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Overexpression of Caffeic Acid *O*-Methyltransferase 1 (*COMT1*) Increases Melatonin Level and Salt Stress Tolerance in Tomato Plant

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Abstract

Melatonin is a natural phytohormone that occurs in most plants. It can regulate not only plant growth and development but also alleviate biotic and abiotic stress in plants. However, no studies have reported on endogenous melatonin increasing tomato tolerance to salt stress. In the present study, we observed that the overexpression of caffeic acid *O*-methyltransferase 1 (*SlCOMT1*) increased melatonin levels in tomato plants. We also observed that transgenic plants exhibited higher salt stress tolerance. *SlCOMT1* overexpression could maintain the balance of Na^+/K^+ and decrease ion damage by activating salt overly sensitive (SOS) pathway under salt treatment. In addition, *SlCOMT1* overexpression significantly enhanced the antioxidant capability, with higher antioxidant enzyme activity observed, including superoxide dismutase, peroxide and catalase activity, and higher ascorbic acid (AsA) and glutamate (GSH) accumulation levels. *SlCOMT1* overexpression also maintained good nutrient homeostasis in the tomato plants. In addition, *SlCOMT1* overexpression upregulated some stress-related genes (*AREB1*, *AIM1*, *MAPK1*, *WRKY33* and *CDPK1*), which resulted in the activation of downstream signaling pathways and could be partly responsible for the improvement in salt stress tolerance.

Keywords Tomato · Salt stress · *SlCOMT1* · Melatonin · Antioxidant capacity · Ion homeostasis

Introduction

Tomato is a cash vegetable crop cultivated globally. However, in the course of tomato cultivation, it is often affected negatively by environmental stress, including high and low temperatures, salinity, and drought. Among the above stress factors, the injury caused by salt stress is particularly severe in arid and semi-arid areas, where about 6% of the world's land areas experience salt stress (Hakeem et al. 2013). Under high-salinity conditions, plants absorb excessive salt ions and ion toxicity and osmotic stress cause damage, which results in the inhibition of plant growth and reduced plant yield (Kong et al. 2017; Moles et al. 2016). The accumulation of high concentrations salt ions would also lead to oxidative stress and nutrient disorders (Shalata and Tal 2010; Hussain et al. 2018; Farooq et al. 2018; Gong et al. 2014). Therefore, it is essential to find a strategy for mitigating the damage caused by salt stress.

With regard to the ionic aspect of salt stress, the SOS pathway is the key pathway for regulating ion homeostasis, particularly the maintenance of Na^+ balance. Excessive Na^+ from external sources could activate SOS1, a Na^+/H^+ antiporter, which would cause the subsequent extrusion of excessive Na^+ from the cytosol. The role of SOS1 in maintaining low concentrations of Na^+ in cells has been demonstrated through a combination of biochemical, genetic, and physiological analyses (Qiu et al. 2002; Shi et al. 2002). Vacuolar Na^+/K^+ exchanger (NHX) pumps excessive Na^+ efficiently into vacuoles (Shi et al. 2002). The high-affinity K^+ transporter (HKT1) controls Na^+ translocation from the root to the stem. Numerous studies have demonstrated that they are all induced by salinity (Vadim 2015; Hazzouri et al. 2018). In addition to ionic toxicity, salt stress causes the accumulation of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) in plants (Chawla et al. 2011),

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which destroy nucleic acids and oxidizes protein, cause lipid peroxidation (Gill and Tuteja 2010). Antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), catalases (CAT), and their antioxidants could effectively eliminate the ROS produced by stress (Bowler 1992; Anandan and Arunachalam 2012; Noctor et al. 1998). In addition, antioxidants such as AsA and GSH can eliminate excess ROS. Liu et al. (2015) observed that exogenous melatonin could increase the concentration of AsA and GSH and alleviate the oxidative stress caused by sodic alkaline.

Melatonin (*N*-acetyl-5-methoxytryptamine) is a hormone found widely in plants (Tan et al. 2010). In higher plants, exogenous melatonin not only regulates plant growth and development, such as seed germination (Zhang et al. 2014), lateral root formation (Wen et al. 2016), delayed flowering (Kolář et al. 2010), and yield promotion (Liu et al. 2016), but could also relieve biotic and abiotic stress induced under extreme temperatures (Bajwa et al. 2014; Gong et al. 2017), drought (Antoniou et al. 2017; Wang et al. 2017), salinity (Li et al. 2012), ultraviolet radiation (Ghobadi et al. 2017), and pathogens (Mandal et al. 2018). *N*-acetylserotonin methyltransferase (ASMT) is the last enzyme in melatonin synthesis and it is considered the rate-limiting step in the melatonin synthesis process (Byeon et al. 2014a; Park et al. 2013). However, only a few monocotyledonous plants have the homologous *ASMT*; therefore, there must be other enzymes exhibiting functions similar to those of *ASMT* and regulating melatonin synthesis. It has been reported that *Arabidopsis thaliana* caffeic acid *O*-methyltransferase (AtCOMT) could be a substitute of the ASMT enzyme in the transformation of *N*-acetylserotonin into melatonin (Byeon et al. 2014b; Lee et al. 2014). Byeon et al. (2014b) observed that the catalytic activity of COMT was more than 700-fold that of ASMT during melatonin synthesis in rice, and another study demonstrated that the overexpression of *COMT* could increase the concentration of melatonin in rice (Choi et al. 2017), while silencing *COMT* decreased the melatonin accumulated in tomato (Ahammed et al. 2019; Cai et al. 2017). All the findings above indicate that *COMT* is critical for melatonin synthesis.

Based on the results of previous studies above, we speculated that the overexpression of *COMT* could play a vital role in tomato tolerance to stress. Therefore, in the present study, *SlCOMT1*-overexpressing tomato plants were generated and transgenic lines used to demonstrate the relationships among *SlCOMT1* expression, melatonin accumulation, and salt tolerance in tomato plants. The results could enhance our understanding of *SlCOMT1* function in theory and facilitate practical applications to improve tomato tolerance to salt stress.

Materials and Methods

Plant Materials and Growth Condition

The phylogenetic tree was reconstructed using MEGA 6.0 (MEGA Inc., Englewood, NJ). The full-length *SlCOMT1* sequence was amplified using PCR with the forward primer 5′–3′: TCTAGACAATTTCAAAAAGATGGGTTC and the reverse primer 5′–3′: GAGCTCGCCAATCCACTCAGA CAAAG. The DNA fragment of the *SlCOMT1* was inserted into the vector pROKII, which contains the *CaMV*35S promoter. The reconstructed construct was transformed into *Agrobacterium tumefaciens* strain LBA4404 using the electroporation method. Six *SlCOMT1* overexpressing lines were obtained (OE1–OE6) from the cotyledons of tomatoes that self-pollinated homozygous lines (wild type, WT) for tissue culture. The seeds of T1 generations were obtained from T0, and T2 generation seeds were collected from the T1 generation.

After germination, the tomato (*Solanum lycopersicum* L.) seedlings were transferred to a growth tray filled with vermiculite. Fifteen-day-old seedlings were transplanted into 3 L black plastic containers to be grown hydroponically using Hoagland nutrient solution. The control (NaCl not added) and the salt treatment (100 mmol L^{-1} of NaCl added) were set up. After 7 days of salt treatment, phenotype photographs and samples were obtained. Samples were stored at − 80 °C for further analyses. In addition, the roots of the WT were harvested at 0, 6, 12, 24, 48, 72 h and the leaves were harvested at 0, 12, 24, 36, 48, 72, 96 h after treatment with 150 mmol L^{-1} NaCl for *SlCOMT1* expression measurements. Moreover, at the third day after treatment, the leaves of the WT and the transgenic plants were obtained for stress-related gene expression measurements. Plants were cultured in a glass greenhouse at 25–30 °C during the day, 15–20 °C at night, and with a 12/14 h (day/night) photoperiod.

Determination of Plant Growth Index

After 7 days of salt treatment, the tomato seedlings were washed with distilled water, the surfaces dried using absorbent paper, and the fresh weights measured. Plant height was the distance from the bottom of the stem to the apical meristem. Plant stem diameter was measured at the base of the stem. They were measured using a tape and Vernier caliper, respectively.

Quantitative RT-PCR

Leaf total RNA of the tomatoes was extracted using TRIzol (Invitrogen) according to Chang et al. (1993) and cDNA

synthesis was performed using the RevertAid First Strand cDNA Synthesis Kit (Vazyme: R233-01, Nanjing, China). All the primers used in the present study are listed in Gong et al. (2015) and Cai et al. (2017). A Power SYBR Green PCR Master Mix (TransGen Biotech, Beijing, China) was used for detection on an ABI Prism 7900 HT device (Applied Biosystems, Foster City, CA, USA). The relative expression levels of the genes were calculated using the $2^{-\Delta\Delta C}{}_{T}$ method (Livak and Schmittgen 2001).

Quantification of Endogenous Melatonin

The extraction and determination of melatonin concentrations in tomato leaves were carried out according to Byeon and Back (2014). Briefly, tomato leaves (0.2 g) were ground into powder with appropriate amounts of liquid nitrogen, and then 1 mL of methanol was added and the solutions transferred to centrifuge tubes and stored overnight at 4 °C. The supernatant were transferred into new tubes after centrifugation at 10,000×*g* for 10 min. After the supernatant were evaporated, the residues were dissolved in 0.5 mL methanol and a needle filter (0.22 μm) used to filter the solution. The melatonin concentrations were determined using high-performance liquid chromatography using a fluorescence detector system (Rigol L3000) and calculated based on the peak areas. The samples were separated on a Kromasil C18 column (250 mm × 4.6 mm, 5 μmmol L^{-1}, Eka Nobel, Bohus, Sweden). Methanol and ultra-pure water were mixed into a mobile phase at a ratio of 40:60. The injection volume was 10 μL, the flow rate was 0.8 mL min^{-1}, the column temperature was 30 °C, the fluorescence detection wavelength was 280 nm, and the emission wavelength was 340 nm. The concentrations of melatonin in the samples were calculated according to the standard curve generated using the known concentrations of melatonin and peak areas.

Determination of Photosynthesis and Chlorophyll Concentrations

The third true leaves of tomato plant were selected for use in the measurement of photosynthetic parameters and chlorophyll concentrations. The net photosynthetic rate (Pn) was determined using an open-flow gas-exchange system (LI-6400, LI-COR Biosciences, Lincoln, NE, USA), and the environmental conditions were as follows: the CO_2 concentration was 380 μmol mol^{-1} and the photo flux density was 850 μmol m^{-2} s^{-1}. The chlorophyll fluorescence parameters were analyzed using an FMS-2 pulse-modulated fluorometer (Hansatech Instruments Ltd., UK). The PSII maximal photochemical efficiency (Fv/Fm) was measured form the third true leaves under a light intensity of 600 μmol m^{-2} s^{-1} and the PSII actual photochemical efficiency (ΦPSII) was measured after 30 min in the dark (Maxwell and Johnson 2000).

Chlorophyll a, b, and carotenoid concentrations were analyzed according to the method of Inskeep and Bloom (1985). Briefly, fresh leaves (0.2 g) were weighted accurately and extracted using 25 mL 80% acetone (v/v) for 36 h in the dark (temperature approximately 25 °C). After centrifuging at 4000×*g* for 10 min, the supernatant was used to determine the chlorophyll concentrations at 663.2 nm, 646.8 nm, and 470 nm. The method of determining the chlorophyll a, chlorophyll b, and total carotenoid concentrations was according to Lichtenthaler (1987) and the following formulas:

$$C_a = 12.25A_{663.2} - 2.79A_{646.8}$$

$$C_b = 21.50A_{646.8} - 5.10A_{663.2}$$

$$C_{x+c} = \left(1000A_{470} - 1.82C_a - 85.02C_b\right)/198,$$

C_a, C_b and C_{x+c} represent chlorophyll a, chlorophyll b and carotenoids, respectively.

Element Determination

The WT and *SlCOMT1* overexpression plants were divided into roots, stems, and leaves, which were washed with deionized water and dried at 75 °C to constant weight after fixation at 105 °C for 20 min in the oven. Dry samples (0.2 g) of the roots, stems, or leaves were digested in a H_2SO_4–H_2O_2 solution, and then the extracts were used to determine the nitrogen (N), phosphorus (P), potassium (K) and sodium (Na) concentrations in the tissues. N and P concentrations were determined using the Kjeldahl method and the vanadomolybdate colorimetric procedure, respectively (Wang et al. 2010). Na^+ and K^+ concentrations were determined based on spectra released following light energy absorption by the electronic states of respective ions in a flame photometer (TAS-990, Purkinje General, China).

ROS Accumulation and MDA Concentration Analysis

The $O_2^{\cdot-}$ production rate was measured using the hydroxylamine oxidation method (Ksk and Naithani 2010). Tomato leaves (1 g) were ground with 3 mL of 65 mmol L^{-1} phosphate buffer solution (PBS, pH 8). The homogenates were centrifuged for 15 min at 10,000×*g*. The supernatant was fixed to 3 mL and used for the determination of the $O_2^{\cdot-}$ production rate. PBS (0.5 mL, 65 mmol L^{-1}, pH 8) was mixed with 0.1 mL 10 mmol L^{-1} hydroxylamine hydrochloride and incubated at 25 °C for 10 min, and then 0.5 mL of the supernatant was added the mixture incubated. After 20 min, 58 mmol L^{-1} sulfonamide and 7 mmol L^{-1} alpha naphthalene were added and the solution was incubated for an additional 20 min. Finally, an equal volume of trichloromethane was added. The sample

was centrifuged at 10,000×*g* for 3 min, and the absorbance of the supernatant measured at 530 nm. The H_2O_2 concentration was measured according to Patterson et al. (1984), and the absorbance of the titanium peroxide complex was measured at 415 nm.

The malondialdehyde (MDA) concentration was measured according to Wang et al. (2010). Leaves (0.5 g) were ground in an ice-cold mortar with 4 mL of ice-chilled PBS (50 mmol L^{-1}, pH 7.8) and then the homogenate centrifuged at 12,000×*g* (4 °C) for 20 min and 2 mL thiobarbituric acid added to 1 mL of the supernatant. The mixture was incubated in a boiling water bath for 15 min and then quickly placed in an ice-bath to terminate the reaction. The cooled mixture was centrifuged at 4000×*g* for 20 min, and the absorbance of the supernatant was measured at 600 nm, 532 nm and 450 nm, respectively. The MDA concentrations were calculated using the following equations:

$$C_{MDA}\left(\mu mol\, L^{-1}\right) = 6.45 * \left(A_{532} - A_{600}\right) - 0.56A_{450},$$

$$MDA\left(\mu mol\, g^{-1}\, FW\right) = C * V/W = 0.1548 * \left(A_{532} - A_{600}\right) - 0.01344A_{450}.$$

In the above formula, *V* represents the volume of the supernatant, and *W* represents the weight of the sample.

Antioxidant Enzyme Activity Determination

The method used to extract the supernatant for enzyme activity measurement was according to Mishra et al. (2006). Leaf samples (0.5 g) were ground with 4 ml 50 mmol L^{-1} PBS (pH 7.8) in an ice-bath. After centrifugation of the homogenates at 12,000×*g* for 20 min at 4 °C, the supernatants were transferred into new centrifuge tubes for enzyme activity determination. The nitro-blue tetrazolium (NBT) method was used to determine SOD activity (Stewart and Bewley 1980). POD activity was measured spectrophotometrically at 470 nm using the guaiacol oxidation method (Shah and Nahakpam 2012). CAT activity was analyzed based on the rate of conversion of H_2O_2 to H_2O and absorbance at 240 nm (Patra et al. 1978).

Glutathione (GR) activity was measured according to Foyer and Halliwell (1976). The enzyme activity was dependent on the rate of decline in nicotinamide adenine dinucleotide phosphate (NADPH) and absorbance was measured at 340 nm. Dehydroascorbate (DHAR) activity was determined by measuring the rate of increase in AsA at 265 nm (Nakano and Asa 1981). Monodehydroascorbate reductase (MDHAR) activity was measured based on the rate of NADH oxidation at 340 nm (Sánchez-Rodríguez et al. 2010).

Antioxidant Metabolite Concentration Determination

The AsA and dehydrogenated ascorbic acid (DHA) concentrations were analyzed according to Arakawa et al. (1981). Leaf samples (0.5 g) were ground with 4 mL of 5% (v/v) m-phosphoric acid and then centrifuged at 12,000×*g* for 15 min. The supernatant was used to determine the concentrations of AsA and DHA. Total AsA was determined by mixing 100 µL of the supernatant, 3 ml PBS (pH 6.8, 100 mmol L^{-1}) and ascorbate oxidase (AAO) (1.25 U mL^{-1}), and was quantified spectrophotometrically at 265 nm. The DHA concentration was determined by mixing 100 µL of enzyme extract, 3 mL of PBS (pH 6.8, 100 mmol L^{-1}) and 10 µL of 1 mol L^{-1} dithiotreitol at 265 nm. The concentration of reduced AsA was total AsA minus DHA concentration. The GSH- and glutathione-oxidized (GSSG) concentrations were determined according to Griffith (1980). Leaf samples (0.5 g) were ground with 4 mL 10% 5-sulfosalicylic acid dihydrate extract and centrifuged at 12,000×*g* at 4 °C for 15 min. The total glutathione concentration was determined by the addition of a GR solution to catalyze the conversion of GSSG to GSH at 412 nm. In addition, vinyl pyridine was added to inhibit GSH reaction and the concentration of GSSG was determined using a GR catalytic reaction. The concentration of GSH was the remaining concentration after GSSG was eliminated from the total glutathione concentrations.

Statistical Analysis

Microsoft Excel 2007 (Microsoft Corp., Redmond, WA, USA) and SPSS 10.0 software (SPSS, Chicago, IL, USA) for data processing and analysis. Different letters represent significant differences ($P < 0.05$). All data are mean ± standard deviation.

Results

Analysis of *SlCOMT1* Response to Salt Stress and Genetically Modified Validation

The phylogenetic tree analysis revealed that *Solyc03g080180.2.1* (*SlCOMT1*) in tomato had the closest kinship with *At5g54160* (Fig. 1). To determine whether *SlCOMT1* was involved in salt stress, we measured *SlCOMT1* expression levels in salt-treated tomato plants,

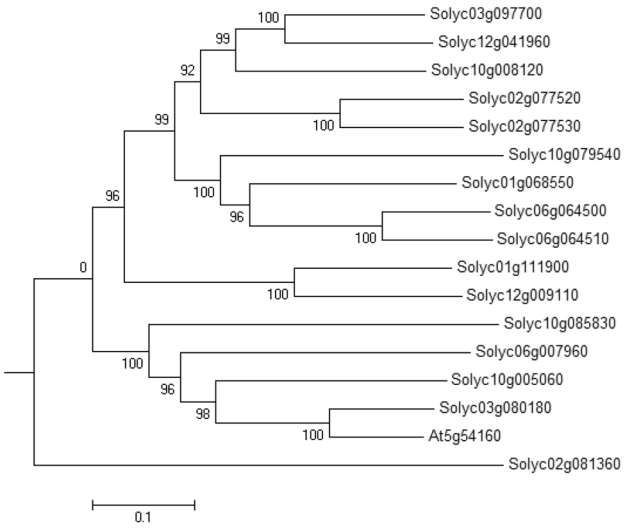

Fig. 1 Phylogenetic tree constructed based on the alignment of *Arabidopsis* AT5G54160 with 16 tomato *O*-methyltransferase proteins. The tree was constructed using MEGA6

and the qPCR analysis revealed that the expression of *SlCOMT1* in the WT in both the leaves and the roots was upregulated significantly ($P < 0.05$) by salt treatment. The highest expression was observed at 72 h in the leaves and at 48 h in the roots after treatment (Fig. 2a, b).

Under normal growth conditions, *SlCOMT1* expression levels in transformed lines (OE1, OE2, and OE3) were two to ten–fold the levels in the non-transformed lines (Fig. 2c), and the concentration of melatonin in the transformed lines increased from 5.7 to 35.3% compared to the concentrations in the non-transformed lines ($P < 0.05$) (Fig. 2d).

SlCOMT1-Overexpressing Tomato Seedlings Exhibited Higher Tolerance to Salt Treatment

Under the control conditions, there were no significant differences in plant growth between the *SlCOMT1* overexpressed lines and the WT, while the transgenic lines exhibited higher growth potential than the WT under salt treatment (Fig. 3). In the salt treatment conditions, the transgenic lines exhibited lower degrees of leaf wilting than the WT (Fig. 3a). In addition, compared with the WT plants, the plant heights, the stem diameters, and the fresh weights of the *SlCOMT1*-overexpressing plants increased significant ($P < 0.05$) under salt treatment conditions (Fig. 3b–d).

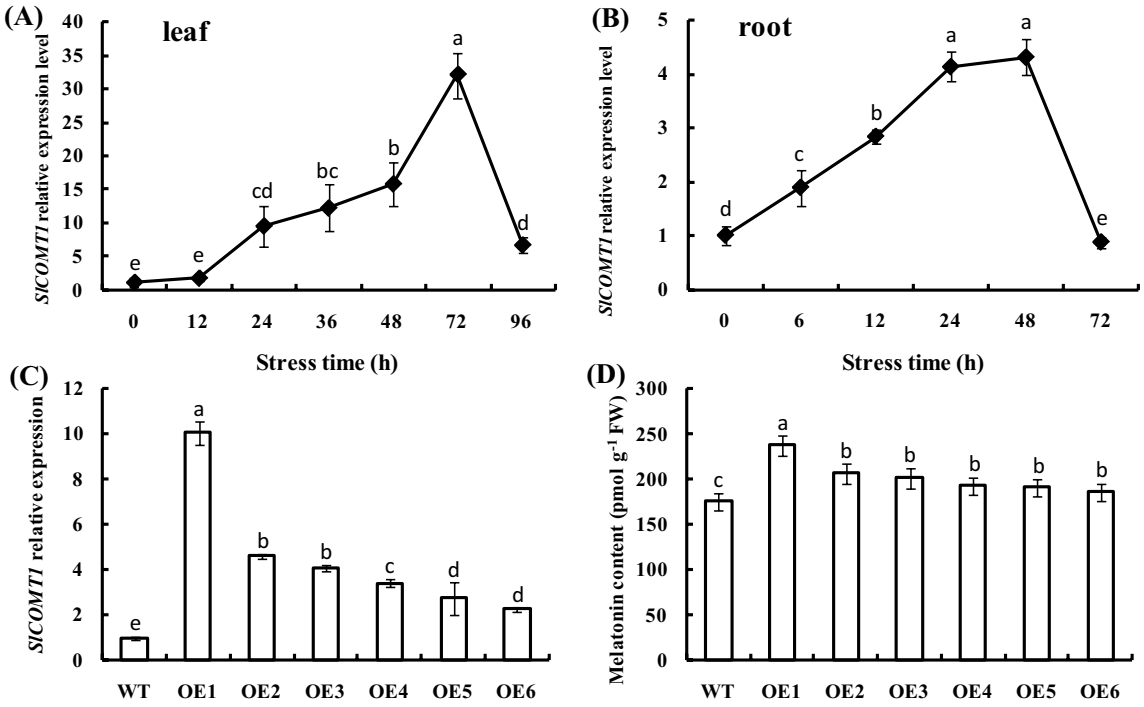

Fig. 2 *SlCOMT1* response to salt treatment in the leaves (**a**) and roots (**b**) of WT; the *SlCOMT1* expresssion levels (**c**) and melatonin concentrations (**d**) in *SlCOMT1*-overexpressing tomato lines and WT under normal conditions. Data are the means of three replicates (± SD). Different letters denote significant differences at $P < 0.05$

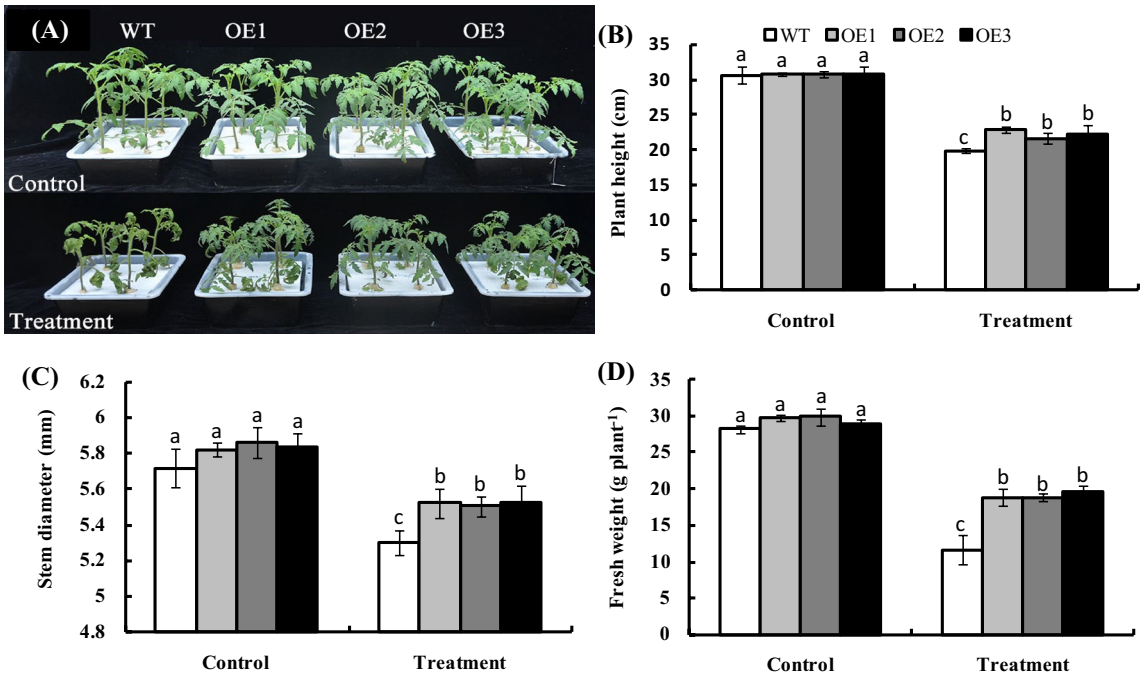

Fig. 3 Effect of salt treatment on tomato seedlings. (**a**) The WT and transgenic seedling phenotypes in the control and salt stress conditions. Photographs were taken after 7 days of salt treatment. Plant height (**b**), stem diameter (**c**), and fresh weight (**d**) of the WT and the transgenic tomato seedlings were taken after 7 days of salt treatment. Data are the means of three replicates (±SD). Different letters denote significant differences at $P < 0.05$

SlCOMT1 Over-Expression Enhanced Chlorophyll Concentration and Photosynthetic Capacity of Tomato Plants Under Salt Treatment

The concentrations of chlorophyll a and b decreased significantly ($P < 0.05$) both in the transgenic lines and the WT under salt treatment. However, the *SlCOMT1* overexpressing lines had much higher chlorophyll a and chlorophyll b concentrations ($P < 0.05$) compared to the WT under salt treatment (Fig. 4a, b). The change in carotenoid concentrations was consistent with the change in chlorophyll concentration. Salt treatment decreased the concentrations of carotenoids in the WT and the transgenic lines, but the carotenoids concentrations in the transgenic lines were higher than the carotenoid concentrations in the WT (Fig. 4c).

The measurement of photosynthetic parameters revealed that the Pn, ΦPSII, and Fv/Fm decreased significantly ($P < 0.05$) in both the WT and the transgenic lines under salt treatment (Fig. 4d–f). Nevertheless, the declines in the photosynthetic indices in the transgenic lines were lower than in the WT plants (Fig. 4d–f).

SlCOMT1 Overexpression Maintained Ionic Balance in Tomato Plants Under Salt Treatment

As shown in Table 1, salt treatment interfered with the ion uptake of the tomato plants, and the concentrations of Na^+ in the roots, stems, and leaves of the WT tomato plants increased significantly under salt treatment ($P < 0.05$) (Table 1), while *SlCOMT1* overexpression decreased Na^+ accumulation in the roots, stems, and leaves compared to in the WT, particularly in the leaves (Table 1). Compared to the WT plants, the transgenic lines increased the K^+ concentrations in the roots, stems and leaves significantly under salt treatment ($P < 0.05$). Therefore, *SlCOMT1* overexpression resulted in lower Na^+/K^+ ratio compared to the ratio in the WT plants (Table 1). The concentrations of N in the leaves of the transgenic lines were higher than that the concentrations of N in the WT plants under both the control and salt treatment. In addition, in the stems, the N concentrations were higher under salt stress in the *SlCOMT1*-overexpressing plants than in the WT plants. There were no significant differences in N concentration in the roots between the transgenic and WT plants under both the control and salt treatment conditions. Under the control conditions, the P

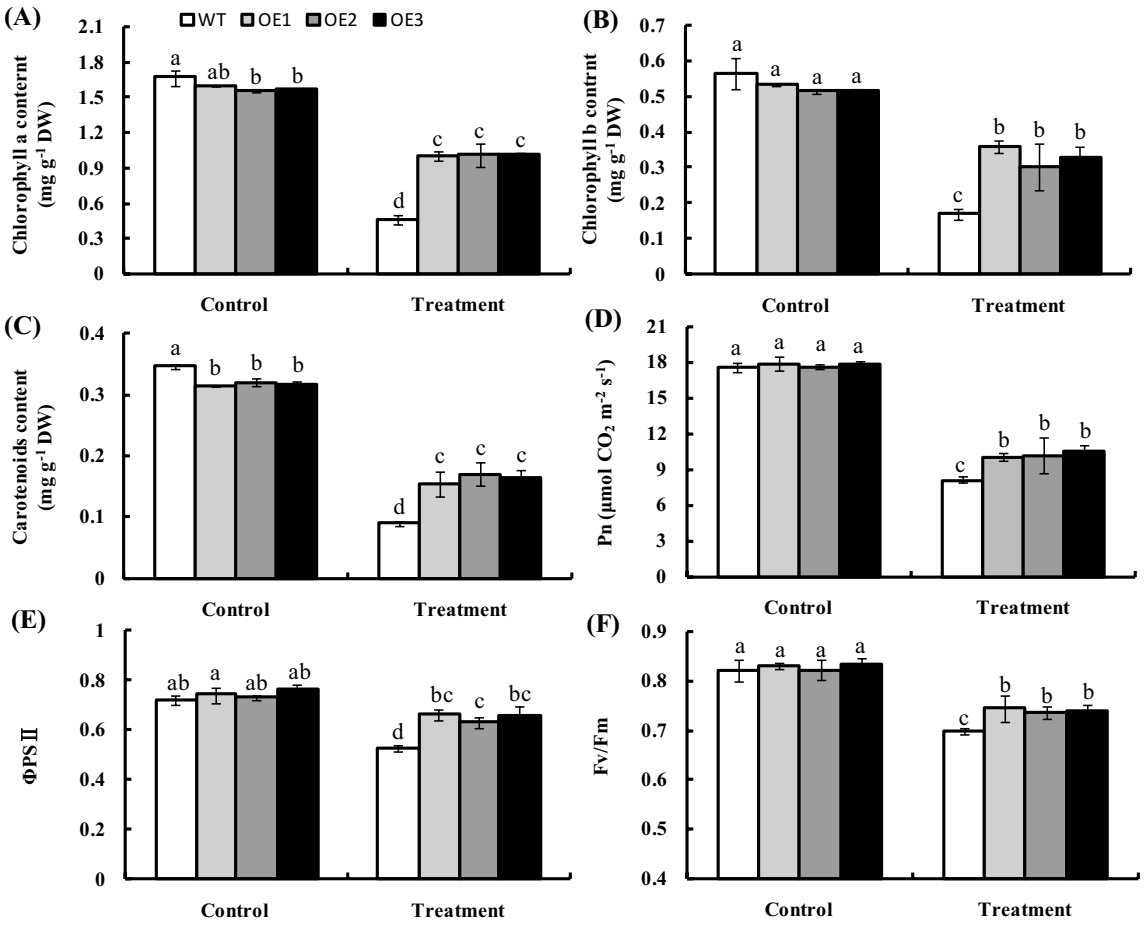

Fig. 4 Effects of salt stress on chlorophyll a (**a**), chlorophyll b (**b**), carotenoids concentrations (**c**), and the Pn (**d**), ΦPSII (**e**), and Fv/Fm (**f**) in both the WT and the transgenic tomato leaves. Data are the means of three replicates (± SD). Different letters denote significant differences at $P < 0.05$

concentrations in the roots were similar between the transgenic lines and the WT, but the P concentrations in the stems and the leaves of the transgenic lines were higher than the concentrations in the WT. In addition, salt treatment resulted in much less P accumulation, while *SlCOMT1* overexpression increased the concentrations of P in the roots, stems, and leaves significantly ($P < 0.05$) (Table 1).

SlCOMT1 Overexpression Increased the SOS Pathway Genes and Stress-Related Genes Under Stress

Under the normal conditions, there were no differences in the leaves of expression of *NHX1*, *NHX2* and *HKT1.2* between the WT and transgenic lines (Fig. 5a–e). Only *SOS1* and *HKT1.1* in OE2 and OE3 exhibited higher levels of expression in the transgenic plants (Fig. 5a, d). Compared to the control, five genes associated with the SOS pathway, which included *SOS1*, *NHX1*, *NHX2*, *HKT1.1*, and *HKT1.2*, all increased in both the WT and the transgenic lines under salt treatment, particularly in the transgenic plants (Fig. 5a–e). Salt stress induced the upregulation of mitogen-activated protein kinase (*MAPK*), calcium-dependent protein kinase (*CDPK1*), and some transcription factors, including ABA-response element-binding protein (*AREB*), ABA-induced MYB transcription factor (*AIM1*), and WRKY transcription factor 33 (*WRKY33*) (Fig. 5f–j). Notably, the levels of expression of the genes in the *SlCOMT1*-overexpressed lines were significantly higher ($P < 0.05$) than the levels in the WT under salt treatment (Fig. 5f–j).

Overexpression of *SlCOMT1* Improved Antioxidant Capacity

Under normal conditions, the $O_2^{\cdot-}$ and H_2O_2 accumulation, and the MDA concentrations were not significantly different between the WT and the transgenic lines (Fig. 6); however, salt treatment induced the significant accumulation of $O_2^{\cdot-}$, H_2O_2, and MDA in both the WT and transgenic lines (Fig. 6). In addition, ROS and MDA accumulation were significantly lower in the transgenic lines compared to in the WT (Fig. 6).

Table 1 Effects of salt treatment on Na, K, Na/K, N and P concentrations both in the WT and the transgenic tomato roots, stems, and leaves

	Root					Stem					Leaf				
	Na (mmol/g DW)	K (mol/g DW)	Na/K	N (g/g DW)	P (mmol/g DW)	Na (mmol/g DW)	K (mol/g DW)	Na/K	N (g/g DW)	P (mmol/g DW)	Na (mmol/g DW)	K (mol/g DW)	Na/K	N (g/g DW)	P (mmol/g DW)
Control															
WT	0.10^{c}	1.65^{b}	0.06	5.31^{a}	2.05^{a}	0.12^{c}	2.05^{a}	0.06	3.89^{a}	0.90^{bcd}	0.27^{c}	0.79^{b}	0.34	5.42^{b}	0.80^{b}
OE1	0.12^{c}	1.76^{a}	0.07	5.81^{a}	1.83^{a}	0.13^{c}	2.08^{a}	0.06	4.07^{a}	1.22^{a}	0.55^{c}	1.00^{a}	0.55	6.46^{a}	1.00^{a}
OE2	0.10^{c}	1.75^{a}	0.06	5.62^{a}	1.74^{ab}	0.13^{c}	2.08^{a}	0.06	4.21^{a}	1.08^{ab}	0.41^{c}	0.99^{a}	0.42	6.32^{a}	1.00^{a}
OE3	0.11^{c}	1.77^{a}	0.06	5.67^{a}	1.83^{a}	0.13^{c}	2.02^{a}	0.07	4.12^{a}	1.25^{a}	0.62^{c}	0.97^{a}	0.64	6.52^{a}	0.98^{a}
Treatment															
WT	1.80^{a}	0.91^{d}	1.98	3.80^{c}	1.10^{d}	2.99^{a}	1.05^{c}	2.84	2.14^{c}	0.73^{d}	25.35^{a}	0.52^{d}	48.88	2.83^{d}	0.62^{c}
OE1	1.67^{b}	1.08^{c}	1.54	4.31^{bc}	1.47^{bc}	2.43^{b}	1.27^{b}	1.91	2.55^{b}	0.97^{bc}	16.45^{b}	0.65^{c}	25.32	4.07^{c}	0.77^{b}
OE2	1.67^{b}	1.08^{c}	1.55	4.35^{bc}	1.40^{c}	2.41^{b}	1.25^{b}	1.94	2.54^{b}	0.85^{cd}	16.45^{b}	0.64^{c}	25.59	3.91^{c}	0.70^{b}
OE3	1.68^{b}	1.09^{c}	1.55	4.50^{b}	1.41^{c}	2.39^{b}	1.21^{b}	1.97	2.41^{b}	1.00^{bc}	16.59^{b}	0.64^{c}	25.81	4.11^{c}	0.77^{b}

Data are the means of three replicates (±SD). Different letters denote significant differences at $P < 0.05$

Salt treatment in both the WT and the transgenic lines, and particularly in the transgenic lines, induced significant SOD, POD, and CAT activity ($P < 0.05$) (Fig. 7a–c). In addition, similar changes were observed in the expression of antioxidant enzyme genes, including *Cu/Zn SOD*, *POD,* and *CAT* (Fig. 7d–f).

Overexpression of *SlCOMT1* Promoted the AsA-GSH Cycle and Enhanced Plant Antioxidant Activity

In the present study, under normal growth conditions, the concentrations of AsA, GSH, and GSSG did not exhibit significant differences ($P < 0.05$) between the WT and the transgenic lines (Fig. 8a, c, d). However, the DHA concentrations were higher in the transgenic lines (Fig. 8b). After salt treatment, the concentrations of AsA and GSH in the transgenic lines were higher than in the WT (Fig. 8a, c), while the concentrations of DHA and GSSG were lower in the transgenic lines (Fig. 8b, d). In addition, salt treatment inhibited DHAR, MDHAR, and GR activity, while enzyme activity in the transgenic lines was higher than in the WT (Fig. 8e–g).

Discussion

Numerous studies have reported that *COMT* participates in the synthesis of melatonin in some plants. *COMT* overexpression can promote the synthesis of melatonin in vivo (Byeon et al. 2014b; Lee et al. 2014; Choi et al. 2017), while inhibiting the expression of *COMT* decreases the concentration of melatonin (Choi et al. 2017; Ahammed et al. 2019; Cai et al. 2017). The result of the studies above are similar to the findings of our experiment, in which *SlCOMT1* overexpression increased melatonin concentrations in transgenic lines (Fig. 2d).

SlCOMT1 overexpression enhanced salt tolerance in tomato, assessed based on plant height, stem diameter, and fresh weight (Fig. 3), which could be due to the role of melatonin as a growth regulator. Hernández-Ruiz et al. (2004) proposed for the first time that melatonin was a hormone-like growth regulator in plants, and could promote the growth of the hypocotyl of lupin in vitro, and its mode of action and distribution gradient in plant tissue was similar to that of indole acetic acid. Similar results have also been observed in monocotyledonous plants, such as barley, wheat, and oat (Park and Back 2012).

Excessive Na^+ accumulation and nutrition disorder are observed following plant growth inhibition by salt stress. Salt stress disturbs ion homeostasis due to the absorption of excessive Na^+ (Table 1). To cope with ion imbalance, plants usually maintain low levels of Na^+ and increase the K^+/Na^+ ratio (Li et al. 2017). The present study demonstrated

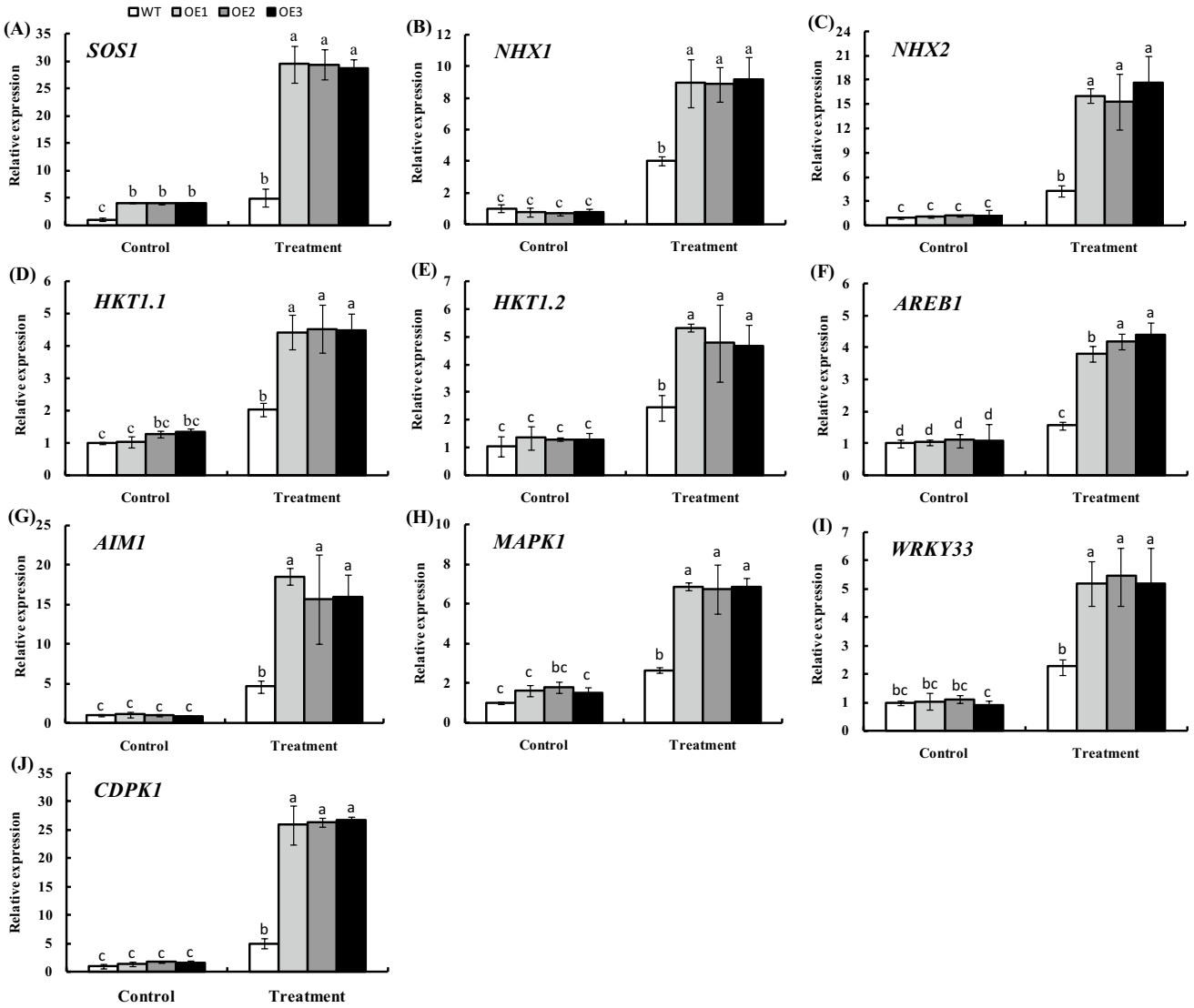

Fig. 5 Effects of salt stress on SOS-signaling pathway and stress-related gene expression. The levels of expression of the Na extraction gene (*SOS1*) (**a**), the Na detoxification genes (*NHX1* and *NHX2*) (**b**, **c**), the Na transporter genes (*HKT1.1* and *HKT1.2*) (**d**, **e**), and the stress-related genes including *AREB1* (**f**), *AIM1* (**g**), *MAPK1* (**h**), *WRKY33* (**i**), and *CDPK1* (**j**) in tomato leaves after 3 days of treatment. Data are the means of three replicates (±SD). Different letters denote significant differences at $P < 0.05$

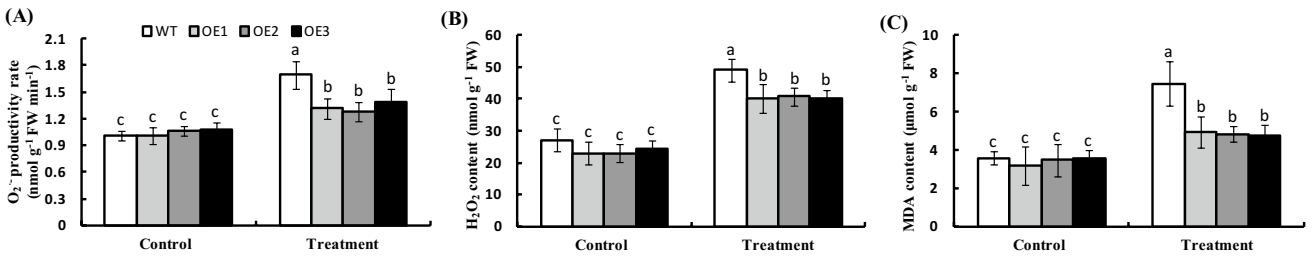

Fig. 6 Effects of salt treatment on $O_2^{\cdot-}$ accumulation (**a**), H_2O_2 (**b**) and MDA concentrations (**c**) in both the WT and the transgenic tomato leaves. Data are the means of three replicates (±SD). Different letters denote significant differences at $P < 0.05$

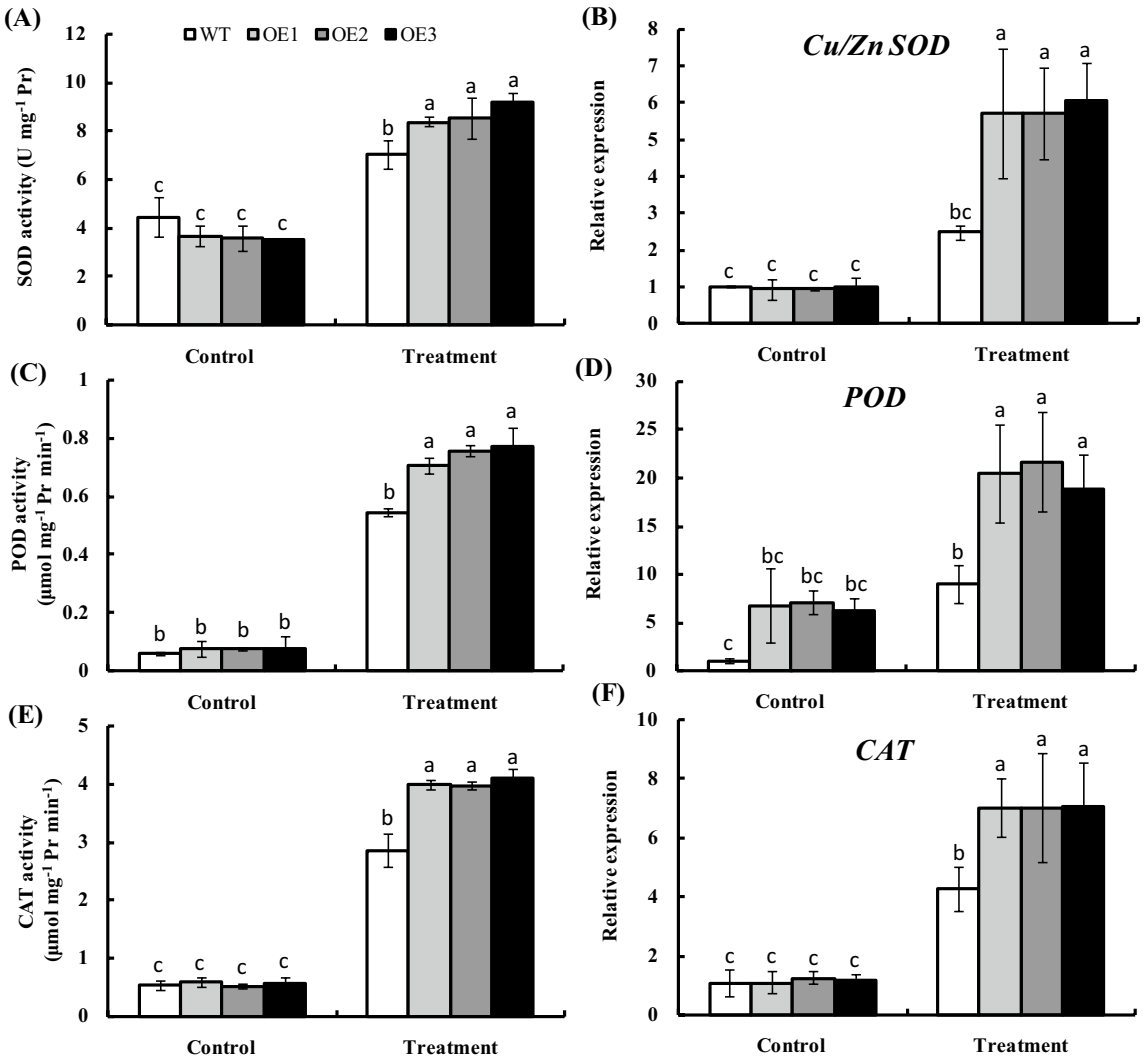

Fig. 7 Effects of salt treatment on SOD (**a**), POD (**c**), and CAT (**e**) activity and the levels of expression of their genes (**b**, **d**, **f**) in both the WT and the transgenic tomato leaves. Data are the means of three replicates (±SD). Different letters denote significant differences at $P < 0.05$

that *SlCOMT1* overexpression upregulated the expression of *SOS1*, *NHX1*, *NHX2*, *HKT1.1* and *HTK1.2* significantly (Fig. 5a–e), which could be an key mechanism via which *SlCOMT1* alleviates salt stress by decreasing the absorption and transport of Na^+, and in turn maintaining Na^+/K^+ homeostasis in plants (Zhu 2002). The finding is consistent with the result reported by Liu et al. (2015) that melatonin could reduce Na^+ toxicity. Chen et al. (2018) suggested that the melatonin-triggered *SOS*-mediated Na^+ efflux is mediated by *AtrbohF*-dependent ROS. In addition to regulating the accumulation of K, *SlCOMT1* regulated the accumulation of N and P, and maintained them at relatively high levels in the roots, stems, and leaves of tomato plants under salt treatment (Table 1). The root system architecture plays an important role in the absorption of nutrients. Nawaz et al. (2016) reported that melatonin could influence N and P absorption due to its function root structure regulation function. Similarly, Zhang et al. (2013) found that melatonin could stimulate root growth and enhance root vigor. In addition, Zhang et al. (2017) demonstrated that pretreatment with melatonin enhanced the activity of enzymes involved in N metabolism. Consequently, we speculated that melatonin could indirectly promote plants uptake of N and P by altering root structure and increasing the activity of nitrogen metabolizing enzymes.

Chlorosis is a common symptom observed following salt treatment, and nitrogen deficiency can decrease the photosynthetic capacity of plants, which may be one of the reasons for chlorosis (Parkhill et al. 2001). It has been reported that exogenous melatonin could slow down the decrease in Mg^{2+} concentration under cold stress conditions, which affect chlorophyll synthesis and increases the photosynthetic

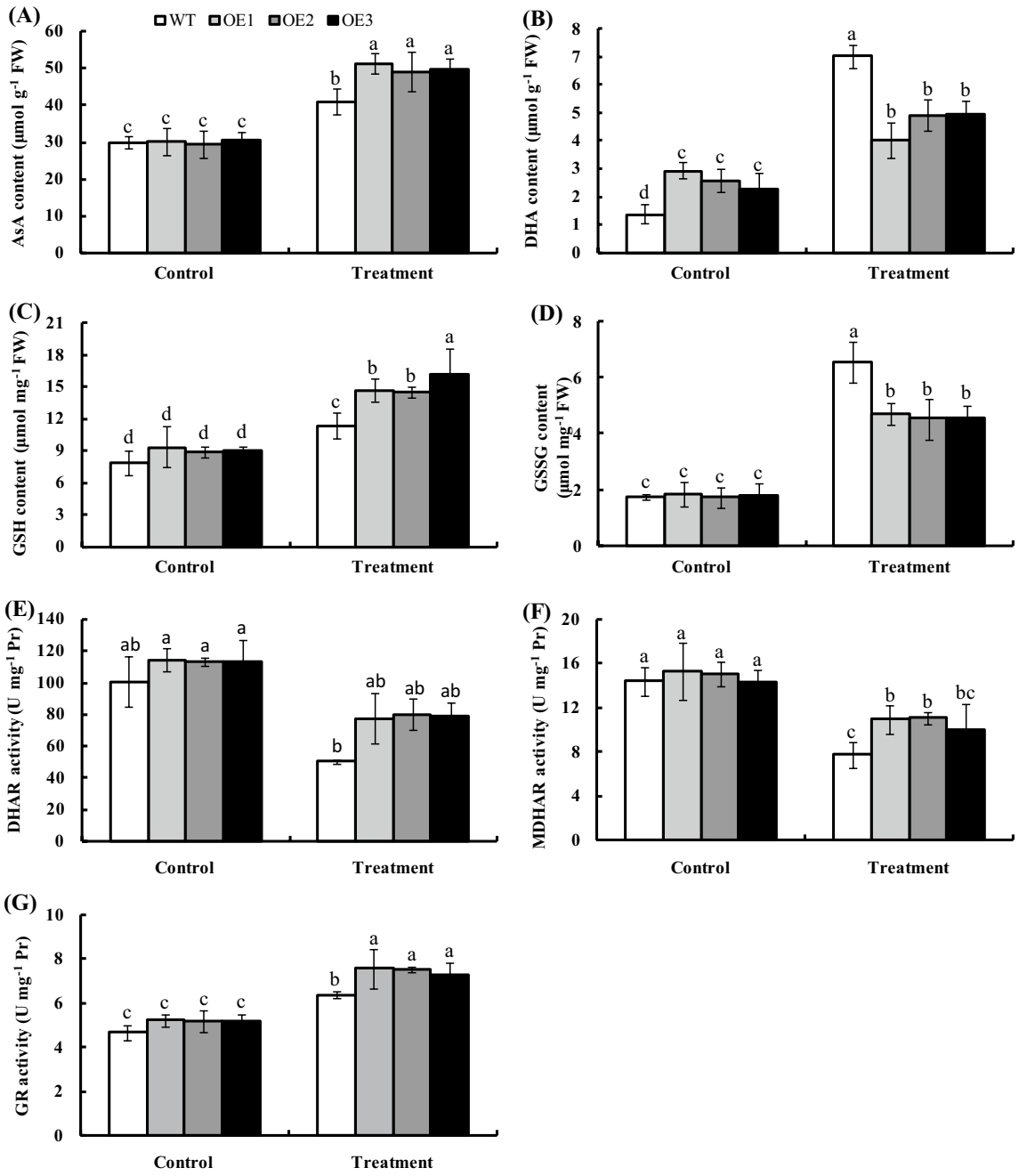

Fig. 8 Effects of salt treatment on the AsA (**a**), DHA (**b**), GSH (**c**) and GSSG (**d**) concentration, and on DHAR (**e**), MDHAR (**f**), and GR (**g**) activity in both the WT and transgenic tomato leaves. Data are the means of three replicates (± SD). Different letters denote significant differences at $P < 0.05$

rate (Turk and Erdal 2015). A decrease in photosynthesis under salt stress conditions could be due to both a decrease in chlorophyll accumulation and the degradation of chloroplast structure (Dekov et al. 2000). In the present study, chlorophyll concentrations in the transgenic lines were relatively high under the salt stress conditions, which may be partly responsible for the higher Pn in the transgenic lines following salt treatment (Fig. 4d). *SlCOMT1* overexpression reduced the damage to the photosynthetic system caused by salt stress, improved light energy absorption and transformation by the leaves, and minimized leaf chlorosis (Figs. 3 and 4). Previous studies have also showed that exogenous melatonin could enhance photosynthetic efficiency by increasing light use efficiency and electron transport in PSII (Li et al. 2017).

ROS are beneficial to plant growth and are key signal molecules for plants. However, excessive ROS would damage membrane systems and negatively affect normal metabolism (Yang et al. 2017). Impaired photosynthetic systems could impede photosynthetic electron transport, resulting in the accumulation of excessive ROS (H_2O_2 and $O_2^{\cdot-}$) under salt stress conditions (Gong et al. 2013). In the present study, compared to in the WT plants, low ROS and MDA concentrations were detected in the *SlCOMT1* overexpressing lines after salt treatment (Fig. 6). Although melatonin can scavenge H_2O_2, $O_2^{\cdot-}$, and other free radicals directly, it can also regulate the antioxidant system to maintain redox homeostasis in plants under stress conditions (Allegra et al. 2003). Ahammed et al. (2019) found that silencing tomato *COMT1* led to the production of more ROS, indicating that endogenous melatonin plays an important role in eliminating ROS. In our experiment, *SlCOMT1* overexpression alleviated antioxidant enzyme activity inhibition by salt stress (Fig. 7), which could have been due to the accumulation of endogenous melatonin. Our findings are consistent with the findings of Chen et al. (2018), who observed that exogenous melatonin increased the activity of antioxidant enzymes, including SOD, POD, CAT, and GR, in maize seedlings under salt stress. In contrast, silencing the tomato *COMT* decreased the activity of antioxidant enzymes and gene expression, which further demonstrated the melatonin regulation of ROS homeostasis (Ahammed et al. 2019). In addition, the AsA-GSH cycle is a key antioxidant system that relieves stress in plants. In the present study, *SlCOMT1* overexpression increased the accumulation of AsA and GSH significantly, while suppressing the accumulation of DHA and GSSG. In addition, the activity of some key enzymes, including DHAR, MDHAR, and GR, were enhanced in transgenic lines compared to in the WT plants after salt treatment, and the enzymes were responsible for the regeneration of AsA and GSH (Fig. 8). Similarly, multiple studies have reported similar results: endogenous or exogenous melatonin could increase the concentrations of antioxidants under stress conditions, and improved stress tolerance (Wang et al. 2012; Cui et al. 2017; Li et al. 2017). According to Gao et al. (2018), melatonin-mediated induction of antioxidant responses might require the activation of ROS and MAPK.

The *MAPK* cascade participates extensively in stress signal transduction in plants, further activating the expression of the stress resistance genes facilitating adaptation to stress (Pan et al. 2012). A previous study reported that *MAPK* is upregulated by exogenous melatonin (Gong et al. 2017), and similar results were observed based on endogenous melatonin in the present study: salt treatment induced the upregulated expression of *MAPK1*, and its level of expression in transgenic lines was higher than in the WT plants (Fig. 5h). In addition, *SlCOMT1* overexpression increased *AREB1* overexpression after salt treatment (Fig. 5f), which could bind ABA-response elements and induce the expression of ABA-responsive genes and enhance osmotic stress, in turn increasing the tolerance of the plants to salt stress (Kim 2006). SlAIM1 transcription factors have been reported to be induced by pathogens, plant hormones, salinity, and oxidative stress (Abuqamar et al. 2009). The results of the present study demonstrated that *AIM1* was involved in salt stress in tomato plants, and *AIM1* expression in transgenic lines with greater salt tolerance was higher than in the WT (Fig. 5g). Li et al. (2013) reported that *WRKY33* was induced by salt stress, and *WRKY33* overexpression in *Arabidopsis thaliana* could activate stress-induced gene expression and improve salt tolerance, which was consistent with our results: under salt treatment, the *WRKY33* expression in transgenic lines was higher than in the WT plants, and the salt tolerance of the transgenic lines was superior than that of the WT plants (Figs. 3 and 5). *SlCOMT1* increased the expression of stress-related genes in tomato plants significantly after salt treatment, which could be one of the major mechanisms of improving salt tolerance in tomato plants.

Conclusions

This study revealed that the overexpression of tomato *SlCOMT1* could increase the concentrations of endogenous melatonin. The transgenic lines demonstrated higher salt tolerance capacity, which could partly be due to endogenous melatonin inducing the higher expression of some stress-related genes. As a result, ion homeostasis was well maintained, the photosynthetic system was protected, and oxidative stress was minimized. The results of the present study provide a theoretical basis for the application of *SlCOMT1* and endogenous melatonin in increasing plant tolerance to salt stress.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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