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Mechanism of calcium in melatonin enhancement of functional substance-phenolic acid in germinated hulless barley†

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Phenolic acid is a physiologically active substance that has a variety of effects on humans. Barley sprouts are often used as food ingredients to enrich phenolic acids and to further produce functional foods rich in phenolic acids. In this study, the mechanism of Ca^{2+} involvement in regulating phenolic acid biosynthesis and plant growth in barley by melatonin (MT) under NaCl stress was investigated. According to the studies, MT (25 μM) increased total calcium content, induced Ca^{2+} burst, and up-regulated the gene expression of calcium-regulated protein-dependent protein kinase and calcium-binding protein transcription-activating protease in NaCl-stressed (60 mM) barley. Exogenous MT and its combined $CaCl_2$ (0.4 mM) significantly promoted phenolic acid biosynthesis by increasing the activity of C4H and PAL, and induced gene expression of *PAL* and *F5H*. The addition of exogenous $CaCl_2$ and MT caused systemic tolerance in NaCl-stressed barley, as determined by a decrease in the fluorescence intensity of hydrogen peroxide and oxygen radical anions as well as an enhancement in the antioxidant enzyme, thus significantly increasing sprout length and fresh weight. In addition, combined use of MT with Ca^{2+} antagonists (lanthanum chloride or ethylene glycol tetraacetic acid), impaired all impacts as mentioned above. These findings imply that Ca^{2+} participated in MT-induced phenolic acid biosynthesis and growth improvement in NaCl-stressed barley.

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1. Introduction

Barley is among the most significant cereal crops worldwide and contains a variety of bioactive substances, along with phenolic acids, tocopherols, and folic acid.[1] Among them, phenolic acids have various physiological impacts on the body, including antioxidant, anticancer, and antibacterial.[2] Because the human body is unable to manufacture phenolic acids, they must be obtained from the diet,[3] the development of functional foods rich in phenolic acids has received much attention from scholars. Studies have shown that environmental stimuli, especially salt stress, can significantly increase the phenolic acid content in barley, but also cause inhibition of barley growth.[4]

Melatonin (MT), as a plant hormone, is efficient in regulating development of plants[5] as well as the detrimental effects of abiotic stress on plants.[6] MT has been discovered in recent years to have a significant regulatory function in forming secondary metabolites in plants. Exogenous MT treatment could enhance levels of flavonoids, carotenoids, and isoflavones in citrus,[7] tomato,[8] and soybean,[9] respectively. Previously, we also found that MT considerably stimulated the biosynthesis of phenolic acids in barley under salt stress[10] and effectively improved plant stress resistance. These MT effects are frequently linked to higher antioxidant enzyme activity and transcriptional activation of relevant secondary metabolite genes.[11] However, MT-related growth, defense responses, and metabolite synthesis of plants are complex, and the signaling pathway that MT regulates these responses is poorly understood.

Ca^{2+} has a vital role in biological signaling as a second messenger. Plant responses to environmental stimuli, such as NaCl stress, include a spike in Ca^{2+} levels that activates Ca^{2+} signaling networks and receptors.[12] Ca^{2+} signaling alleviates plant injury through proline accumulation, enhancement of antioxidant enzyme activity, and regulation of ion homeostasis in tissues.[13] By promoting the flavonoid signaling system's gene expression, the calcium signaling route has also been shown to be essential for the anthocyanin production in grapes.[14] Notably, an amount of recent studies have revealed that MT can exert a regulatory effect on the calcium signaling pathway in plants. MT induced the relative genes expression involved in

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Ca^{2+} signaling in plants.[15] Meanwhile, combined MT and Ca^{2+} treatment could promote photosynthesis and salt tolerance of *Dracocephalum kotschyi*,[16,17] improve postharvest quality of cassava,[12] and enhances resilience against arsenic toxicity of *Vicia faba*.[18] The only two studies showed that MT induced the synthetic of apigenin, luteolin, and rosmarinic acid in NaCl-stressed *Dracocephalum kotschyi*[19] and astaxanthin in *Haematococcus pluvialis*,[20] respectively, dependent on Ca^{2+}/CaM signaling. To determine if Ca^{2+} is involved in the MT-induced production of specific metabolites, more research is required. In particular, the role of Ca^{2+} in the MT-induced phenolic acid enrichment and growth improvement in NaCl-stressed barley remains to be further elucidated.

Lanthanum chloride ($LaCl_3$) and ethylene glycol tetraacetic acid (EGTA) are Ca^{2+} plasmalemma channel blockers and Ca^{2+} chelators, respectively. $LaCl_3$ inhibits the release of extracellular calcium stores by blocking the opening of Ca^{2+} plasmalemma channel, and attenuates Ca^{2+} flux on protoplasts, cytoplasmic membranes, and tonoplast vesicles. Therefore, the effects of MT combined with exogenous $CaCl_2$ and its antagonist ($LaCl_3$ and EGTA) treatment on growth characteristics, phenolic acid, and calcium content, activities, as well as gene expressions of critical enzymes required for the synthesis of phenolic acids and Ca^{2+} signaling metabolic pathways in barley were investigated. This made it possible to learn more about the intrinsic function of Ca^{2+} signaling involved in MT-induced phenolic acid biosynthesis and growth improvement in germinated barley under NaCl stress.

2. Materials and methods

2.1 Plant material and treatments

Barley seeds should be soaked in distilled water for 6 h at 25 °C, then put them in a germination box and germinate for 6 days at 25 °C in the dark. Different treatments were used:

(1) N: 60 mM NaCl; (2) NM: 60 mM NaCl plus 20 μM MT; (3) NC: 60 mM NaCl plus 0.4 mM $CaCl_2$; (4) NMC: 60 mM NaCl plus 20 μM MT plus 0.4 mM $CaCl_2$; (5) NL: 60 mM NaCl plus 5 mM $LaCl_3$; (6) NML: 60 mM NaCl plus 20 μM MT plus 5 mM $LaCl_3$; (7) NE: 60 mM NaCl plus 0.4 mM EGTA; (8) NME: 60 mM NaCl plus 20 μM MT plus 0.4 mM EGTA. The concentrations of NaCl, MT, $CaCl_2$, $LaCl_3$ and EGTA were selected according to the previous experiment. Random samples of barley sprouts from different treatments were collected at 4 and 6 days after germination for biochemical examination. NaCl and $CaCl_2$ were procured from Macklin (Shanghai, China), MT, $LaCl_3$ and EGTA were procured from Sigma-Aldrich Co. (St. Louis, USA). All other chemicals and reagents used were analytical grade.

2.2 Sprout length and fresh weight

According to Yin *et al.* (2022b),[9] a sample of thirty sprouts were selected to measure sprout length and fresh weight.

2.3 Total phenolic acid

The Folin-technique Ciocalteu's was used to calculate the total phenolic acid content.[3]

2.4 Intracellular free calcium, H_2O_2 and $O_2^{\cdot-}$

The intracellular free calcium, H_2O_2 and $O_2^{\cdot-}$ fluorescence staining of 4 day-old germinated barley according to the method of Yin *et al.*[9]

2.5 Total calcium

According to the method of Callicott *et al.*[21] determined the total calcium content.

2.6 Antioxidant enzyme activity

Superoxide dismutase (SOD) activity was measured as described by Kochs *et al.*[22] Catalase (CAT) and peroxidase (POD) activities were measured as described by Wang *et al.*[23]

2.7 Phenylpropanoid metabolism-related enzyme activity

Phenylalanine ammonia lyase (PAL) and cinnamic acid 4-hydroxylase (C4H) activities were determined according to the method of Ma *et al.*[24]

2.8 Gene expression assay

Total RNA extraction, reverse transcription, and fluorescence quantitative PCR analysis refer to the method of Yin *et al.*[25] The primers used were listed in ESI Table S1.† Using the $2^{-\Delta\Delta C_t}$ technique, relative gene expression levels were determined.

2.9 Statistical analysis

The data were all reported as mean ± standard deviation, and each experiment was run in triplicate. Using Tukey's multiple-range test, variables from three replicates were compared, and a p-value of 0.05 was deemed significant.

3. Results

3.1 The total phenolic acid content of barley sprouts

Decreasing phenolic acid content in barley sprouts with increasing germination time (Fig. 1). Compared with NaCl stress, at the same germination time, exogenous MT, $CaCl_2$ and the combination of both significantly increased phenolic acid content in sprout ($p < 0.05$), and the MT–$CaCl_2$ treatment resulted in the greatest phenolic acid content, 1.13 and 1.11 times that of the NaCl–MT treatment. However, the addition of $LaCl_3$ and EGTA reversed MT-induced phenolic acid synthesis. The above results suggest that exogenous MT was used to increase the total phenolic acid content of barley sprouts through the synthesis of the Ca^{2+} pathway.

3.2 The physiology and biochemistry of barley sprouts

Exogenous MT, $CaCl_2$ and MT–$CaCl_2$ promoted the length and fresh weight of barley sprouts under NaCl stress at the same growth time (Fig. 2I–III, $p < 0.05$). EGTA also promoted sprouts length of barley under NaCl stress, however, the addition of $LaCl_3$ not only inhibited the physiological status (Fig. 2I–III) of barley sprouts under NaCl stress but also reversed the alleviating effect of NaCl–MT treatment.

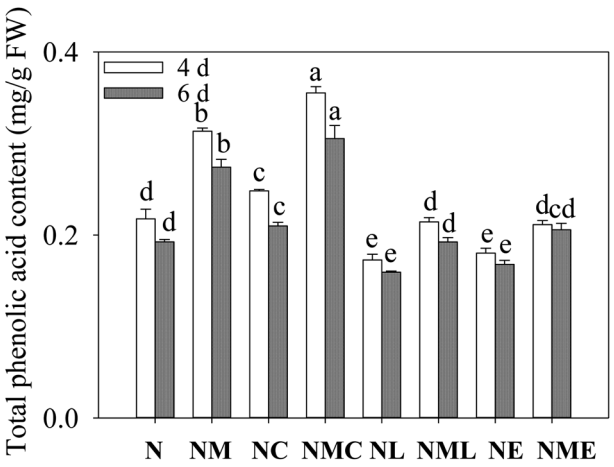

Fig. 1 Effect of MT–Ca^{2+} on the total phenolic acid content of barley sprout under NaCl stress. Different letters indicate the significant differences in indicators among treatments at the same germination time using Tukey's test ($p < 0.05$). N: NaCl; NM: NaCl + MT; NC: NaCl + $CaCl_2$; NMC: NaCl + MT + $CaCl_2$; NL: NaCl + $LaCl_3$; NML: NaCl + MT + $LaCl_3$; NE: NaCl + EGTA; (8) NME: NaCl + MT + EGTA.

MT and MT–$CaCl_2$ groups significantly attenuated blue ($O_2^{\bullet-}$) and red (H_2O_2) (Fig. 2IV and V) fluorescence intensities, as well as distributions, compared to NaCl treatment alone, which suggests that MT–$CaCl_2$ reduces the extent of cell membrane damage. $LaCl_3$ and EGTA dramatically increased the fluorescence intensity of H_2O_2 and $O_2^{\bullet-}$ in the root tips of sprouts treated with NaCl combined with MT, indicating that the Ca^{2+} inhibitor enhanced the cumulation of reactive oxygen species (ROS) in the cells.

3.3 The antioxidant enzyme activity of barley sprouts

MT significantly increased the activity of antioxidant enzyme in sprouts under NaCl treatment (Fig. 3I–III), and the activities of POD, SOD and CAT increased by 37%, 69.3% and 97.5% on the fourth day, and 34.3%, 72.4% and 114.4% on the sixth day. $CaCl_2$ addition significantly increased POD activity by 10.7% and 10.2% compared to NaCl–MT treatment (Fig. 3I). However, the addition of $LaCl_3$ and EGTA reversed the effects of MT-induced salt stress on POD, SOD and CAT activities in barley, and the inhibitory effect of $LaCl_3$ was greater than that of EGTA.

3.4 Effect of MT on Ca^{2+} metabolism of barley sprouts

The addition of exogenous MT considerably raised the total calcium concentration in the sprouts compared to the NaCl treatment alone (Fig. 4I, $p < 0.05$) and rose by 41.5% and 81.9%. Furthermore, the intensity of green (Ca^{2+}) fluorescence in the root tip obviously increased (Fig. 4II). $CaCl_2$ addition resulted in 2.05- and 1.56-fold higher total calcium content in sprouts than the NaCl plus MT treatment (Fig. 4I), as well as enhanced fluorescent staining (green) and expanded distribution of root tips (Fig. 4II). In contrast, EGTA and $LaCl_3$ addition significantly inhibited the total calcium content in the sprouts, and the inhibition effect of $LaCl_3$ was significantly greater than that of

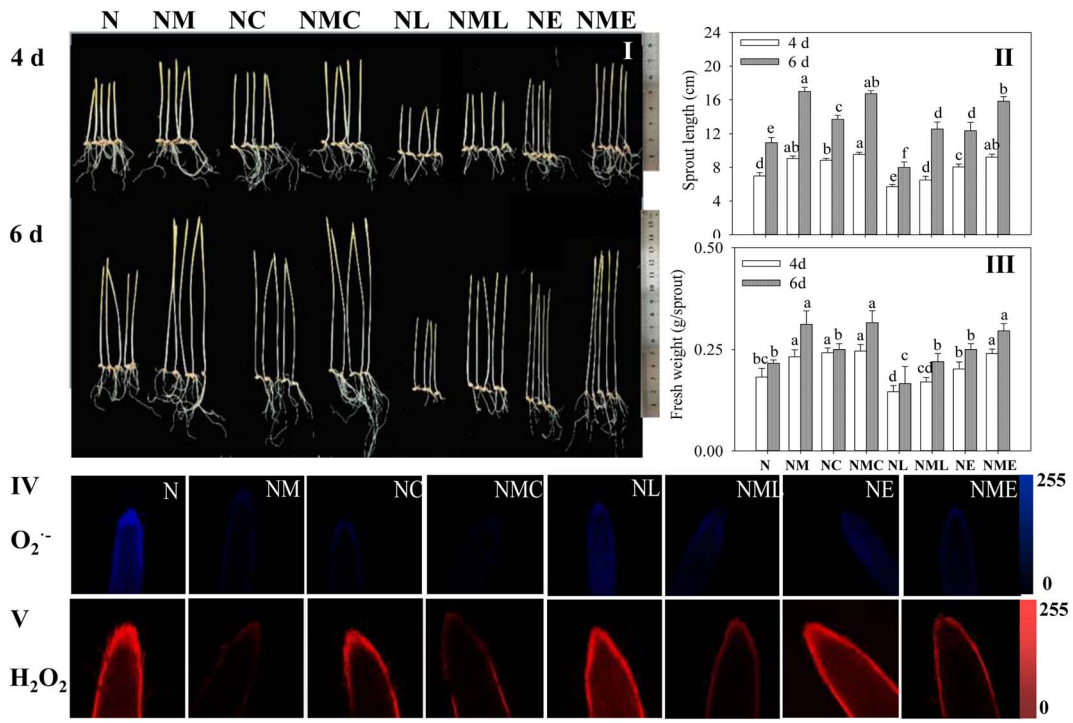

Fig. 2 Effects of MT–Ca^{2+} on the (I) growth performance, (II) sprout length, (III) fresh weight, and root tip staining of (IV) $O_2^{\bullet-}$ and (V) H_2O_2 of barley sprout under NaCl stress. The scale length is 100 μm. Different letters indicate the significant differences in indicators among treatments at the same germination time using Tukey's test ($p < 0.05$). N: NaCl; NM: NaCl + MT; NC: NaCl + $CaCl_2$; NMC: NaCl + MT + $CaCl_2$; NL: NaCl + $LaCl_3$; NML: NaCl + MT + $LaCl_3$; NE: NaCl + EGTA; (8) NME: NaCl + MT + EGTA.

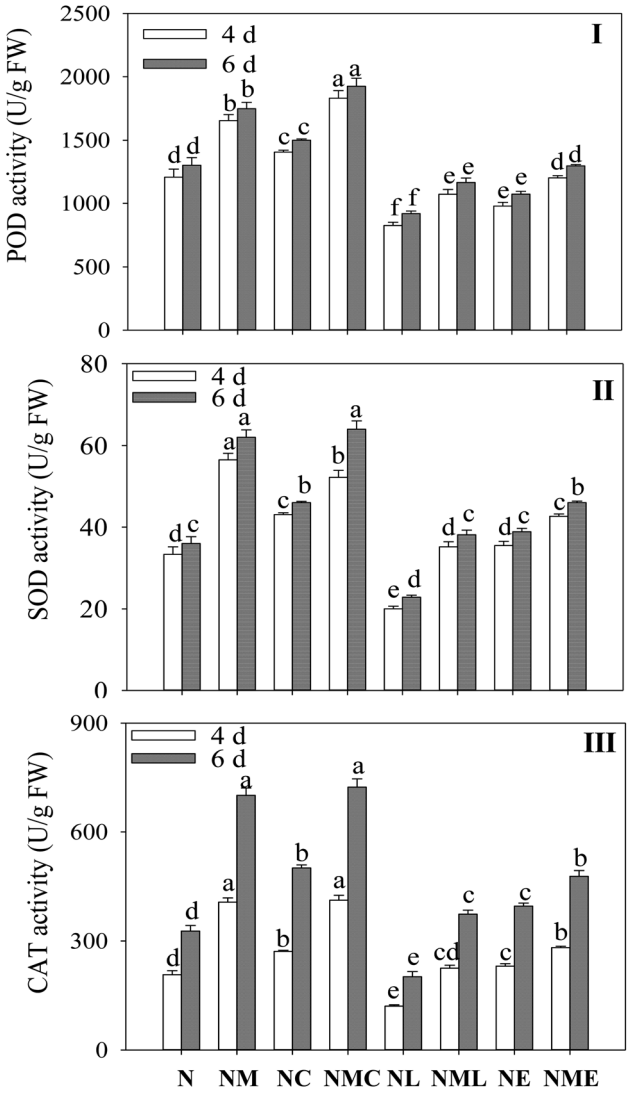

Fig. 3 Effects of MT–Ca^{2+} on the activities of (I) POD, (II) SOD and (III) CAT of barley sprout under NaCl stress. Different letters indicate the significant differences in indicators among treatments at the same germination time using Tukey's test ($p < 0.05$). N: NaCl; NM: NaCl + MT; NC: NaCl + $CaCl_2$; NMC: NaCl + MT + $CaCl_2$; NL: NaCl + $LaCl_3$; NML: NaCl + MT + $LaCl_3$; NE: NaCl + EGTA; (8) NME: NaCl + MT + EGTA.

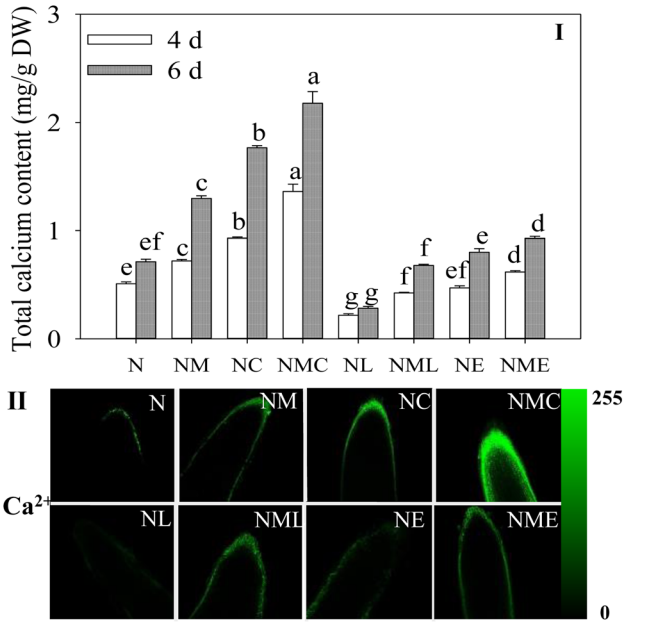

Fig. 4 Effect of MT on Ca^{2+} metabolism of barley sprouts under NaCl stress. (I) Total calcium content and root tip staining of (II) intracellular free calcium. The scale length is 100 μm. Different letters indicate the significant differences in indicators among treatments at the same germination time using Tukey's test ($p < 0.05$). N: NaCl; NM: NaCl + MT; NC: NaCl + CaCl2; NMC: NaCl + MT + $CaCl_2$; NL: NaCl + $LaCl_3$; NML: NaCl + MT + $LaCl_3$; NE: NaCl + EGTA; (8) NME: NaCl + MT + EGTA.

EGTA (Fig. 4I). The findings suggest that MT can cause Ca^{2+} spikes in cell root tips.

3.5 The expression of calcium target protein genes of barley sprouts

Exogenous MT substantially increased *CaMT* expression in 6 day-old barley sprouts exposed to NaCl ($p < 0.05$), and this increase was 2.43 times more than that of NaCl treatment (Fig. 5V). In 6 day-old barley sprouts, $CaCl_2$ addition significantly induces the levels of *Ca^{2+}-ATP*, *CaMK1*, *CaMK2* and *CDPK* (Fig. 5I–IV, $p < 0.05$), which was 2.9-, 3.6-, 12.1- and 13-fold greater than that of the NaCl–MT treatment, respectively. Similarly, the addition of EGTA and $LaCl_3$ significantly up-regulated the expression of *Ca^{2+}-ATP*, *CaMK1*, *CaMK2* and *CDPK* on the sixth day compared to the NaCl plus MT treatment ($p < 0.05$).

3.6 The expression of phenolic acid metabolizing enzymes and related genes of barley sprouts

As shown in Fig. 6I and II, exogenous MT, $CaCl_2$ and the combination of both markedly enhanced the activity of PAL and C4H in wheat seedlings under NaCl stress ($p < 0.05$). At 4 d of NaCl stress, PAL and C4H activities in sprouts under MT–$CaCl_2$ treatment were the highest, 1.23 and 1.19 times higher than those in the MT-treated group. The stimulatory effect of MT was markedly reversed under NaCl stress by applying the $LaCl_3$ and EGTA ($p < 0.05$).

To deeply investigate the mechanism of MT-induced phenolic acid synthesis, we analyzed the gene expression of key enzymes in the phenolic acid synthesis pathway (Fig. 6III–VIII). According to Fig. 6, compared with NaCl treatment, MT addition substantially up-regulated the expressions of *PAL*, *C4H* and *4CL*. Additionally, the NaCl–MT treatment significantly increased the expression of *C3H* and *COMT* in sprouts that were 6 days old ($p < 0.05$). The application of $CaCl_2$ significantly up-regulated the expression of *PAL* (Fig. 6III) on germination 4 d and *F5H* (Fig. 6VII) on germination 6 d, and which was 2.1 and 29.3 times higher than that of NaCl combined with MT treatment, respectively. However, compared with NaCl combined with MT treatment, the addition of EGTA significantly up-regulated the expression of *F5H*, but did not affect the

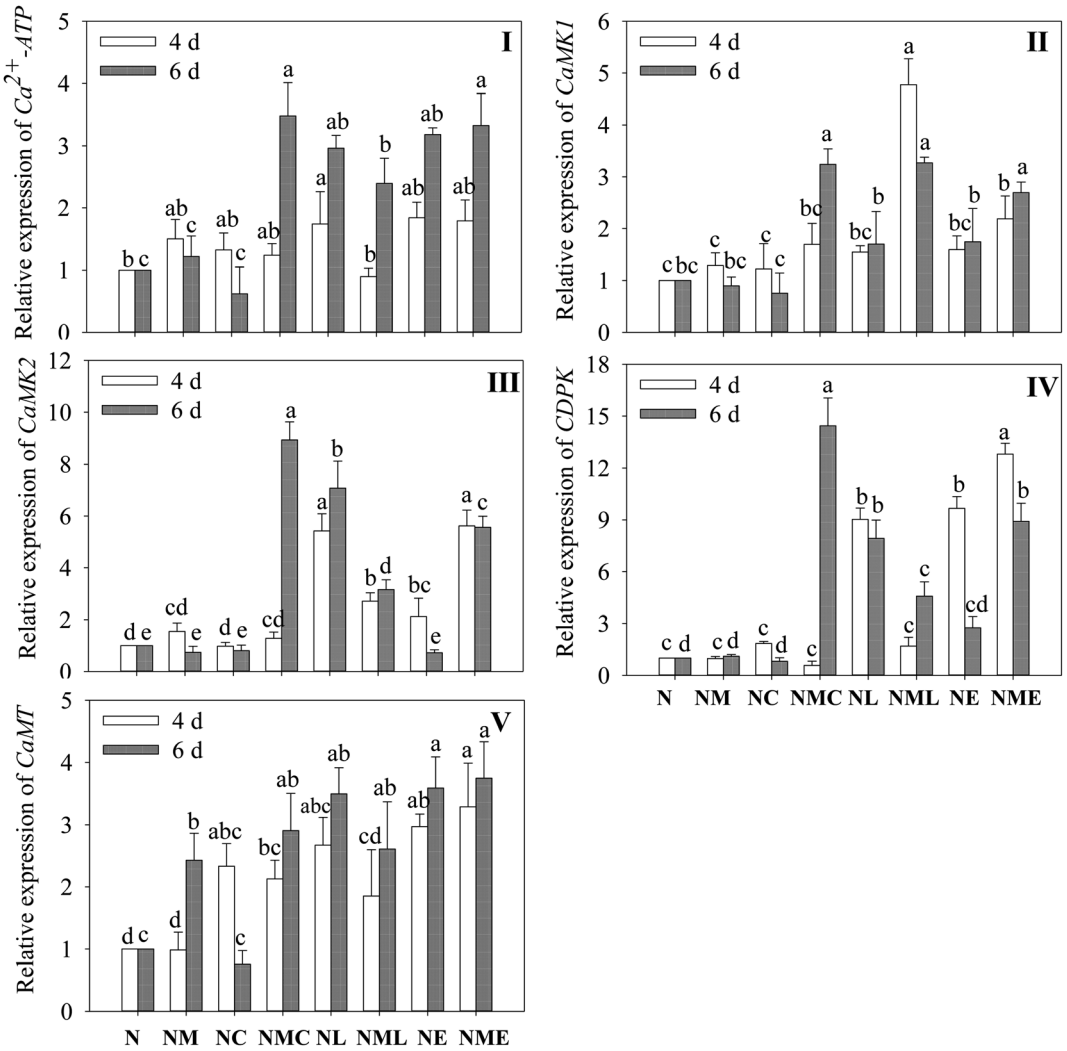

Fig. 5 Effect of MT–Ca^{2+} on the gene expression of *Ca^{2+}-ATP* (I), *CaMK1* (II), *CaMK2* (III), *CDPK* (IV), and *CaMT* (V) of barley sprouts. The gene expression in germinating barley treated with NaCl was used as the control. Different letters indicate the significant differences in indicators among treatments at the same germination time using Tukey's test ($p < 0.05$). N: NaCl; NM: NaCl + MT; NC: NaCl + $CaCl_2$; NMC: NaCl + MT + $CaCl_2$; NL: NaCl + $LaCl_3$; NML: NaCl + MT + $LaCl_3$; NE: NaCl + EGTA; (8) NME: NaCl + MT + EGTA.

expression of *C4H*, *C3H*, and *4CL*. In contrast, $LaCl_3$ treatment suppressed the expression of *C4H* as well as *PAL*, *C3H*, *4CL* and *COMT* for 6 days of germination. The above results indicated that $CaCl_2$ and MT promoted the synthesis of phenolic acids by enhancing the metabolic activity of key enzymes and the expression levels of enzyme genes in barley sprouts under NaCl stress through the interaction relationship.

4. Discussion

Barley is considered a good food material for the enrichment of phenolic acids. However, abiotic stress, although able to increase phenolic acids in germinated barley, can inhibit the growth and development.[4] Exogenous MT, a novel plant regulator, participates in the biosynthesis of secondary metabolites in plants and is efficient in reducing the negative effects of abiotic stress on them.[7,8] Exogenous MT is not only effective in alleviating the growth inhibition of barleys by NaCl stress, but also further promotes phenolic acid biosynthesis under salt stress, according to prior research.[10] However, the mechanisms by which MT regulates phenolic acid metabolism and NaCl tolerance in barleys are still unknown. Ca^{2+}, an essential messenger of plant signaling,[26] has been shown to interact with MT to activate antioxidant enzyme systems, reduce ROS, and promote secondary metabolite anabolism.[27] However, studies on the control of phenolic acid metabolism in barley sprouts by MT–Ca^{2+} interaction under NaCl stress are few.

Ca^{2+} is a widely distributed secondary messenger in plant signaling networks.[28] Plants' ability to regulate their reactions to their environment and adapt to it depends heavily on the concentration and redistribution of Ca^{2+}. The findings of the current investigation shown that, in comparison to NaCl treatment, the addition of exogenous MT considerably raised Ca^{2+} fluorescence intensity and increased the total calcium content in cells (Fig. 4I), indicating that MT not only induced Ca^{2+}

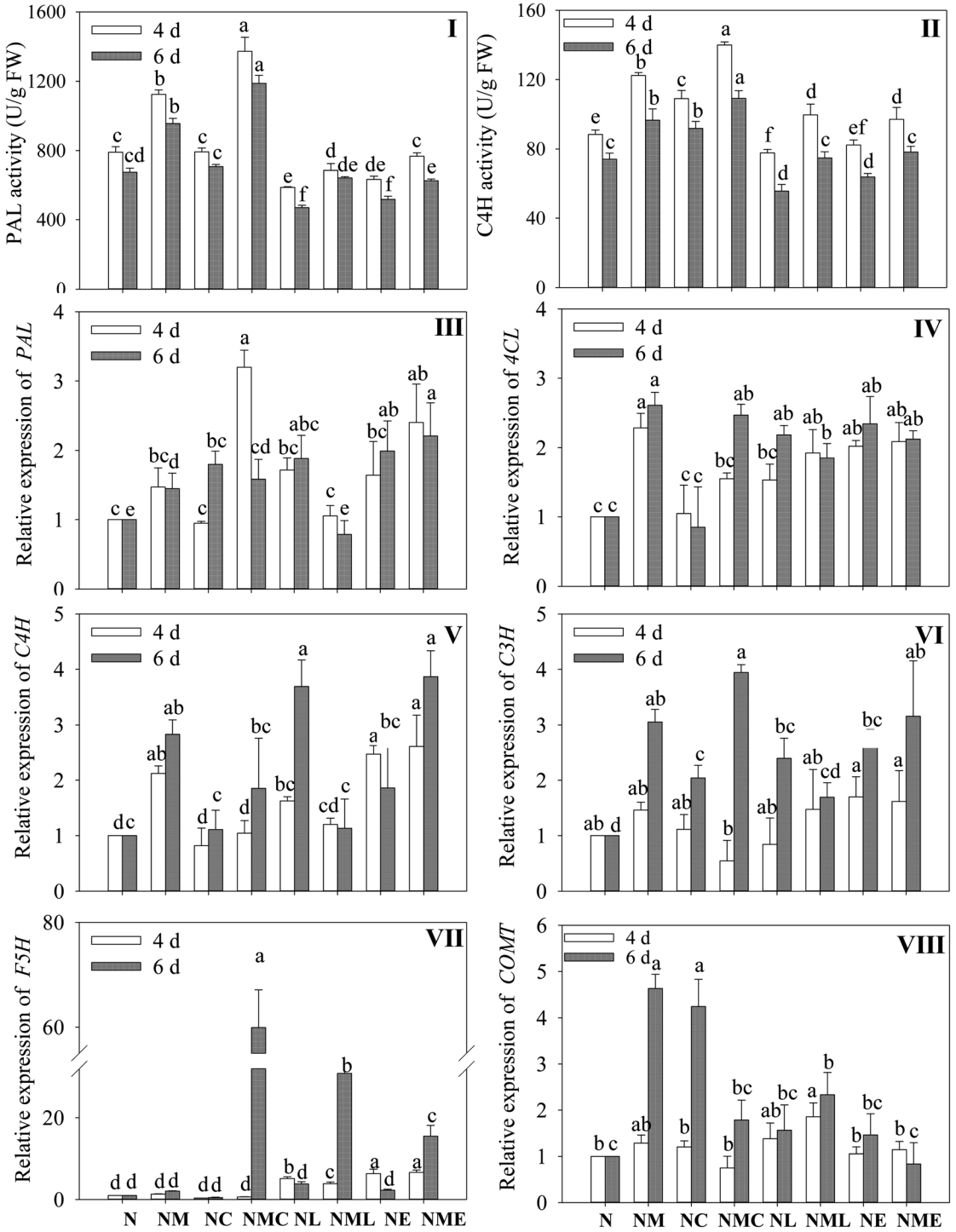

Fig. 6 Effects of MT–Ca^{2+} on activity of *PAL* (I) and *C4H* (II) and gene expression of *PAL* (III), *4CL* (IV), *C4H* (V), *C3H* (VI), *F5H* (VII), and *COMT* (VIII) in barley sprouts. The gene expression in germinating barley treated with NaCl was used as the control. Different letters indicate the significant differences in indicators among treatments at the same germination time using Tukey's test ($p < 0.05$). N: NaCl; NM: NaCl + MT; NC: NaCl + $CaCl_2$; NMC: NaCl + MT + $CaCl_2$; NL: NaCl + $LaCl_3$; NML: NaCl + MT + $LaCl_3$; NE: NaCl + EGTA; (8) NME: NaCl + MT + EGTA.

relocalization and distribution in root tip cells but also promoted the release of Ca^{2+} from root tips. We also found that the inhibitory effect of $LaCl_3$ on total calcium was significantly greater than that of EGTA, indicating that Ca^{2+} transport by MT under NaCl stress was mainly *via* calcium channels. Environmental stresses induce Ca^{2+} with different amplitude, temporal and spatial distribution, producing so-called "Ca^{2+} signaling" with different characteristics. In addition, Ca^{2+} and CaM can interact in plants to create Ca^{2+}–CaM complexes.[29] The addition of $CaCl_2$ in this study significantly increased the

relative gene expression of *CaMK*1, *CaMK*2, *CDPK*, and *Ca*$^{2+}$*-ATP* under MT treatment (Fig. 5). Through the activation of flavonoid biosynthesis genes, the Ca^{2+} signaling pathway has also been demonstrated to be involved in anthocyanin production.[30] Salicylic acid-induced[31] and abscisic acid[32] secondary metabolite synthesis are likewise controlled by the Ca^{2+} signaling. In the current investigation, $CaCl_2$ addition further greatly enhanced the phenolic acid content in MT-treated seedlings, while the addition of calcium antagonists ($LaCl_3$ and EGTA) completely reversed it (Fig. 1). These findings imply that MT most likely to regulates the amount of extracellular Ca^{2+} that enters the plasma membrane, thereby increasing the cytosolic Ca^{2+} level and inducing Ca^{2+} signaling, further regulating phenolic acid anabolism. To our knowledge, there aren't many research on how MT and Ca^{2+} interactions affect the synthesis of phenolic chemicals. However, consistent with our hypothesis, methyl jasmonate[33] and cytokinin[34] have all been reported to promote alkaloid synthesis by increasing cytosolic Ca^{2+} and thus alkaloid synthesis in cell culture *in vitro*. Meanwhile, study by Vafadar *et al.*[19] has demonstrated that extracellular Ca^{2+} influx and Ca^{2+}/CaM complex formation are necessary for the MT-induced accumulation of phenolic compounds in plants.

The accumulation of phenolic acid is closely related to the activity and relative gene expression levels of major enzymes involved in the pathway of phenolic acid synthesis. The activity of PAL, a rate-limiting enzyme for the production of phenols in plants, is correlated with the concentration of phenols.[35] The results showed that both exogenous MT alone and its combined $CaCl_2$ treatment significantly enhanced PAL and C4H activity and the corresponding phenolic acid content in NaCl-stressed barley. In contrast, the addition of $LaCl_3$ and EGTA inhibited PAL and C4H activity and reduced phenolic acid enrichment. This suggests that during phenolic acid synthesis in barley seedlings, Ca^{2+} may act downstream of MT to increase metabolic enzyme activity and thus induce phenolic acid accumulation. This agrees with the findings of Vafadar *et al.*[19] that exogenous MT enhances PAL activity through modulation of Ca^{2+} signaling and ultimately increases phenolics in salt-stressed *Dracocephalum kotschyi*. Additionally, the addition of $CaCl_2$ dramatically up-regulated the expression levels of related phenolic acid synthesis genes under NaCl combined with MT treatment. Exogenous MT and $CaCl_2$ promoted phenolic acid enrichment under NaCl stress by inducing expression levels of *PAL*, *COMT*, *4CL*, and *C3H* in 6 day-old barley. This is consistent with the findings reported in the literature that exogenous Ca^{2+} enhanced the expression of *PAL* and thus the phenolic content of wheat plants in a saline environment.[36] It is shown that under NaCl stress, MT acts on Ca^{2+} signaling to induce the activity (PAL and C4H) and gene expression levels (*PAL*, *C3H*, *4CL*, and *F5H*) of enzymes involved in the phenolic acid metabolic pathway, thereby promoting phenolic acid accumulation.

Plants accumulate large amounts of ROS in the body under abiotic stress,[37] which can induce a ROS scavenging system of enzymatic antioxidants in plants. Studies have demonstrated the ability of MT to modulate multiple antioxidant enzyme activities through the ROS scavenging system composed of enzymatic antioxidants and improve plant tolerance to a variety of environmental stresses.[38] The results demonstrated that MT greatly increased POD, SOD, and CAT activities in sprouts (Fig. 3). The addition of exogenous $CaCl_2$ further increased antioxidant enzyme activities in seedlings, compared with NaCl combined MT treatment, which was inhibited by adding $LaCl_3$ and EGTA. Furthermore, exogenous MT significantly reduced the H_2O_2 and $O_2^{\bullet-}$ fluorescence intensity in barley root tips under NaCl stress, compared with NaCl stress, and $CaCl_2$ addition further reduced the fluorescence intensity (Fig. 2). Previous studies reported that co-treatment of MT and $CaCl_2$ increased the antioxidant enzyme system and decreased the accumulation of ROS in low temperature-stressed cotton[39] and drought-stressed *Dalbergia odorifera*,[40] respectively.

Based on the above results, Fig. 7 shows a possible molecular mechanism by which MT–Ca^{2+} affects the enhancement of phenolic acid accumulation and growth, and we conclude that (1) exogenous MT induced Ca^{2+} influx and expression levels of calmodulin-related genes. (2) Ca^{2+} plays a critical part in MT regulation of activities and gene expression levels of the key enzyme in the phenolic acid synthesis pathway, leading to phenolic acid enrichment. (3) Ca^{2+} is involved in MT activation of antioxidant enzyme systems and maintenance of ROS homeostasis, thus improving barley growth. However, further in-depth studies are needed to confirm the details of Ca^{2+} signaling involvement in MT regulates phenolic acid biosynthesis and improves growth.

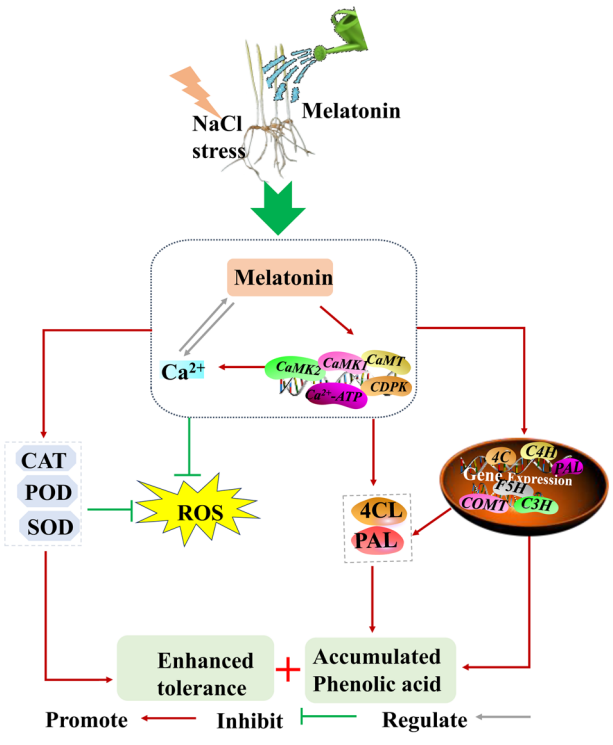

Fig. 7 A simulation of how combined MT and Ca^{2+} affected the phenolic acid biosynthesis and enhanced tolerance in barley under NaCl stress. CAT: catalase; SOD: superoxide dismutase; POD: peroxidase; PAL: phenylalanine ammonia lyase; 4CL: 4-coumarate-CoA ligase; ROS: reactive oxygen species.

Author contributions

Xin Tian: performed experiments and drafted manuscript. Xudong He: reviewed writing, Jinpeng Xu: performed experiments. Zhengfei Yang: reviewed writing. Weiming Fang: reviewed writing. Yongqi Yin: designed study and wrote manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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