

Melatonin Enhanced Short-term Salt Tolerance Through Improving Water Status in Maize Seedlings

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Abstract

Background: The beneficial effects of melatonin (MT) on enhancing plant salt stress tolerance has been widely studied. However, the underlying mechanism that involved in MT-mediated the detailed process of plant water uptake and transport under salt stress condition remains largely unknown. To detect the underlying mechanisms that exogenous melatonin application enhances plant salt resistance, the influence of MT (1 μ M) on maize (*Zea mays* L.) seedlings growth under short-term salt stress (2h, 100 mM NaCl) was investigated.

Result: Results showed exogenous MT had increased the endogenous MT content in maize root. Under normal condition exogenous MT application did not affected the growth of seedlings significantly, whereas under salt stress condition MT application produced 99% in root and 20.2% in biomass higher than that without MT. Salt stress decreased the leaf net photosynthetic rate, leaf stomatal conductance, leaf transpiration rate, root hydraulic conductance and whole plant hydraulic conductance (Lp) significantly, but exogenous application of MT mitigated these salt stress-induced decreases. Plants treated with MT held higher photosynthetic rate (24.3%), stomatal conductance (39.4%), and transpiration rate (40.7%) compared with untreated plants under salt stress. Compared with normal condition, salt decreased Lp by only 61% in melatonin-treated seedlings and decreased by 97% in untreated seedlings. RT-PCR and the aquaporin inhibitor mercury were used to detect role of aquaporin in this process. Under salt stress, melatonin increased specific aquaporin gene (*ZmPIP1;6*, *ZmPIP2;3* and *ZmPIP2;7*) expression, and under HgCl₂ treatment, there was no significant difference in transpiration rates of seedlings with and without MT, which confirmed that the aquaporin played an important role in exogenous MT-induced improved maize root water uptake capacity under salt stress.

Conclusions: Taking all these results into consideration, this study indicates that exogenous melatonin pre-treatment enhances maize resistance to short-term salt stress by regulating Lp through improving expression and activity of root aquaporin.

Background

Soil salinization, which affects up to 7% of arable land worldwide, is one of the major influencing factors which has severely limited crop growth and grain yield production due to the sensitivity of crops to high concentrations of salt [1, 2]. A high salt concentration could affect plants by increasing osmotic stress and ionic toxicity, which destroyed many plant physiology and metabolic processes, including leaf photosynthesis, chloroplast electron-transfer, integrity of cell membrane and even the protein synthesis, and to several morphological changes, which reduce crop grain yield production [3, 4]. Furthermore, long-term groundwater irrigation also salinizes the soil of inland farmland [5]. In consideration of damage of salt stress, many method including crop cultivation and growth regulating agent were researched to reduce the salt damage [2, 6, 7].

Glycinebetaine, salicylic acid, nitric oxide, jasmonic acid, silicon and melatonin were found to enhance salt tolerance of plant [8-10]. Melatonin, with the [chemical formula](#) as N-acetyl-5-methoxytryptamine, was found to take part in numerous physiological processes of life of animal [11]. Lastly, studies have confirmed that melatonin has vital functions in maintaining normal vegetative growth, reproductive development, and making various stress responses of plants [12]. In the past decades, melatonin has been studied mainly in relation to the scavenging of reactive oxygen species (ROS) via regulating antioxidant enzyme system. In maize (*Zea mays* L.), melatonin application decreased accumulation of H_2O_2 to decrease the oxidative damage to produce less malondialdehyde (MDA), thereby increasing maize seedling drought tolerance [13]. In addition, melatonin enhanced maize seedlings salt stress tolerance by activating antioxidant enzymes and alleviating salt-induced photosynthetic inhibition and oxidative damage [14]. In rice (*Oryza sativa* L.) melatonin could delay cell death, slow rice leaf senescence and enhance abiotic stress tolerance through counteracting the cellular accumulation of hydrogen peroxide (H_2O_2) [15]. Melatonin prevented accumulation of ROS caused by salt stress by improving the photosynthetic activities of tomato (*Solanum lycopersicum*) seedlings [16]. However, all these studies were conducted to detect the role of melatonin in alleviating salt damage under long-term stress (>7 days). In fact, the short-term salt stress (<3days) is also important for plant, in which plants undergo double stress including salt induced osmotic stress and ion stress and plant make initial reaction to stress. Therefore, the mechanism by which melatonin improves plant short term salt resistance remains still unclear.

In saline soil, plants immediately sense osmotic stress, which affects plant physiological activity immediately by decreasing the stomatal conductance, transpiration rate, root hydraulic conductance (L_p) and the whole plant hydraulic conductance (K_{plant}), which induced water shortage in plant [2, 17, 18]. K_{plant} represents the fluidity of water transport at the whole plant level, and L_p is important index that measure the root water absorption capacity, which plays an important role in guaranteeing the water status of the entire plant [19]. In the other side, previous study also confirmed that under abiotic stress condition L_p was affected by aquaporins like its expression and activity [20]. under short-term salt stress, silicon could alleviate the decrease in L_p by mediating aquaporin activity via eliminating aquaporin oxidant gating H_2O_2 , which could guarantee root water supply to shoot at the beginning of salt stress [2]. These observations started the research on the whole plant water balance and root water uptake involved in plant responses to short-term salt stress.

Under short-term salt stress, exogenous melatonin application has been found to enhance the stomatal conductance, transpiration rate and plant leaf relative water content (RWC) [13, 14, 21]. Taking all these findings together, under salt stress, melatonin may maintain a better leaf water status (higher RWC), which is not achieved by decreasing the leaf transpiration rate due to declines in leaf water loss but is achieved by improving the root water uptake capacity. Therefore, here, we proposed the hypothesis that melatonin could enhance aquaporin activity to improve root water uptake to enhance plant salt resistance under short-term salt stress. To confirm this hypothesis, we conducted a set of experiments including investigations of the relative transcriptional expression level of root aquaporin genes, RWC,

transpiration rates, aquaporin activity, L_p , K_{plant} , and leaf growth rates in maize seedlings with a hydroponic system under short-term salt stress. According to previous study, the dosage of melatonin used here was $1\mu\text{M}$ and NaCl was 100 mM [2, 22]. There were four treatments: normal condition 1/4 Hoagland's solution (CK), 1/4 Hoagland's solution+ $1\mu\text{M}$ melatonin (CK+MT), salt stress 1/4 Hoagland's solution+100 mM NaCl (S), and 1/4 Hoagland's solution+100 mM NaCl+ $1\mu\text{M}$ melatonin (S+MT).

Results

Growth status of seedlings

No significant difference was detected in the biomass and leaf elongation rate between CK and CK+MT ($P>0.05$, Fig.1) after seven-day salt treatment, salt reduced the maize plant biomass accumulation compared with CK (reduced 41.4% in biomass and 68.1% in root) (Fig.1A), while the MT treatment had reduced this decline in biomass ($P<0.05$) and S+MT held 99% in root and 20.2% in biomass higher than that of S. Salt stress significantly reduced the leaf elongation rate ($P<0.05$). The leaf elongation rate was 65.9% higher in the S+MT treatment than that in the S treatments ($P<0.05$). These results confirmed that melatonin application alleviated salt-induced decreases in the leaf elongation rate.

Endogenous MT content

After salt treatment for seven days, the endogenous MT content was significantly increased in maize root with exogenous MT application with or without salt stress (86.6% under normal condition and 305% under salt stress, $P<0.05$). And we also could find that the salt stress reduced the endogenous MT content in S+MT compared with CK+MT treatment (19.3%, Fig. 2).

Leaf gas parameter

Under normal condition, melatonin application have not affected the leaf net photosynthetic rate, leaf stomatal conductance, and leaf transpiration rate significantly ($P>0.05$, Fig. 3). Similar to the leaf elongation rate, salt stress reduced all these parameters, and melatonin application significantly reduced the decrease in these parameters ($P<0.05$), and S+MT held higher photosynthetic rate (24.3%), stomatal conductance (39.4%), and transpiration rate (40.7%) than that of S treatment. These results show that exogenous melatonin application could be beneficial for maintaining the net photosynthesis rate, leaf transpiration rate, and stomatal conductance of seedlings in maize under short-term salt stress.

Leaf water status

The RWC was 91.0% in CK and 91.2% in CK+MT, and almost not affected by application of melatonin without salt addition (Fig. 4A). Under short-term salt stress, non-melatonin-treated plants held the significantly lower leaf RWC (75.6%) than the MT-treated plants (84.0%) ($P<0.05$), indicating exogenous melatonin application was rewarding to the maintenance of plant leaf water status. Under well-water conditions, the leaf water potential was from -0.24 to -0.21 MPa, and no significant difference was found

between the CK and CK+MT treatments ($P>0.05$, Fig. 4B). The leaf water potential was decreased to -0.68 MPa when the maize seedlings were exposed to salt stress, but in the presence of melatonin this parameter was maintained at -0.57 MPa. These results showed that exogenous melatonin application could improve the water status of plants.

After 1 h of salt treatment, the salt-treated maize seedlings without MT treatment held the lowest whole plant transpiration rate among these treatments ($P<0.05$, Fig. 5A). This result showed that melatonin improved the plant water status at the whole plant level in the early treatment period, i.e., 1 h.

Plant hydraulic parameters

No significant difference was detected in K_{plant} in the presence ($1.88 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$) or absence ($2.18 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$) of melatonin treatment maize seedlings under normal water condition ($P>0.05$, Fig. 5B). Under short-term salt stress, a drastic decrease in K_{plant} was observed in both the presence and absence of melatonin application compared with that of normal conditions, but melatonin-treated seedlings held 68% higher in K_{plant} than the untreated seedlings.

Under the normal water condition, melatonin application did not affect the L_p significantly ($P>0.05$, Fig. 6). However, under salt stress, L_p decreased by only 61% in melatonin-treated seedlings and decreased by 97% in untreated seedlings indicating that MT application increased the root water uptake capacity. The root area did not exhibit any difference among these treatments ($P>0.05$, Fig. S1).

Root aquaporin genes

Almost all *ZmPIPs* were down-regulated by melatonin application under normal conditions, except for *ZmPIP1;6* and *ZmPIP2;7* (Fig.7). Compared with none melatonin-treated plant, *ZmPIP1;6*, *ZmPIP2;3* and *ZmPIP2;7* in melatonin-treated seedlings were significantly up-regulated by 1.95-, 3.41- and 5.32-fold, respectively, under salt stress conditions.

HgCl₂ experiment

With HgCl₂ application, compared to the absence of HgCl₂, the whole plant transpiration rate was decreased (Fig. 8 and Fig. 4). Similar to the absence of HgCl₂, no significant difference was found between CK and CK+MT ($P>0.05$). While the absence of HgCl₂ had different effects under salt stress, we had not detected significant difference in the transpiration rate of S and S+MT seedlings ($P>0.05$). These results showed that under short-term salt stress conditions, aquaporins had important function in maintaining a higher root water uptake capacity in the melatonin-induced improvement of maize salt resistance.

Discussion

As one of the most important and widely cultivated cereal crops in the world, multiple abiotic stresses is limiting maize productivity (e.g. salinity, flooding and drought) [23, 24]. In the past decades, numerous studies have been conducted to investigate the underlying mechanisms involved in alleviating salt stress, and researchers have also conducted many excellent studies to explain how melatonin relieves long-term salt stress induced Na^+ toxicity [22, 25]. At the present study, exogenous melatonin was found that could improve the plant short-term salinity tolerance through regulating root water uptake. Elucidating the underlying mechanism in which melatonin regulates plant growth and development under salt stress condition could accelerate the application of melatonin in the development of saline-alkali agriculture greatly.

Aquaporins are involved in the melatonin-mediated increase in hydraulic conductance

The dynamic balance between plant root water uptake and water loss from leaf was important not only for plant growth but also for plant adaptation to osmotic stress induced by salt and drought [17]. Osmotic stress, including short-term salt stress and drought induced osmotic stress, causes leaf cells water loss, which leads to a drastically decreased leaf water potential, and could reduce cell elongation and division, ultimately affected the plant growth and grain yield production [2]. leaf RWC and water potential was significantly increased with melatonin application under salt stress (Fig. 4), indicating that application of melatonin could alleviate salt-induced stress damage by improving the water status of leaves.

Salt stress significantly reduced K_{plant} and stomatal conductance, but melatonin application reduced these declines (Fig. 5 and Fig. 3). Previous studies have found that stomatal conductance and photosynthesis was affected by changes in K_{plant} [19, 26], which is consistent with the observations of our study. In general, K_{plant} was affected by leaf hydraulic conductance, stem hydraulic conductance and root hydraulic conductance. According to previous study, the main whole plant hydraulic resistance rooted in the leaves and roots, and due to higher flow of stem hydraulic conductance it is not the main water transport limiting factor [27, 28]. In fact, as the first stress receptor, root usually was the main limiting organ in the soil–plant–air continuum under stress condition [28]. In the present study, exogenous melatonin was found to significantly reduce the decrease in L_p under short-term salt stress. L_p , which was determined by root surface area and root water permeability, represents the water uptake capacity of plant root. No difference was found in root surface area (Supplementary Fig. S1), which indicates that melatonin did not influence root surface area to improve water uptake capacity under short-term salt stress. Previous studies have confirmed that aquaporins play an important role in the “cell-to-cell” water transport pathway under water deficit conditions [29, 30]. To detect the role of aquaporins in melatonin-mediated increases in hydraulic conductance, we studied the expression of root aquaporin genes and found that *ZmPIP1;6*, *ZmPIP2;3* and *ZmPIP2;7* was considerably up-regulated (Fig. 7). Previous studies showed PIP1;6 and PIP2;3 was highly up-regulated in *Sorghum bicolor* with silicon-mediated increase in root water uptake under osmotic and salt stress [2, 19]. This finding indicates that these two PIPs also function in melatonin-mediated increases in hydraulic conductance. *ZmPIP1;5* and *ZmPIP2;6* were found to be highly responsive to salt stress, which was consistent with the observations of a previous study [31], while *ZmPIP1;5* and *ZmPIP2;6* expression in S+MT plants was low; these results

showed that melatonin specifically regulates *ZmPIPs* and that the expression of *ZmPIPs* might be involved in melatonin-mediated enhanced root water uptake capacity under short-term salt stress.

Here, the exogenous melatonin induced the increased relative expression of PIPs under salt stress. Many studies confirmed under abiotic stress, ABA, Si and Ca application increased the PIPs' expression [19, 32, 33]. Exogenous melatonin increased ABA production and ABA biosynthesis inhibitor application caused severe membrane lipid peroxidation and lowered melatonin-induced antioxidant defense responses under cold stress in *Elymus nutans* indicating that ABA-dependent pathways might contribute to melatonin-induced cold stress response [34]. It is inferred from results that under salt stress, melatonin induced the increased relative expression of PIPs through ABA-dependent pathways.

In general, exogenous substances regulate aquaporins to improve water uptake under stress mainly by regulating aquaporin activity. To detect the participation of aquaporins in melatonin-induced improved water uptake, we performed a HgCl₂ application test. Under the HgCl₂ treatment, the significant differences in the measured characteristics between S and S+MT disappeared (Fig. 4, Fig. 5 and Fig. 8). These results confirmed the viewpoint that higher aquaporin activity is necessary for the melatonin-induced improvement in Lp under short term salt stress and that melatonin could regulate aquaporin. The improved aquaporin activity could be regulated by ROS, especially the hydrogen peroxide (H₂O₂). Environment stress induced the ROS accumulation e.g. H₂O₂ and exogenous melatonin was found to stimulate antioxidant defense systems alleviating ROS accumulation [21, 35]. In plant, hydrogen peroxide was oxidative gating of aquaporins, which could lead on to inhibition of root water uptake [36]. Here, it is inferred from results that under salt stress, melatonin application could increase the antioxidant capacity and decrease the ROS accumulation to reduce the H₂O₂ induced oxidative gating of aquaporins to increase Lp.

Melatonin mitigates short-term salt stress in maize

Photosynthesis, physico-chemical process by light energy was translated to organic compounds, is the basis of plant growth, development and production [37]. Photosynthesis was repressed by multiple types of abiotic stress, e.g. salinity, drought, and oxidative stress [38]. Here, after 2 h of salt treatment, plant in the S+MT group had a higher net photosynthetic rate than that in the S group (Fig. 3), which induced a quicker leaf growth rate and larger biomass accumulation (Fig. 1). These results were confirmed by Li et al [21] who found exogenous melatonin enhanced watermelon salt stress tolerance through improving photosynthesis associated with the inhibition of stomatal closure. In general, plants undergoing the drought and salt stress would close stoma to decrease leaf transpiration to reduce water loss [39, 40], which induced stomatal limitation of photosynthesis [41]. In this study, plants of S+MT treatment held higher stomatal conductance than that of S treatment, indicating that plant of S+MT treatment could hold larger stomatal opening. This result was consistent with previous study, which showed that exogenous melatonin alleviated salt induced the decrease of stomatal conductance in maize seedlings [14]. These remissions in stomatal conductance could decrease the harmful effects of short-term salt stress on photosynthesis.

Conclusions

Taking all these results into conclusion, the present study suggests that exogenous melatonin pre-treatment enhances maize resistance to short-term salt stress by improving L_p , which held a lesser decrease extent through the up-regulation of specific aquaporin genes and thereby improves the aquaporin activity. Based on this conclusion, the underlying mechanism by which melatonin improves maize resistance to short-term salt stress could be speculated. Under short-term salt stress, melatonin application could increase the root water uptake capacity by regulating aquaporin activity to maintain a higher leaf water potential and leaf relative water content. As a result, melatonin-treated maize seedlings could hold improved leaf water status and reduced stomatal limitation to maintain a higher photosynthetic rate and leaf growth rate.

Methods

Plant materials and growth conditions and treatments

Seeds of *Zea mays* L. cv. Zhengdan 958, which is one of the most widely used varieties in P.R. China and provided by Beijing Doneed Seed Industry Co. LTD, Beijing, P.R.China, were disinfected with 3% hypochlorite solution for ten minutes, washed with double distilled water six times and then germinated in the dark at 25°C. Subsequently, seedlings of the same size were transplanted to a hydroponic system and grown in a growth chamber (MGC-800HP, Yiheng Instrument, Shanghai) under fixed cycles of 14 h of light (450 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) and 10 h of darkness, with relative humidity and temperature of 40–50% and 25°C. Per hydroponic system contained six seedlings. The hydroponic system included 4 litres of one-quarter strength Hoagland's solution with pH6.0, which was adjusted every day, and Hoagland's solution was changed every other day. After transplantation for 7 days, 1 μM melatonin was supplied to half of these seedlings, which was dissolved in ethyl alcohol and tween 20, and rest of seedlings were supplied the same doses of ethyl alcohol and tween 20. After transplantation for ten days, 100 mM sodium chloride (NaCl) was added into the Hoagland's solution to induce salt stress, a dose was commonly used in salt stress studied [20, 22]. Treatments were control normal Hoagland's solution (CK), Hoagland's solution +melatonin (CK+MT), Hoagland's solution +NaCl (S) and Hoagland's solution +NaCl +melatonin (S+MT).

Biomass, leaf elongation rate and melatonin content

After seven-day treatment, the biomass dry weight was measured as described previously [30]. Each treatment included ten replications. The leaf elongation rate was measured as in previous study [2]. Each treatment included ten replications.

Melatonin content of maize root after salt treat for seven days was measured by HPLC according to method of Byeon and Back [42].

Leaf gas parameter

After 2 h of NaCl treatment, leaf gas exchange parameters were detected using a photosynthesis system (Li-6400; LI-COR Inc., Lincoln, NE, USA) with the new fully expanded leaves. The air temperature, CO₂ concentration and photosynthetic photon flux density in the leaf chamber were set at 25°C, 450 μmol·mol⁻¹, 1000 μmol m⁻² s⁻¹, respectively. Each treatment included ten replications.

Leaf water data measurement

Ten fully expanded leaves similar to leaves used in leaf gas measurement were sampled to measure the leaf RWC under Barrs and Weatherley's method [43]. New fully expanded leaves after 2 h of salt treatment were used to detect leaf water potential by a pressure chamber (Model 3115, Soil moisture Corp., Santa Barbara, CA, USA). Each treatment included ten replications.

Whole plant transpiration rate and K_{plant}

Single plant was inset 50 ml conical flask, which contained four kinds of solutions respectively. After 1 h adaptation, the whole plant transpiration rate was determined gravimetrically every ten min for 6 times. The whole plant transpiration rate was calculated as:

$$\text{whole plant transpiration rate} = (W_{\text{time } n+1} - W_{\text{time } n}) / \text{leaf area}$$

where the leaf area was determined by a perfection V800 photo scanner (Seiko Epson Corporation, Japan) and then analysed by WinRHIZO PRO 2015 software (Regent Inc., Canada). The K_{plant} was calculated as:

$$K_{\text{plant}} = \text{whole plant transpiration rate} / (\text{hydroponic water potential} - \text{leaf water potential})$$

Root hydraulic conductance (L_p)

Root hydraulic conductance, L_p , was determined based on the root surface area and was measured using a pressure chamber [30, 44].

After measuring the volume of sap exuded, the root was sampled using a scanner to determine the root surface area. The data were analysed using WinRHIZO PRO 2015 software (Regent Inc., Canada). Each treatment included ten replications.

Expression analysis of maize root aquaporin genes

After salt treatment for 2 hours, root tips (<5 cm) were collected and frozen in liquid nitrogen to measure the expression of aquaporin genes. Total RNA was extracted from the samples using an EasyPure Plant RNA Kit (Transgen, Beijing, China). The RNA extract was digested with DNase I and examined using a dissociation curve to ensure that DNA was eliminated. cDNA was synthesised in vitro using TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgen, Beijing, China) according to the manufacturer's instructions. Quantitative real-time PCR was performed on a LightCycler 480 using

TransStart Top Green qPCR SuperMix (Transgen, Beijing, China). The relative expression levels of thirteen genes encoding putative intrinsic plasma-membrane aquaporins were investigated, and the primers used in Hachez [45] research are used here. The data were analysed using the $2^{-\Delta\Delta C_t}$ method [46] and normalised against the data from the CK treatment as a relative unit.

Transpiration rate responds to aquaporin inhibitor (HgCl₂)

In accordance with the method of Knipfer et al [47], the effect of aquaporin on the root water uptake was investigated through measuring changes in the transpiration rate under salt stress with or without MT application under the application of the aquaporin inhibitor HgCl₂. Seedlings were treated with 50 μM HgCl₂, and the transpiration rate was detected with the weighing method. Briefly, the seedling was transplanted to the triangular flask, which contained the four-treatment solution with HgCl₂ and then parafilm was used to seal. After 1h adaption, we weighted the whole triangular flask+seedling+solution weight every ten min for 6 times. Then the leave of seedling was collected to detect surface area using WinRHIZO PRO 2015 software (Regent Inc., Canada). Each treatment included ten replications.

Statistical analyses

The software SPSS version 18.0. was used to detect the difference among these treatments with One-way ANOVA analysis. Differences between the means were compared by the least significant difference (LSD) test at $P < 0.05$. The figures were produced using SigmaPlot version 12.01 (Systat Software, Inc). All experiments were repeated thrice.

Abbreviations

MT: melatonin; RWC: relative water content; K_{plant} : whole plant hydraulic conductance; L_p : root hydraulic conductance.

Declarations

Ethics approval and consent to participate

No applicable.

Consent for publication

All authors agreed to publish.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JY conceived and designed the study. NZ and ML carried out the experiments and analysed the data. ML drafted the manuscript. JY and NZ contributed to the editing of the manuscript. All authors read and approved the final manuscript.

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Figures

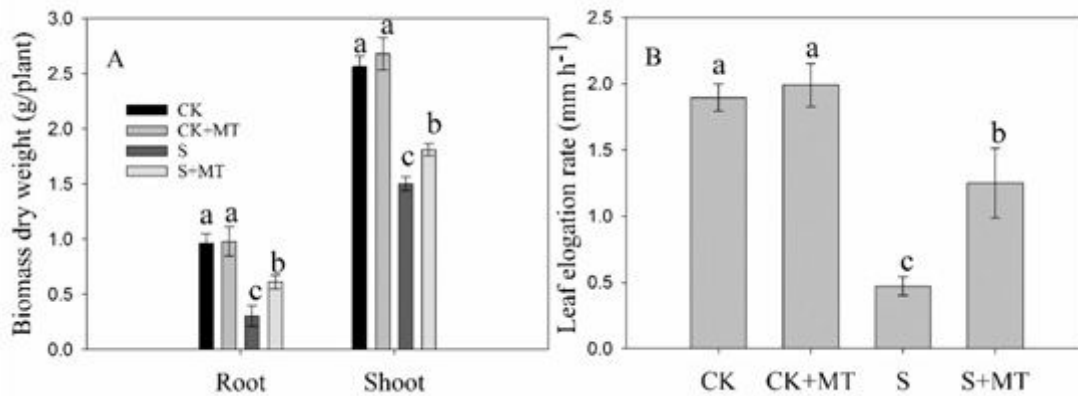


Figure 1

The biomass (A) and leaf elongation rate (B) of maize seedlings with or without melatonin (MT) application under normal (CK) and salt (S) treatments. New fully appeared leaves were used to measure the leaf elongation rate after 24 h of S treatment. Values are the means \pm SD of ten independent replicates. Different letters indicate significant differences ($p < 0.05$).

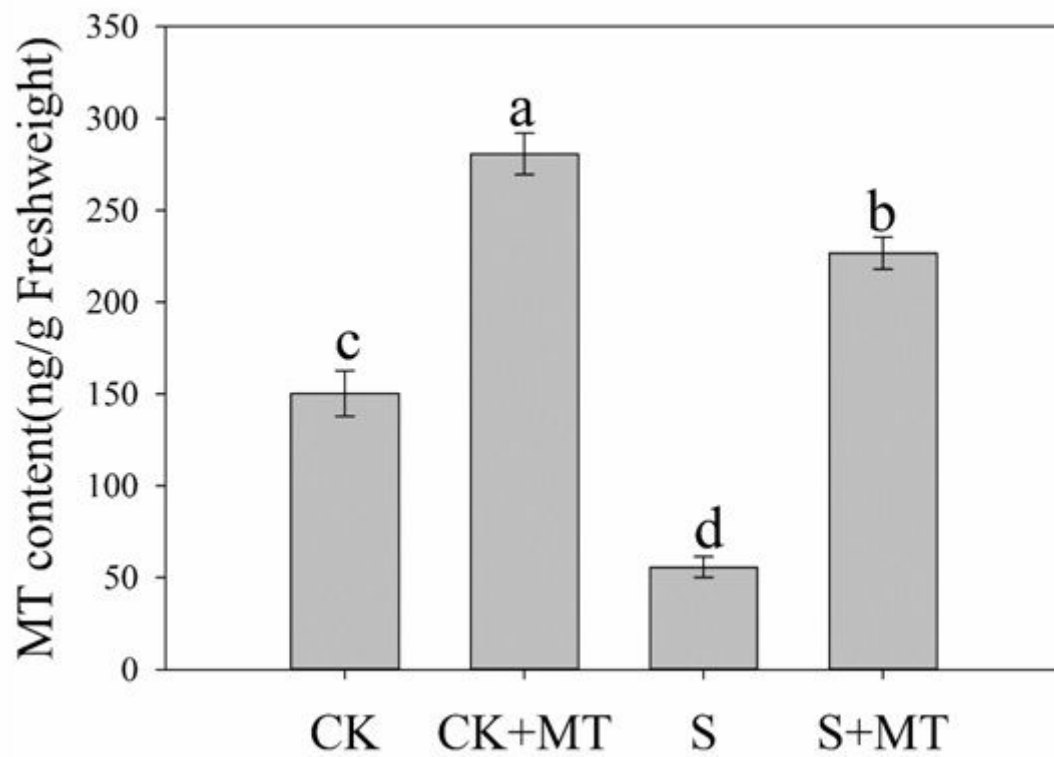


Figure 2

The root MT content after salt treat for seven days. means \pm SD of ten replicates was showed. Different letters indicate a significant difference ($P < 0.05$).

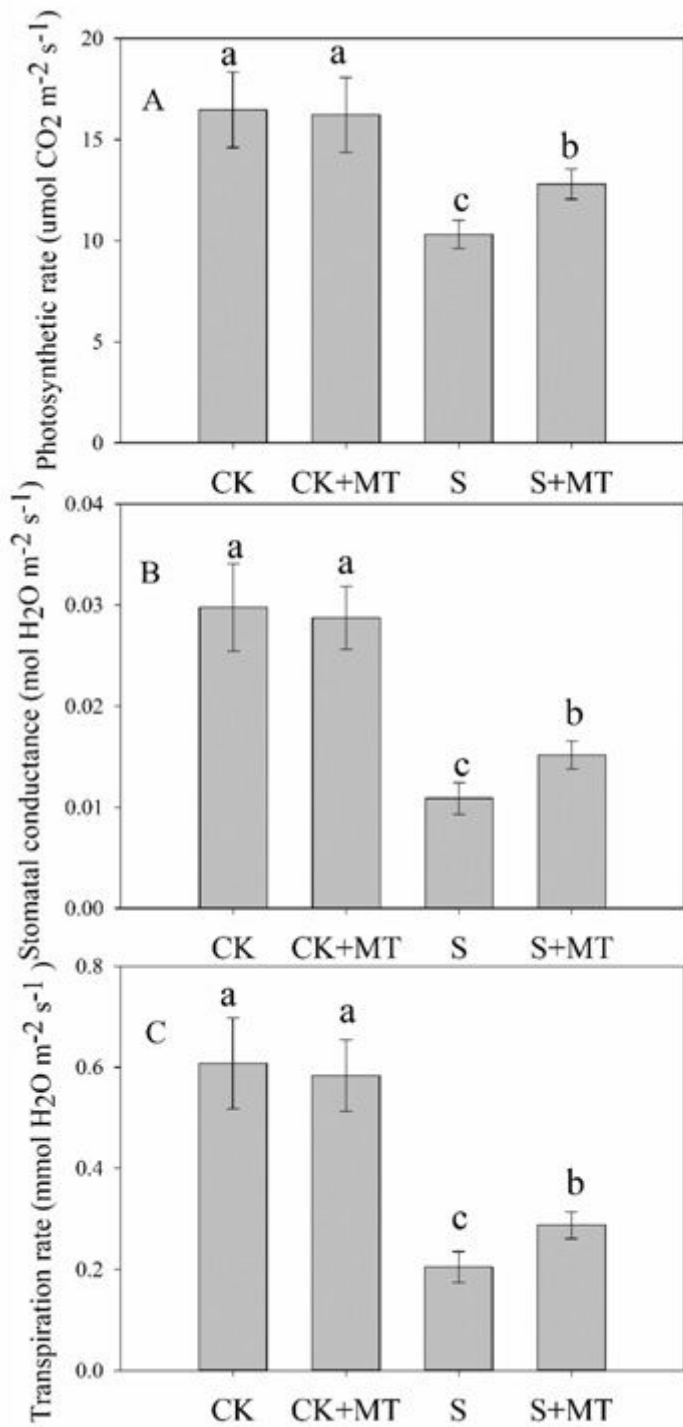


Figure 3

Effects of melatonin application on the photosynthesis rate (A), stomatal conductance (B), and transpiration rate (C) of maize seedlings under normal and salt treatments. Values are the means \pm SD of ten replicates. Different letters indicate significant differences ($p < 0.05$).

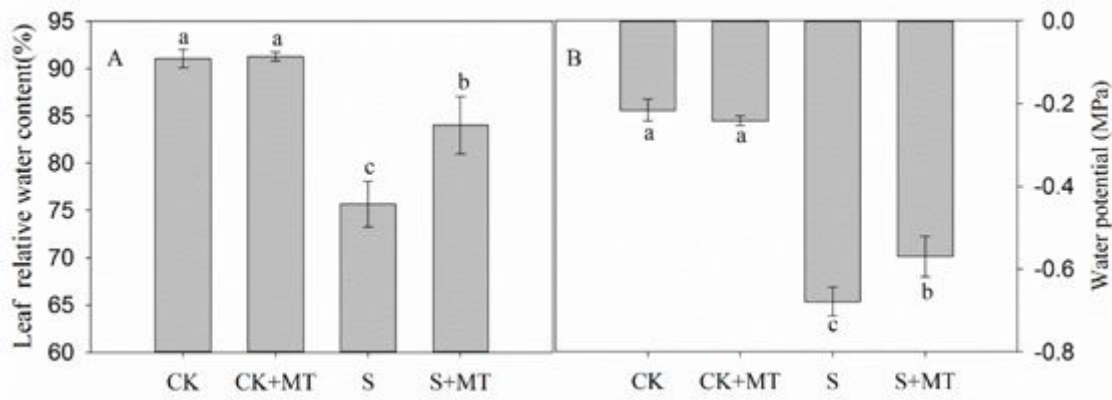


Figure 4

Effects of melatonin application on the leaf relative water content (RWC) (A) and water potential (B) of maize seedlings under normal and salt treatments. Values are the means \pm SD of ten replicates. Different letters indicate significant differences ($p < 0.05$).

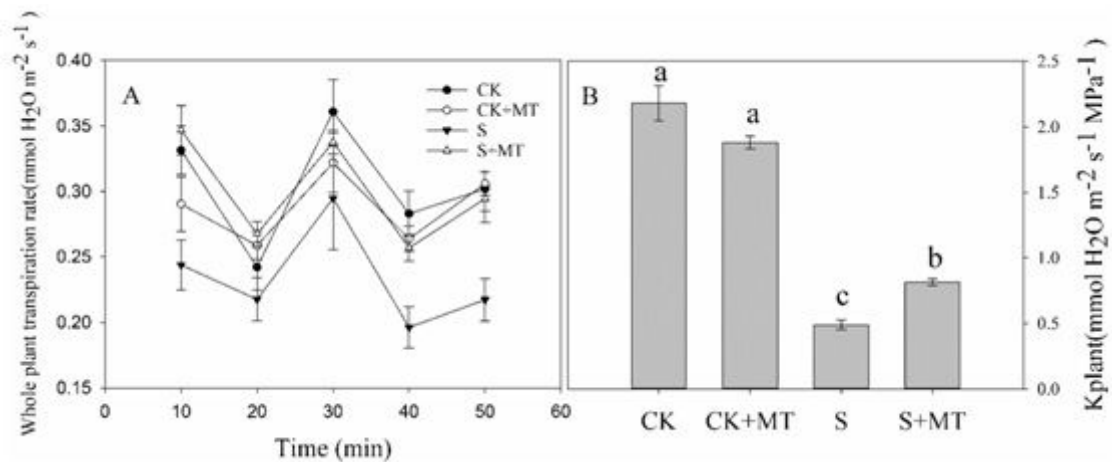


Figure 5

Effects of melatonin application on transpiration rate and whole plant hydraulic conductance (K_{plant}) of hydroponic culture under normal and salt treatments. Values are the means \pm SD of five replicates.

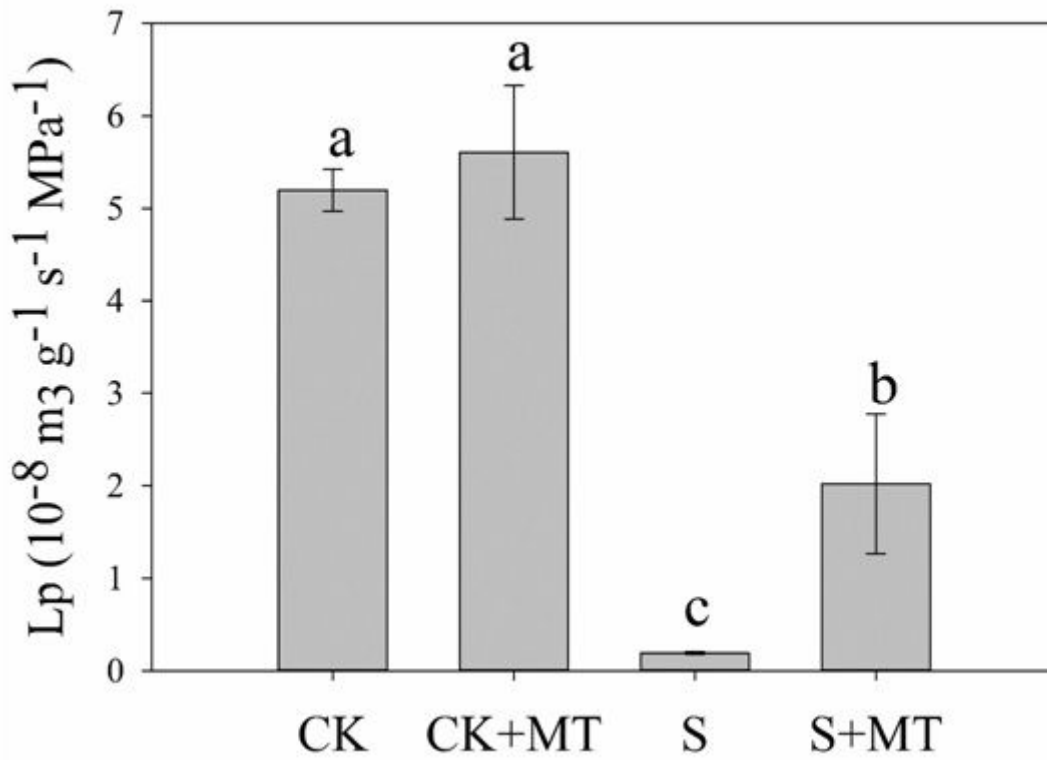


Figure 6

Effects of melatonin application on the root hydraulic conductance (L_p) of maize seedlings under normal and salt treatments. Values are the means \pm SD of ten replicates. Different letters indicate significant differences ($p < 0.05$).

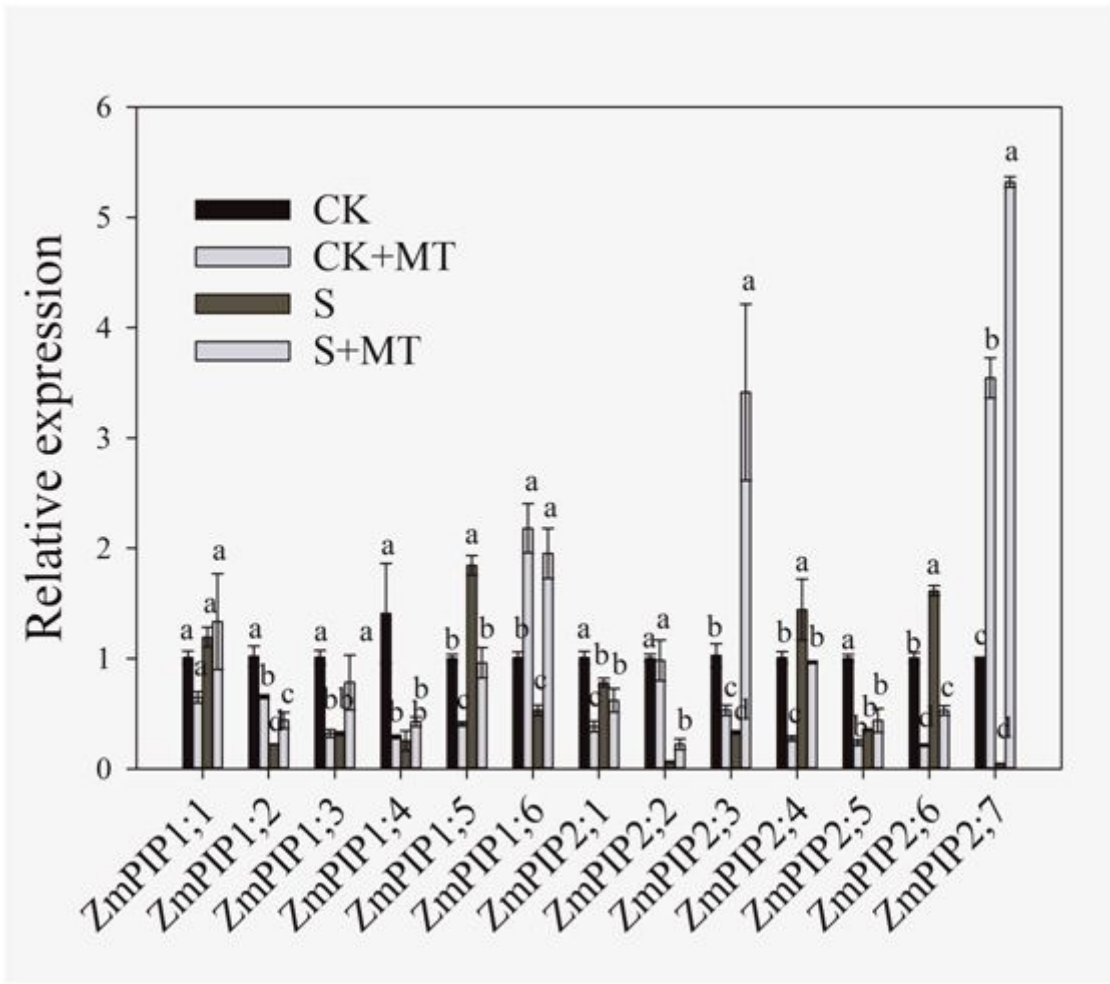


Figure 7

Effects of melatonin application on the expression levels of root ZmPIP aquaporin genes. Values are the means±SD of three replicates. Different letters indicate a significant difference (P<0.05).

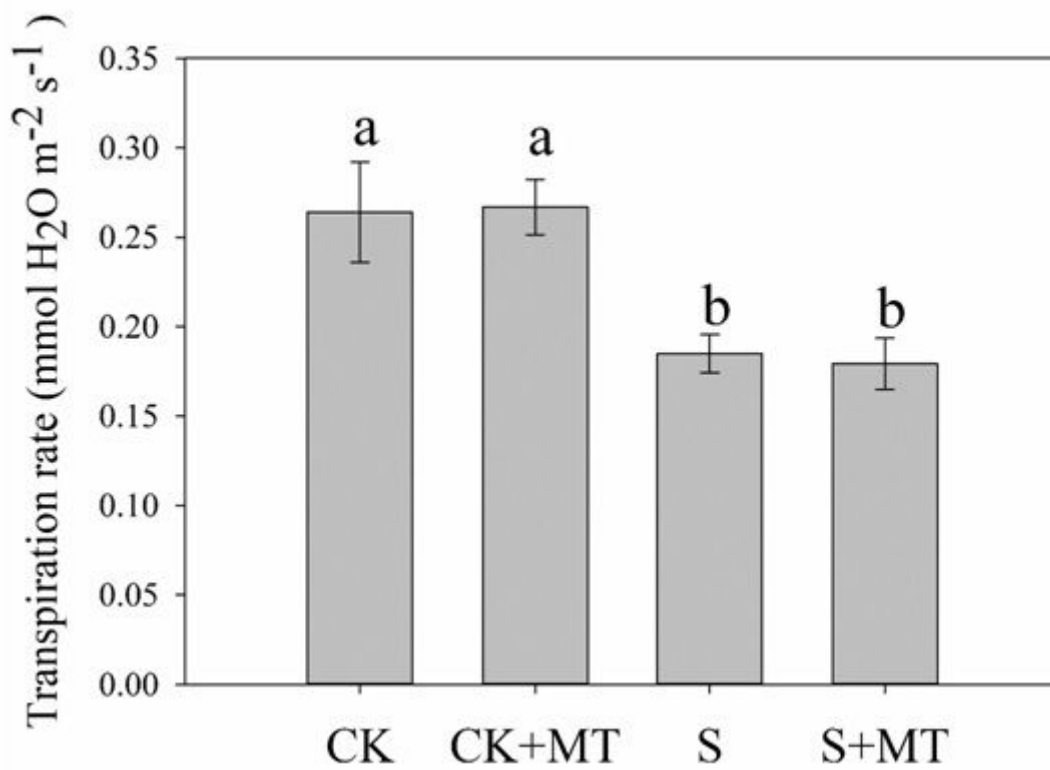


Figure 8

Effect of the aquaporin inhibitor HgCl₂ on the transpiration rate with and without melatonin application under salt stress. Values are the means±SD of ten replicates. Different letters indicate a significant difference (P<0.05).

Supplementary Files

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- [FigS1.jpg](#)