**MELATONIN ENHANCED SHORT-TERM SALT TOLERANCE BY IMPROVING THE WATER STATUS OF MAIZE SEEDLINGS**

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**Abstract** To determine the underlying mechanisms by which exogenous melatonin (MT) application enhances plant resistance to salt, the effects of melatonin (1 μM) on maize (*Zea mays* L.) seedling growth under short-term salt stress (2 h, 100 mM sodium chloride (NaCl)) were investigated. The results showed that exogenous MT increased the endogenous MT content in maize roots. Under normal conditions, seedling growth was not significantly affected by exogenous MT application, whereas under salt stress conditions, compared with no MT application, MT application resulted in 99% greater root biomass and 20.2% greater plant biomass. Salt stress significantly decreased the photosynthesis rate, stomatal conductance, transpiration rate, whole-plant hydraulic conductance (Kplant) and root hydraulic conductance (Lp), but MT application reduced these stress-induced decreases. Furthermore, compared with untreated seedlings, seedlings treated with MT had a greater photosynthesis rate (24.3%), stomatal conductance (39.4%), and transpiration rate (40.7%) under salt stress. Compared with normal conditions, salt stress conditions decreased the Lp by only 61% in MT-treated seedlings but decreased it by 97% in untreated seedlings. The contribution of aquaporins to Lp was characterized via RT-PCR and HgCl2, an aquaporin inhibitor. Under salt stress, MT increased the expression of specific aquaporin genes (*ZmPIP1;6*, *ZmPIP2;3* and *ZmPIP2;7*), and exogenous application of HgCl2 reduced the transpiration rates of seedlings treated with and without MT to the same level. Taken together, the results of this study indicate that exogenous MT pre-treatment enhances maize resistance to short-term salt stress by regulating Lp via increased expression and activity of root aquaporins.

**Keywords:** Melatonin, Aquaporin, Transpiration rate, Root hydraulic conductance, salt stress

1. **Introduction**

Soil salinization, which affects up to 7% of arable land worldwide, is one of the major influencing factors that severely limits crop growth and grain yield production because of the sensitivity of plants to high concentrations of salt (Wu et al.,2012a; Liu et al.,2015). A high salt concentration can affect plants by increasing osmotic stress levels and ionic toxicity, both of which hinder many plant metabolic processes and systems, including photosynthesis, electron transfer, cell membrane integrity and protein synthesis, and cause several morphological changes, ultimately reducing crop grain yield production (Ruizlozano et al.,2012; Luo et al.,2017). Furthermore, long-term groundwater irrigation also salinizes the soil of inland farmland (Ghazaryan and Chen,2016). In consideration of salt stress damage, many methods, including crop cultivation and the use of growth-regulating agents, have been studied for their effectiveness at reducing salt damage (Liu et al.,2015; Zhu et al.,2015; Huang,2018).

Glycinebetaine, salicylic acid, nitric oxide, jasmonic acid, Si and melatonin (MT) have been found to enhance the salt tolerance of plants (D. et al.,2017; Jian et al.,2018; Farhangi-Abriz and Ghassemi-Golezani,2018). MT (N-acetyl-5-methoxytryptamine) has been shown to play important roles in numerous physiological processes in animals (Rodriguez et al.,2004). Moreover, studies have confirmed that MT has vital functions in maintaining normal plant growth and development and in various responses to stress (Arnao and Hernández-Ruiz,2015). In recent decades, MT has been studied mainly with respect to the scavenging of reactive oxygen species (ROS). In maize (*Zea mays* L.), MT application decreased the accumulation of hydrogen peroxide (H2O2) and malondialdehyde (MDA), thereby increasing maize seedling drought tolerance (Ye et al.,2016). In addition, MT enhanced the salt stress tolerance of maize seedlings by activating antioxidant enzymes and by alleviating salt-induced photosynthesis inhibition and oxidative damage (Chen et al.,2018). In rice (*Oryza sativa* L.), MT could delay cell death, slow leaf senescence and enhance abiotic stress tolerance by counteracting the cellular accumulation of H2O2 (Liang et al.,2015), and MT prevented the accumulation of ROS caused by salt stress by improving the photosynthesis activity of tomato (*Solanum lycopersicum*) seedlings (Zhou et al.,2016). However, all these studies were conducted to determine the role of MT in alleviating salt damage under long-term stress (>7 days). In fact, short-term salt stress (<3 days) is also important for plants; during short-term salt stress, plants are subjected to two types of stress, salt-induced osmotic stress and ion stress, and plant make initial reaction to stress. However, the mechanism by which MT improves plant short-term salt resistance remains unclear.

In saline soil, plants immediately sense osmotic stress, which immediately affects plant physiological activities by causing reductions in stomatal conductance, transpiration, root hydraulic conductance (Lp) and whole-plant hydraulic conductance (Kplant) (Horie et al.,2011; Liu et al.,2015; Yin et al.,2013). Kplant represents the fluidity of water transport at the whole-plant level, and Lp represents the water uptake capacity of the roots, which plays an important role in maintaining the water status of the entire plant (Liu et al.,2014). On the other hand, a previous study also confirmed that under stress conditions, Lp was affected by aquaporins, similar to that which occurs with respect to its expression and activity (Qian et al.,2014). Under short-term salt stress, Si could alleviate the decrease in Lp by mediating aquaporin activity by eliminating aquaporin oxidant-gating H2O2, leading to increased water uptake and resistance to salt-induced osmotic stress (Liu et al.,2015). 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 salt stress, MT application has been found to enhance stomatal conductance, the transpiration rate and the leaf relative water content (RWC) of plants (Ye et al.,2016; Chen et al.,2018; Li et al.,2017). Taken together, these findings indicate that, in plants under salt stress, MT may maintain a better leaf water status (greater RWC); this status is not achieved by decreasing the leaf transpiration rate to reduce leaf water loss but rather is achieved by improving the root water uptake capacity. Therefore, we proposed the hypothesis that MT could enhance aquaporin activity to improve root water uptake to increase the salt resistance of plants under short-term salt stress. To test this hypothesis, we conducted a set of experiments including investigations of the transcript level of aquaporin genes, RWC, transpiration rates, aquaporin activity, Lp, Kplant, and leaf growth rates of maize seedlings under short-term salt stress in a hydroponic system. According to a previous study, 1µM MT was used, and 100 mM sodium chloride (NaCl) was used (Liu et al.,2015; Ke et al.,2018). There were four treatments: normal conditions, which involved 1/4-strength Hoagland’s solution (CK); 1/4-strength Hoagland’s solution+1 μM MT (CK+MT); 1/4-strength Hoagland’s solution+100 mM NaCl (S); and 1/4-strength Hoagland’s solution+100 mM NaCl+1 μM MT (S+MT).

1. **Materials and methods**

**2.1 Plant materials, 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, were disinfected with 3% hypochlorite solution for 10 min, washed with double-distilled water six times and then germinated in the dark at 25°C. Seedlings of the same size were subsequently transplanted into a hydroponic system and grown in a growth chamber (MGC-800HP, Yiheng Instrument, Shanghai) under a photoperiod consisting of 14 h of light (450 µmol photons m-2 s-1) and 10 h of darkness, a relative humidity of 40–50% and a temperature of 25°C. The hydroponic system contained six seedlings and consisted of 4 litres of one-quarter-strength Hoagland’s solution whose pH was 6.0; the pH was adjusted daily, and Hoagland’s solution was changed every other day. At seven days after transplantation, half of these seedlings were treated with 1 μM MT, which was dissolved in ethyl alcohol mixed with Tween 20, and the rest of the seedlings were treated the same doses of ethyl alcohol mixed with Tween 20. The MT solution was prepared as follows: 0.23 g of MT was dissolved in 50 mL ethyl alcohol as a stock solution. Afterwards, 0.1 mL of the stock solution was diluted to 2 L with deionized water and 0.05% (v/v) Tween-20 as a surfactant. At ten days after transplantation, 100 mM NaCl was added to Hoagland’s solution to induce salt stress; this dose is commonly used in salt stress studies (Qian et al.,2014; Ke et al.,2018). The treatments included a normal Hoagland’s control solution (CK), Hoagland’s solution + MT (CK+MT), Hoagland’s solution + NaCl (S) and Hoagland’s solution + NaCl + MT (S+MT).

**2.2 Biomass, leaf elongation rate and MT content**

After seven days of treatment, the biomass dry weight was measured as described previously (Yan et al.,2016). Each treatment included ten replications. The leaf elongation rate was measured as described in a previous study (Liu et al.,2015). Each treatment included ten replications.

The MT content of maize roots after salt treatment for seven days was measured via HPLC according to the methods of Byeon and Back (2014).

**2.3 Leaf gas exchange parameters**

Leaf gas exchange parameters such as the leaf photosynthesis rate, stomatal conductance, and transpiration rate of new fully expanded leaves were measured via a photosynthesis system (LI-6400; LI-COR Inc., Lincoln, NE, USA) after 2 h of NaCl treatment. The air temperature, CO2 concentration and photosynthetic photon flux density in the leaf chamber were set at 25°C, 450 µmol mol-1, and 1000 µmol m-2 s-1, respectively. Each treatment included ten replications.

**2.4 Leaf water content measurements**

Ten fully expanded leaves similar to those used for leaf gas exchange measurements were sampled to measure the leaf RWC according to the methods of Barrs and Weatherley (1962). New fully expanded leaves of plants after 2 h of salt treatment were used to measure leaf water potential via a pressure chamber (Model 3115, Soil moisture Corp., Santa Barbara, CA, USA). Each treatment included ten replications.

**2.5 Whole-plant transpiration rate and Kplant**

A single plant was inserted into a 50 mL conical flask that contained one of four different kinds of solutions. After 1 h of acclimation, the whole-plant transpiration rate was determined gravimetrically every ten min for a total of 6 times. The whole-plant transpiration rate was calculated as follows:

whole-plant transpiration rate = (Wtime n+1-Wtime n)/leaf areawhere the leaf area was measured by a scanner and analysed by WinRhizo Pro 2015 software (Regent Inc., Canada). The Kplant was calculated as follows:

Kplant = whole-plant transpiration rate/ (hydroponic water potential-leaf water potential)

**2.6 Lp**

Lp was determined on the basis of the root surface area and was measured by a pressure chamber (Zhao et al.,2005; Yan et al.,2016).

After measuring the volume of sap exuded, the roots were measured with a scanner to determine the root surface area. The data were subsequently analysed using WinRhizo Pro 2015 software (Regent Inc., Canada). Each treatment included ten replications.

**2.7 Analysis of maize root aquaporin gene expression**

After 2 h of salt treatment, root tips (<5 cm in length) were collected and subsequently 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 then digested with DNase I and evaluated via a dissociation curve to ensure that all DNA was eliminated. cDNA was synthesized in vitro via TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgen, Beijing, China) according to the manufacturer’s instructions. Quantitative real-time PCR (qRT-PCR) was performed on a LightCycler 480 instrument in conjunction with 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 by Hachez (2006) (Hachez et al.,2006) were used. The data were analysed according to the 2-ΔΔCt method (Pfaffl 2001) (Pfaffl,2001) and normalized against the data from the CK treatment (relative units).

**2.8 Changes in the transpiration rate in response to an aquaporin inhibitor (HgCl2)**

In accordance with the method of Knipfer et al. (2011), we investigated aquaporin-mediated water transport by measuring changes in the transpiration rate in response to the application of the aquaporin inhibitor HgCl2. Seedlings were treated with 50 μM HgCl2, after which the transpiration rate was measured according to the weighing method. Briefly, the seedlings were transplanted into a triangular flask that contained one of the four treatment solutions together with HgCl2, after which the flask was then sealed with parafilm. After 1 h treatment, we weighed the whole triangular flask+seedling+solution every ten min a total of 6 times. Afterwards, the leaves of the seedlings were collected to measure their surface area via WinRhizo Pro 2015 software (Regent Inc., Canada). Each treatment included ten replications.

**2.9 Statistical analyses**

The statistical data were analyzed with SPSS version 18.0. One-way ANOVA was used to determine the effects of MT under non-stressed conditions and stressed conditions. Differences between the means were compared by the least significant difference (LSD) test at P<0.05, and figures were produced using SigmaPlot version 12.01 (Systat Software, Inc.). All the experiments were repeated three times.

1. **Results**

**3.1 Biomass and leaf elongation rate**

There was no significant difference in the biomass and leaf elongation rate between the CK and CK+MT treatments (P>0.05, Fig. 1). After seven days of salt treatment, salt reduced the maize plant biomass accumulation compared with that of the CK treatment (a 41.4% decrease in whole-plant biomass and a 68.1% decrease in root biomass) (Fig. 1A), while the MT treatment reduced this decrease in biomass (P<0.05). In addition, compared with the S treatment, the S+MT treatment resulted in 99% greater root biomass and 20.2% greater whole-plant biomass. Moreover, salt stress significantly reduced the leaf elongation rate (P<0.05); the leaf elongation rate was 65.9% greater in the S+MT treatment than in the S treatment (P<0.05). Taken together, these results indicated that MT application alleviated the salt-induced decrease in the leaf elongation rate.

**3.2** **Endogenous MT content**

After seven days of salt treatment, the endogenous MT content significantly increased in maize roots with of seedlings exogenously treated with MT in the absence or presence of salt stress (an increase of 86.6% under normal conditions and an increase of 305% under salt stress, P<0.05). We also found that salt stress reduced the endogenous MT content in the S+MT treatment compared with the CK+MT treatment (19.3%, Fig. 2).

**3.3 Leaf gas exchange parameters**

Under normal conditions, MT application did not significantly affect the photosynthesis rate, stomatal conductance, or transpiration rate (P>0.05, Fig. 3). Similar to that which occurred for the leaf elongation rate, salt stress decreased all these parameters, and MT application significantly reduced the decrease in these parameters (P<0.05); compared with the S treatment, the S+MT treatment had a greater photosynthesis rate (24.3%), stomatal conductance (39.4%), and transpiration rate (40.7%). These results show that exogenous MT application could be beneficial for maintaining the photosynthesis rate, stomatal conductance, and transpiration rate of maize seedlings under short-term salt stress.

**3.4 Leaf RWC, water potential and whole-plant transpiration rate**

The leaf RWC of the seedlings was 91.0% in the CK treatment and 91.2% in the CK+MT treatment and was nearly unaffected by the application of MT in the absence of salt (Fig. 4A). However, under salt stress, the leaf RWC was significantly greater for the MT-treated seedlings (84.0%) than for the non-MT-treated seedlings (75.6%) (P<0.05), indicating that exogenous application of MT was beneficial to the maintenance of the leaf water status of the seedlings. Under normal conditions, the leaf water potential ranged from -0.24 to -0.21 MPa, and there was no significant difference between the CK and CK+MT treatments (P>0.05, Fig. 4B). However, the exposure of the maize seedlings to salt stress reduced the leaf water potential to -0.68 MPa, but this parameter was maintained at -0.57 MPa when the seedlings were treated with MT. Taken together, these results showed that MT application could improve the water status of plants.

After 1 h of salt treatment, the whole-plant transpiration rate of the salt-treated maize seedlings was lower than that of the untreated seedlings (P<0.05, Fig. 5A). These results showed that MT improved the plant water status at the whole-plant level during the early treatment period, i.e., 1 h.

**3.5 Kplant and Lp**

Under normal conditions, there was no significant change in the Kplant of maize seedlings treated with (1.88 mmol H2O m-2 s-1 MPa-1) or without (2.18 mmol H2O m-2 s-1 MPa-1) MT (P>0.05, Fig. 5B). Under salt stress, however, a sharp decrease in Kplant was observed in seedlings either treated or not with MT compared with that in seedlings under normal conditions, but the Kplant was 68% greater in the MT-treated seedlings than in the untreated seedlings.

The Lp was not affected by MT application under normal conditions (P>0.05, Fig. 6), but it was significantly affected under salt stress. Under salt stress, the Lp decreased by only 61% in the MT-treated seedlings but decreased by 97% in the untreated seedlings, indicating that MT application increased the root water uptake capacity. The root area of the seedlings did not differ among these treatments (P>0.05, Fig. S1).

**3.6 Expression of root aquaporin genes**

With the exceptions of *ZmPIP1;6* and *ZmPIP2;7*, whose expression was upregulated, the expression of nearly all *ZmPIP*s was downregulated in response to MT application under normal conditions (Fig. 7). Under salt stress conditions, compared with that in non-MT-treated seedlings, the expression of *ZmPIP1;6*, *ZmPIP2;3* and *ZmPIP2;7* in MT-treated seedlings was significantly upregulated – 1.95-, 3.41- and 5.32-fold, respectively.

**3.7 Changes in the transpiration rate in response to HgCl2**

Compared with that in the absence of HgCl2, the whole-plant transpiration rate in the presence of HgCl2 decreased (Fig. 8 and Fig. 4). Similar to that which occurred in the absence of HgCl2, there was no significant difference between the CK and CK+MT treatments (P>0.05). While the absence of HgCl2 had different effects under salt stress, there was no significant difference in the transpiration rate between seedlings in the S treatment and S+MT treatment (P>0.05). Taken together, these results showed that under salt conditions, aquaporins play an important role in maintaining a greater root water uptake capacity in the MT-induced improvement of maize salt resistance.

**4 Discussion**

As one of the most important cereal crop species worldwide, maize productivity is limited by multiple abiotic stresses (e.g., salinity, flooding and drought) (Messmer et al.,2009; Chalivendra and Martin,2003). In the past few decades, numerous studies have investigated the underlying mechanisms involved in alleviating salt stress, and researchers have also conducted many excellent studies to explain how MT relieves Na+ toxicity after long-term salt stress (Ke et al.,2018; Kostopoulou et al.,2015). In the present study, we found that exogenous MT can improve plant salinity tolerance by regulating root water uptake under short-term salt stress. Elucidating the underlying mechanism through which MT regulates plant growth and development by alleviating salt stress would greatly accelerate the application of MT in the development of saline-alkali agriculture.

**4.1 Aquaporins are involved in the MT-mediated increase in hydraulic conductance**

Water homeostasis plays a crucial role not only in plant growth but also in adaptation to osmotic stress induced by salt and drought (Horie et al.,2011). Osmotic stress causes water loss in leaf cells and leads to a decrease in leaf water potential, which can result in reduced cell elongation and division, ultimately leading to decreased leaf growth (Liu et al.,2015). MT application significantly increased the leaf water potential and leaf RWC (Fig. 4) of treated seedlings compared with those of untreated seedlings, indicating that MT alleviates salt-induced stress by improving the water status of leaves.

Salt stress significantly reduced the Kplant and stomatal conductance, but MT application reduced these declines (Fig. 5 and Fig. 3). Previous studies have shown that changes in Kplant affect stomatal conductance and photosynthesis (Hubbard et al.,2001; Liu et al.,2014), which is consistent with the observations in our study. In general, Kplant is affected by leaf hydraulic conductance, stem hydraulic conductance and Lp. According to a previous study, the main whole-plant hydraulic resistance is grounded in the leaves and roots, and stem hydraulic conductance is not the limiting factor for water transport because of its greater flow (Javot and Maurel,2002; Martre et al.,2002). In fact, as the first plant organs to perceive stress, roots are usually the main limiting organ within the soil–plant–air continuum under stress conditions (Martre et al.,2002). In the current study, we found that exogenous MT significantly reduced the decrease in Lp under short-term salt stress. Lp represents the root water uptake capacity and is determined by the root surface area and root water permeability. No difference was found in root surface area (Supplementary Fig. S1), which indicates that MT did not affect the water uptake by influencing the root surface area 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 (Wang et al.,2013; Yan et al.,2016). To determine the role of aquaporins in the MT-mediated increase in hydraulic conductance, we studied the expression of root aquaporin genes and found that the expression of *ZmPIP1;6*, *ZmPIP2;3* and *ZmPIP2;7* was considerably upregulated (Fig. 7). Previous studies have shown that the expression of PIP1;6 and PIP2;3 is highly upregulated in *Sorghum bicolor* via Si-induced increased root water uptake under osmotic stress and salt stress conditions (Liu et al.,2014; Liu et al.,2015). These findings indicate that these two PIPs also function in the MT-mediated increase in hydraulic conductance. *ZmPIP1;5* and *ZmPIP2;6* were found to be highly responsive to salt stress, which was consistent with the results of a previous study (Zhu et al.,2005); however, the expression of *ZmPIP1;5* and *ZmPIP2;6* in seedlings in the S+MT treatment was low. These results showed that MT specifically regulates *ZmPIP*s and that the expression of *ZmPIP*s might be involved in MT-induced improved water uptake under short-term salt stress.

Here, exogenous MT induced an increase in the relative expression of PIPs under salt stress. Many studies have confirmed that, under abiotic stress, abscisic acid (ABA), Si and Ca applications increase PIP expression (Liu et al.,2014; Wu et al.,2012b; Mahdieh and Mostajeran,2009). Exogenous MT increased ABA production, and application of an ABA biosynthesis inhibitor caused membrane lipid peroxidation and reduced MT-induced antioxidant defence responses in *Elymus nutans* under cold stress, indicating that ABA-dependent pathways may contribute to the MT-induced stress response (Fu et al.,2017). Taken together, these results suggest, that under salt stress, MT induced an increase in the 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 determine the participation of aquaporins in MT-induced increased water uptake, we performed a HgCl2 application test. Under HgCl2 treatment, the significant differences in the measured characteristics between the S treatment and the S+MT treatment disappeared (Fig. 4, Fig. 5 and Fig. 8). These results confirmed the viewpoint that greater aquaporin activity is necessary for the MT-induced improvement in Lp under short-term salt stress and that MT could regulate aquaporins. The improved aquaporin activity could be regulated by ROS, especially H2O2. Environmental stress-induced ROS accumulation, e.g., H2O2 and exogenous MT, was found to stimulate the antioxidant defence system, alleviating ROS accumulation (Li et al.,2017; Jiang et al.,2016). In plants, H2O2 causes an oxidative blockage of aquaporins, which ultimately inhibits water uptake by the roots (Ye and Steudle,2006). Here, the results suggest that, under salt stress, MT application could increase the antioxidant capacity and decrease ROS accumulation to reduce the H2O2-induced oxidative blockage of aquaporins to increase Lp.

**4.2 MT mitigates the effects of short-term salt stress in maize**

Photosynthesis is the physico-chemical process by which plants use light energy to drive the synthesis of organic compounds and is the basis of plant production (Barnawal et al.,2017). Various types of stress, such as salinity stress, drought stress, and oxidative stress, severely repress photosynthesis (Gombos et al.,1994). After 2 h of salt treatment, seedlings in the S+MT treatment had a greater net photosynthesis rate than did those in the S group (Fig. 3); however, the leaves of the plants in the S group grew more quickly, and the plants accumulated more biomass (Fig. 1). These results are in agreement with those of Li et al. (Li et al.,2017), who reported that exogenous MT enhanced watermelon salt stress tolerance by improving photosynthesis and inhibiting stomatal closure. In general, plants under drought and salt stress close their stoma to decrease leaf transpiration to reduce water loss (Yan et al.,2020; Shahid et al.,2019), which subsequently induces stomatal limitation of photosynthesis (Kimura et al.,2020). In the present study, seedlings in the S+MT treatment presented greater stomatal conductance than did those in the S treatment, indicating that the degree of stomatal opening in seedlings in the S+MT treatment was greater. These results are consistent with those of a previous study showing that exogenous MT alleviated salt-induced decreases in stomatal conductance in maize seedlings (Chen et al.,2018). These decreases in stomatal conductance could reduce the harmful effects of short-term salt stress on photosynthesis.

Taking all these results into consideration, the present study suggests that exogenous MT pre-treatment enhances maize resistance to short-term salt stress by regulating Lp, which decreases to some extent by the upregulated expression of specific aquaporin genes and thereby improves aquaporin activity. On the basis of this conclusion, the underlying mechanism by which MT improves maize resistance to short-term salt stress can be speculated. Under short-term salt stress, MT application could increase the root water uptake capacity by regulating aquaporin activity to maintain a greater leaf water potential and leaf RWC. As a result, MT-treated maize seedlings maintained an improved leaf water status and a decreased stomatal limitation to maintain a relatively high photosynthesis rate and leaf growth rate.

**Conflicts of interest:** The authors declare that they have no competing interests.

**Author contributions:** Jiakun Yan: Conception and design, analysis and data processing; Final approval of the article, obtainment of funding. Ningning Zhang and Huiling Chen: Collection of data, analysis and data processing. Meng Li: Collection of data, analysis and data processing. Rong Yan: Collection of data, analysis and data processing. All authors took part in writing the manuscript.

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Figures



Figure. 1 Changes in the biomass and leaf elongation rate of maize seedlings in response to MT application under CK and salt treatments. New fully expanded leaves were used to measure the leaf elongation rate after 24 h of NaCl treatment. The values are the means±standard deviations (SDs) of ten independent replicates. The different letters indicate significant differences (P<0.05).



Figure. 2 Changes in root MT content after salt treatment for seven days. The values are the means ± SDs of ten replicates. The different letters indicate significant differences (P<0.05).



Figure. 3 Changes in the photosynthesis rate (A), stomatal conductance (B), and transpiration rate (C) of maize seedlings in response to MT application under CK and salt treatments. After 2 h of NaCl treatment, fully expanded leaves were used for measurements via a portable photosynthesis system (LI-6400). The values are the means±SDs of ten replicates. The different letters indicate significant differences (P<0.05).



Figure. 4 Changes in the leaf RWC (A) and water potential (B) of maize seedlings in response to MT application under CK and salt treatments. The RWC and leaf water potential were measured after 2 h of NaCl treatment. Ten leaves were removed to measure the RWC. The water potential of new fully expanded leaves was measured in a pressure chamber (Model 3500, Soil Moisture Corp., Santa Barbara, CA, USA). The values are the means±SDs of ten replicates. The different letters indicate significant differences (P<0.05).



Figure. 5 Effects of MT application and salt stress on the transpiration rate and Kplant of hydroponically cultivate seedlings. The transpiration rate of the maize seedlings was determined gravimetrically after 1 h of NaCl treatment. Kplant was calculated by the gravimetrically determined transpiration rate divided by the difference between the hydroponic water potential and leaf water potential. The values are the means±SDs of five replicates. The values are the means±SDs of ten replicates.



Figure. 6 Changes in the Lp of maize seedlings in response to MT application under CK and salt treatments. After 2 h of NaCl treatment, the entire root system of each ten-day-old seedling was removed near the base of the roots, leaving 4 cm of mesocotyl tissue, after which root system was inserted into a pressure chamber. To determine the driving force, the volume exuded from the root system was plotted as a function of time. The slope of these relationships in reference to the root dry mass was denoted by Lp. The values are the means±SDs of ten replicates.



Figure. 7 Effects of MT application and salt stress on the expression levels of root *ZmPIP* aquaporin genes. The roots were sampled after 2 h of salt osmotic treatment with and without MT application. The relative expression was determined by qRT-PCR. The values are the means±SDs of three replicates. The different letters indicate a significant difference (P<0.05).



Figure. 8 Effects of the aquaporin inhibitor HgCl2 on the transpiration rate with and without MT application under salt stress. The transpiration rate was measured after 50 μM HgCl2 was added gravimetrically. The values are the means±SDs of ten replicates. The different letters indicate a significant difference (P<0.05).



Figure. S1 Effects of MT application and salt stress on the root surface. The values are the means ± SD of ten replicates. Different letters indicate significant differences (P<0.05).