Exogenous melatonin improves salt tolerance in tomato by regulating photosynthetic electron flux and the ascorbate–glutathione cycle

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ABSTRACT
Melatonin (MT) can protect plants against abiotic stress. In order to explore whether melatonin can improve photosynthetic function under NaCl stress, Solanum lycopersicum L. cv. Liaoyuanduoli were exposed to 150 mmol L\(^{-1}\) NaCl stress with or without pretreatment with 150 mmol L\(^{-1}\) melatonin. The results showed that NaCl stress can lead to reduced chlorophyll content, lower photosynthetic function, increased reaction oxygen species (ROS) levels, and decreased PSII activity. These changes were mainly due to the reduction in oxygen-evolving complex (OEC) activity on the donor side of PSII and the blockage of electron transfer from QA to QB on receptor side of PSII. The donor side of PSII was more sensitive to NaCl stress relative to the receptor side of PSII. Interestingly, application of MT enhanced tomato NaCl tolerance. MT reduced the production of ROS by balancing the distribution of photosynthetic electron flux, facilitated the repair of PSII by maintaining the abundance of Psb O and D1, and promoting the ability of the donor and acceptor sides of PSII to deliver electrons. MT also enhanced the scavenging ability of ROS by stimulating the activity of enzymes involved in the AsA–GSH cycle.

Introduction
Soil salinity is a common abiotic stress that limits agricultural yield and productivity worldwide (Munns and Tester 2008). Salt stress results in ion imbalance, hyperosmotic stress, photosynthesis, oxidative damage, and other physiological disturbances in plants (Zhu 2002). In particular, salt stress can reduce the activities of photosystem II (PSII) and photosystem I (PSI), which leads to a decrease in electron receptor production (Takahashi and Murata 2008). The inhibition of photosynthesis electron transport (PET) may be caused by the degradation of D1 (Zhu 2002; Zhang et al. 2012). In general, electrons are transferred from the primary to secondary quinone electron acceptor of PSII, then go to PSI and interact with the electron acceptor NADP\(^+\) through ferredoxin (Fd). However, superfluous electrons induced by reduced OEC activity either on the donor or the receptor sides of PSII can be transferred either to oxygen in PSI or generate reactive oxygen species (ROS) through a Mehler reaction (Foyer and Noctor 2009). These excessive electrons can leak and attack free oxygen molecules. Thus, salt stress generates reactive oxygen species (ROS) and damages PSI reaction centers, further leading to the peroxidation and dissociation of the thylakoid membranes (Zhang et al. 2012). ROS production and scavenging balance is necessary for photosynthetic function. Salinity-induced photoinhibition and excessive ROS often underlie oxidative damage. Excessive ROS damages cells by reducing the function of membrane transporters and associated enzymes (Zhan et al. 2014; Zhang et al. 2015a). Exogenous substances can be used to enhance plant stress tolerance and improve the crop’s productivity under stress (Yang et al. 2015; Han et al. 2016). Melatonin (MT) is an effective exogenous substance that is widely used to ameliorate the effects of stress on crops (Galano et al. 2011; Zhang et al. 2015b).

MT is a natural antioxidant that can effectively scavenge ROS in animals and plants (Arnao and Hernández-Ruiz 2015). MT protects plants against various environmental stressors, including extreme temperatures (Lei et al. 2004; Kang et al. 2010; Shi and Chan 2014), heavy metals (Tan et al. 2007; Posmyk et al. 2008), UV radiation (Afreen et al. 2006), and salt stress (Tal et al. 2011; Li et al. 2012; Fan et al. 2014). Moreover, MT regulates plant development, including, seed germination (Tiryaki and Keles 2012; Zhang et al. 2013), leaf senescence (Wang et al. 2013a; Wang et al. 2013b; Wang et al. 2014), root development (Park and Back 2012; Pelagio-Flores et al. 2012; Zhang et al. 2013), and circadian rhythm (Kolář et al. 1997; Arnao and Hernández-Ruiz 2009). MT’s intermediate product, N1-acetyl-N2-formyl-5-methoxykynuramine (AMFK), can scavenge ROS in a direct and efficient manner (Tao et al. 2007; Manchester et al. 2015). Furthermore, MT can stimulate the activities of antioxidant enzymes involved in the ascorbate–glutathione (AsA–GSH) cycle to scavenge excess ROS in apple and cucumber (Wang et al. 2012; Li et al. 2015; Li et al. 2016a). Tomato (Solanum lycopersicum L. cv Liaoyuanduoli) is an important crop, and its growth and development can be hindered due to salt stress. Although, the salt tolerance response in tomato has been studied previously (Zheng et al. 2015; Zhou et al. 2016),
the role of MT in regulating the distribution of the PSII electron transport system and ROS scavenging ability of the AsA-GSH cycle to enhance salt tolerance in tomato remains unclear. This study aims to provide insights into improving the quality and yield of tomato cultivation in soil with high levels of salt.

Here, we investigated whether MT can alleviate salt stress in tomato by regulating the distribution of electron fluxes and ROS balance. We determined the changes on the donor and acceptor sides of PSII using the polyphasic rise of fluorescence transients (OJIP fluorescence induction curve), which is the process that generally gives rise to increased ROS levels in high salt environments. In addition, we performed western blots to analyze the changes in the core proteins in the donor and acceptor sides. Moreover, we evaluated membrane integrity and activity of ROS-related antioxidative enzymes involved in the AsA–GSH cycle. This information is critical for improving our understanding of how MT may alleviate salt stress in tomato.

Materials and methods

Plant materials and NaCl treatment

Tomato (S. lycopersicum L. cv Liaoyuanduoli) seeds were germinated and grown hydroponically in a growth chamber. Plants were placed in Hoagland solution at 23°C under fluorescent light (300 μmol m\(^{-2}\) s\(^{-1}\), 13 h light/11 h dark) and 60% relative humidity. The nutrient solution was renewed every day in order to provide a stable nutrient supply. To investigate the effects of MT in tomato seedlings in response to NaCl stress, the leaves of tomato seedlings at the four-leaf stage were pretreated with exogenous MT that was irrigated into the Hoagland solution at 0, 10, 50, 100, 150, 250 μM. Among these treatments, 150 μM MT was selected as most effective in relieving salinity stress (unpublished data). After three days of MT pretreatment, the control and NaCl stressed seedlings were treated with NaCl (150 mM) for seven days, where each treatment was conducted over 12 plants. The treatments as were follows: CK-Hoagland’s solution only as control; CK+MT-Hoagland’s solution with 150 μM MT; NaCl-Hoagland’s solution with 150 mM NaCl; and NaCl +MT-Hoagland’s solution with 150 μM MT and 150 mM NaCl. Leaves were harvested seven days after treatment and either used freshly or collected into liquid nitrogen and preserved at −80°C. At least three biological replicates were collected for each treatment.

Measurement of chlorophyll content

Chlorophyll a (Chla), chlorophyll b (Chlb) and total chlorophyll (Chls) contents were determined by acetone, anhydrous ethanol and distilled water (4:5:4:5:1:V:V:V) in the dark at 4°C until leaves turned completely white. The absorbance of the supernatant was measured at 450, 332, and 600 nm, and recorded as OD\(_{450}\), OD\(_{332}\) and OD\(_{600}\) respectively (Porra 2002).

Measurement of net CO\(_2\) assimilation rate

Photosynthesis parameters, such as net photosynthetic rates (P\(_n\)), stomatal conductance (G\(_s\)), intercellular CO\(_2\) concentration (G\(_i\)), and transpiration rates (T\(_r\)) of leaves were measured at 10:00–11:00 am using the portable photosynthesis system LI-6400 (LI-COR Biosciences, Lincoln, USA). The radiation that was used to activate photosynthesis was 600 μmol m\(^{-2}\) s\(^{-1}\) (saturation light). The air temperature and humidity were 25°C and 50–60 %, respectively (Zhang et al. 2018). Concentration of CO\(_2\) was changed at 3 min intervals in a sequence of 1600, 1200, 1000, 800, 600, 400, 300, 200, 150, 100, and 0 μmol mol\(^{-1}\). Irradiance and CO\(_2\) concentration were controlled by the system. CE was obtained from the slope of the Pn–Ci response curve (Cheng et al. 2016).

Membrane integrity

To detect lipid peroxidation and membrane integrity, the malondialdehyde (MDA) content was determined using the thiobarbituric acid (TBA) reaction (Wang et al. 2010). The relative electrolyte leakage (REL) ratio was measured following previously published protocols (Gong et al. 1998).

Analysis of superoxide anion (O\(_2^−\)) and hydrogen peroxide (H\(_2\)O\(_2\))

To detect the levels of ROS in tomato leaves, the rate of O\(_2^−\) generation and H\(_2\)O\(_2\) content were measured. The generation rate of O\(_2^−\) was measured following Yan et al. (1996).

The H\(_2\)O\(_2\) content was measured by spectrophotometry measurements at 415 nm after potassium iodide treatment, as previously described (Ibrahim and Jaafar 2012). Briefly, tissues were ground in 0.1% trichloroacetic acid, and the homogenate was centrifuged at 15,000 g for 15 min at 4°C, and the supernatant was used to measure H\(_2\)O\(_2\) levels.

Analysis of antioxidant enzymes

Assay of antioxidant enzymes, including superoxide (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione S-transferase (GST), glycolate oxidase (GO) and glutathione peroxidase (GPX) were performed following published protocols (Yin et al. 2014; Suo et al. 2015; Yin et al. 2017; Zhang et al. 2019). The protein content was measured in all the enzymatic preparations using the Bradford method (Bradford 1976).

Measurements of chlorophyll a fluorescence transient (OJIP)

The polyphasic rise of fluorescence transients (OJIP fluorescence induction curve) were measured using the third fully expanded leaf from plants in different treatment groups using a Plant Efficiency Analyser (PEA, Hansatech Instruments Ltd., King’s Lynn, Norfolk PE32 1JL, UK) following protocols from Strasser et al. (Strasser and Srivastava 1995; Strasser and Strasser 1995). The leaves were adapted to darkness for 30 min before testing. The characteristic points O, J, I and P on the OJIP curve corresponded to time points 0, 2, 30 and 1000 ms, and the corresponding relative fluorescence intensities were expressed as F\(_{O}\), F\(_{I}\), F\(_{I}\) and F\(_{m}\). The 0.15 and 0.3 ms time points on the OJIP curve were defined as L and K, respectively, and the corresponding relative fluorescence intensities were expressed as F\(_{L}\) and F\(_{K}\), respectively.
Standardizations of O-P, O-J and O-K on the OJIP curves of different treatments were carried out by defining the relative fluorescence intensity of O as 0, and the relative fluorescence intensities of P, J and K as 1. The formulas for the standardization were: \( V_{O,P} = (F_{t} - F_{o})/(F_{t} - F_{p}) \), \( V_{O,J} = (F_{t} - F_{o})/(F_{t} - F_{p}) \), \( V_{O,K} = (F_{t} - F_{o})/(F_{t} - F_{p}) \), where \( F_{t} \) is the relative fluorescence intensity at different time points. The relative variable fluorescence at the three characteristic points L, K and J on the standardized curve were expressed as \( V_{L} \), \( V_{K} \) and \( V_{J} \), respectively, and were calculated using the following formulas: \( V_{L} = (F_{t} - F_{o})/(F_{t} - F_{p}) \), \( V_{K} = (F_{t} - F_{o})/(F_{t} - F_{p}) \), \( V_{J} = (F_{t} - F_{o})/(F_{t} - F_{p}) \). The differences between CK and \( V_{O,P} \), \( V_{O,J} \) and \( V_{O,K} \) curves of the plant leaves under different treatment conditions were determined and expressed as \( \Delta V_{O,P} \), \( \Delta V_{O,J} \) and \( \Delta V_{O,K} \) respectively (Strasser et al. 1997; Strasser, 1997) (Strasser and Strasser 1995). The PSI maximum photochemical efficiency (\( F_{p}/F_{m} \)) was obtained through JIP-test analysis of OJIP curves following methods described in Strasser and Strasser 1995.

### Protein extraction and western blotting

Total protein from tomato leaves that were subjected to different treatments were extracted using the Minute™ total protein extraction kit (Invent Biotechnologies, USA). Protein samples were boiled for 5 min in a water bath and separated by 10% SDS-PAGE gel, then transferred onto polyvinylidene difluoride (PVDF) membranes using the Trans-Blot Turbo™ (Bio-Rad, USA) transfer system. Subsequently, the PVDF membranes were blocked by 5% skimmed milk and incubated for 2 h with the primary antibody (1:1000) at 4°C overnight and secondary antibody (1:5000) for 1 h. The primary antibodies against Psb O and D1 antibodies obtained from Agrisera (Umeo, Sweden), and anti-β-actin and secondary antibodies were obtained from Kangwei (Beijing, China). The chemical coloration Azure c600 Western blot imaging system (Azure, USA) was used after the DAB Horseradish Peroxidase Color Development Kit to visualize the bands on the Western blots.

### Statistical analysis

Data were analyzed with SPSS 17.0 (SPSS Inc., Chicago, USA) using ANOVA and LSD (p < 0.05). All the results are presented as means ± standard error (SE) and all experiments had more than three biological replicates.

### Results

#### Effects of MT on chlorophyll content and carbon assimilation under NaCl stress

To test whether MT treatment reduced the effects of NaCl stress in tomato plants, chlorophyll content of the leaves was measured by evaluating changes in leaf color (Figure 1). In NaCl treated plants, Chls, Chl a and Chl b contents in tomato leaves decreased by 20.68%, 25.82% and 8.44%, respectively. Compared to the NaCl treatment alone, plants that were treated with MT and NaCl had increased Chls, Chl a and Chl b levels by 33.06%, 32.04%, and 35.03%, respectively, (Figure 1A–C), suggesting that the MT treatment mitigated the effects of NaCl. Seedlings that were treated with only MT had significantly higher Chls, Chl a and Chl b levels compared to the control (Figure 1A–C). The ratio of chl a/b were similar across all treatments (Figure 1D), which may be due to MT’s ability to mitigate changes in Chl a and Chl b at a similar level.

Seedlings that were treated with NaCl for seven days showed significant 40.65% reduction in net photosynthetic rate (\( P_{n} \)) compared to untreated plants, while \( P_{n} \) decreased by 20.33% in seedlings that were exposed to MT and NaCl compared to the untreated control. These results suggest that the adverse effects of NaCl stress on tomato photosynthesis were significantly reduced by pre-treatment with MT. Compared to untreated seedlings, stomatal conductance (Gs) and intercellular CO₂ concentration (Ci) in NaCl treated plants decrease by 31.35% and 30.42%, respectively (Table 1), suggesting that stomatal limitation was an important factor that reduced photosynthetic rates in tomato plants undergoing NaCl stress. Nevertheless, the decrease in Ci was lower than Gs, suggesting that the decline of \( P_{n} \) was caused by non-stomatal limitation, suggesting that NaCl stress inhibited the light system, carbon assimilation and enzyme activation to some extent. In contrast, Gs and Ci were decreased by 13.43% and 17.12%, respectively, in plants that were treated with NaCl and compared to control seedlings (Table 1), indicating that MT can alleviate NaCl-induced reductions in the photosynthetic rate of leaves by overcoming stomatal factors (Table 1).

Carboxylation efficiency (CE) is usually used to measure the amount of Rubisco in leaves, and NaCl stress often influences CE of vegetables more than other species (Cheng et al. 2016). When compared to the untreated controls, the CE of tomato seedlings decreased by 27.28% under NaCl stress and by 13.64% in seedlings that were pre-treated with MT and exposed to NaCl stress (Table 1), indicating that MT lessened the effect of \( P_{n} \) in tomato seedlings caused by NaCl stress by enhancing the activity of Rubisco.

#### Effects of MT on membrane integrity and key enzymes of the Ascorbate–Glutathione (AsA–GSH) cycle

To evaluate the effects of MT on membrane stability under NaCl stress, we measured the MDA content and REL in leaves. The MDA and REL contents of tomato leaves were significantly increased under NaCl stress, however, the application of exogenous MT significantly reduced the MDA and REL contents (Figure 2A). Specifically, the MDA content increased from 10.32 nmol g⁻¹ fresh weight (FW) to 28.73 nmol g⁻¹ FW under NaCl stress, but the MDA content was 17.99 nmol g⁻¹ FW in seedlings that were pretreated with MT and exposed to NaCl stress (Figure 2A). The REL content increased from 41.48 nmol g⁻¹ FW to 85.35 nmol g⁻¹ FW under NaCl stress, but it was also decreased to 71.95 nmol g⁻¹ FW in seedlings that were pretreated with MT and exposed to salt stress (Figure 2A). The results indicated that pretreatment with 150 mM MT was sufficient to improve the salt tolerance of tomato seedlings, reduce the damage on the cell membrane caused by NaCl stress, and inhibit further oxidation of membrane lipids, thereby maintaining the integrity of the cell membrane under salt stress.

To monitor the effect of MT on ROS homeostasis in tomato leaves under NaCl stress, the \( \text{O}_{2}^{-} \) generation rate, \( \text{H}_{2}\text{O}_{2} \) content and the activity levels of enzymes involved in the ascorbate–glutathione cycle were analyzed. The \( \text{O}_{2}^{-} \) generation
rate and H$_2$O$_2$ content were higher under NaCl treatment, while samples that were treated with both MT and NaCl showed reverted values (Figure 2(B)), indicating that MT can effectively alleviate oxidative stress caused by NaCl stress.

Various antioxidant enzymes were involved in ROS scavenging (Figure 2(C)). In particular, SOD activity increased by 20.91% under NaCl treatment compared to the untreated control, yet SOD activity levels were similar between the control samples treated with or without MT (Figure 2(C)). In addition, among the enzymes involved in reducing H$_2$O$_2$ levels, POD, CAT and GPX activities were significantly increased by 10.95%, 10.74% and 94.07% (Figure 2(C,D,F)), respectively, under NaCl treatment. Compared to the NaCl treated leaves, MT and NaCl treatment resulted in additional increases in POD, CAT, and GPX activities by 3.71, 7.77 and 5.12 %, respectively, compared with those treated with NaCl alone (Figure 2(D,E,F)). These results indicated that exogenous applications of MT could promote the activity of key enzymes in the AsA–GSH cycle under salt stress.

Effects of MT on donor and acceptor sides of photosystem II (PS II) under NaCl stress

The fluorescence transient showed a polyphasic rise when leaves are illuminated by a high density of actinic light, which provides measurements for phase O, J, I, and P of PSII, such as electron transport levels in the donor and acceptor sides (Srivastava et al. 1997; Strasser and Strasser 1995). As shown in Figure 3(A), the shapes of the OJIP curves of the tomato leaves were significantly different under the four treatments, and the ranges of the variations of the relative fluorescence intensity were also different at each characteristic point on the OJIP curve. OJIP curves were normalized to the ($F_m$–$F_o$) level (Figure 3(B,D,F)), and $\Delta V_t$ was obtained by subtracting the kinetics of plants that were untreated from kinetics of MT or NaCl treated plants (Figure 3(C,E)). The shape of the OJIP transient changed over time, where the J,
K and L points increased significantly under NaCl stress, and this increase was not as pronounced in the MT and NaCl treated samples when compared to the NaCl treated samples (Figure 3(B,C)). This suggests that NaCl stress blocked electron transport from QA to QB, and MT treatment alleviated this effect. The K step (at 300 μs) of the chlorophyll a fluorescence transient (quantified as $V_K$) is an indicator for OEC damage (Strasser, 1997; TóTh et al. 2005). We observed a 14.34% decrease in $V_K$ in samples treated with NaCl alone, while NaCl and MT treatment led to a 11.86% increase in $V_K$ compared with the NaCl only treatment (Figure 3(D,E)), suggesting that NaCl stress led to the inhibition of the donor side of PSII activity and damaged OEC, while MT alleviated PSII inhibition and OEC damage. According to the Grouping Concept (Strasser and Strasser1995) and JIP test (Strasser et al. 2004; Lin et al.2009), a positive step in JIP indicates low grouping or low energy exchange between PSII units. Because the grouped conformation is more stable than the ungrouped one (Strasser et al. 2004; Lin et al. 2009), the decreased grouping indicates that PSII units may have become unstable or more fragile. This observation implies that NaCl stress damaged the PSII units, and exogenous application of MT reduced this NaCl-induced damage of PSII.

**Effects of MT on thylakoid membrane proteins D1 and PsbO under NaCl stress**

Western blots were performed to determine the effects of photoinhibition induced by NaCl stress on the degradation of D1 and PsbO proteins. Compared to the untreated controls, NaCl stress led to a decrease in D1 and PsbO levels by 58.67% and 35.72%, respectively. However, plants treated with MT and NaCl had 29.27% and 26.56% higher D1 and PsbO levels compared to NaCl stress (Figure 4).

**Discussion**

**Inhibitory effect of MT on chlorophyll degradation and photosynthesis reduction in tomatoes under NaCl stress**

MT is a natural agent that has been widely used to promote growth and photosynthesis of plants under NaCl stress (Li et al. 2012; Wang et al. 2016). In this study, MT effectively lessened the decrease of Chls, Chl a and Chl b contents caused by NaCl stress (Figure 1) and promoted the increase of Pn and CE (Table 1), indicating that MT could alleviate chlorophyll degradation induced by NaCl stress (Kudoh and Sonoike 2002). In addition, Chls, Chl a and Chl b contents were much higher when plants were treated with MT than under normal conditions, suggesting that MT may facilitate the chloroplast gene expression and protein turnover to promote the accumulation of chlorophyll (Suo et al.2015). However, further research into the connection between the photosynthetic machinery and chlorophyll metabolic pathways is needed to further understand under NaCl stress.
Stomatal limitation and non-stomatal limitation factors can both decrease the photosynthetic rate in plants under stressful conditions (Zhu 2002). Stomatal limitation is usually caused by the partial closure of the stomata, which can reduce the CO₂ concentration in mesophyll cells and inhibit photosynthesis. Non-stomatal factors restrict the diffusion of CO₂ in carboxylation sites in mesophyll cells or reduce enzymatic activities during carbon assimilation (Sharkey et al. 2007). The lower levels of GS and Ci that were observed under NaCl stress indicated that the decrease of Pn may be due to the effect of NaCl stress on root activity, leading to the partial closure of stomata, thus resulting in the decrease of Ci (Table 1). This finding suggested that stomatal limitation under NaCl stress was the main reason for the decrease of Pn that was observed in tomato seedling leaves. However, GS and Ci increased with increasing Pn in MT and NaCl treated plants, indicating that application of MT led to maintaining of high potential PSII activity. The above results showed that MT regulated photosynthesis and enhanced the adaptability of tomato leaves in response to NaCl tolerance (Wang et al. 2016). This may be through maintaining higher Rubisco activity and solar utilization efficiency, and overcoming the stomatal limitation, thereby reducing the adverse effects caused by NaCl stress (Zhang et al. 2013).

MT promoted ROS scavenging by increasing the activities of enzymes in the AsA–GSH cycle

NaCl stress reduced the efficiency of photosynthesis, resulting in accumulation of excess light energy and obstruction of

![Figure 3](image-url)
electron transfer. Excess light energy and electrons can influence the metabolic balance of ROS in plants, leading to an accumulation of ROS (Wilhelm and Selmar 2011). Excess ROS causes peroxidation of lipids and pigments on the cell membrane, resulting in increased cell membrane permeability and destruction of its functions (Zhang et al. 2012). The antioxidant capacity of MT has been widely reported, and it can significantly reduce the adverse effects of abiotic stress, such as cold-induced injury, heavy metals, UV radiation and NaCl injury (Hasan et al. 2015; Shi et al. 2015; Li et al. 2016b). This study showed that MT could improve the activity of SOD under NaCl stress (Figure 2 (C)), which was consistent with a previous study that showed that application of MT on apple leaves reduced the effects of NaCl stress (Li et al. 2012). In addition, under NaCl stress, MT promoted the activities of enzymes in the AsA–GSH cycle (APX, MDHAR, DHAR and GR), which can effectively increase ROS scavenging (Palma and Rio 2006). Previous results showed that MT promoted the activities of enzymes in the AsA–GSH cycle in cucumber chloroplasts exposed to cold stress (Zhao et al. 2016). Interestingly, the activities of these enzymes increased more than POD, CAT and GPX (Figure 2). The results are consistent with a recent study on tomato under NaCl stress, which showed that exogenous application of MT induced the AsA–GSH cycle by activating antioxidant enzymes and non-antioxidant enzymes, and these changes led to higher tolerance to environmental stress (Siddiqui et al. 2019). Therefore, application of MT alleviated

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**Figure 4.** Effects of MT on light energy absorption and utilization parameters under salt stress in tomato. The values were determined after plants were treated with 150 mM NaCl for three days, and were presented as means ± standard deviation (n ≥ 3).
The oxidative damage caused by NaCl stress by promoting the activities of SOD and enzymes involved in the AsA–GSH cycle, thereby balancing ROS generation and scavenging and reducing the damage on PSII. This may be a special protective mechanism that MT plays a role in tomato under NaCl stress.

**MT inhibited ROS production by promoting PSII repair**

The information on the photochemistry of the donor and acceptor sides of PSII can be obtained using chlorophyll fluorescence technology (Strasser and Strasser 1995; Strasser, 1997; Lin et al. 2009). In this study, chlorophyll fluorescence was conducted to analyze the possible mechanism of MT in alleviating PSII from photosynthetic non-stomatal factors under NaCl stress. The increase of the relative variable fluorescence $V_J$ at the K point (300 μs) was due to the inhibition of the water-splitting system and the partial suppression of the acceptor side before QA (Figure 3(D,E)). The K step of the chlorophyll a fluorescence transient (quantified as $V_K$) is an indicator of OEC injury in the photosynthetic apparatus (Strasser 1997; Zhang et al. 2017). The K phase was clearly visible in the standardized O-J curve (Figure 3(D,E)), and $V_K$ also increased significantly under NaCl stress (Figure 3(D,E)). The increase in $V_K$ is considered to be a sign of damage to the oxygen-evolving complex (OEC) on the electron donor side of PSII (Zhang et al. 2017). However, the increase in $V_K$ was reduced when exogenous MT was applied (Figure 3(D,E)). In addition, O₂ release capacity and the degradation of the PsbO protein (OEC) in plant leaves...
decreased significantly under NaCl stress (Figure 3(D,E)). The decrease in the activity of OEC at the electron donor side of PSII would lead to incomplete cleavage of water, producing $\text{H}_2\text{O}_2$. The blockage of the electron transfer at the acceptor side of PSII would lead to leakage of the excess electrons, which can attack the free $\text{O}_2$ in cells and generate superoxide anions. Reactive oxygen species such as $\text{H}_2\text{O}_2$ and superoxide anions can increase the peroxidation of cell membranes, leading to electrolyte extravasation, which can affect the normal functioning of cell membranes (Venkatesh et al. 2012).

The relative variable fluorescence at the J-step ($V_f$) represents the kinetic bottleneck of the electron transport chain, resulting in the momentary maximum accumulation of $Q_V$ (Li et al. 2009; Strasser and Strasser 1995). The increase of $V_f$ in the OJIP curve (Figure 3(B,C)) indicated that NaCl stress suppressed the acceptor side of PSII (Figure 3(B,C)), while the application of exogenous MT lessened the damage on the donor and acceptor sides of PSII (Figure 3(B,C)). The degradation of D1 and PsbO in PSII were alleviated in plants that were pre-treated with MT under NaCl stress (Figure 4). This confirmed that MT might promote the electron transport of the PSII acceptor side by protecting OEC function of the PSII donor side and prevent the degradation of D1 (Zhou et al. 2016).

**Conclusion**

This study evaluated the response mechanism of PSII to NaCl stress in tomato leaves (Figure 5). The donor and acceptor sides of the photosystems were damaged by NaCl stress, indicating that photoinhibition and photoinhibition-like damage had occurred. PsbO and D1 proteins in donor and acceptor sides of PSII were degraded and there was an over-accumulation of ROS. Application of exogenous MT increased NaCl tolerance in tomato by reducing chlorophyll, balancing the distribution of photosynthetic electrons, thereby suppressing ROS production, and by promoting the activities of enzymes involved in the ascorbate–glutathione cycle to enhance ROS scavenging. Taken together, these mechanisms suppressed damages caused by salt stress in plants that were treated with MT (Figure 6).

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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