions. However, under salt stress, the RWC of leaves increased by 9.8% and the REC of leaves decreased by 16.2% in Se-treated tomato seedlings compared to seedlings without Se treatment (Fig. 1C, D).

Effects of Se on the photosynthetic pigment contents

Under control conditions, Se treatment increased the contents of chlorophyll b and carotenoids but did not affect the chlorophyll a content (Table 2). Salt stress decreased the contents of all these pigments. However, Se supplementation significantly increased the chlorophyll a, chlorophyll b, and the total chlorophyll contents of tomato leaves by 20.8, 43.8, and 26.4%, respectively (Table 2).

Effects of Se on photosynthesis and chlorophyll fluorescence parameters

We investigated the photosynthetic parameters of various treatment groups, such as the net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci) to ascertain the impact of exogenous Se on plant photosynthesis under salt stress (Table 2). Under normal conditions, Se addition did not affect the photosynthetic characteristics of tomato plants compared to the controls. However, salt stress dramatically reduced the Pn, Tr, Gs, and Ci in tomato seedlings, and Se
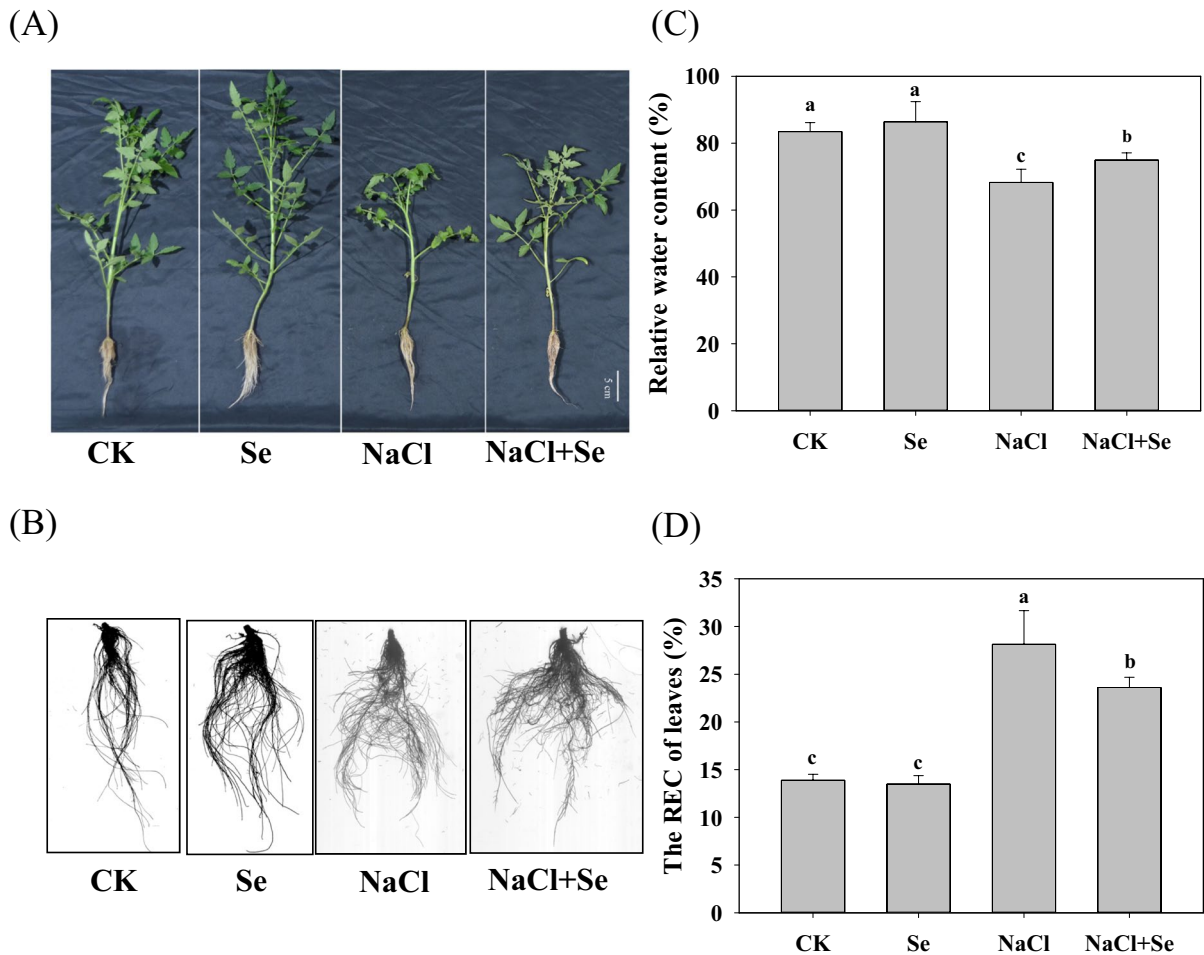
(A)

(B)

(C)

(D)

Fig. 1 Influence of exogenous Se on tomato plant growth and development under salt stress treatment with/without Se supplementation for 15 d. (**A**) Plant phenotype observation. (**B**) The root morphology of tomato seedlings. (**C**) The relative water content (RWC) of tomato seedlings. (**D**) The relative electrical conductivity (REC) of tomato seedlings. CK, control; Se, selenium; NaCl: salt treatment; NaCl + Se: salt + selenium treatment. Data are presented as mean ± SD of six biological replicates. Different small letters indicate significant differences between treatment groups at $P < 0.05$

treatment significantly recovered the Pn, Tr, Gs, and Ci by 27.9, 11.0, 73.4, and 10.6%, respectively, compared to the seedlings without Se treatment (Table 2). Under salt stress conditions, Se treatment also increased the WUE of tomato leaves by 14.6%. Chlorophyll fluorescence is a non-intrusive and accurate indication of photosynthetic performance (Murchie and Lawson 2013). Under non-saline conditions, exogenous Se did not change the maximum quantum yield of photosystem II (Fv/Fm) and the actual photochemical efficiency of photosystem II (Φ_{PSII}) in tomato seedlings (Table 2). However, under salt stress conditions, Se application significantly increased the Fv/Fm and Φ_{PSII} values by 5.3% and 8.5%, respectively (Table 2).

Se contents in different parts of tomato plants

We measured the Se content in tomato shoots and roots with exogenous Se addition under normal and salt stress (Table 3). Applying Se to the culture solution increased the Se content in tomato tissues under

Table 1 Influence of exogenous Se on plant growth and root characteristics of tomato seedlings under salt stress

Treatment		CK	Se	NaCl	NaCl + Se
Growth indices	Plant height (cm)	20.67 ± 2.25^b	22.44 ± 1.24^a	15.67 ± 1.12^c	18.44 ± 1.33^b
	Stem diameter (cm)	5.28 ± 0.39^a	5.02 ± 0.35^{ab}	3.96 ± 0.20^c	4.87 ± 0.50^b
Biomass (g/plant)	Fresh weight of shoots	17.99 ± 0.79^a	18.22 ± 0.90^a	7.82 ± 0.90^c	$8.91 + 0.69^b$
	Fresh weight of roots	2.73 ± 0.12^a	2.79 ± 0.08^a	2.31 ± 0.09^b	2.40 ± 0.07^b
	Dry weight of shoots	1.55 ± 0.14^a	$1.59 + 0.19^a$	0.65 ± 0.03^b	0.80 ± 0.05^b
	Dry weight of roots	0.20 ± 0.03^a	0.22 ± 0.04^a	0.15 ± 0.03^b	0.17 ± 0.02^b
	Total dry weight	1.75 ± 0.16^a	1.81 ± 0.22^a	0.80 ± 0.05^b	0.98 ± 0.06^b
Root morphological traits	Total root length (cm)	452.5 ± 41.9^b	545.9 ± 46.1^{ab}	459.4 ± 26.9^b	593.3 ± 86.3^a
	Total surface area (cm^2)	61.4 ± 5.1^b	75.1 ± 10.8^a	54.9 ± 5.0^b	67.4 ± 8.1^{ab}
	Total root Volume (cm^3)	0.46 ± 0.03^{bc}	0.66 ± 0.09^a	0.41 ± 0.07^c	0.57 ± 0.06^b
	Average root diameter (mm)	0.29 ± 0.01^c	0.46 ± 0.03^a	0.28 ± 0.02^c	0.36 ± 0.02^b

Different lowercase letters denote significant differences among different treatments at $P < 0.05$

Table 2 Influence of Se on photosynthetic pigments contents, chlorophyll fluorescence parameters and photosynthetic characteristics in tomato plants under salt stress

Treatment		CK	Se	NaCl	NaCl + Se
Leaf pigments	Chl a (mg g^{-1} DW)	1.93 ± 0.06^a	2.14 ± 0.25^a	1.49 ± 0.11^b	1.80 ± 0.29^a
	Chl b (mg g^{-1} DW)	0.68 ± 0.1^{bc}	1.08 ± 0.1^a	0.48 ± 0.04^c	0.69 ± 0.12^b
	Total Chl (mg g^{-1} DW)	2.61 ± 0.06^{bc}	3.22 ± 0.35^a	1.97 ± 0.15^c	2.49 ± 0.78^b
	Carotenoids (mg g^{-1} DW)	0.43 ± 0.01^b	0.52 ± 0.07^a	0.3 ± 0.02^c	0.32 ± 0.05^{bc}
Chlorophyll fluorescence parameters	Fv/Fm	0.737 ± 0.01^a	0.731 ± 0.01^a	0.704 ± 0.05^b	0.741 ± 0.02^a
	Φ_{PSII}	0.65 ± 0.04^a	0.62 ± 0.01^{ab}	0.59 ± 0.04^b	0.64 ± 0.03^a
Photosynthetic parameters	Pn (µmol CO$_2$ m^{-2} s^{-1})	4.95 ± 0.2^a	4.73 ± 0.25^a	2.72 ± 0.2^c	3.48 ± 0.37^b
	Tr (mmol H$_2$O m^{-2} s^{-1})	3.86 ± 0.87^a	3.71 ± 0.28^a	1.73 ± 0.08^c	1.92 ± 0.06^b
	Gs (mmol H$_2$O m^{-2} s^{-1})	111.74 ± 5.6^a	95.13 ± 7.3^a	40.68 ± 3.34^c	70.52 ± 15.2^b
	Ci (µmol CO$_2$ mol^{-1} air)	329.49 ± 34.09^a	303.08 ± 22.13^{ab}	260.5 ± 8.85^c	288.2 ± 10.92^b
	WUE (mmol CO$_2$ mol^{-1}H$_2$O)	1.28 ± 0.26^c	1.27 ± 0.13^c	1.58 ± 0.17^b	1.81 ± 0.16^a

Different lowercase letters denote significant differences among different treatments at $P < 0.05$

Chl a chlorophyll a, Chl b chlorophyll b, Total Chl chlorophyll a + b, Fv/Fm maximum quantum yield of PSII, $\Phi PSII$ photon yield of PSII, Pn net photosynthetic rate, Tr Transpiration rate, Gs stomatal conductance, Ci Intercellular CO$_2$ concentration, WUE Water use efficiency

Table 3 Influence of exogenous Se on the contents of Se and various inorganic ions in tomato seedlings under salt stress

Tissues	Treatment	Se (mg g^{-1})	K$^+$ (mg g^{-1})	Na$^+$ (mg g^{-1})	K$^+$/ Na$^+$	Cl$^-$ (mg g^{-1})
Shoots	CK	ND	70.83 ± 4.35^a	2.55 ± 0.49^c	27.73 ± 4.306^a	0.61 ± 0.18^d
	Se	0.61 ± 0.16^a	57.74 ± 3.95^b	4.38 ± 0.33^c	13.26 ± 0.98^b	0.81 ± 0.05^c
	NaCl	ND	23.39 ± 3.276^d	99.43 ± 6.10^a	0.24 ± 0.04^c	2.70 ± 0.13^a
	NaCl + Se	0.58 ± 0.10^a	32.43 ± 2.23^c	82.99 ± 9.75^b	0.39 ± 0.04^c	2.30 ± 0.16^b
Roots	CK	0.29 ± 0.07^c	43.99 ± 7.73^a	3.07 ± 0.76^b	15.00 ± 3.96^b	0.49 ± 0.07^c
	Se	17.98 ± 0.89^a	40.79 ± 1.68^a	2.02 ± 0.08^b	20.18 ± 1.07^a	0.59 ± 0.27^c
	NaCl	0.15 ± 0.06^c	25.83 ± 0.53^b	21.36 ± 3.48^a	1.30 ± 0.12^c	2.19 ± 0.23^a
	NaCl + Se	9.33 ± 0.78^b	27.76 ± 0.83^b	19.54 ± 2.64^a	1.45 ± 0.019^c	1.57 ± 0.74^b

Different lowercase letters denote significant differences among different treatments at $P < 0.05$

non-stress and salt stress conditions (Table 3). This demonstrates that plants absorb Se via their roots and is an effective strategy to enhance Se content in different tomato tissues.

Effects of Se on inorganic ions concentrations under salt stress

Under normal conditions, there were no significant differences in the Na^+ contents in the shoots and roots of Se-treated plants compared with control plants (Table 3). However, Se treatment resulted in a decrease in K^+ content and an increase in Cl^- content in tomato shoots. Under salt stress, the K^+ in the shoots and roots decreased significantly, but Se treatment increased the K^+ content in tomato shoots by 38.6% compared to plants without Se (Table 3). The Na^+ and Cl^- contents were markedly increased by NaCl addition compared to control plants. The addition of Se decreased the Na^+ and Cl^- contents in tomato shoots by 16.5 and 14.8%, respectively (Table 3). However, the presence of Se did not significantly affect the Na^+ content in tomato roots under salt stress.

Effects of Se on lipid peroxidation and osmoprotectants under salt stress

Under non-saline conditions, the malondialdehyde (MDA), soluble sugar, and proline contents of tomato leaves or roots were not changed by Se treatment (Fig. 2). However, their contents were significantly induced after 15 days of salt stress. Se treatment decreased the MDA and soluble sugar contents of leaves under salt stress by 30.1 and 37.6%, respectively, compared to the seedlings under salt stress without Se treatment (Fig. 2A, B). Se treatment also reduced the MDA and soluble sugar contents in tomato roots in response to salt stress by 44.7 and 29.8%, respectively (Fig. 2A, B). The addition of Se also slightly decreased the proline content in leaves but did not change its content in roots under salt stress (Fig. 2C).

H_2O_2 and $O_2^{•-}$ accumulation and antioxidant defense

Histochemical staining showed that Se application did not affect H_2O_2 and $O_2^{•-}$ accumulation in tomato leaves under non-stress conditions (Fig. 3A). Salt stress increased the reactive oxygen species (ROS) accumulation in tomato leaves, which was significantly decreased by Se application (Fig. 3A, upper). On the other hand, Se supplementation decreased the H_2O_2 and $O_2^{•-}$ contents in tomato leaves under salt stress by 22.2 and 47.2%, respectively (Fig. 3A, lower).

We investigated the activities of the antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) to ascertain the effect of Se treatment on antioxidant defense in tomato plants in response to salt stress (Fig. 3B). Under non-saline conditions, Se treatment did not affect SOD and POD activity in leaves or roots or CAT activity in roots, but did reduce CAT activity in leaves (Fig. 3B). Salt stress increased SOD, POD, and CAT activities, and Se treatment decreased their activities by 19.2, 34.1, and 39.6% in leaves and 21.7, 15.1, and 34.1% in roots compared to seedlings without Se treatment (Fig. 3B).

Phytohormones contents

To investigate the influence of Se addition on the contents of phytohormones under salt stress treatment, we measured the level of several phytohormones in different tomato tissues after 5 days of salt stress with or without Se addition, including SA, abscisic acid (ABA), melatonin (MT), and jasmonic acid (JA) (Fig. 4). Se treatment increased the SA content in tomato leaves and roots under non-stress and salt stress environments (Fig. 4A). Under salt stress conditions, the SA contents in the leaves and roots of the Se-treated tomatoes were 80.7 and 35.2% higher, respectively, than tomatoes without Se. The ABA content of leaves did not differ significantly between treatment groups, but it was lower in Se-treated tomato roots than control plants (Fig. 4B). Se addition did not significantly affect the MT contents in the tomato tissues under non-stress or stress conditions (Fig. 4C). Both under non-stress and stress conditions, Se application did not change the JA content in tomato leaves. However, the root JA content was decreased by Se treatment under non-stress and stress conditions (Fig. 4D).

Expression of genes associated with SA biosynthesis and transformation

The qRT-PCR was performed to analyze the transcripts of genes involved in SA biosynthesis and

Fig. 2 Influence of exogenous Se on lipid peroxidation and osmoprotectants content of tomato seedlings under salt stress treatment with/without Se supplementation for 15 d. (**A**) The malondialdehyde (MDA) content. (**B**) The soluble sugar content. (**C**) The proline content. Data are presented as mean ± SD of three biological replicates, each of which contains three seedlings. Different small letters indicate significant differences between treatment groups at $P < 0.05$
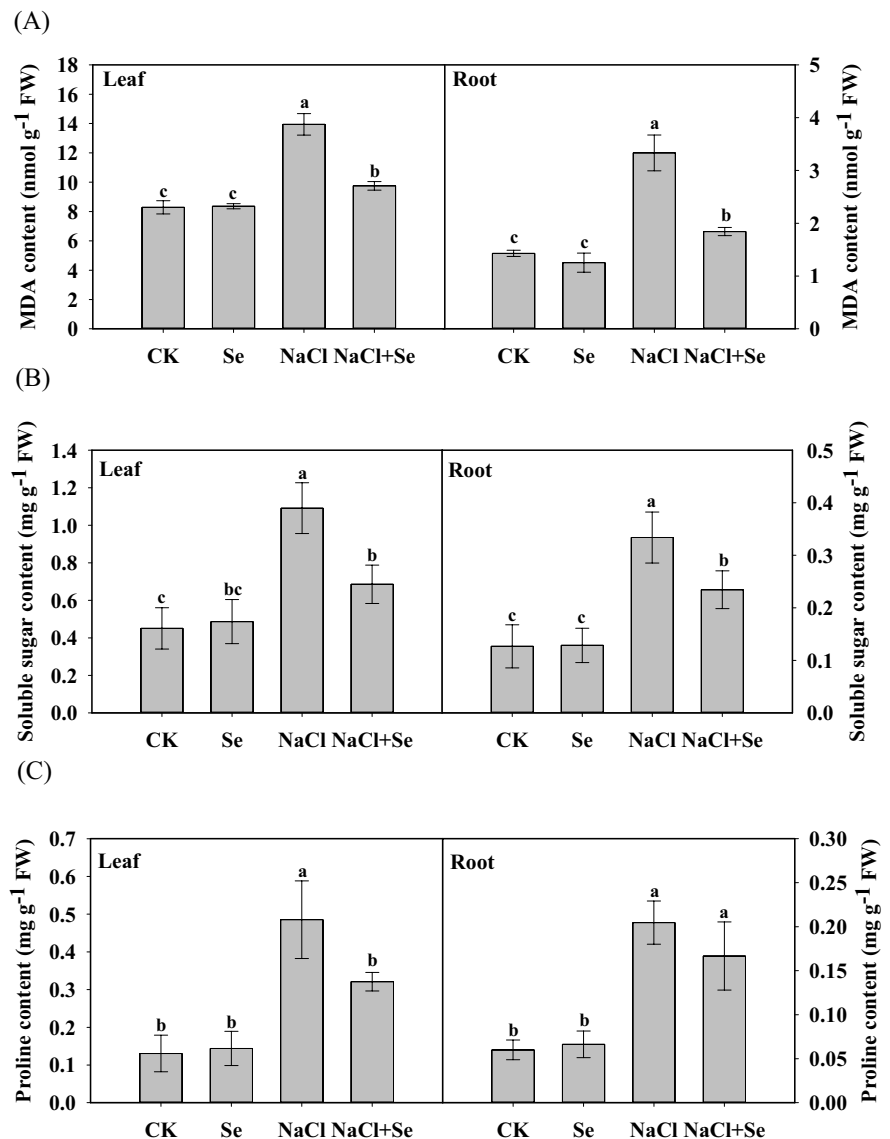
transformation (Fig. 4E). The transcripts of phenylalanine ammonia-lyase 1 (*PAL1*) were upregulated in tomato leaves in response to Se application under normal and salt stress conditions for 5 days. However, in tomato roots, the addition of Se induced the expression of *PAL1* under salt stress conditions but did not change its expression under normal conditions. Under normal condition, Se application did not change the transcripts of isochorismate synthase (*ICS*) in tomato leaves, but it downregulated its expression in the roots. In addition, Se treatment increased *ICS* gene

expression in tomato leaves in response to salt stress (Fig. 4E). Se treatment did not affect salicylate carboxymethyltransferase (*SAMT*) gene expression in tomato leaves under normal and salt stress conditions. However, Se treatment upregulated *SAMT* expression in tomato roots under normal conditions but not under salt stress (Fig. 4E). Finally, Se treatment downregulated salicylic acid-binding protein 2 (*SABP2*) gene expression in different tomato tissues under normal conditions, but it had no apparent impact under salt stress (Fig. 4E).

(A)

(B)
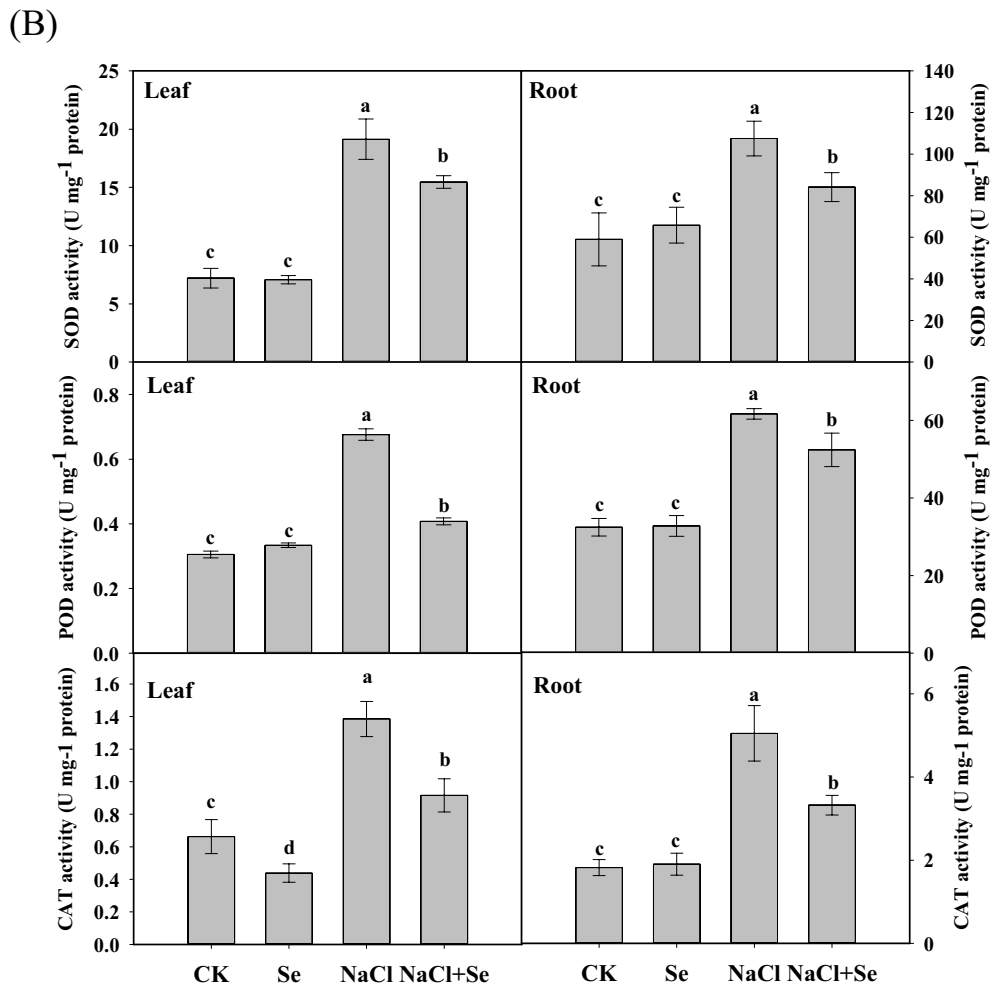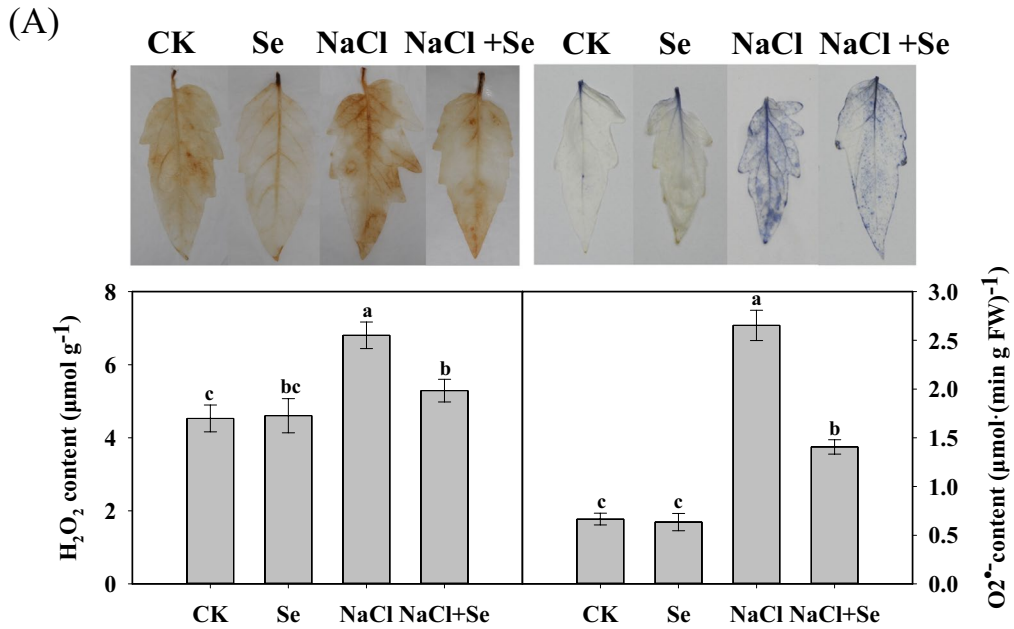
Fig. 3 Effect of exogenous Se on ROS accumulation and activities of antioxidant enzymes in tomato seedlings under salt stress for 15 d. (**A**) H_2O_2 and $O_2^{\bullet-}$ observation in tomato leaves (upper) and H_2O_2 and $O_2^{\bullet-}$ contents in tomato leaves (lower). (**B**) The activity of SOD, POD and CAT in tomato leaves and roots. Data are presented as mean ± SD of three biological replicates. Different small letters indicate significant differences between treatment groups at $P < 0.05$

Roles of SA in Se-induced salt stress tolerance in tomato seedlings

As shown in Fig. 5A, under normal conditions, pre-treatment with 100 µM ABT (a SA biosynthesis inhibitor) reduced the plant height but had no apparent effect on the stem diameter, fresh weight, or dry weight of tomato seedlings, indicating that ABT had no apparent inhibitory effect on plant growth under non-stress conditions. In contrast, under salt stress, ABT treatment decreased the plant height and biomass of tomato plants compared to the plants grown without ABT. Se treatment improved the plant height, stem diameter, and biomass of tomato seedlings impacted by salt stress (Fig. 5A); however, pre-spraying with ABT weakened these beneficial effects of Se (Fig. 5B). In addition, we detected the SA content of tomato leaves in each treatment group. Figure 5C showed that ABT treatment did significantly reduce the SA content under normal and salt stress treatments. Se treatment significantly increased the SA content under salt stress, however, the induction effect of Se on SA content was inhibited by ABT treatment (Fig. 5C).

Then the effects of ABT on plant photosynthesis after Se application in response to salt stress were studied (Table 4). Pre-treatment with ABT reduced the Tr and Gs of plants under normal conditions but inhibited the *Fv/Fm* of plants under salt stress. Pn was significantly reduced under salt stress, which was significantly improved by Se treatment (Table 4). Meanwhile, ABT-pre-treated plants had 53% lower Pn compared to those treated only with Se under salt stress. ABT also reduced the promoting effects of Se on Tr, Gs, and WUE under salt stress, although these findings were not statistically significant when comparing them within groups (Table 4). Under salt stress, ABT pre-treatment raised the REC by 52.3% and reduced the RWC by 12.7% compared to plants with Se treatment alone (Fig. 5B). Finally, histochemical staining of H_2O_2 and $O_2^{\bullet-}$ in plant leaves revealed that ABT pre-treatment abolished the Se-mediated decrease in H_2O_2 and $O2^{\bullet-}$ accumulation under salt stress (Fig. 5D).

Discussions

Exogenous Se promoted plant photosynthesis and growth under salt stress

Excess salinity stress leads to osmotic stress and ion toxicity, which increases the Na^+ content in plant tissue, aggravates the cell membrane peroxidation, causes oxidant damage, and suppresses plant photosynthesis, thus leading to nutrient deficiency, retarded plant growth, and reduced biomass (Gupta and Huang 2014). In our study, we analyzed the impact of Se application in enhancing tomato plant growth through its involvement in the regulation of key physiological and biochemical parameters (Table 1). Under normal conditions, exogenous Se application raised the plant height of tomato seedlings, since Se had been found to play important roles in the production and signaling of some phytohormones involved in plant internode elongation, such as cytokinin and gibberellin (Jiang et al. 2019; Shan et al. 2021). Moreover, Se supplementation boosted plant development under salt stress, as evidenced by an increase in plant height, stem diameter, and biomass. Se also relieved the wilting state of tomato plants caused by salt stress (Table 1).

Studies have shown that Se could improve growth and development in many plants exposed to various abiotic stresses, partly attributed to the promotion of plants' photosynthetic efficiency (Diao et al. 2014; Fan et al. 2022), which was also supported by our results. The reduction in photosynthesis under salinity was probably due to the lower Gs and Pn rates caused by salt stress (Jiang et al. 2017). In this study, adding Se increased plant Pn and Gs under salt stress, protecting the photochemical efficiency and increasing Tr, and further enhanced photosynthesis and promoted plant growth compared to tomato seedlings without Se (Fig. 1, Table 1).

Under saline conditions, Gs rapidly decreases to protect the water supply (Lotfi et al. 2020). However, exogenous Se increased the plants' Gs under salt stress. This could be related to the high SA content induced by Se supplementation (Fig. 4), since SA reportedly increased the Gs of several plant
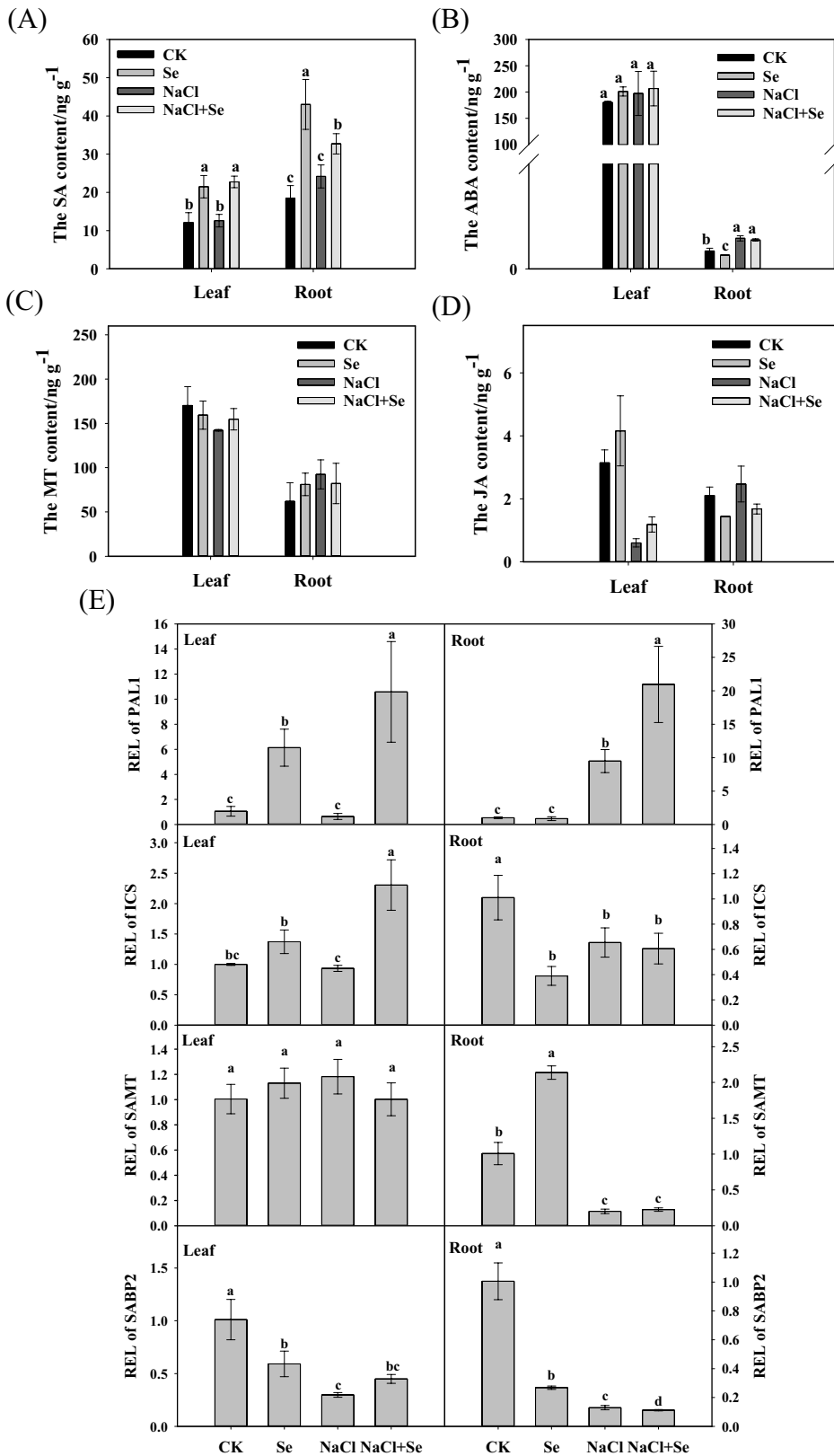
◄**Fig. 4** Influence of exogenous Se on phytohormone contents and the expression of some genes involved in salicylic acid (SA) biosynthesis in tomato tissues under salt stress for 5 d. (**A**) SA concentration. (**B**) Abscisic acid (ABA) concentration. (**C**) Melatonin (MT) concentration. (**D**) Jasmonic acid (JA) concentration. (**E**) The relative expression level (REL) of several genes involved in biosynthesis and transformation. Data are presented as mean $\pm$ SD of three biological replicates, each of which contains three seedlings. Different small letters denote significant differences between various treatments at $P < 0.05$

species in response to various abiotic stresses (Stevens et al. 2006; Khan et al. 2014; Ma et al. 2017; Lotfi et al. 2020; Khalvandi et al. 2021). In addition, K^+ deficiency may induce stomatal closure, so the increased K^+ concentration (Table 3) under Se treatment might also have contributed to the increase in Gs under salt stress with Se supplementation (Zhang et al. 2020). Interestingly, Benlloch-Gonzalez et al. (2010) found that K^+ accumulation in leaves after SA treatment was due to the inhibition of ethylene formation, which regulated Gs. Therefore, we expect an interplay among SA, ethylene, and K^+ in regulating stomatal movement and photosynthesis. Restoration of the photosynthetic capacity in salt-treated tomato plants by Se application may also be related to the increased chlorophyll and carotenoid contents under salt stress (Jiang et al. 2017).

Exogenous Se impacted osmotic regulation in tomato plants under salt stress

Plants produce and regulate a variety of organic (soluble sugars, soluble proteins, proline, etc.) and inorganic (Na^+, Cl^-, K^+, Ca^{2+}, etc.) osmolytes or osmoprotectants under salt stress, which have biological functions in the plant response to salt stress (Riffat and Ahmad 2018). Na^+ and Cl^- are the most toxic ions in plants produced under salt stress, and their excessive accumulation in plants eventually leads to arrested plant growth (Khare et al. 2015). Salt stress often leads to excessive Na^+ and Cl^- and increased K^+ leakage from cells due to membrane damage, resulting in increased Na^+ and Cl^-, and decreased K^+, contents in plant tissues, and a decreased K^+/Na^+ ratio (Gupta and Huang 2014). In this study, Se application increased the K^+ content and reduced the Na^+ and Cl^- contents in shoots compared to tomato plants without Se treatment under salt stress, which was consistent with the findings of Jiang et al. (2017) in maize. Se application maintained K^+ and Na^+ homeostasis under salt stress, which is essential for cell osmoregulation and to reduce ion toxicity caused by salt stress. Se application increased intracellular K^+ levels, which are essential to maintain the membrane potential, osmoticum production, and the action of numerous cytosolic enzymes (Zhu 2003). Moreover, Se significantly reduced the Na^+ content in tomato shoots under salt stress but did not change its content in roots (Table 3), indicating that Se application might reduce the transfer of Na^+ from tomato roots to shoots. It was reported by Nemat Alla et al. (2020) that exogenous Se could up-regulate the expression of *SOS1* and *NHX1* genes under salt stress, while inhibiting the accumulation of Na^+. These results indicated that Se application could improve the expression of H^+-ATPase and Na^+/H^+ antitransporter in the vacuolar membrane of root cells under salt stress, thereby limiting the distribution of Na^+ in the upper tissue and alleviating its toxicity.

Soluble sugars (sucrose, glucose and fructose), a kind of important osmoprotectants, play crucial roles in regulating osmotic adjustment, maintaining the water balance of plants, scavenging excess ROS, and protecting cell membranes under various adverse environmental stresses (Dien et al. 2019). In addition, soluble sugars act as a kind of chelating agent to entrap Na^+ within starch granules, helping to neutralize Na^+ toxicity in plants under salt stress (Kanai et al. 2007). Proline is a vital amino acid that benefits plants in response to salinity (El Moukhtari et al. 2020). It not only acts as an osmoprotectant to regulate the water balance of plant cells, but also plays important roles in ROS scavenging and membranes stabilization to prevent electrolyte leakage (Shamsul et al. 2012; Naliwajski and Skłodowska 2021). Several studies reported that the soluble sugar and proline contents were enriched when plants were subjected to salt stress, potentially contributing to plants' osmotic and salt stress tolerance (Boriboonkaset et al. 2013; El-Katony et al. 2021). However, other studies reported opposite or inconsistent results. For example, Watanabe et al. (2000) reported that the total soluble sugar content increased in young *Populus euphratica* leaves but decreased in the mature leaves after salt stress treatment. Xu et al. (2015) reported that the soluble sugar content in stress-resistant rice decreased in response to drought stress. Naliwajski
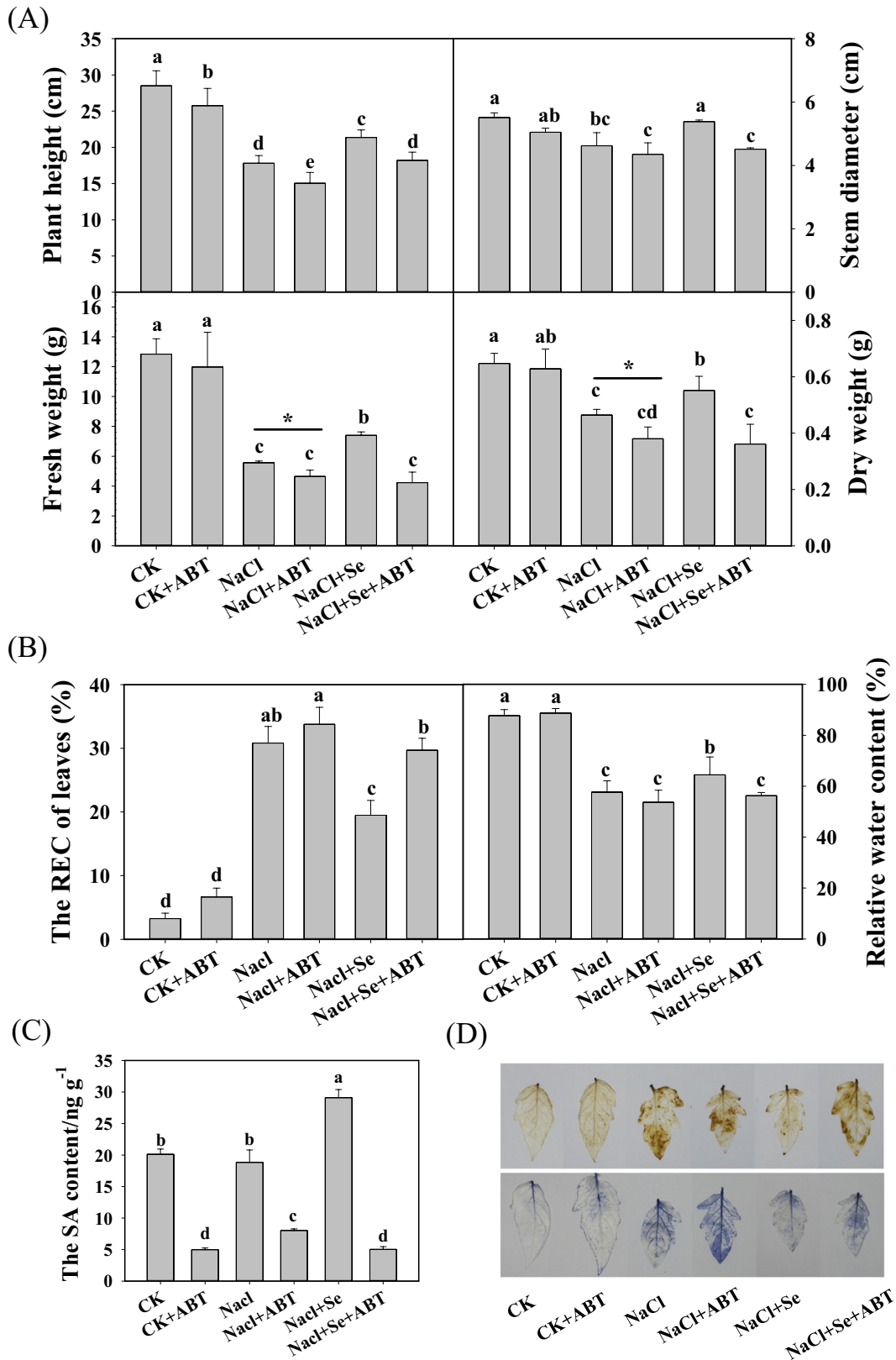
(A)

(B)

(C)

(D)

and Skłodowska (2021) reported that acclimated cucumber plants accumulated lower proline levels under salt stress than non-acclimated plants under normal and stress conditions. Therefore, changes in plants' soluble sugar and proline contents in response to salt stress vary among plant species, varieties, and growth stages (Xu et al. 2015; Naliwajski and Skłodowska 2021). Furthermore, in our study, the content of soluble sugars and proline of Se-treated tomato seedlings under salt treatment for 15 d was lower compared to the tomato plants without Se supplementation (Fig. 2B, C), which suggested that the duration of Se application in plants under salt stress might also be one of the factors affecting the accumulation of soluble sugars and proline, since the toxicity and damage to plants caused by salt stress were basically mitigated by Se addition for 15 d (Fig. 1A) and it was not necessary for plants to produce continual high level of osmotic protective substance, which indirectly indicated the promoting effect of Se on plant salt stress tolerance.

Exogenous Se alleviated the oxidative damage caused by salt stress

Salt stress is often accompanied with the secondary oxidative damage for plants, since high salinity leads to the accumulation of excessive ROS, such as H2O2, O^{2-}, and $O^{\bullet-}$, which potentially causes cell membrane lipid peroxidation and even cell death (Zhang et al. 2016). Moreover, MDA levels and relative electrolyte leakage in plants can represent the integrity of their cellular membranes in response to salt stress (Gou et al. 2020b). Plants have evolved an efficient antioxidant system, including enzymes such as SOD, POD and CAT

to scavenge ROS to avoid oxidative damage caused by various environmental stresses (Gill and Tuteja 2010). It was reported that Se treatment could regulate the antioxidant defense system by up-regulating the activities of several antioxidant enzymes, reflected by decreased H_2O_2 and MDA contents in the cells of different plant species under various environmental stresses (Kaur and Nayyar 2015; Ashraf et al. 2018). In this study, Se treatment mitigated the oxidative damage caused by salt stress (Fig. 3A), whereas the activity of various antioxidant enzymes was lower in Se-treated tomato seedlings under salt stress than in tomato plants in the absence of Se addition (Fig. 3B). This was probably due a decrease of the oxidative damage induced by the salt stress treatment following Se supplementation for 15 days, and the reduced need for antioxidant enzymes to eliminate ROS. Furthermore, Se is an integral component of enzymes like glutathione peroxidase and other seleno-compounds, which have vital roles in reducing cell peroxidation and oxidative damage (Puccinelli et al., 2017), suggesting that Se might regulate other antioxidant mechanisms under salt stress.

SA was involved in Se-induced salt tolerance in tomato plants

Previous studies reported that SA could regulate various plant metabolic processes, and hence regulate the plant stress resistance, including regulating the stomatal and water potential, enhancing plant nutrient uptake and photosynthesis, and promoting plant growth and development (Khan et al. 2015). *PAL* and *ICS* encode phenylalanine ammonia-lyase and isochorismate synthase respectively, which are the two key enzymes of the two SA biosynthesis pathways (Ding and Ding 2020). It has been observed that the transcripts of *PAL1* were remarkably upregulated by Se treatment in tomato leaves and roots under salt stress whereas the ICS expression was only induced by Se addition in tomato leaves (Fig. 4E). Moreover, the SA content was obviously induced by Se application in different tomato tissues under salt stress conditions (Fig. 4A). These results suggest that the accumulation of SA in tomato leaves under salt stress is probably attributed to the upregulation of both PAL and ICS pathways following Se application, while its accumulation in roots might only be related to the upregulation of PAL pathway. As described by Park et al. (2007), SAMT1 catalyzes SA to become methylated

SA (MeSA), which is hydrolysed by *SABP2* and re-converted into SA again. Under salt stress conditions, the expression of *SAMT1* and *SABP2* was not changed by exogenous Se treatment, indicating Se did not regulate the mutual transformation between SA and MeSA in response to salt stress (Fig. 4E). Moreover, Se treatment increased the SA content but decreased the JA content in tomato roots under control and salt stress conditions, which was probably because SA was an important antagonist of the JA response (Koornneef et al. 2008). SA can suppress JA signaling through the downregulation of JA-responsive gene expression in various plant species (Van der Does et al. 2013; Wang et al. 2022). Previous studies have revealed the characteristics of the trade-off between JA and SA signaling (Leon-Reyes et al. 2010).

ABT inhibits the SA synthesis by inhibiting the activity of benzoic acid 2-hydroxylase (BA2H), a key enzyme in the formation of SA via the PAL pathway (Leon et al. 1995). Under non-stress conditions, ABT did not affect plant growth, but it had an apparent inhibitory effect on plant growth under salt stress (Fig. 5A), confirming the critical role of SA in plant salt stress tolerance (Wassie et al. 2020; Ahmed et al. 2020). Interestingly, being pre-sprayed with ABT reduced the stimulating effect of Se on plant growth and photosynthesis, increased the REC, worsened the damage caused by oxidative stress, and eventually abolished the beneficial effect of Se application in plant salt resistance (Fig. 5, Table 4). Meanwhile, we detected the content of SA of tomato plants in each treatment groups, which was consistent with the resistance of different tomato plants to salt stress (Fig. 5C). These results further proved the conclusion that Se application played an important role in enhancing salt stress resistance of plants, which was partly due to the induction of SA content by Se addition. Wang et al. (2018) reported that exogenous treatment with SeO_3^{2-} induced a constitutively higher expression of genes involved in SA synthesis in the Se hyperaccumulator *Stanleya pinnata* compared to its related non-hyperaccumulator, *Stanleya elata*. Furthermore, SA played a central role in the high Se tolerance in *S. pinnata* (Freeman et al. 2010). These results suggest complex interactions between Se and SA in plants, which might depend on the concentration and duration of Se treatment, as well as the plant species and different ability of Se accumulation.

Table 4 Influence of a SA inhibitor (1-aminobenzotriazole, ABT) on the photosynthetic characteristics in tomato plants under salt stress

Treatment	CK	CK + ABT	NaCl	NaCl + ABT	NaCl + Se	NaCl + Se + ABT
Fv/Fm	0.795 ± 0.003^a	0.786 ± 0.01^{ab}	0.752 ± 0.028^c	0.712 ± 0.02^d	0.783 ± 0.012^{ab}	0.762 ± 0.028^{bc}
Pn ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	2.73 ± 0.57^a	2.38 ± 0.26^{ab}	1.08 ± 0.38^c	0.83 ± 0.03^c	1.85 ± 0.24^b	0.87 ± 0.04^c
Tr ($mmol\ H_2O\ m^{-2}\ s^{-1}$)	2.18 ± 0.32^a	1.33 ± 0.28^b	0.18 ± 0.01^c	0.17 ± 0.04^c	0.27 ± 0.05^c	0.15 ± 0.02^c
Gs ($mmol\ H_2O\ m^{-2}\ s^{-1}$)	133.12 ± 23.22^a	75.77 ± 9.81^b	18.30 ± 7.61^c	9.26 ± 2.67^c	22.16 ± 4.81^c	4.51 ± 1.80^c
Ci ($\mu mol\ CO_2\ mol^{-1}\ air$)	345.86 ± 6.73^a	311.56 ± 19.55^a	192.14 ± 69.45^{bc}	155.35 ± 24.07^c	230.26 ± 23.40^b	134.31 ± 32.40^c
WUE ($mmol\ CO_2\ mol^{-1}\ H_2O$)	1.24 ± 0.12^b	1.86 ± 0.53^a	5.84 ± 1.82^a	5.20 ± 1.63^a	7.02 ± 1.35^a	5.75 ± 1.05^a

Different lowercase letters denote significant differences among different treatments at $P < 0.05$

Conclusions

In conclusion, this study demonstrated that low level of exogenous Se (25 μM) generally stimulated plant growth and enhanced the salt stress tolerance of tomato seedlings, which might be due to several mechanisms: (a) exogenous Se application increased the accumulation of photosynthetic pigments, improved the Pn, thereby enhanced the photosynthesis and plants growth under salt stress; (b) Se application maintained K^+ and Na^+ homeostasis under salt stress, which was essential for cellular osmoregulation and the reduction of ion toxicity caused by salt stress; (c) Se treatment also reduced the REC and decreased the H_2O_2, $O_2^{\bullet-}$, and MDA contents, and mitigated the oxidative damage caused by salt stress. (d) Exogenous Se increased SA content in tomato leaves and roots via up-regulating the PAL pathway of SA biosynthesis, which played vital roles in plant salt stress resistance. Our results clarify the roles and regulatory mechanisms of Se in plant salt stress tolerance and highlight the importance of SA in this process. These findings help support the application of Se in agricultural production to enhance crop growth and yield under salt stress.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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