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ENHANCING SALT TOLERANCE IN MELON BY EXOGENOUS APPLICATION OF MELATONIN AND CA^{2+}

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Abstract

Melatonin and Ca^{2+} have been implicated in growth, development and stress responses in plants. Here, we report the effects of exogenous melatonin and Ca^{2+}on growth, photosynthetic capacity, antioxidant potential and ion homeostasis in melon (*Cucumis melon* L. cv. Yangjiaosu) seedlings under salt stress. It was observed that salt stress significantly inhibited growth in melon seedlings, while exogenous application of melatonin and Ca^{2+} alleviated the inhibition. Further analysis showed that exogenous application of melatonin or Ca^{2+} promoted photosynthetic rate and water use efficiency in salt-stressed melon seedlings. In addition, exogenous melatonin or Ca^{2+} enhanced accumulation of Ca^{2+}, but reduced accumulation of Na^{+} in both leaves and roots of melon seedlings under salt stress. Furthermore, exogenous melatonin or Ca^{2+} reduced oxidative damage, as demonstrated by decreased electrolyte leakage and MDA content, by stimulating activities of antioxidant enzymes, including SOD, POD, CAT and APX, in salt-stressed melon seedlings. Collectively, these results suggest that exogenous melatonin or Ca^{2+} acts in the alleviation of salt stress by improving photosynthesis, maintaining ion homeostasis and elevating antioxidant capacity in melon seedlings. Our work also provides a case study and melatonin or Ca^{2+} may serve as useful agent to relieve salt stress in agricultural production.

Key words: Melon (*Cucumis melo* L.); Salt stress; Melatonin; Ca^{2+}; Antioxidative capacity.

Introduction

Salinity is a widespread environmental stress factor, limiting growth and development in plants and influencing species distribution (Qin *et al*., 2010). It has been a major issue in agricultural production in many arid and semiarid regions (Kaya *et al*., 2013). Excessive NaCl reduces growth, impairs photosystems and accelerates production of reactive oxygen species (ROS) in crops (Khan and Hemalatha, 2016; Liu *et al*., 2018). To cope with the deleterious effects of salt stress, plants have evolved with multiple strategies, including increased activities of antioxidant enzymes and enhanced accumulation of non-enzymatic antioxidants (Mittler, 2002; Jan *et al*., 2017).

Melatonin was first identified as a hormone playing diverse roles in animals and humans. In recent years, it has been found present in a large number of plant species and has been demonstrated to play crucial roles in growth, development and responses to a variety of environmental stresses in plants(Hardeland *et al.*, 2011; Janas & Posmyk, 2013). Extensive studies have been done to elucidate the role of melatonin in plants. Previous studies have shown that melatonin functions as a growth promoter possibly due to its structural similarity with auxin. Further, accumulating evidence is supporting that this ubiquitous molecule plays a key role in the protection of plants against a plethora of biotic and abiotic stresses (Zhang *et al.*, 2015).

Ca^{2+} is not only required as a nutritional element, but also acts as a second messenger, mediating a series of physiological responses to environmental stimuli (Chinnusamy, 2004). It has been widely accepted that Ca^{2+} plays a role in alleviating abiotic stresses, such as drought, extreme temperatures and salinity (Kang & Saltveit, 2001). Salt stress seriously affects development, growth and yield in plants, while Ca^{2+} improve performance in salt-stressed plants (Chen *et al*., 2007).

Edible melon (*Cucumis melo* L.) is an important and widely cultivated fruit-type vegetable crop. Salt stress is a major environmental stress that limits melon production worldwide. This study was aimed to investigate the effects of MT and Ca^{2+} on growth, andphysiological and biochemical changes of melon under salt stress. This study may provide useful information for the application of melatonin and Ca^{2+} in agricultural production in regions with soil salinity.

Materials and Methods

Plant materials: Melon (*Cucumis melo* L. cv. Yangjiaosu) was used for this study which is a widely cultivated variety in China. The experiments were performed from March to September 2017 in a greenhouse at cultivation Station of science and technology of Anhui Science and Technology University, Fengyang, China.

Melon seeds were germinated in petri dishes at 28°C in thermotank for 2 days. The germinated seeds were then sown into a 30-hole tray and maintained in greenhouse. Daytime temperature was controlled from 25°C to 30°C, and relative humidity was 80% with a photoperiod of 14 h. After 25 days, seedlings with two fully expanded true leaves were transferred to growth chambers with the following growth conditions: 28°C/20°C day/night, 75% relative humidity, 14 h photoperiod.

Salt stress treatment: After 4 days recovery in growth chamber, seedlings were randomly assigned to different treatment groups. CK: the control; SS: 100 $mmol \cdot L^{-1}$ NaCl treatment; SS+MT: 100 $mmol \cdot L^{-1}$ NaCl + 100 $\mu mol \cdot L^{-1}$ MT; SS+Ca: 100 $mmol \cdot L^{-1}$ NaCl + 3 $mmol \cdot L^{-1}$ Ca^{2+}; SS+Ca+MT: 100 $mmol \cdot L^{-1}$ NaCl + 100 $\mu mol \cdot L^{-1}$ MT+ 3 $mmol \cdot L^{-1}$ Ca^{2+}. The seedlings were pre-treated with 100 $\mu mol \cdot L^{-1}$ MT, 3 $mmol \cdot L^{-1}$ Ca^{2+}, or mock solutions. Two days after, seedlings with three true leaves were transferred into nutrient solution containing 100 $mmol \cdot L^{-1}$ NaCl for salt stres, or the same solution without salt as a control. The leaves and roots were harvested at 5th and 10th day for further analysis. The net photosynthetic rate (Pn) and values for chlorophyll content were recorded.

Measurement of Chlorophyll content: Chlorophyll of melon leaves was extracted in 80% acetonein the dark and chlorophyll content was measured as previously described (Arnon, 1949).

Measurement of photosynthetic characteristics: After 10 days salt stress treatment, the net photosynthetic rate (Pn) and transpiration rate (Tr) of the second functional leaf of muskmelon seedlings were measured using a photosynthetic system (CIRAS-2, PPSYTEMS) with 400 ± 4 $\mu L \cdot L^{-1}$ CO2, 25 ± 0.5ºC and 800 ± 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photons flux density.

Water use efficiency was calculated using used the following formula: Water use efficiency = Pn/Tr

Measurements of growth: Stem diameter, plant height, root and shoot dry weights, and total dry weight were measured at day 10 following salt stress.

Mineral analysis: Dry plant tissues (leaves and roots) were ground separately in Wiley mill and screened by 20 meshes. Then 0.5 g dry plant tissues were used for analysis of Na and Ca. Na and Ca concentrations were determined by dry ashing at 400°C for 24 h, dissolving the ash in 1:20 HNO_3 , and assaying the solution obtained by using of an inductively coupled plasma emission spectrophotometer (ICP Iris; Thermo Optek, Milano, Italy) (Isaac & Johnson, 1998).

Measurement of activities of antioxidant enzymes: Frozen leaf were ground into fine powder in a mortar and pestle in liquid N_2. The powder was used for enzyme extraction. to extract antioxidant enzymes. The extraction homogenate was centrifuged at 15,000g for 15 min at 4°C, which was used for the subsequent enzyme assays (Jiang & Zhang, 2002).Peroxidase (POD) and catalase (CAT) activities were determined by Rao *et al*., (1996). Superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities were determined by Zhu *et al*., (2004).

Measurements of MDA content and electrolyte leakage: Malondialdehyde (MDA) content was determined according to Sun *et al*., (2006). Electrolyte leakage was determined as previously described.

Measurements of $O_2^{\cdot-}$ and H_2O_2 content: $O_2^{\cdot-}$ content was measured according to the method of Liu *et al*., (2015). H_2O_2 content was measured according to the method of Patterson *et al*., (1984).

Results and Discussion

The influence of exogenous MT and Ca^{2+} on growth of melon seedlings under salt stress: The melon seedlings were pre-cultivated with different concentrations of MT and Ca^{2+}, and after 2 days, the seedlings were treated with 100 mM NaCl for 10 d. Data are presented as the mean values (±SE) of six independent measurements (n=6). Different lowercase letters indicate significant differences according to Duncan's multiple range tests ($p<0.05$). CK; SS: 100 mM NaCl; SS+MT: 100 mM NaCl +100 $\mu mol \cdot L^{-1}$ MT; SS+Ca: 100 mM NaCl +3 $mmol \cdot L^{-1}$ Ca^{2+}; SS+Ca+MT: 100 mM NaCl +100 $\mu mol \cdot L^{-1}$ MT+3 $mmol \cdot L^{-1}$ Ca^{2+}.

Salt stress substantially restricts plant growth and biomass production (Jampeetong & Brix, 2009). Under salt stress, key processes in photosynthesis, growth and development are adversely affected in plants (Zhang *et al*., 2013, Wang, *et al.*, 2016). In this study, salt stress significantly inhibited plant growth as exhibited by a marked decline in the dry weight of shoot and root in melon seedlings (Table 1). The exogenous application of MT or Ca^{2+} alleviated the adverse effect of salt stress on seedlings growth. Particularly, SS+Ca+MT treatment led to an increased in root dry weight by 40.5% and shoot dry weight by 37.9% compared with salt stress without MT or Ca^{2+} application. Similar results have been observed in cucumber plants. Thus, our results suggest that exogenous MT and Ca^{2+} aides in the alleviation salt-stress induced inhibition in plants.

Effects of exogenous MT and Ca^{2+} on photosynthetic characteristics of melon seedlings under salt stress: The chlorophyll content and photosynthetic parameters are generally assessed to investigate the effect of abiotic stress on the functioning of photosynthetic system (Maxwell & Johnson, 2000; Öquist & Huner, 2003). In this study, we observed that salt stress influenced chlorophyll anabolism, Pn, Tr and water use efficiency. On 10^{th} day following salt treatment, leaves from salt-stressed seedlings retained significantly lower chlorophyll, Pn, Tr, and water use efficiency compared with those from control seedlings (Fig. 1). However, exogenous application of MT or Ca^{2+} alleviated the adverse effects of salt stress chlorophyll anabolism, Pn, Tr and water use efficiency. Notably, SS+Ca+MT treatment resulted in a dramatic increase in Pn by 145.2%, Tr rate by 61.9%, and water use efficiency by 50.2 %, as compared with salt stress without MT and Ca^{2+} application, suggesting that exogenous MT and Ca^{2+} were involved in the alleviation of SS-inhibited net photosynthetic rate in plants.

Effects of exogenous MT and Ca^{2+} on antioxidant enzymes of melon seedlings under salt stress: A number of studies have established that activities of antioxidant enzymes, such as SOD, CAT, POD and APX were boosted in plants by salt stress (Zhang *et al*., 2014; Li *et al*., 2012). The effects of MT and Ca^{2+} on the activities of significant antioxidant enzymes in melon leaves were reported in Fig. 2. Exogenous MT and Ca^{2+} enhanced activities of antioxidant enzymes in melon leaves. On 5th day, SS+Ca+MT significantly increased SOD activity by 24.8 %, compared with SS treatment (Fig. 2A). Compared with NaCl stress treatment, the SS+Ca+MT treatment increased POD, CAT and APX activity by 78.6, 55.9 and 52.2% in leaves, respectively (Fig. 2B, C, D). The activities of antioxidant enzymes of 5^{th} day melon leaves were lower than those of 10th day, indicating that MT and Ca^{2+} benefits activities of antioxidant enzymes. The increased activities of antioxidant enzymes are important in the mitigation of oxidative stress induced by salinity (Jiang *et al.*, 2016).

Table 1. Effects of 100 $\mu mol \cdot L^{-1}$ MT and 3 $mmol \cdot L^{-1}$ Ca^{2+} on growth of melon seedlings under salt stress.

Treatment	Plant height (cm)	Stem diameter (cm)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
CK	13.6 ± 0.64a	0.34 ± 0.01a	8.34 ± 0.42a	2.46 ± 0.22a
SS	9.5 ± 0.47b	0.35 ± 0.02a	5.12 ± 0.26d	1.48 ± 0.07d
SS + Ca	10.9 ± 0.54b	0.35 ± 0.02a	6.18 ± 0.32c	1.74 ± 0.08c
SS + MT	11.2 ± 0.53b	0.34 ± 0.01a	6.25 ± 0.31c	1.82 ± 0.09c
SS + Ca + MT	12.3 ± 0.62a	0.36 ± 0.02a	7.06 ± 0.36b	2.08 ± 0.10b

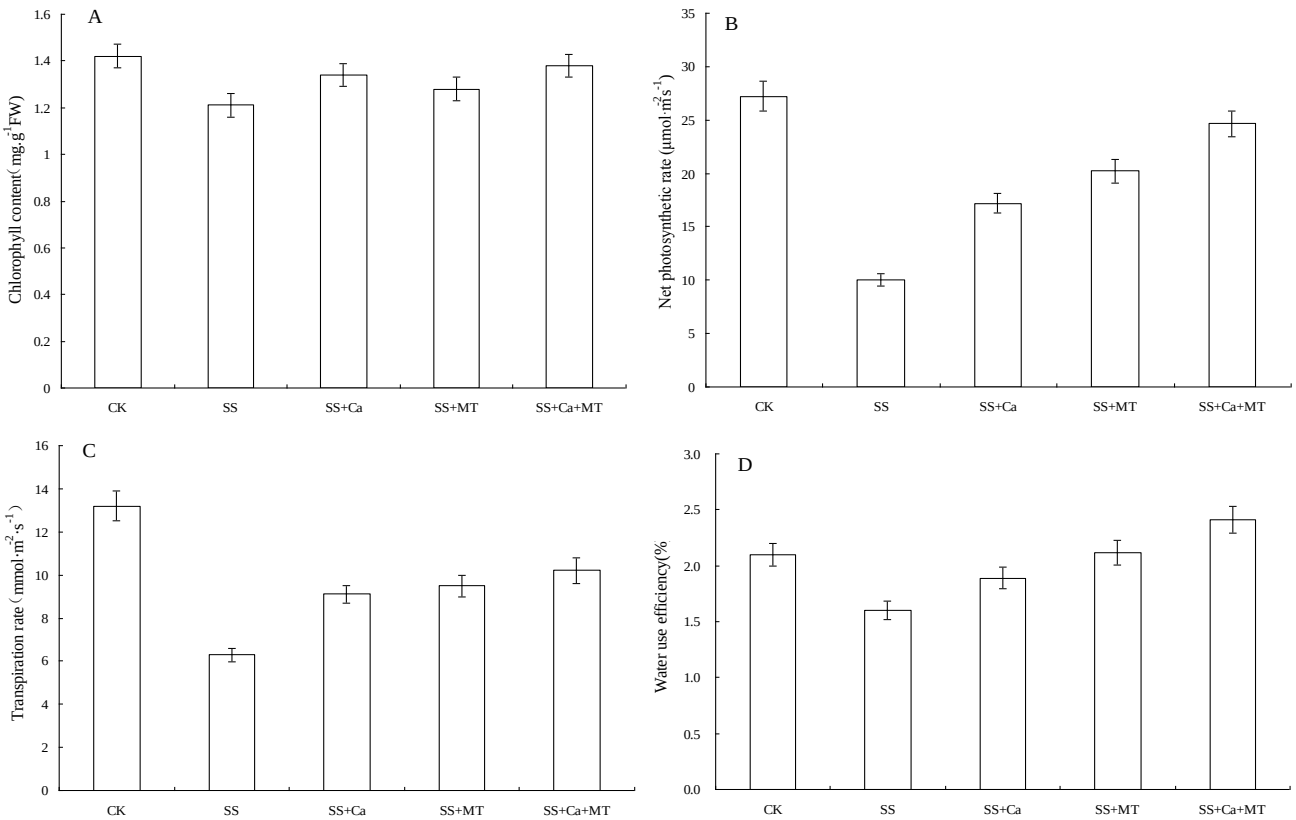

Fig. 1. Influence of exogenous 100 μmol·L^{-1} MT and 3 mmol·L^{-1} Ca^{2+} on photosynthetic characteristics of melon seedlings under salt stress. Data are presented as the mean values (±SE) of six independent measurements (n=6). CK; SS: 100 mM NaCl; SS+MT: 100 mM NaCl +100 μmol·L^{-1} MT; SS+Ca: 100 mM NaCl +3 mmol·L^{-1} Ca^{2+}; SS+Ca+MT: 100 mM NaCl +100 μmol·L^{-1} MT+3 mmol·L^{-1} Ca^{2+}.

Effects of MT and Ca^{2+} on $O_2^{\cdot-}$, H_2O_2 content, relative conductivity and MDA content under salt stress: Under salinity stress condition, the balance between production and elimination of ROS is disrupted, leading to excessive accumulation of cellular free radicals (Ardic *et al*., 2009). We found that salt stress increased the generation of $O_2^{\cdot-}$ and H_2O_2 in the leaves of salt-stressed melon seedlings, however, exogenous MT and Ca^{2+} could depress this trend (Fig. 3A, B). As compared with NaCl alone, the treatment of exogenous MT and Ca^{2+} decreased $O_2^{\cdot-}$ and H_2O_2 contents in leaves under salt stress by 42.1 and 39.8% at the 10th day, respectively (Fig. 3A, B). These results were consistent with a previous study on plants under oxidative stress (Zhang *et al*., 2012). The membrane damage and increased electrolyte leakage were as a consequence of lipid peroxidation caused by the excessive accumulation of $O_2^{\cdot-}$ and H_2O_2. (Al-Mureish *et al*., 2014). Under salt stress, MDA content and electrolyte leakage were significantly increased (Fig. 3C, D), however, addition of MT and Ca^{2+} significantly reduced both MDA content and electrolyte leakage (Fig. 3C). Among treatments, combined application of MT or Ca^{2+} was most effective in the reduction of MDA content and electrolyte leakage, suggesting the synergistic effects of MT and Ca^{2+} in the mitigation of salt stress in melon seedlings.

Effects of exogenous MT and Ca^{2+} on Na^+ and Ca^{2+} content of melon seedlings under salt stress: In the present study, it was observed that Na^+ content increased significantly, while Ca^{2+} content decreased markedly in both leaves and roots of melon seedlings under salt stress, compared with control (Fig. 4). However, MT and Ca^{2+} application significantly decreased Na^+ content and considerably increased Ca^{2+} content in leaves and roots under salt stress, especially those treated with MT and Ca^{2+} (Fig. 4). Na^+ content in leaves and roots of SS+Ca+MT was 79.5% and 70.5% of those under SS condition (Fig. 4A, B), whereas Ca^{2+} content in leaves and roots of SS+Ca+MT was 148.2% and 125.5% of those under SS (Fig. 4C, D). The Na^+ and Ca^{2+} contents of 5th day melon leaves were lower than those of 10th day. Salinity has been extensively reported to inhibit growth in most species due partly to ion imbalance or disturbance of ion homeostasis (Zhao *et al*., 2007; Jiang *et al*., 2012).

Effects of exogenous MT and Ca^{2+} on root activity and root morphology of melon seedlings under salt stress: Roots are the first organ to sense drought and salinity in soil (Wells & Eissenstat, 2003). It was found in this study that salt stress decreased the root activity of melon seedlings, while exogenous MT or Ca^{2+} could enhance the root activity under salt stress. Particularly, SS+Ca+MT treatment had the most pronounced effect on the alleviation of salt stress (Fig. 5A). The root morphology, including total root length, apical number, and root area were analyzed in control and salt-stressed melon seedlings. The 100 mM NaCl treatment significantly reduced the root growth of melon seedlings (Fig. 5) as manifested by decreased root length of melon seedlings (43.2%) in comparison with the control. Exogenous MT or Ca^{2+} stimulated root development under salt stress, as compared with NaCl alone. Notably, SS+Ca+MT increased total root length, apical number, and root area activities by 59.56, 130.32 and 62.29%, respectively.

On 10th Day, the growth status of melon seedlings with 100 μmol·L^{-1} MT and 3 mmol·L^{-1} Ca^{2+} treatment appeared better than others under salt stress (Fig. 6).

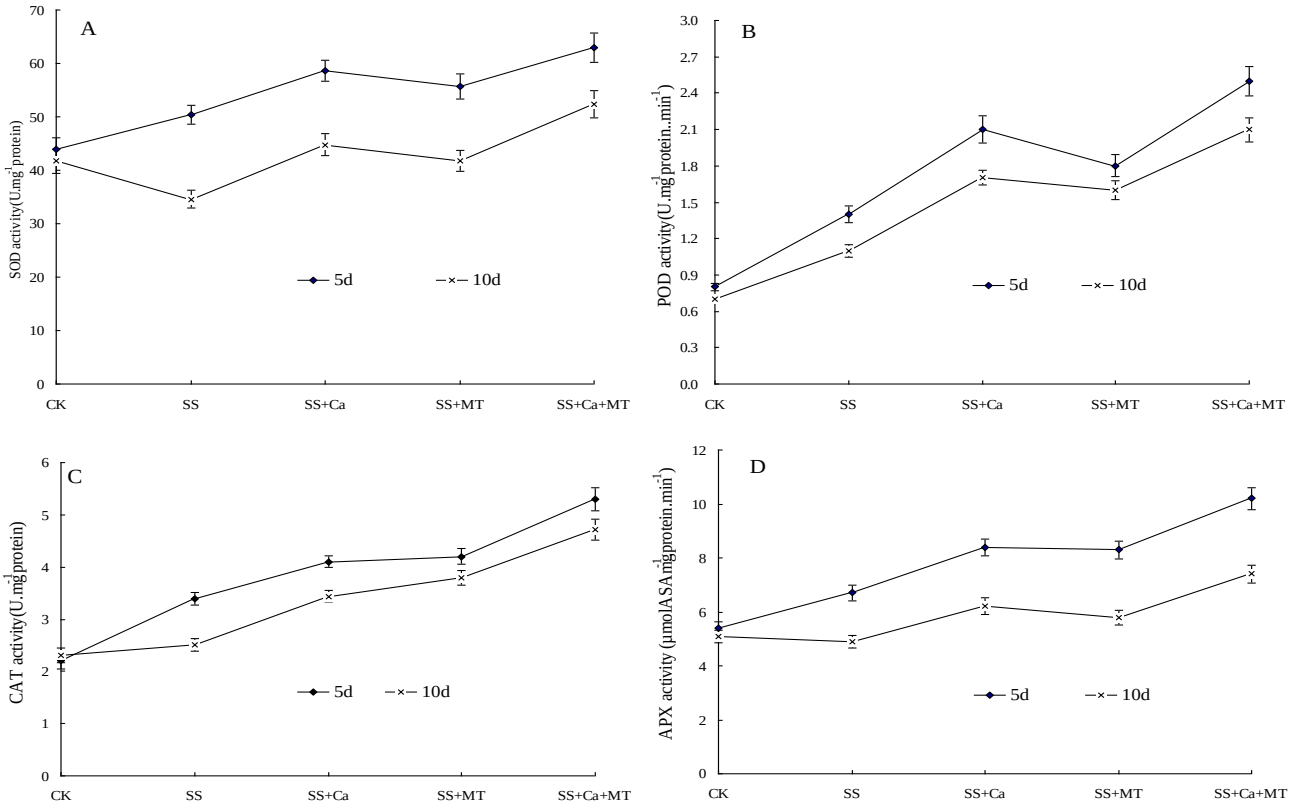

Fig. 2. Influence of exogenous 100 μmol·L^{-1} MT and 3 mmol·L^{-1} Ca^{2+} on antioxidant enzymes of melon seedlings under salt stress. CK; SS: 100 mM NaCl; SS+MT: 100 mM NaCl +100 μmol·L^{-1} MT; SS+Ca: 100 mM NaCl +3 mmol·L^{-1} Ca^{2+}; SS+Ca+MT: 100 mM NaCl +100 μmol·L^{-1} MT+3 mmol·L^{-1} Ca^{2+}.

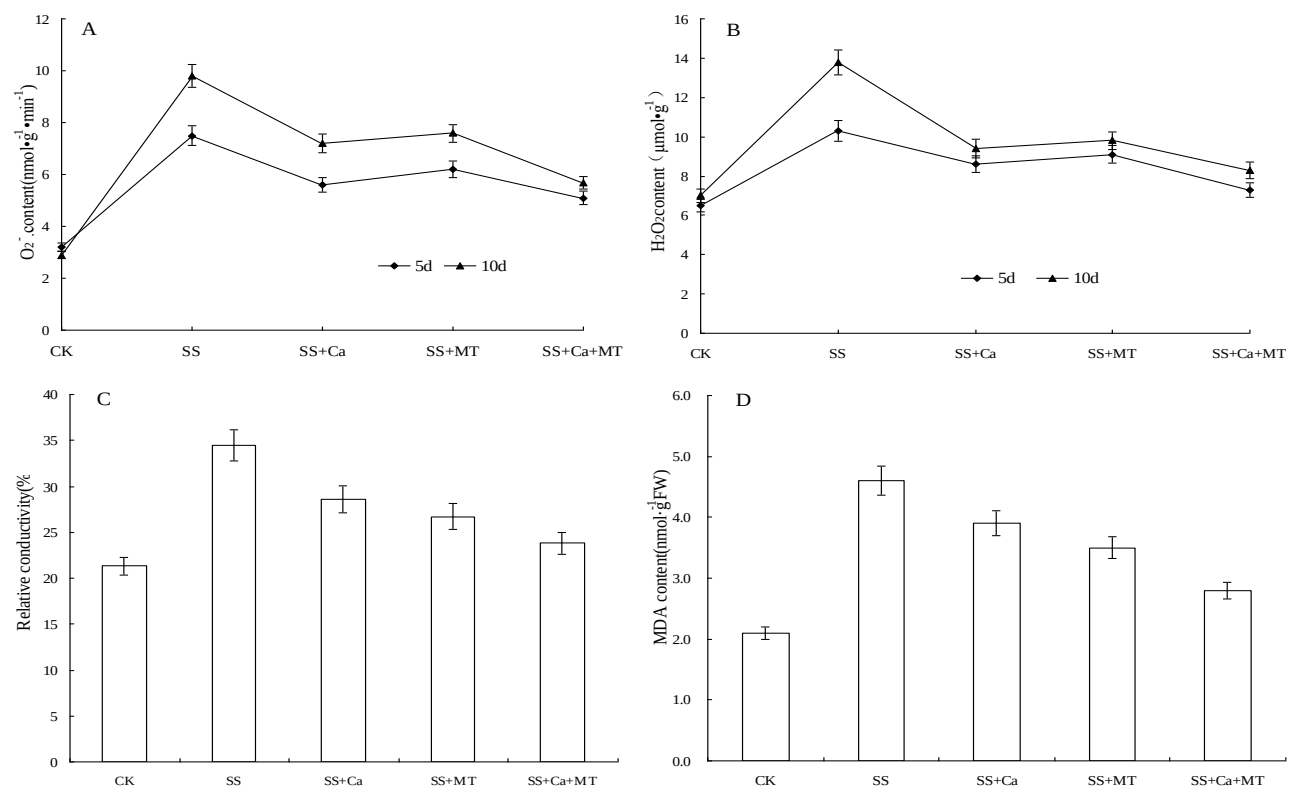

Fig. 3. Influence of exogenous 100 μmol·L^{-1} MT and 3 mmol·L^{-1} Ca^{2+} on $O_2^{\cdot-}$, H_2O_2 content, relative conductivity and MDA content of melon seedlings under salt stress. CK; SS: 100 mM NaCl; SS+MT: 100 mM NaCl +100 μmol·L^{-1} MT; SS+Ca: 100 mM NaCl +3mmol·L^{-1} Ca^{2+}; SS+Ca+MT: 100 mM NaCl +100 μmol·L^{-1} MT+3 mmol·L^{-1} Ca^{2+}.

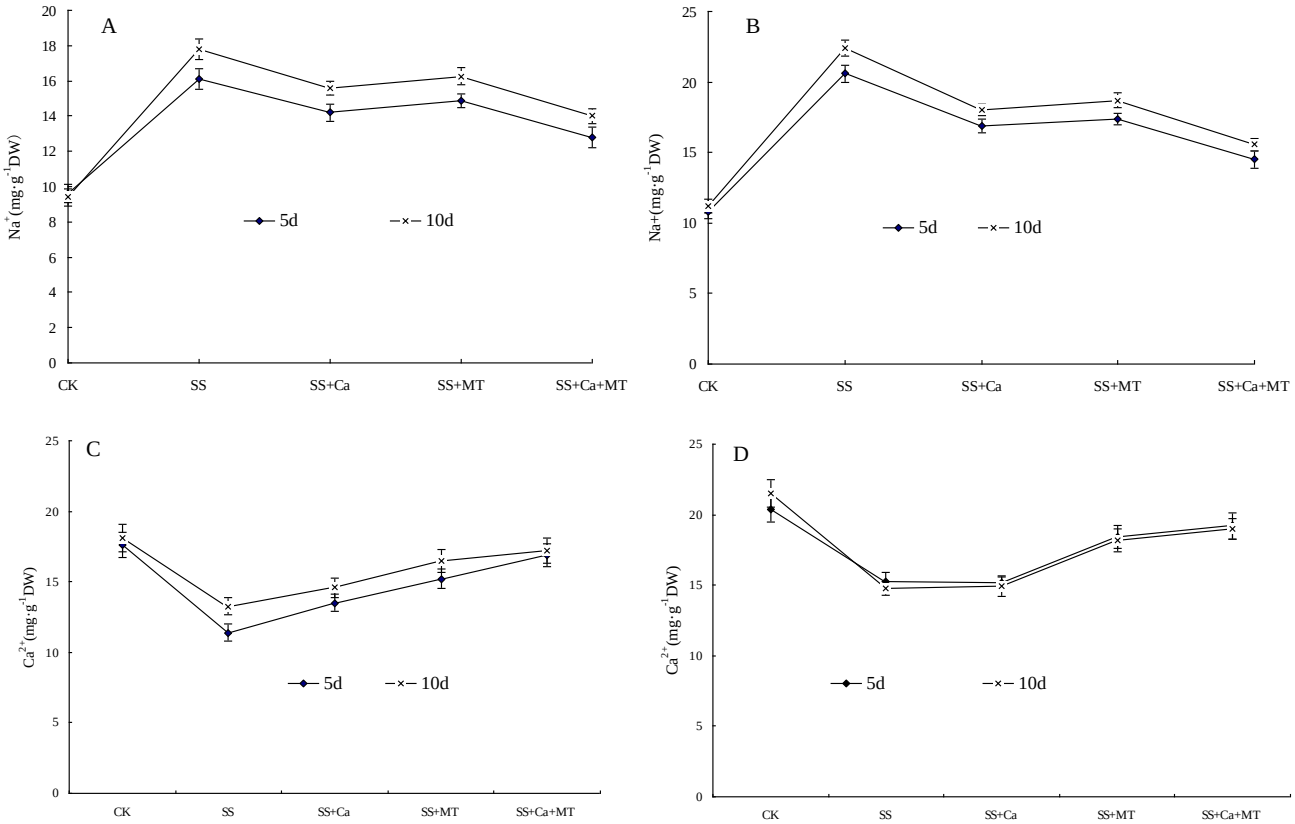

Fig. 4. Influence of exogenous 100 μmol·L^{-1} MT and 3 mmol·L^{-1} Ca^{2+} on Na^{+} and Ca^{2+} of melon seedlings under salt stress. CK; SS: 100 mM NaCl; SS+MT: 100 mM NaCl +100μmol·L^{-1} MT; SS+Ca: 100 mM NaCl +3 mmol·L^{-1} Ca^{2+}; SS+Ca+MT: 100 mM NaCl +100 μmol·L^{-1} MT+3 mmol·L^{-1} Ca^{2+}.

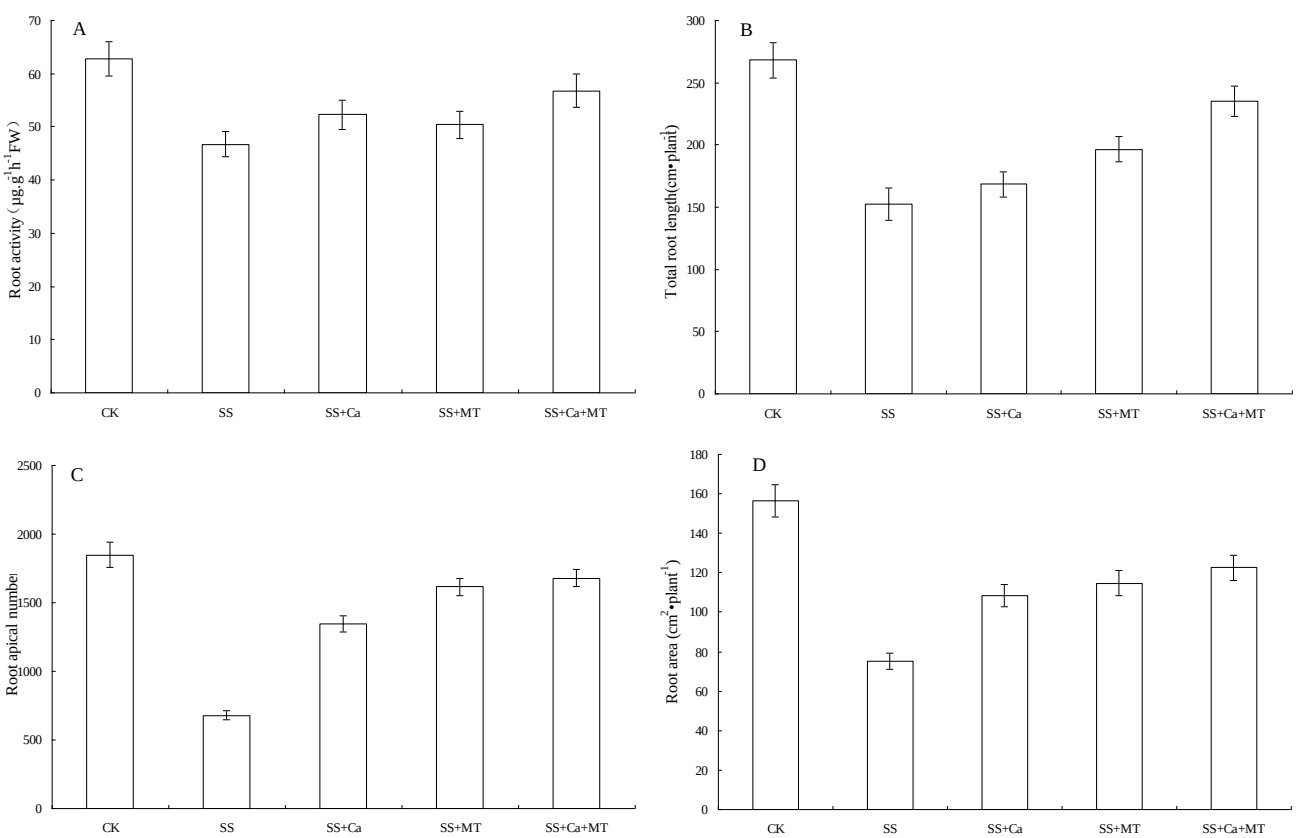

Fig. 5. Influence of exogenous 100 μmol·L^{-1} MT and 3 mmol·L^{-1} Ca^{2+} on root activity and root morphology of melon seedlings under salt stress. CK; SS: 100 mM NaCl; SS+MT: 100 mM NaCl +100 μmol·L^{-1} MT; SS+Ca: 100 mM NaCl +3 mmol·L^{-1} Ca^{2+}; SS+Ca+MT: 100 mM NaCl +100 μmol·L^{-1} MT+3 mmol·L^{-1} Ca^{2+}.

Fig. 6. Effects of MT and Ca^{2+} pretreatment on melon plant on 10th day of salt stress.

Conclusions

We conclude that exogenous MT or Ca^{2+} treatment improves salt tolerance of melon seedlings and the evidence leading to this conclusion is presented. In the first place, MT or Ca^{2+} improves antioxidant potential, thereby reducing ROS accumulation and alleviating oxidative damage in melon seedlings under salt stress. Secondly, MT or Ca^{2+} enhances photosynthetic capacity possibly by protecting photosystems against salt induced oxidative damage in melon seedlings. o Lastly, MT or Ca^{2+} adjusts ion homeostasis of melon seedlings under salt stress. However, it must be noted that further studies are required to elucidate the underlying mechanisms of MT or Ca^{2+} in the alleviation of salt stress in melon seedlings.

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