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Exogenous melatonin improves growth and photosynthetic capacity of cucumber under salinity-induced stress

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Abstract

Melatonin mediates many physiological processes in animals and plants. To examine the potential roles of melatonin in salinity tolerance, we investigated the effects of exogenous melatonin on growth and antioxidant system in cucumber under 200 mM NaCl stress conditions. The results showed that the melatonin-treated plants significantly increased growth mass and antioxidant protection. Under salinity stress, the addition of melatonin effectively alleviated the decrease in the net photosynthetic rate, the maximum quantum efficiency of PSII, and the total chlorophyll content. Our data also suggested that melatonin and the resistance of plants exhibited a concentration effect. The application of 50–150 μM melatonin significantly improved the photosynthetic capacity. Additionally, the pretreatment with melatonin reduced the oxidative damage under salinity stress by scavenging directly H_2O_2 or enhancing activity of antioxidant enzymes (including superoxide dismutase, peroxidase, catalase, ascorbate peroxidase) and concentrations of antioxidants (ascorbic acid and glutathione). Therefore, the melatonin-treated plants could effectively enhance their salinity tolerance.

Additional key words: antioxidant enzymes; chlorophyll fluorescence; gas exchange; growth analysis; melatonin; salt tolerance.

Introduction

Soil secondary salinization is one of the major environmental factors limiting agricultural production (Zhu 2001). High salinity stress results in ion toxicity, osmotic stress, and production of reactive oxygen species (ROS) (Sreenivasulu *et al*. 2000). The imbalance of ROS was the important reason for oxidative damage and programmed cell death under salinity conditions (Roncarati *et al*. 2008). Plants improve their salinity resistance on the basis of a decreasing salt content, ion compartmentation, osmotic adjustment, and induction of antioxidant enzymes (Mittler 2002, Munns and Tester 2008).

Salinity stress affects the photosynthesis, the integrity of cellular membranes, and the activities of enzymes in plants (Smirnoff 1993). A large number of ROS, such as singlet oxygen, superoxide, hydrogen peroxide, and hydroxyl radicals, were generated under salinity conditions (Radyukina *et al*. 2007). Excess of ROS triggers phytotoxic reactions, such as lipid peroxidation, protein degradation, and DNA mutation (Smirnoff 1993, McKersie *et al*. 1996). To resist salt-induced oxidative stress, plants decrease ROS by upregulating activity of the antioxidative enzymes [superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), *etc*.] and enhancing the antioxidant production (ascorbate, glutathione, and polyphenolic compounds). Studies demonstrated that the ROS-scavenging mechanism can improve the resistance of plants (McKersie *et al*. 1996, Noctor and Foyer 1998).

Melatonin (MT), a tryptophan derivative, is present in almost all organisms (Hardeland *et al*. 2011). The wide distribution of MT shows that it plays an important role in the function and survival of organisms. There are many functions of MT in animals including circadian rhythm

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Abbreviations: APX – ascorbate peroxidase; AsA – ascorbate; CAT – catalase; Chl – chlorophyll; DAT – days after treatment; DM – dry mass; DTNB – 5,5'-dithio-bis-2-nitrobenzoic acid; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; FM – fresh mass; GR – glutathion reductase; GSH – reduced glutathione; GSSH – oxidized glutathione; IAA – indole acetic acid; MDA – malondialdehyde; MT – melatonin; NSC – unstressed control group; P_N – net photosynthetic rate; POD – peroxidase; ROS – reactive oxygen species; SOD – superoxide dismutase; ST – salt treatment.
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and photoperiodic reaction, elimination of different forms of free radicals, other ROS, and related products (Tan *et al*. 2007, Jung-Hynes *et al*. 2010, Motilva *et al*. 2011). In the recent decades, some functions of MT in plants were found (Arnao 2014). In plants, MT acts as a kind of plant growth regulator, similar to the role of indole acetic acid (IAA), in boosting cell expansion to promote growth (Zhang 2012). MT also plays a role in regulating a photoperiod and circadian rhythms in plants (Kolář *et al.* 2003), it attenuates photooxidation of the photosynthetic apparatus, and, at moderate concentrations, protects chlorophyll (Chl) during senescence. Additionally, many reports demonstrated that MT, as its primary function in widespread antioxidant actions (Galano *et al*. 2011, Tan *et al*. 2013, Arnao and Hernández-Ruiz 2013b, 2014), was able to alleviate the effects of environmental stress, such as low temperature (Lei *et al.* 2004), drought (Wang *et al*. 2013, Zhang *et al*. 2013), the UV-B (Afreen *et al*. 2006), and heavy metal pollution (Xu *et al*. 2010). Meanwhile, environmental stress can increase the content of endogenous MT in plants (Arnao and Hernández-Ruiz 2013a, Zhang *et al*. 2015).

Cucumber is the major vegetable in protected cultivation which is easily influenced by high salinity. Therefore, improving the salt tolerance of cucumber is important for practice in production facilities. Li *et al*. (2012) have reported that MT eased the inhibition in *Malus hupehensis* under salinity stress. But there is no report on a pretreatment by MT of cucumber during the salinity condition. Therefore, we studied the effects of melatonin on cucumber growth, photosynthetic rates, and the response to oxidative stress under salinity conditions. The results could bring a new insight into MT function in plants and provide guidance for facility vegetable cultivation of high yield and good quality.

Materials and methods

Plants and treatment: Cucumber (*Cucumis sativus* L.) cv. Jin Chun No. 4, a widely cultivated variety in China, was used for this study. In 2013, the experiments were performed from April to August in plastic greenhouses at the Horticultural Experimental Station of Northwest A&F University, Yangling, China. After germination, two seeds were planted in each black plastic pot (12 × 13 cm) filled with soil and sand. Seedlings of a similar size (two leaves) were selected and transferred to the plastic tubs (180 × 260 cm) containing 3 kg of soil. The seedlings were precultivated for 7 d to allow adaption for new conditions. After precultivation, the seedlings were treated with different concentrations of MT solution (0, 50, 100, 150, and 200 μM) and the addition of MT continued until the whole experiment was finished. After 7 d of treatment with MT, the seedlings were treated with 200 mM NaCl. Each treatment contained three replicates of ten plants. Seedlings were treated for 12 d. After 0, 3, 6, 9, and 12 d (DAT) after salt treatment (ST), net photosynthetic rate (P_N) and maximal quantum yield of PSII photochemistry (F_v/F_m) were recorded, while leaves were sampled from plants. All leaf samples were quickly frozen in liquid nitrogen and stored at –80°C.

Growth of the seedlings: To verify the mitigative effects of MT on salinity stress, we measured the growth status. When the second leaf expanded, the seedlings were divided into two groups. The control group was just kept sprinkling normally, the other group was treated with 100 μM MT (this concentration was found to be quite effective in relieving salinity stress), and the application of MT continued until the end of the whole experiment. After the precultivation, the groups of control and MT-pretreated seedlings were randomly divided into unstressed control group (NSC) and 200 mM NaCl-stressed group (ST). This resulted in four new experiment groups:

Normal control	-
Salinity treatment	200 mM NaCl
Melatonin control	100 μM melatonin
Combined treatment	100 μM melatonin + 200 mM NaCl

Each treatment contained three replicates of ten plants. Seedlings were treated by ST for 12 d. After 12 DAT, the growth index was measured.

After MT and ST for 12 d, the plant height and leaf area were measured. The second to fifth leaves from the top were sampled from ten plants per treatment. After recording the fresh mass (FM), dry mass (DM) was determined by drying the tissues at 80°C for three days.

Photosynthetic rates and Chl fluorescence: P_N was measured with a portable system (*Li-6400*, *LICOR*, Lincoln, NE, USA), on the second leaves from top between 9:00 and 11:00 h. All measurements were carried out at 1,000 μmol(photon) m^{-2} s^{-1}, and constant airflow rate of 500 μmol s^{-1}. The cuvette CO_2 concentration was 380 ± 10 μM, the temperature was 25 ± 1°C, and a vapor pressure deficit of 2.0 to 3.4 kPa. Measurements of P_N and Chl fluorescence were carried out on the same leaf. Chl fluorescence was measured between 22:20 and 23:00 h (samples were dark-adapted for 2 h). F_0 was measured under weak modulated irradiation (< 0.1 μmol m^{-2} s^{-1}). A 600-ms saturating flash (> 7,000 μmol m^{-2} s^{-1}) was then applied to determine F_m and to calculate F_v/F_m, where $F_v = F_m – F_0$.

Measurements of Chl, malondialdehyde (MDA), electrolyte leakage, and H_2O_2: Chl was extracted with 80% acetone, analyzed for the contents on a spectrophotometer (*UV-1750*, *SHIMADZU*, Japan) according to the method of Lichtenthaler and Wellburn (1983). MDA was determined according to the methods described by

Hodges *et al*. (1999). Electrolyte leakage of the leaves was determined according to the method of Dionisio-Sese *et al*. (1998). H_2O_2 was extracted with 5% (w/v) trichloroacetic acid and measured according the methods of Patterson *et al*. (1984).

Extraction and assays of antioxidant enzymes: Leaf samples (0.1 g) were ground in a chilled mortar with 1% (w/v) polyvinylpolypyrrolidone and then homogenized with 1.2 mL of 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM EDTA-Na_2 and 0.3% Triton X-100. For the APX assay only, 1 mM ascorbate was added to this mixture. Each homogenate was centrifuged at 13,000 × *g* for 20 min at 4°C and the supernatant was used for the following assays.

SOD (EC 1.15.1.1) activity was determined by the methods of Zhang *et al*. (2013). The reaction mixture (3 mL) contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 mM NBT, 2 mM riboflavin, 0.1 mM of EDTA, and 100 µL of the enzyme extract. The reaction mixtures were illuminated for 25 min at a light intensity of 90 µmol(photon) m^{-2} s^{-1}. The SOD activity was assayed by determining the ability of the enzyme extracts to inhibit the photochemical reduction of NBT as monitored at 560 nm. One unit of SOD activity was defined as the amount of enzyme needed to contain NBT photochemical reduction 50%. SOD activity of the extracts was expressed in µmol min^{-1} g^{-1}(FM).

CAT (EC 1.11.1.6) activity was measured by monitoring the decrease in absorbance at 240 nm because of the decomposition of H_2O_2 (the extinction coefficient of 39.4 mM^{-1} cm^{-1}) in 1.0 mL of reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2, and 20 µL of enzyme extract. This reaction also was initiated by adding H_2O_2 (Aebi 1984). CAT activity of the extracts was expressed in µmol min^{-1} g^{-1}(FM).

POD (EC 1.11.1.7) was determined at 470 nm (the extinction coefficient of 25.2 mM^{-1} cm^{-1}) in 1.0 mL of a reaction mixture containing 100 mM potassium phosphate buffer (pH 6.0), 16 mM guaiacol, 5 µL of 10% (w/v) H_2O_2, and the enzyme extract. This reaction was started by adding the enzyme extract (Rao *et al*. 1996). POD activity of the extracts was expressed in µmol min^{-1} g^{-1}(FM).

APX (EC 1.11.1.11) activity was assayed by the decrease in absorbance at 290 nm as reduced ascorbate was oxidized (the extinction coefficient of 2.8 $mM^{-1}\cdot cm^{-1}$) in 1.0 mL of a reaction mixture containing 50 mM Hepes-KOH (pH 7.6), 0.1 mM EDTA-Na_2, 0.5 mM ascorbate, 0.2 mM H_2O_2, and 20 µL of enzyme extract. This reaction was initiated by adding H_2O_2 (Nakano and Asada 1981). APX activity of the extracts was expressed in µmol min^{-1} g^{-1}(FM).

Extraction and assays of antioxidants: Ascorbic acid (AsA) was measured by the method of Logan *et al*. (1998). Leaf samples (0.1 g) were ground in a chilled mortar and then homogenized with 1.5 mL of ice-cold 6% (w/v) $HClO_4$. The homogenate was centrifuged at 10,000 × *g* for 10 min at 4°C and the supernatant was used for the assays. AsA was assayed spectrophotometrically at 265 nm in 200 mM sodium acetate buffer (pH 5.6), before and after 15 min incubation with 1.5 units of AsA oxidase.

Glutathione was determined according to the methods of Griffith (1980). Leaf samples (0.1 g) were ground in a chilled mortar, homogenized with 1.5 mL of 5% sulfosalicylic acid and then centrifuged at 14,000 × *g* for 10 min at 4°C and the supernatant was used for the assays. For total reduced glutathione (GSH), the 1 mL of the reaction mixture including 20 µL extract, 200 µL of potassium phosphate buffer (pH 7.7), 560 µL of 10 mM EDTA, 100 µL of 6 mM 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) (dissolved in potassium phosphate buffer), and 100 µL of 2.1 mM NADPH. The reaction was initiated by adding 1 unit of glutathion reductase (GR) and monitored the increase in absorbance at 412 nm. For oxidized glutathione (GSSG), 20 µL of the extract was mixed with 200 µL of buffer A and 4 µL of 2-vinylpyridine, and the mixture was incubated at room temperature for 30 min to remove GSH by derivatization. GSSG was assayed by the same way as for total GSH previously. GSH content was determined by subtracting the value for GSSG from the total GSH content.

Statistical analysis: The data were analyzed by one-way analysis of variance (*ANOVA*), followed by *Duncan*'s multiple range tests. A *P*-value <0.05 was regarded as significant, and data were presented as the means ± SD, $n = 5$.

Results

Effects of MT treatment on growth of cucumber under salinity stress: As shown in Table 1, the growth index of cucumber seedlings significantly improved after MT treatment for 19 d (precultivation for 7 d, ST for 12 d), compared with nontreated controls. Salt stress induced by 200 mM NaCl significantly inhibited the growth of cucumber seedlings. However, the application of 100 µM MT could effectively alleviate this phenomenon. This feature was proved by reductions in the plant height and leaf area (Table 1). The results indicated that applied MT not only increased the resistance of cucumber to salt stress, but also improved the growth of the plants without stress.

Effects of MT on P_N under salinity stress: To investigate the influence of MT application on salt stress in cucumber, MT of different concentrations was discontinuously applied to roots for one week before ST. As shown in Fig. 1, ST declined P_N by 15.7%, whereas it was alleviated in the plants precultivated with MT. MT alleviated the decline of P_N in evident concentration-dependent manner.

Table 1. Effects of 100 μM melatonin (MT) treatment on growth of cucumber under salt stress. The cucumber seedlings were precultivated with different concentrations of melatonin, and after 7 d of pretreatment with melatonin, the seedlings were treated 12 d with 200 mM NaCl. Data represent means ± SD, n = 5. Different *lowercase letters* mean significant difference according to a *Duncan*'s multiple range test (P<0.05).

Treatment	Plant height [cm]	Leaf area [cm^2]	Fresh quality [g]	Dry quality [g]
0 μM MT	14.07 ± 0.60^b	189.56 ± 3.47^b	30.00 ± 1.02^b	2.63 ± 0.23^b
100 μM MT	16.48 ± 1.14^a	213.88 ± 17.36^a	33.37 ± 1.35^a	3.17 ± 0.31^a
0 μM MT + NaCl	10.27 ± 0.24^d	118.16 ± 13.24^d	16.56 ± 0.36^d	1.47 ± 0.04^d
100 μM MT + NaCl	11.47 ± 0.89^c	163.64 ± 5.16^c	25.85 ± 1.25^c	2.09 ± 0.09^c

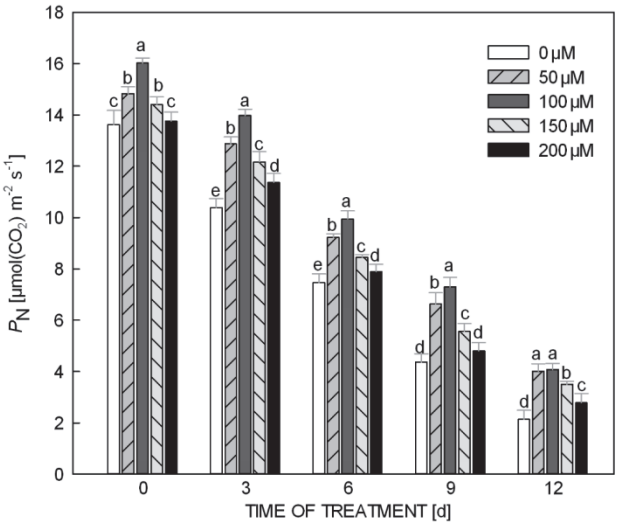

Fig. 1. Effects of different concentrations of melatonin on net photosynthetic rate (P_N) during 200 mM NaCl treatment. Data represent means ± SD, n = 5. Different *lowercase letters* mean significant differences according to a *Duncan*'s multiple range test (P<0.05).

The addition of 100 μM MT showed the greatest reversal of ST-induced inhibition of P_N, followed by 200 μM MT with values close to the nontreated plants (Fig. 1).

Effects of MT on F_v/F_m and Chl content under salinity stress: F_v/F_m (Fig. 2*A*) decreased under salinity conditions. At day 0 of ST, different concentrations of MT did not affect the F_v/F_m in the precultivated plants. However, the application of MT alleviated the decline in the MT-treated plants with prolonged duration of the experiment. Salinity stress seriously inhibited the ability of cucumber plants to take up water, causing significant degradation of Chl. At 12 DAT with NaCl, the total Chl content decreased by 27.7% in the ST-treated plants, whereas it was 20.6% in the 100 μM MT-treated plants (Fig. 2*B*). This showed that the application of MT may significantly inhibit the Chl degradation.

Effects of MT on relative electrolyte leakage, MDA and H_2O_2 content under salinity stress: The electrolyte leakage and MDA concentration both act as indicators in assessing an extent of membrane damage in leaves. Compared with the control, both the electrolyte leakage and MDA concentration were reduced in leaves treated with MT at 0 DAT of ST, especially those treated with 100 μM MT (Fig. 3*A*,*B*). Under ST condition, the plants watered with MT showed significantly lower indexes than the control. The exogenous MT significantly inhibited the increase of the MDA under ST (Fig. 3*B*).

Additionally, H_2O_2 concentration is an indicator of the status of the ROS-scavenging capacity in plants under oxidative stress. ST increased the H_2O_2 concentration by 37.5% on 12 DAT; however, the MT-treated plants showed relatively low content of H_2O_2 during the whole experiment (Fig. 3*C*). This demonstrated that exogenous MT retarded the increase in the H_2O_2 content in the ST-treated plants.

Effects of MT on the activity of SOD, POD, CAT and APX under salinity stress: The main protective enzymes in an enzymatic defense system, SOD, POD, CAT can effectively scavenge the active oxygen species. The activity of antioxidant enzymes changed evidently and presented similar trends during the ST period. At 0 DAT, the precultivation with MT for one week significantly increased the activities of the antioxidant enzymes. The activities of SOD, POD, CAT, and APX under ST condition decreased by at least 31.7% in the non-MT-treated plants at 12 DAT (Fig. 4). Additionally, the data also showed that 50–150 μM of the MT-treated plants had significantly higher activities than the non-MT-treated leaves under ST. Compared with the non-MT-treated plants, 200 μM MT-treated plants did not show significant differences in the activities of SOD, POD, CAT, and APX.

Effects of MT on AsA and GSH content under salinity stress: AsA and GSH, two important antioxidants, play a main role in maintaining the balance of free radicals. As

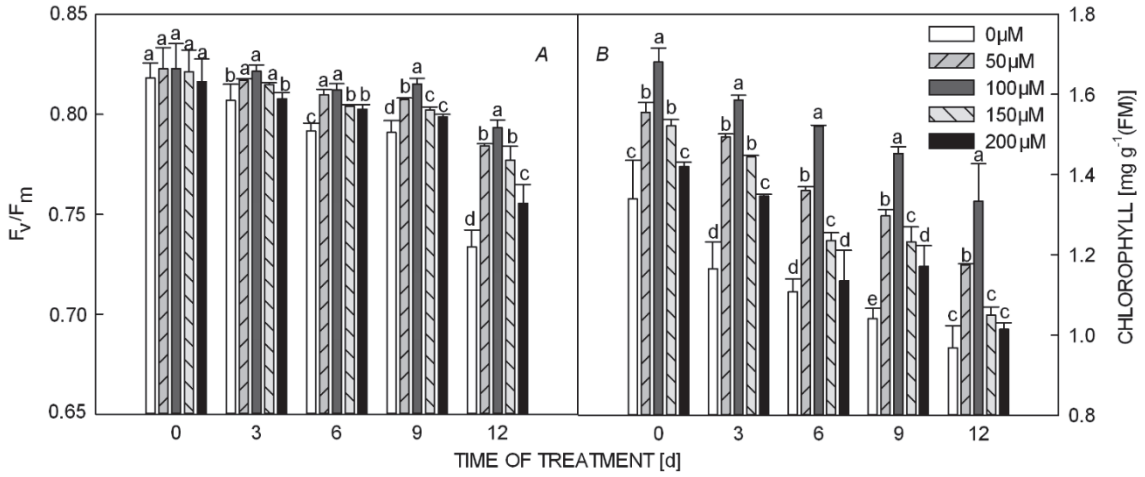

Fig. 2. Effects of different concentrations of melatonin on maximal quantum yield of PSII photochemistry (F_v/F_m) (*A*) and chlorophyll content (*B*) during 200 mM NaCl treatment. Data represent means ± SD, $n = 5$. Different *lowercase letters* mean significant differences according to a *Duncan*'s multiple range test ($P<0.05$).

shown in Fig. 5, the AsA and GSH concentrations were reduced in the leaves as the stress was prolonged. However, the addition of 50–100 μM exogenous MT could significantly alleviate the decline of AsA and GSH contents under the salinity conditions. At 12 d DAT of NaCl treatment, AsA and GSH concentrations in leaves treated with 100 μM MT were 1.7 and 1.3-folds, respectively, of those in the non-MT-treated plants. Thus, our data indicated that the application of MT could help maintain higher concentrations of AsA and GSH in leaves during ST.

Discussion

Salinity is one of the main abiotic stresses limiting plant growth and biomass production (Jampeetong and Brix 2009, Gómez-Pando *et al*. 2010). Under salinity stress conditions, all important processes, such as photosynthesis, and energy and lipid metabolism, are affected (Zhang *et al*. 2013). In this study, we found that almost all of the growth parameters measured significantly decreased in the plants during the ST period. However, the application of MT significantly alleviated a decline of those indexes. Several studies have shown that MT can act as a potential modulator of plant growth and development in a dose-dependent manner (Hernández-Ruiz *et al*. 2004, Afreen *et al*. 2006, Arnao and Hernández-Ruiz 2007, Hernández-Ruiz 2008). We showed that 100 μM MT could effectively increase the growth and biomass production (Table 1). It suggested that exogenous MT possesses the ability to alleviate the ST-inhibited growth in plants.

Photosynthesis is a basic physiological process to maintain plant life activities (Parida and Das 2005). In our study, we found that P_N lowered quickly under ST conditions, but after the application of MT, this trend could be significantly slowed down (Fig. 1). Our results suggested that MT helped maintain high photosynthetic efficiency in plants (Wang *et al*. 2012).

In this study, the MT treatment improved F_v/F_m (Fig. 2*A*) and the total Chl content (Fig. 2*B*). It indicated that MT could alleviate the effect of salinity stress on photosynthesis. Chl play an essential role in photosynthesis. In the present study, the Chl content decreased by 27.7% under salinity stress. Whereas it was 20.6% in the 100 μM MT treatment groups. This indicated that the MT treatment enhanced synthesis and slowered decomposition of Chl under the salinity stress.

The balance between formation and elimination of ROS was destroyed under salinity stress conditions, causing the accumulation of free radicals and cellular damage (Foyer and Noctor 2000). Our data showed that ST increased the generation of H_2O_2 in the leaves, but applying MT could depress this trend. Similar conclusions have been reported with plants under oxidative stress (Zhang 2012). The accumulation of H_2O_2 resulted in the lipid peroxidation, which causes membrane damage and electrolyte leakage (Khalid *et al*. 2014). Electrolyte leakage is a good indicator of assessing the integrity of membrane during abiotic stress. A reduction of electrolyte leakage, concentrations of MDA and H_2O_2 suggested that MT showed the protective effect against membrane damage under ST-induced conditions.

To reduce the oxidative stress, plants possess defense systems including antioxidants and antioxidant enzymes to scavenge excessive ROS. As the part of this system, antioxidant enzymes play the dominant role in the defense mechanisms. The activities of antioxidant enzymes, such as SOD, POD, CAT, and APX, first increased in the leaves before decreasing gradually as the stress was prolonged. Those observed that the activities of scavenging H_2O_2 enzymes decreased clearly under the salinity stress. MT may directly scavenge H_2O_2 and maintain H_2O_2

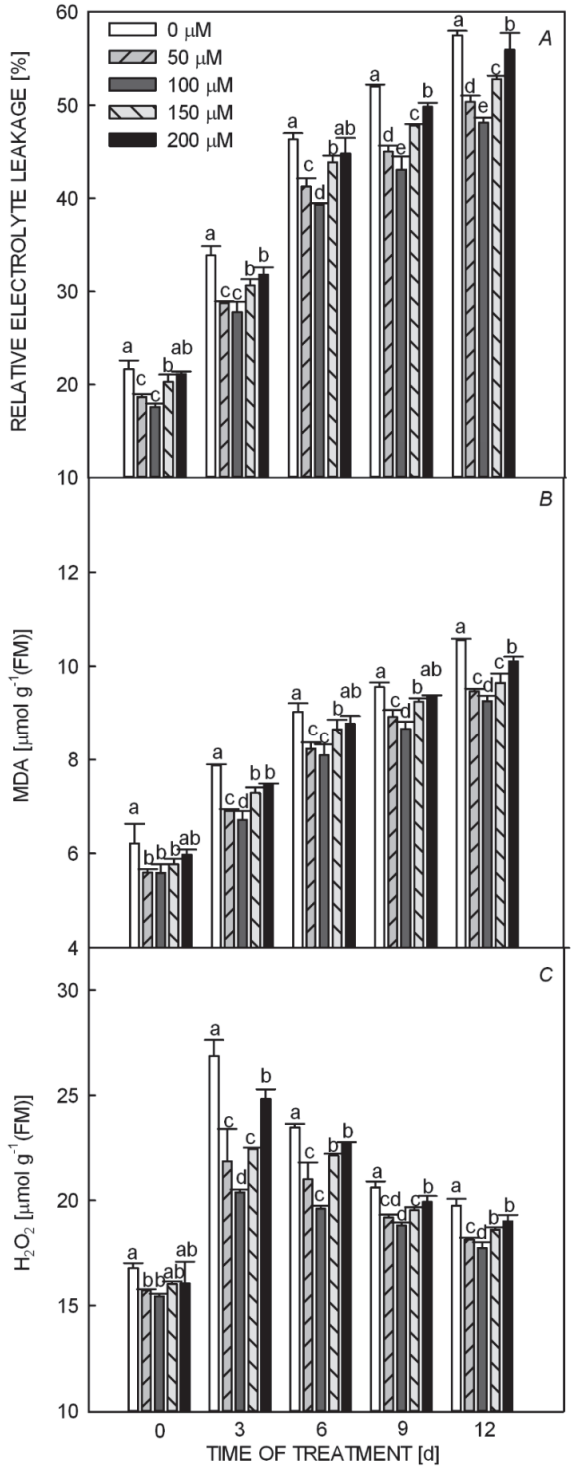

Fig. 3. Effects of different concentrations of melatonin on electrolyte leakage (*A*), malondialdehyde (MDA) content (*B*), and H_2O_2 content (*C*) during 200 mM NaCl treatment. Data represent means ± SD, $n = 5$. Different *lowercase letters* mean significant differences according to a *Duncan*'s multiple range test ($P<0.05$).

concentrations at steady levels (Choi *et al*. 2011). In our study, the application of exogenous MT inhibited the accumulation of H_2O_2, which might be a result of direct ROS-scavenging by MT and the elevated CAT and POD activities. Our data confirmed that MT was involved in ROS-scavenging in the ST leaves. The melatonin molecule is well known as an endogenous free radical scavenger (Reiter *et al*. 2007) and a broad-spectrum antioxidant (Arnao and Hernández-Ruiz 2014). The results of the present study indicated that the activities of SOD, POD, CAT, and APX were significantly raised in the MT-treated plants. Also, the application of MT elevated P_N, contents of MDA and H_2O_2, activities of antioxidant enzymes, and contents of antioxidants at 0 DAT. These results supported the theory that MT might protect the plants from stress conditions and prevent injuries induced by oxidative stress at the cellular level.

AsA, a widespread antioxidant in plant tissues, shows a direct response to ROS. GSH is also one of the ROS scavengers. The contents of AsA and GSH changed significantly when the plants encountered environmental stress (Wang *et al*. 2013). In cucumber leaves, AsA and GSH contents were reduced during the salinity stress. However, MT pretreatment suppressed the reduction of AsA and GSH contents. We suggest that MT might accelerate the regeneration of AsA and GSH and maintain the higher concentrations of AsA and GSH.

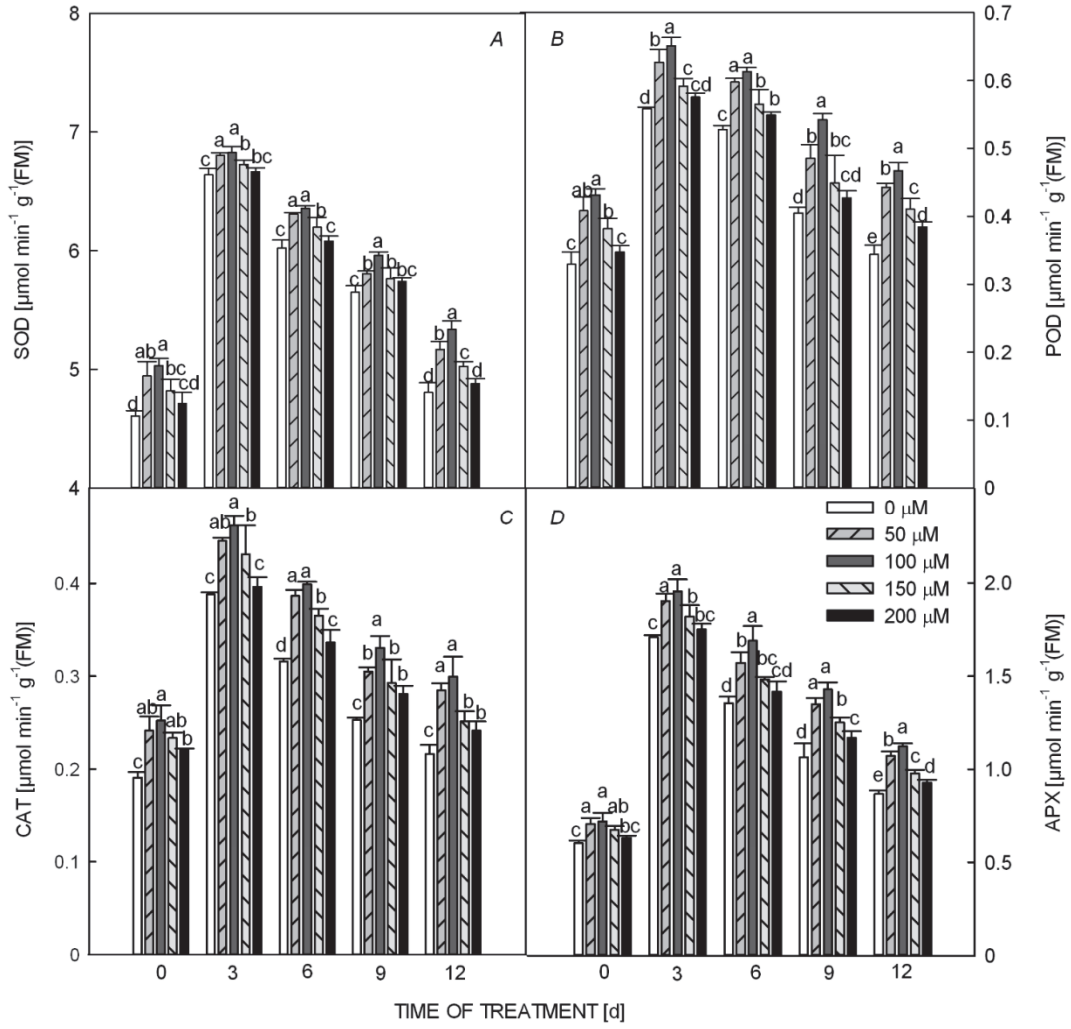

Fig. 4. Effects of different concentrations of melatonin on the activity of superoxide dismutase (SOD) (*A*), peroxidase (POD) (*B*), catalase (CAT) (*C*), and ascorbate peroxidase (APX) (*D*) during 200 mM NaCl treatment. Data represent means ± SD, $n = 5$. Different *lowercase letters* mean significant differences according to a *Duncan*'s multiple range test ($P<0.05$).

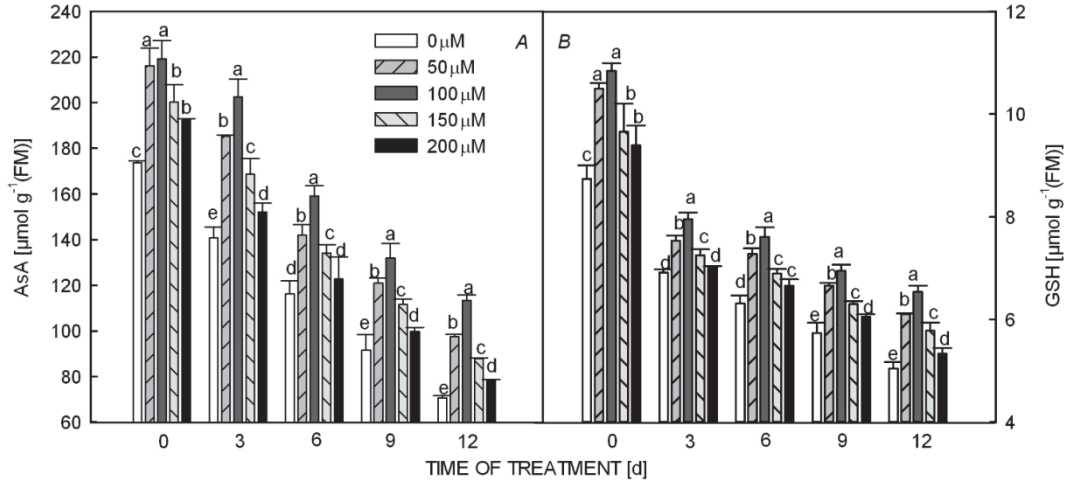

Fig. 5. Effects of different concentrations of melatonin on ascorbate (AsA) (*A*) and glutathione (GSH) (*B*) content during 200 mM NaCl treatment. Data represent means ± SD, $n = 5$. Different *lowercase letters* mean significant differences according to a *Duncan*'s multiple range test ($P<0.05$).

In summary, the application of melatonin increased salinity tolerance in cucumber plants. On one hand, the addition of melatonin improved the growth and biomass production and enhanced photosynthesis. On the other hand, melatonin might effectively scavenge enhanced ROS and improve the activities of antioxidant enzymes. We hope that the positive effect of melatonin on salinity tolerance offers new opportunities for its use in agriculture.

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