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Effects of melatonin on seedling growth, mineral nutrition, and nitrogen metabolism in cucumber under nitrate stress

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Abstract

In China, excessive use of nitrogen fertilizers in greenhouses leads to nitrate accumulations in soil and plants, which then limits productivity. Melatonin, an evolutionarily highly conserved molecule, has a wide range of functions in plants. We analyzed the effects of melatonin pretreatment on the growth, mineral nutrition, and nitrogen metabolism in cucumber (*Cucumis sativus* L. 'Jin You No. 1') when seedlings were exposed to nitrate stress. An application of 0.1 mM melatonin significantly improved the growth of plants and reduced their susceptibility to damage due to high nitrate levels (0.6 M) during the ensuing period of stress treatment. Although excess nitrate led to an increase in the concentrations of nitrogen, potassium, and calcium, as well as a decrease in levels of phosphorus and magnesium, exogenous melatonin generally had the opposite effect except for a further rise in calcium concentrations. Pretreatment also significantly reduced the accumulations of nitrate nitrogen

and ammonium nitrogen and enhanced the activities of enzymes involved in nitrogen metabolism. Expression of *Cs-NR* and *Cs-GOGAT*, two genes that function in that metabolism, were greatly down-regulated when plants were exposed to 0.6 M nitrate, but was up-regulated in plants that had received the 0.1 mM melatonin pretreatment. Our results are the first evidence that melatonin has an important role in modulating the composition of mineral elements and nitrogen metabolism, thereby alleviating the inhibitory effect on growth normally associated with nitrate stress.

1 | INTRODUCTION

Nitrate is the major source of useable nitrogen for most plants.[1] In China, application rates for nitrogen fertilizers have recently increased dramatically in intensive agricultural systems, especially those used for protected vegetable production.[2] However, their misuse has resulted in the secondary salinization of soils and the accumulation of NO_3^-,[2,3] which reduces photosynthesis and enzyme activity in plants[4] but increases ion toxicity,[5] osmotic stress, and the production of reactive oxygen species.[6] These environmental factors limit agricultural productivity.

Excess nitrogen is generally applied so that adequate levels will be maintained in the rhizosphere. In fact, NO_3^- accounts for approximately 67% to 76% of all anions in the soil.[7] However, for vegetable crops, this disrupts the balance of elements, alters the assimilation of calcium and magnesium, and increases the susceptibility to disease due to calcium deficiency.[8] Nitrate accumulations enhance the concentrations of NH_4^+, so that plants have shorter and narrower stems, lower fresh and dry weights,[9] and weaker root systems.[10] By comparison, the accumulation of NO_3^- leads to increased proline concentrations, severe oxidative damage and

nitrogen metabolic disorders, inhibited photosynthesis, and considerable declines in biomass production in cucumber (*Cucumis sativus*) and tomato (*Lycopersicon esculentum*).[6,11]

Melatonin (N-acetyl-5-methoxytryptamine), a low-molecular-weight molecule with an indole ring in its structure, has been discovered in evolutionarily distant organisms. This conserved substance is ubiquitous, existing in living organisms ranging from bacteria to animals.[12-14] It has numerous functions in plants. For example, melatonin can stimulate seedling shoot growth and the production of adventitious and lateral roots,[15,16] while slowing the rate of chlorophyll degradation,[17] improving photosynthesis rates,[18] and delaying the processes of flowering and leaf senescence.[19, 20] Most importantly, melatonin protects plants against biotic and abiotic stresses[21-25] by activating various physiological systems and mechanisms, including those that involve antioxidants,[26] the ascorbate–glutathione cycle,[27] ascorbic acid,[28] and photosynthesis.[4]

Cucumber is one of the most valuable horticultural crops in China. Because these plants tend to absorb nitrogen fertilizer, especially the NO_3^- form, excess levels in the soils severely inhibit growth and development in greenhouses.[29] Although numerous recent studies have examined the effects of salt stress on plants, most have focused on NaCl and few have investigated the interaction between nitrate salt stress and horticultural crops. Earlier research demonstrated that melatonin, primarily functioning as an antioxidant,[30-33] can alleviate the effects of such environmental stresses. However, little is known about the relationship between melatonin and nitrogen metabolism. Therefore our objective was to examine how melatonin influences seedling growth, mineral nutrition, and nitrogen metabolism in cucumber when subjected to nitrate stress.

2 | MATERIALS AND METHODS

2.1 | Plant materials and treatments

Experiments were conducted at Northwest A & F University, Yangling, China (34°20′N, 108°24′E). Germinated seeds of *Cucumis sativus* L. 'Jin You No. 1' were sown in black plastic pots (12 × 13 cm) filled with soil and sand. At the three-leaf stage, 270 seedlings of uniform size were transferred to larger plastic pots (38 cm × 28 cm). After 6 d of adaptation to greenhouse conditions, 90 seedlings were assigned to a pre-treatment group that received 0.1 mM melatonin in the irrigation water while others continued to receive standard irrigation (normal). The most appropriate melatonin level had been determined in our previous experiment.[34] Particularly, alcohol was used to dissolve melatonin, and other groups were treated with the same alcohol. These treatments were applied every other day for a total of three times. After the pre-treatment period was completed, the plants in each group were randomly assigned to new treatment groups (n=30 each, with three replicates), in which they were exposed to either a normal level (0.05 M) of nitrate (averagely from calcium nitrate, $Ca(NO_3)_2$ and potassium nitrate, KNO_3) or excess nitrate (0.6 M). These three treatments were designated as 'N': normal control, 0 mM MT + 0.05 M nitrate; 'HN': high-nitrate stress, 0 mM MT + 0.6 M nitrate; and 'MT-HN': melatonin pre-treatment followed by high-nitrate stress, i.e., 0.1 mM MT + 0.6 M nitrate. On day 12 of the nitrate treatment, growth parameters and mineral element concentrations were measured. On Days 0, 3, 6, 9, and 12 of the nitrate treatment, leaves were collected from selected plants in each treatment group to analysis nitrate nitrogen (NO_3^--N), ammonium nitrogen (NH_4^+-N) and enzymes involved in nitrogen metabolism. All samples were quickly frozen in liquid nitrogen and stored at –80°C.

2.2 | Growth parameters and seedling healthy index

On Day 12 of the nitrate treatment, seedlings were harvested from each group to determine final shoot heights and stem diameters. Afterward, the leaves, stems, and roots were immersed in deionized water and blotted carefully with tissue paper. Root lengths and fresh weights (FWs; shoot, root, and total plant) were recorded. Dry weights (DWs) were determined after each tissue type was oven-dried at 80°C for 2 d. The dried samples were used for analyzing the composition of mineral elements. To calculate the Seedling Health Index, we used the following formula: $(\text{stem diameter/shoot height} + \text{root DW/shoot DW}) \times \text{total DW}$.[35]

2.3 | Calculations of root activity and the salt injury index

The deoxidization capacity of the roots (mg per g FW per h) was monitored via the TTC method.[36] After 12 days of nitrate stress, the Salt Injury Index for seedlings in different treatment groups was investigated as described by Zhang et al.[37] The following grades were assigned: 0, no damage; 1, one-third of the leaf edge showing damage; 2, two-thirds of the leaf edge showing damage; 3, entire leaf edge damaged or one-third of the lamina desquamated; 4, two-thirds of the lamina desquamated; or 5, entire lamina desquamated. The Salt Injury Index (%) was calculated as equal

to $\Sigma\ (\text{grade} \times \text{number of plants}) \times 100 / (\text{the highest grade} \times \text{total number of plants})$.

2.4 | Determination of mineral element concentrations

On Day 12 of the stress experiment, selected seedlings were harvested from each treatment, washed three times with distilled water, and separated into leaf, stem, and root portions. The samples were then fixed at 105°C for 30 min, dried for 48 h at 80°C, and ground to pass through a 1-mm sieve. Concentrations of mineral elements were determined after wet digestion using H_2SO_4-H_2O_2. The volume of white colorless digested material was made up to 100 mL with distilled water and then filtered. Levels of nitrogen, phosphorus (P), and potassium (K)

were determined with a Continuous Flow Analyzer (Flowsys, Italy) while those of calcium (Ca) and magnesium (Mg) were determined after the samples were treated with strontium chloride on a Polarized Zeeman Atomic Absorption Spectrophotometer (Z-2000; Hitachi Instrument, Tokyo, Japan).

2.5 | Analysis of nitrate nitrogen and ammonium nitrogen concentrations

NO_3^--N concentrations were measured in fresh samples (0.5 g) according to the method of Gao.[38] Tissues were ground in 10 mL of Millipore-filtered water and then held in a boiling water bath for 30 min. Afterward, 0.1 mL of the supernatant was mixed with 0.4 mL of 5% salicylic-H_2SO_4 to react for 20 min before 9.5 mL of 8% NaOH was added. Absorbance was read at 410 nm.

NH_4^+-N was extracted and analyzed by the ninhydrin hydrate (NIN) method described by Hao.[39] Briefly, 1 g of tissue was ground in 10 mL of 10% acetic acid and the final volume was made up to 25 mL with distilled water. After 2 mL of the supernatant was mixed with 3 mL of NIN and 0.1 mL of ascorbic acid, the mixture was placed in a boiling water bath for 15 min and then cooled in an ice bath. Absorbance was read at 580 nm.

2.6 | Calculation of nitrate reductase activity

Nitrate reductase (NR) activity was monitored in the leaves according to the in vitro method described by Gao,[38] with minor modifications. Samples (0.5 g) were ground in a chilled mortar with cysteine, ethylene diamine tetraacetic acid (EDTA), and phosphate buffered saline (pH 8.7). The reaction mixture contained 0.5 mL of 100 mM KNO_3 and 0.3 mL of 2.5 mM nicotinamide adenine dinucleotide hydrate. The assay was initiated by adding 0.20 mL of leaf extract and was conducted at 25°C for 30 min. The reaction was stopped by the addition of 1 mL of 30% trichloroacetic acid (TCA). Afterward, 2 mL of sulphonamide reagent and 2 mL of 1-naphthylamine were added and the reaction continued for 15 min. Absorbance of the supernatant was read at 520 nm. The level of NR activity was expressed as the amount of NO_2^- (μg per h per g) obtained from the fresh plant material.

2.7 | Calculation of glutamine synthetase activity

Glutamine synthetase (GS) activity was determined by the hydroxamate biosynthesis method.[39] Leaf samples (1.0 g) were ground in a chilled mortar with 50 mM potassium phosphate buffer (pH 8.0) containing 2 mM $MgSO_4$, 2 mM dithiothreitol (DTT), and 0.4 M sucrose. The homogenate was centrifuged (12,000 rpm, 20 min, 4ºC) to produce a supernatant of thick enzyme fluid. The assay mixture contained 100 mM Tris–HCl buffer (80 mM $MgSO_4$, 20 mM glutamine, 20 mM cysteine, and 2 mM ethylene glycol tetraacetic acid; pH 7.4). The above assay mixture with hydroxyl-ammonium chloride was used as a controlled reaction liquid. The chromogenic agent comprised 0.2 mM TCA, 0.37 M $FeCl_3$, and 0.6 M HCl. Activity by GS was determined by calculating the A540 of the clathrate generated in the reaction.

2.8 | Calculation of activities by glutamate synthase and glutamate dehydrogenase

Leaf samples (0.5 g) were homogenized in 100 mM Hepes-KOH buffer (pH 7.5) containing 5 mM $MgCl_2$, 1 mM EDTA-Na_2, 5 mM DTT, 1% (v/v) TritonX-100, 2% (w/v) polyvinylpyrrolidone (PVPP), 1% (w/v) bovine serum albumin (BSA), and 10% (v/v) glycerol, with a chilled pestle and mortar. The homogenate was centrifuged (13,000 g, 10 min, 4ºC), and the supernatant was used for determining the activities of glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH). While GOGAT was assayed by the method described by Jiao et al.[40] and Gajewska and Sklodowska,[41] GDH was assayed according to the method of Debouba et al.[42] and Majerowicz et al.[43]

2.9 | RNA extraction and quantitative real-time polymerase chain reactions

On Days 0, 3, and 9 of nitrate treatment, the leaves were ground into fine powder using liquid nitrogen. Total RNA was extracted from each sample with MiniBEST Plant RNA Extraction Kits (TaKaRa, Dalian, China), according to the manufacturer's instructions. It was then reverse-transcribed into cDNA with a PrimeScript ™

RT reagent Kit and gDNA Eraser (TaKaRa, Dalian, China). Two genes were chosen for further research. *Cucumis sativus-Nitrogen Reductase* (*Cs-NR*) and its specific primers have been described previously.[44] The second gene, *Cucumis sativus-Glutamate Synthase* (*Cs-GOGAT*), was blasted from *AT4G26150.1*, and its specific primer was designed by using the GenScript Real-Time PCR (TaqMan) Primer Design Tool (GenScript, Piscataway, NJ, USA). Actin was chosen as an internal control for data normalization. The primer sequences for quantitative real-time polymerase chain reaction (qRT-PCR) assays are listed in Table 1. All qRT-PCR procedures were performed with PrimeScriptTMRT Reagent Kits (Takara) and conducted on a CFX96™ real-time PCR detection system (Bio-Rad Laboratories, Inc, Hercules, CA, USA). The PCR conditions were as follows: pre-denaturing at 94°C for 5 min, then 40 cycles of 95°C for 15 s and 60°C for 60 s. Relative gene expression was analyzed according to the method of Livak and Schmittgen.[45] These qRT-PCR experiments were repeated three times, based on three separate RNA extracts from three samples.

2.10 | Statistical analysis

All data were analyzed via IBM SPSS Statistics 21 and graphed with Sigma Plot 12.5. Values were presented as the mean ± standard deviation of 30 plants (n = 30) with triplicate for each measurement. The data were evaluated by one-way ANOVA and Duncan's multiple range tests, where differences were considered significant at $P < 0.05$.

3 | RESULTS

3.1 | Growth parameters

After 12 d of induced nitrate stress, cucumber plant growth was assessed directly in terms of shoot height, stem diameter, root and shoot DWs, root length, and root capacity for deoxidization. We then calculated indices of seedling health and susceptibility to salt injury.

In the HN stress treatment, values for shoot height, stem diameter, root DW, and shoot DW were reduced by 38.97%, 21.95%, 52.34%, and 49.46% respectively, when compared with the performance of seedlings exposed to normal conditions (Fig. 1A-D). The Seedling Health Index was also 38.38% lower for stressed plants than for the N group (Fig. 1F). Root lengths and activity were markedly decreased in response to nitrates (Fig. 2) and leaves from stressed plants showed obvious yellowing after 12 d of treatment (Fig. 3A). The Salt Injury Index was 6.85-fold higher for the HN group than for the N group (Fig. 3A). When plants received pretreatment with 0.1 M melatonin (MT-HN group), the growth inhibition found with HN plants was considerably alleviated by comparison, and pretreated plants were 23.49% taller than those exposed only to nitrates (Fig. 1A). Furthermore, the MT-HN plants showed increases of 17.00% in stem diameter (Fig. 1B), 10.49% in shoot DW (Fig. 1C), 57.14% in root DW (Fig. 1D), 14.67% in DW (Fig. 1E), 17.36% in the Seedling Health Index (Fig. 1F), 21.53% in root length (Fig. 2A), 86.96% in root deoxidization activity (Fig. 2B), and 53.59% in the Salt Injury Index (Fig. 3A) when compared with HN seedlings. This indicated that exogenous melatonin significantly offset the decline in plant growth that was associated with nitrate stress.

3.2 | Concentrations of mineral elements

Both nitrate stress and melatonin pretreatment had significant effects on levels of minerals in cucumber tissues. Compared with the control plants, concentrations in tissues from the HN group after 12 d of stress were 9.85% (roots), 36.23% (stems), and 32.46% (leaves) higher for nitrogen (Fig. 4A) and 22.19% (roots), 40.96% (stems), and 74.89% (leaves) higher for K (Fig. 4C). However, pretreatment with melatonin decreased nitrogen concentrations by 7.70% (roots), 19.88% (stems), and 20.54% (leaves) and K concentrations by 10.55% (roots), 11.21% (stems), and 33.10% (leaves) when compared with plants that had not received melatonin.

Ca concentrations were significantly higher in the HN group (rising by 47.68% for roots, 5.90% for stems, and 10.89% for leaves) than in the N group. Melatonin pretreatment led to a further increase in those levels when plants were exposed to high-nitrate conditions (Fig. 4D). Although stress significantly inhibited the accumulation of Mg in roots (by 10.02%) and leaves (by 42.14%) when compared with the N group, exogenous melatonin helped to reverse that response (Fig. 4E). The addition of melatonin increased root and leaf concentrations of Mg by 5.84% and 45.92%, respectively, when compared with levels in stressed plants that were not pretreated. However, a similar trend was not found for the stem samples. For P, the pattern of response was the same as that for Mg (Fig. 4B).

3.3 | Concentrations of nitrate nitrogen and ammonium nitrogen

Nitrate-nitrogen concentrations were much higher in plants under stress conditions (Fig. 5A). On Day 0 of treatment, exogenous melatonin did not affect the level of NO_3^--N. Over time, however, those levels increased slightly in N group plants. On Day 12 d, nitrate concentrations were 3.88-fold higher in HN than in the N group. By contrast, those levels were 25.00% lower in MT-HN plants than in the HN group (Fig. 5A). This demonstrated that the melatonin pretreatment significantly inhibited the accumulation of NO_3^--N.

At nearly all of the measurement points, melatonin-pretreated plants had relatively less NH_4^+-N than those exposed only to nitrate stress. On Day 12, the ammonium-nitrate concentration was 2.89-fold higher in the HN group when compared with the control (Fig. 5B). However, the application of melatonin prior to nitrate stress helped to meliorate that large margin, such that ammonium-nitrogen levels were reduced by 25.98% for the MT-HN plants when compared with the HN group. This again showed that exogenous melatonin could significantly alleviate the toxicity of NH_4^+-N associated with nitrate stress.

3.4 | Enzyme activities

The balance within the nitrogen metabolism system is primarily maintained by NR, GS, GOGAT, and GDH. We found that their activities were noticeably changed in a similar manner over our stress period. For example, on Day 0, pretreatment with melatonin did not appear to have an effect on any enzyme except NR (Fig. 6). Over time, enzyme activities initially increased in the leaves before decreasing gradually. Whereas activities of NR, GS, GOGAT, and GDH were at least 28.94% lower in HN plants than in the normal group, those activities were much higher in the MT-HN group than in the HN plants throughout most of the experimental period. This demonstrated that exogenous melatonin could retard the decline in activities by enzymes for nitrogen metabolism when plants were under high-nitrate stress.

3.5 | Expression of *Cs-NR* and *Cs-GOGAT*

We used qRT-PCR to analyze the expression of *Cs-NR* and *Cs-GOGAT*, two genes that regulate enzyme activity. Changes in their transcriptional expression are shown in Figure 7. Obviously, the transcription level of both genes showed similar trends. Over time, the transcription level in HN and MT-HN group were significantly decreased. Particularly, the gene expression level in HN group was significantly down-regulated (by 84.1% on Day 3, 89.6% on Day 9 for *Cs-NR*; 70.59% on Day 3, 92.79% on Day 9 for *Cs-GOGAT*) than that in N group, while the level was up-regulated (by 2.88 fold on Day 3 and 93.3% on Day 9 for *Cs-NR*; 54.69% on Day 3, 3.22 fold on Day 9 for *Cs-GOGAT*) after melatonin pretreatment.

4 | DISCUSSION

Melatonin is one of the most commonly used substances to improve plant resistance against various environmental stresses.[21-28, 30] However, no previous studies have been conducted to understand how melatonin influences the relationship between the nitrogen metabolism system and the mineral composition of plants under

nitrate stress. The accumulation of nitrate salt is a major factor that severely limits greenhouse production of vegetable crops in China. Therefore, we examined the effect of melatonin pretreatment on growth, mineral nutrition, and nitrogen metabolism in nitrate-stressed cucumber seedlings (Fig. 8).

4.1 | Growth parameters

Inhibited growth is a critical indicator of plants subjected to environmental stresses. We found that values for shoot height, stem diameter, and shoot and root DWs were significantly lower in nitrate-stressed plants than in those exposed to normal conditions. This was similar to previously reported observations.[46, 47] Moreover, values for root lengths and deoxidization activity were lower in stressed plants. However, these negative effects were significantly alleviated when melatonin was applied prior to the induction of stress. Liu et al have shown that exogenous melatonin can significantly improve the health index of tomato seedlings under drought conditions.[35] We also found that pretreatment with 0.1 mM melatonin could effectively increase the Seedling Health Index when nitrate stress was introduced later, and that melatonin also significantly reduced the susceptibility of plants to stress-related cell damage. All of these results indicated that melatonin can alleviate nitrate-associated growth inhibition.

4.2 | Mineral elements

Ion uptake and compartmentalization are crucial not only for promoting normal growth but also for sustaining plant performance under high-salinity conditions because such stress disturbs ion homeostasis.[48] Nitrogen is an essential mineral element that is required in the greatest amount in plants. Although growth is dependent upon having an adequate nitrogen supply,[49] excessive nitrogen fertilization can be harmful. Our data showed dramatic increases in nitrogen concentrations in the roots, stems and leaves after 12 days of nitrate treatment (Fig. 4A). However, the level of nitrogen did not rise as much when melatonin was applied exogenously. The same trend

was noted for leaf concentrations of NO_3^--N and NH_4^+-N (Fig. 5). Under high-nitrogen conditions, melatonin accelerated the growth of plants by decreasing the amount of excrescent nitrogen.

P provides anion equivalents and is responsible for the charge balance in plants,[50] especially those that are salt-stressed. In accord with results previously reported,[51] we found that excess nitrate led to a decrease in P concentrations that might have been related to the high level of NO_3^-. Likewise, concentrations of P were increased in MT-HN plants in parallel with a decline in NO_3^- levels.

K is essential for osmotic regulation.[52] Because the onset of osmotic stress is one of the primary causes of salt stress in plants,[53] this element has a key role in mitigating the effects of such stress, including that related to nitrates. Our results indicated that treatment with $Ca(NO_3)_2$ and KNO_3 caused K concentrations to rise significantly whereas pretreatment with melatonin limited the absorption of K under the nitrate-stress conditions that followed. Therefore, the effect of exogenous melatonin on K accumulations in cucumber roots, stems, and leaves might play a vital role in mediating intracellular ion equilibrium under nitrate stress, thereby acting as a mechanism for adjusting the plant response to osmotic stress.

Ca provides intermolecular linkages and is thought to have a crucial function in stabilizing cell walls and membranes.[54] Similar to the response in K concentrations, Ca levels were significantly increased in plants treated with $Ca(NO_3)_2$ and KNO_3. Furthermore, melatonin pretreatment enhanced those increases in Ca concentrations under stress conditions, similar to results reported previously.[21] The protective effect of melatonin on cell membranes has also been described from earlier studies.[16, 55, 56] This improvement in Ca concentrations in pretreated seedlings suggests that melatonin positively influences the integrity of cells and membranes by increasing the accumulation of Ca.

Mg is a key component of chlorophyll biosynthesis and other physiological and biochemical reactions related to plant growth and development.[57, 58] A deficiency in this element can reduce photosynthesis rates.[58, 59] We found that excess nitrate was associated with a drop in levels of Mg in the roots and leaves but not in the stems. However, exogenous melatonin improved Mg concentrations in the roots and leaves of stressed plants. It may have accelerated the rate of chlorophyll biosynthesis, thereby leading to an increase in biological output, i.e., higher tissue fresh and dry weights as well as greater shoot and root elongation. Melatonin has a protective role against chlorophyll degradation and promotes plant growth in stressful environments, perhaps because it up-regulates the accumulation of Mg under such conditions.[16, 60]

4.3 | Nitrate metabolism

The assimilation of nitrogen is directly responsible for crop biomass production and grain yield.[61] Appropriate levels of NO_3^- can facilitate nitrogen metabolism, thereby benefiting plant growth. Ammonium is a central intermediate in this metabolism.[62] However, excess accumulations of NO_3^--N and NH_4^+-N can lead to salt toxicity.[63] That response disturbs physiological processes, such as photosynthesis,[11] pH regulation,[64] and the balance of essential elements.[65] Cucumber plants are somewhat sensitive to NO_3^- stress and NH_4^+ stress.[50, 62] When the supply of nitrogen exceeds the capacity of its assimilation, many species tend to accumulate the excess nitrogen.[62] We made similar observations here, with a high concentration of nitrate leading to significantly higher concentrations of NO_3^--N and NH_4^+-N. However, those increases measured in stressed seedlings were tempered by pretreatment with melatonin. These findings demonstrated that exogenous melatonin enhances the capacity for nitrate reduction and ammonia assimilation, subsequently alleviating the damage associated with those challenges while also improving biomass production.

Exogenous melatonin can increase tolerance by numerous plant species to various environmental stresses by regulating the activity of some enzymes.[66] However, little has been known about how melatonin influences the systemic activity of enzymes involved in nitrate metabolism, including NR, GS, GOGAT, and GDH. With a key role in the pathway for nitrogen assimilation, NR catalyzes the reduction of NO_3^- to NO_2^-. We found that, under nitrate stress, NR activity was initially increased in the leaves of plants, regardless of pretreatment, but that activity then decreased gradually as the experimental period was prolonged. Compared with plants in the control group, NR activities in HN leaves declined markedly in response to nitrate stress, which possibly due to feedback inhibition of higher concentrations of NO_3^--N. Similar results have been reported by Loque et alia.[67] For MT-HN plants, however, NR activity was increased, which then reversed the accumulation of NO_3^--N.

Higher plants utilize three enzymes – GS, GOGAT, and GDH – for NH_3 assimilation. Ammonium is rapidly assimilated into organic nitrogen through either the GS/GOGAT cycle or the GDH pathway. As the absorption of NO_3^--N increases, NR activity is promoted, which leads to the accumulation of NH_4^+. To prevent ammonia nitrogen from accumulating in plants, GS, GOGAT, and GDH activities rise rapidly at the beginning of the stress period. Over time, however, those activities are gradually reduced by continuing nitrate stress, thus inhibiting nitrogen metabolism. However, pretreatment with melatonin slows that rate of inhibition. Both GS and GOGAT are directly involved in nitrate assimilation, which then catalyzes the assimilation of ammonium into amino acids.[68] Induction of such activity is dependent upon the NH_4^+ concentration. However, high accumulations of endogenous NH_4^+ are toxic to higher plants.[63] Nitrate stress is associated with marked declines in GS/GOGAT activities, possibly due to feedback inhibition of increased NH_4^+. Our results indicated that those activities were significantly greater in melatonin-treated plants that later were exposed to nitrate stress. This positive role for melatonin is probably related to its enhanced antioxidant properties[30] because GS and GOGAT proteins can be oxidatively modified.[69]

As a second alternative pathway, GDH participates in the assimilation of ammonium into organic nitrogen. Here, GDH activity followed the same trend observed for GS/GOGAT in the stress response, with one difference. Whereas GS/GOGAT activities peaked at Day 3 of the stress period and then declined, maximum GDH activity was detected at Day 6, thereby indicating that GS/GOGAT is the main pathway for ammonia assimilation during the initial stage of the stress response. As GS/GOGAT activity began to decrease, ammonia assimilation shifted from the normal GS/GOGAT pathway to the GDH pathway by Day 6. Nevertheless, GDH activity was also significantly inhibited by stress after 6 d, and did not reduce the accumulation of NH_4^+ beyond that time point, suggesting that the GDH cycle has only a small role in ammonia assimilation.

The most accurate method for analyzing gene expression is qRT-PCR.[70] The NR enzyme plays a key role in reducing NO_3^- to NH_4^+, and GS/GOGAT is the main pathway for NH_4^+ assimilation. Therefore, we selected *Cs-NR* and *Cs-GOGAT* for qRT-PCR analysis. Previous research has shown that nitrate strongly stimulates NR genes in the plant response to adverse environmental conditions.[71] Our results showed that *Cs-NR* was down-regulated under HN treatment, an outcome consistent with the report by Loque et alia.[67] Our analysis of *Cs-GOGAT* revealed a similar pattern of expression. Melatonin pretreatment promoted the expression of both genes under nitrate stress, with their patterns somewhat paralleling those of NR and GOGAT activity. Whereas transcript levels for *Cs-NR* and *Cs-GOGAT* had decreased by Day 3, the activity of NR and GOGAT did not decline until Day 6. Thus, changes in the transcriptional expression of those genes might explain why melatonin enhanced plant capacity for nitrate metabolism. It is possible that narrow gene specificity and primer design limited our examination of expression related to nitrogen metabolism. Therefore, further studies are required.

5 | CONCLUSION

This is the first report demonstrating the relationship between melatonin and the system of nitrogen metabolism for alleviating the effects of abiotic stress. We found that pre-treating cucumber seedlings with melatonin prior to the induction of nitrate stress suppressed the accumulation of excess nitrate and ammonium by increasing the activities of enzymes involved in nitrate metabolism and the expression of their related genes. It also made plants less susceptible to stress-related cell damage. Consequently, the Salt Injury Index was reduced while several growth indices were improved. In addition, melatonin regulated the levels of mineral elements that are directly connected to osmotic regulation, membrane structure, and photosynthesis. In conclusion, this alleviation mechanism of melatonin against the effects of nitrate stress is partly due to the enhanced capacity for nitrogen metabolism and regulated uptake of mineral elements in pretreated plants.

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CONFLICT OF INTEREST

The authors declared that they have no competing financial interests.

REFERENCES

1. Scheible WR, Gonzalez-Fontes A, Lauerer M, et al. Nitrate acts as a signal to induce organic acid metabolism and repress starch metabolism in tobacco. *Plant Cell*. 1997; 9:783–798.

2. Yang X, Wang X, Wei M, et al. Changes of nitrate reductase activity in cucumber seedlings in response to nitrate stress. *Agric Sci China*. 2010;9:216-222.

3. Ju XT, Kou CL, Christie P, et al. Changes in the soil environment from excessive application of fertilizers and manures to two contrasting intensive cropping systems on the North China Plain. *Environ Pollut*. 2007;145:497-506.

4. Wang LY, Liu JL, Wang WX, et al. Exogenous melatonin improves growth and photosynthetic capacity of cucumber under salinity-induced stress. *Photosynthetica*. 2016;54:19-27.

5. Wang S, Liu P, Chen D et al. Silicon enhanced salt tolerance by improving the root water uptake and decreasing the ion toxicity in cucumber. *Front Plant Sci*. 2015;6.

6. Zhang G, Liu Z, Zhou J, et al. Effects of $Ca(NO_3)_2$ stress on oxidative damage, antioxidant enzymes activities and polyamine contents in roots of grafted and non-grafted tomato plants. *Plant Growth Regul.* 2008;56:7-19.

7. Li DP, Wu Z, Liang C. Characteristics and regulation of greenhouse soil environment. *Chin J Ecol.* 2004;23:192-197.

8. Schmidt R, Mieulet D, Hubberten HM, et al. SALT-RESPONSIVE ERF1 regulates reactive oxygen species-dependent signaling during the initial response to salt stress in rice. *Plant Cell*. 2013;25:2115-2131.

9. Ingestad T. Mineral nutrient requirements of cucumber seedlings. *Plant Physiol*. 1972;52:332-338.

10. Matsumoto H, Tamura K. Respiratory stress in cucumber roots treated with ammonium or nitrate nitrogen. *Plant Soil*. 1981;60:195-204.

11. Yuan L, Yuan Y, Du J, et al. Effects of 2,4-epibrassinolide on nitrogen metabolism in cucumber seedlings under $Ca(NO_3)_2$ stress. *Plant Physiol Biochem*. 2012;61:29-35.

12. Macias M, Rodriguez-Cabezas MN, Reiter RJ et al. Presence and effects of melatonin in *Trypanosoma cruzi*. *J Pineal Res.* 1999;27:86-94.

13. Hardeland R, Cardinall DP, Srinivasan V et al. Melatonin -- A pleiotropic, orchestrating regulator molecule. *Prog Neurobiol.* 2011;93:350-384.

14. Manchester LC, Poeggeler B, Alvares FL, et al. Melatonin immunoreactivity in the photosynthetic prokaryote Rhodospirillum rubrum: implications for an ancient antioxidant system. *Cell Mol Biol Res.* 1995; 41:391-395.

15. Arnao MB, Hernandez-Ruiz J. Melatonin promotes adventitious and lateral root regeneration in etiolated hypocotyls of *Lupinus albus* L. *J Pineal Res.* 2007;42:147-152.

16. Zhang N, Zhao B, Zhang H, et al. Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber *(Cucumis sativus* L.*)*. *J Pineal Res.* 2013;54:15-23.

17. Arnao MB, Hernandez-Ruiz J. Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. *J Pineal Res.* 2009;46:58-63.

18. Sarropoulou V, Dimassi-Theriou K, Therios I, et al. Melatonin enhances root regeneration, photosynthetic pigments, biomass, total carbohydrates and proline content in the cherry rootstock PHL-C (*Prunus avium* x *Prunus cerasus*). *Plant Physiol Biochem.* 2012;61:162-168.

19. Wang P, Sun X, Chang C, et al. Delay in leaf senescence of *Malus hupehensis* by long-term melatonin application is associated with its regulation of metabolic status and protein degradation. *J Pineal Res.* 2013;55:424-434.

20. Kolar J, Johnson CH, Machackova I. Exogenously applied melatonin (N-acetyl-5-methoxytryptamine) affects flowering of the short-day plant *Chenopodium rubrum*. *Physiol Plant.* 2003;118:605-612.

21. Turk H, Erdal S. Melatonin alleviates cold-induced oxidative damage in maize seedlings by up-regulating mineral elements and enhancing antioxidant activity. *J Plant Nutr Soil Sci*. 2015;178:433-439.

22. Xu W, Cai S, Zhang Y, et al. Melatonin enhances thermotolerance by promoting cellular protein protection in tomato plants. *J Pineal Res*. 2016; 61:457-469.

23. Li MQ, Hasan MK, Li CX, et al. Melatonin mediates selenium-induced tolerance to cadmium stress in tomato plants. *J Pineal Res*. 2016; 61:291-302.

24. Kołodziejczyk I, Dzitko K, Szewczyk R, et al. Exogenous melatonin improves corn (*Zea mays* L.) embryo proteome in seeds subjected to chilling stress. *J Plant Physiol*. 2016;193:47–56.

25. Reiter RJ, Tan DX, Zhou Z, et al. Phytomelatonin: Assisting Plants to Survive and Thrive. *Molecules*. 2015; 20:7396-7437.

26. Zhang H, Zhang N, Yang R, et al. Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA_4 interaction in cucumber *(Cucumis sativus* L.*)*. *J Pineal Res.* 2014;57:269-279.

27. Wang P, Sun X, Li C, et al. Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. *J Pineal Res.* 2013;54:292-302.

28. Xu X, Sun Y, Guo X, et al. Effects of exogenous melatonin on ascorbate metabolism system in cucumber seedlings under high temperature stress. *Chin J Appl Ecol.* 2010;21:2580-2586.

29. Yang X, Wang X, Wei M, et al. Response of ammonia assimilation in cucumber seedlings to nitrate stress. *J Plant Biol*. 2010;53:173-179.

30. Reiter RJ, Mayo JC, Tan DX, et al. Melatonin as an antioxidant: under promises but over delivers. *J Pineal Res*. 2016; 61:253-278.

31. Galano A, Tan DX, Reiter RJ. Melatonin as a natural ally against oxidative stress: a physicochemical examination. *J Pineal Res.* 2011;51:1-16.

32. Tan D, Manchester LC, Liu X, et al. Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. *J Pineal Res.* 2013;54:127-138.

33. Arnao MB, Hernandez-Ruiz J. Functions of melatonin in plants: a review. *J Pineal Res.* 2015;59:133-150.

34. Wang WX, Zhang RM, Sun Y, et al. Effect of exogenous melatonin on the antioxidant system of cucumber seedlings under nitrate stress. *Acta Horticulturae Sinica*. 2016;43 (4):695-703.

35. Liu J, Wang W, Wang L, et al. Exogenous melatonin improves seedling health index and drought tolerance in tomato. *Plant Growth Regul*. 2015;77:317-326.

36. Lichtenhaler. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem Soc Trans*. 1983;11:591-592.

37. Zhang YQ. Study on screening of salt-tolerant watermelon stock and mechanism of salt-tolerance. *Acta Agric Boreali-Occident Sin*. 2003;12:105-108.

38. Gao J. Plant Physiology Experiment Instructor. *Higher Education Press*. Beijing, China, 2000.

39. Hao Z, Cang J, Xu Z. Plant Physiology Experiment. *Harbin Industrial University Press*. Harbin, China, 2004.

40. Jiao DM, Huang XQ, Li X, et al. Photosynthetic characteristics and tolerance to photo-oxidation of transgenic rice expressing C_4 photosynthesis enzymes. *Photosynth Res*. 2002;72:85-93.

41. Gajewska E, Sklodowska M. Nickel-induced changes in nitrogen metabolism in wheat shoots. *J Plant Physiol*. 2009;166:1034-1044.

42. Debouba M, Maaroufi-Dghimi H, Suzuki A, et al. Changes in growth and activity of enzymes involved in nitrate reduction and ammonium assimilation in tomato seedlings in response to NaCl stress. *Ann Bot-London*. 2007;99:1143-1151.

43. Majerowicz N, Kerbauy GB, Nievola CC, et al. Growth and nitrogen metabolism of *Catasetum fimbriatum* (orchidaceae) grown with different nitrogen sources. *Environ Exp Bot.* 2000;44:195-206.

44. Li Q, Wang X, Ma L, et al. Molecular characterization of a cucumber nitrate reductase (*CsNR*) gene under NO_3^- stress. *Mol Biol Rep.* 2012;39:4283-4290.

45. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods.* 2001;25:402-408.

46. Gao Q, Wang X, Shi Q, et al. Effects of lanthanum on the plant growth and leaf anti-oxidative enzyme activities of cucumber seedlings under nitrate stress. *Chin. J. Ecol.* 2008;19:976-980.

47. Jin C, Sun J, Guo S. Effects of exogenous spermidine on growth and active oxygen metabolism in cucumber seedlings under $Ca(NO_3)_2$ stress. *Acta Bot Boreali-Occident. Sin.* 2010;30:1627-1633.

48. Adams P, Thomas J, Vernon D. Distinct cellular and organismic responses to salt stress. *Plant Cell Physiol*. 1992;33:1215-1223.

49. Ruiz JM, Romero L. Cucumber yield and nitrogen metabolism in response to nitrogen supply. *Sci Hortic-Amsterdam*. 1999;82:309-316.

50. Roosta HR, Sajjadinia A, Rahimi A, et al. Responses of cucumber plant to NH_4^+ and NO_3^- nutrition: the relative addition rate technique vs. cultivation at constant nitrogen concentration. *Sci Hortic-Amsterdam.* 2009;121:397-403.

51. Yuan L, Zhu S, Shu S, et al. Regulation of 2,4-epibrassinolide on mineral nutrient uptake and ion distribution in $Ca(NO_3)_2$ stressed cucumber plants. *J Plant Physiol.* 2015;188:29-36.

52. Golldack D, Quigley F, Michalowski CB, et al. Salinity stress-tolerant and -sensitive rice (*Oryza sativa* L.) regulate AKT1-type potassium channel transcripts differently. *Plant Mol Biol.* 2003;51:71-81.

53. Khan MN, Siddiqui MH, Mohammad F, et al. Interactive role of nitric oxide and calcium chloride in enhancing tolerance to salt stress. *Nitric Oxide-Biol Chem*. 2012;27:210-218.

54. Xu L, Dong Y, Kong J, et al. Effects of root and foliar applications of exogenous NO on alleviating cadmium toxicity in lettuce seedlings. *Plant Growth Regul*. 2014;72:39-50.

55. Li C, Wang P, Wei Z, et al. The mitigation effects of exogenous melatonin on salinity-induced stress in *Malus hupehensis*. *J Pineal Res*. 2012;53:298-306.

56. Garcia JJ, Reiter RJ, Guerrero JM, et al. Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. *FEBS Lett*. 1997;408:297-300.

57. Wang C, Zhang SH, Wang PF, et al. The effect of excess Zn on mineral nutrition and antioxidative response in rapeseed seedlings. *Chemosphere*. 2009;75:1468-1476.

58. Shabala S, Hariadi Y. Effects of magnesium availability on the activity of plasma membrane ion transporters and light-induced responses from broad bean leaf mesophyll. *Planta*. 2005;221:56-65.

59. Waraich EA, Ahmad R, Halim A, et al. Alleviation of temperature stress by nutrient management in crop plants: a review. *J Soil Sci Plant Nutr.* 2012;12:221-244.

60. Meng J, Xu T, Wang Z, et al. The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: antioxidant metabolites, leaf anatomy, and chloroplast morphology. *J Pineal Res.* 2014;57:200-212.

61. Bergamo Fenilli TA, Reichardt K, Ocheuze Trivelin PC, et al. Volatilization of ammonia derived from fertilizer and its reabsorption by coffee plants. *Commun Soil Sci Plan.* 2007;38:1741-1751.

62. Roosta HR, Schjoerring JK. Effects of ammonium toxicity on nitrogen metabolism and elemental profile of cucumber plants. *J Plant Nutr.* 2007;30:1933-1951.

63. Britto DT, Kronzucker HJ. $NH4^+$ toxicity in higher plants: a critical review. *J Plant Physiol.* 2002;159:567-584.

64. Walch-Liu P, Neumann G, Bangerth F, et al. Rapid effects of nitrogen form on leaf morphogenesis in tobacco. *J Exp Bot.* 2000;51:227-237.

65. Siddiqi MY, Malhotra B, Min XJ, et al. Effects of ammonium and inorganic carbon enrichment on growth and yield of a hydroponic tomato crop. *J Plant Nutr Soil Sci.* 2002;165:191-197.

66. Arnao MB, Hernandez-Ruiz J. Melatonin: plant growth regulator and/or biostimulator during stress? *Trends Plant Sci.* 2014;19:789-797.

67. Loque D, Tillard P, Gojon A, et al. Gene expression of the NO_3^- transporter NRT1.1 and the nitrate reductase NIA_1 is repressed in *Arabidopsis* roots by NO_2^-, the product of NO_3^- reduction. *Plant Physiol.* 2003;132:958-967.

68. Liu Z, Bie Z, Huang Y, et al. Rootstocks improve cucumber photosynthesis through nitrogen metabolism regulation under salt stress. *Acta Physiol Plant*. 2013;35:2259-2267.

69. Balestrasse KB, Gallego SM, Tomaro ML. Oxidation of the enzymes involved in nitrogen assimilation plays an important role in the cadmium-induced toxicity in soybean plants. *Plant Soil.* 2006;284:187-194.

70. Bustin SA, Benes V, Nolan T, et al. Quantitative real-time RT-PCR -- a perspective. *J Mol Endocrinol.* 2005;34:597-601.

71. Cao Y, Fan X, Sun S, et al. Effect of nitrate on activities and transcript levels of nitrate reductase and glutamine synthetase in rice. *Pedosphere*. 2008;18:664-673.

TABLE 1 Sequences of primers used in quantitative real-time RT-PCR.

Gene	Primer Sequence (5'---3')
Actin	F: CCACGAAACTACTTACAACTCCATC
	R: GGGCTGTGATTTCCTTGCTC
Cs-NR	F: CACAACAAAGGTGGTGGAGGGCAAAA
	R: ACAAAACGAATCCAACGTCCTTTTC
Cs-GOGAT	F: ATGGGAAGCTGCGTTTC
	R: CTAGTTTTGCACAGCCACTG

FIGURE CAPTIONS

FIGURE 1 Effects of exogenous melatonin and nitrate stress on shoot height (A), stem diameter (B), shoot dry weight (C), root dry weight (D), total plant dry weight (E), and Seedling Health Index (F). Data represent means ± SD of 3 replicates. Different letters within a panel indicate significant differences among treatments according to Duncan's multiple range tests (P <0.05). N, normal control treatment: 0 mM MT (melatonin) + 0.05 M nitrate; HN, high-nitrate treatment: 0 mM MT + 0.6 M nitrate; MT-HN, pretreatment with 0.1 mM MT followed by treatment with 0.6 M nitrate.

FIGURE 2 Effects of exogenous melatonin and nitrate stress on root length (A) and root deoxidization activity (B). Data represent means ± SD of 3 replicates. Different letters within a panel indicate significant differences among treatments according to Duncan's multiple range tests (P <0.05). (C) Effects of melatonin on root growth after 12 d of stress treatment. N, normal control treatment: 0 mM MT (melatonin) + 0.05 M nitrate; HN, high-nitrate treatment: 0 mM MT + 0.6 M nitrate; MT-HN, pretreatment with 0.1 mM MT followed by treatment with 0.6 M nitrate.

FIGURE 3 (A) Effects of exogenous melatonin and nitrate on Salt Injury Index. Data represent means ± SD of 3 replicates. Different letters within a panel indicate significant differences among treatments according to Duncan's multiple range tests (P <0.05). (B) Effects of melatonin on damaged leaves on the whole plant after 12 d of stress treatment. N, normal control treatment: 0 mM MT (melatonin) + 0.05 M nitrate; HN, high-nitrate treatment: 0 mM MT + 0.6 M nitrate; MT-HN, pretreatment with 0.1 mM MT followed by treatment with 0.6 M nitrate.

FIGURE 4 Effects of exogenous melatonin and nitrate stress on levels of mineral elements: nitrogen (A), phosphorus (B), potassium (C), calcium (D), and magnesium (E). Data represent means ± SD of 3 replicates. Different letters within a panel indicate significant differences among treatments according to Duncan's multiple range tests (P <0.05). N, normal control treatment: 0 mM MT (melatonin) + 0.05 M nitrate; HN, high-nitrate treatment: 0 mM MT + 0.6 M nitrate; MT-HN, pretreatment with 0.1 mM MT followed by treatment with 0.6 M nitrate.

FIGURE 5 Effects of exogenous melatonin and nitrate stress on levels of nitrate nitrogen (A) and ammonium nitrogen (B). Data represent means ± SD of 3 replicates. Different letters within a panel indicate significant differences among treatments according to Duncan's multiple range tests (P <0.05). N, normal control treatment: 0 mM MT + 0.05 M nitrate; HN, high-nitrate treatment: 0 mM MT (melatonin) + 0.6 M nitrate; MT-HN, pretreatment with 0.1 mM MT followed by treatment with 0.6 M nitrate.

FIGURE 6 Effects of exogenous melatonin and nitrate stress on activities of NR (A), GS (B), GOGAT (C), and GDH (D). Data represent means ± SD of 3 replicates. Different letters within a panel indicate significant differences among treatments according to Duncan's multiple range tests (P <0.05). N, normal control treatment: 0 mM MT (melatonin) + 0.05 M nitrate; HN, high-nitrate treatment: 0 mM MT + 0.6 M nitrate; MT-HN, pretreatment with 0.1 mM MT followed by treatment with 0.6 M nitrate.

FIGURE 7 Effects of exogenous melatonin and nitrate stress on relative expression of *Cs-NR* (A) and *Cs-GOGAT* (B). Total RNA was isolated from samples on Days 0, 3, and 9 of stress treatment, then converted to cDNA and subjected to real-time RT-PCR. Data represent means ± SD of 3 replicates. Different letters within a panel indicate significant differences among treatments according to Duncan's multiple range tests (P <0.05). N, normal control treatment: 0 mM MT (melatonin) + 0.05 M nitrate; HN, high-nitrate treatment: 0 mM MT + 0.6 M nitrate; MT-HN, pretreatment with 0.1 mM MT followed by treatment with 0.6 M nitrate.

FIGURE 8 Schematic showing effects of exogenous melatonin on cucumber growth, based on status of nitrogen metabolism and mineral nutrition under nitrate stress. The green parts represent nitrogen metabolism material. NO_3^-: nitrate nitrogen, NH_4^+: ammonium nitrogen. The blue parts represent nitrogen metabolism enzyme. NR: nitrate reductase, GS: glutamine synthetase, GOGAT: glutamate synthase, GDH: glutamate dehydrogenase. The purple part represents the mineral element. N: Nitrogen, P: Phosphorus, K: Potassium, Ca: Calcium, Mg: Magnesium. Others: NiR: nitrite reductase, Gln: glutamine, Glu: glutamic acid.

Figures

Fig. 1

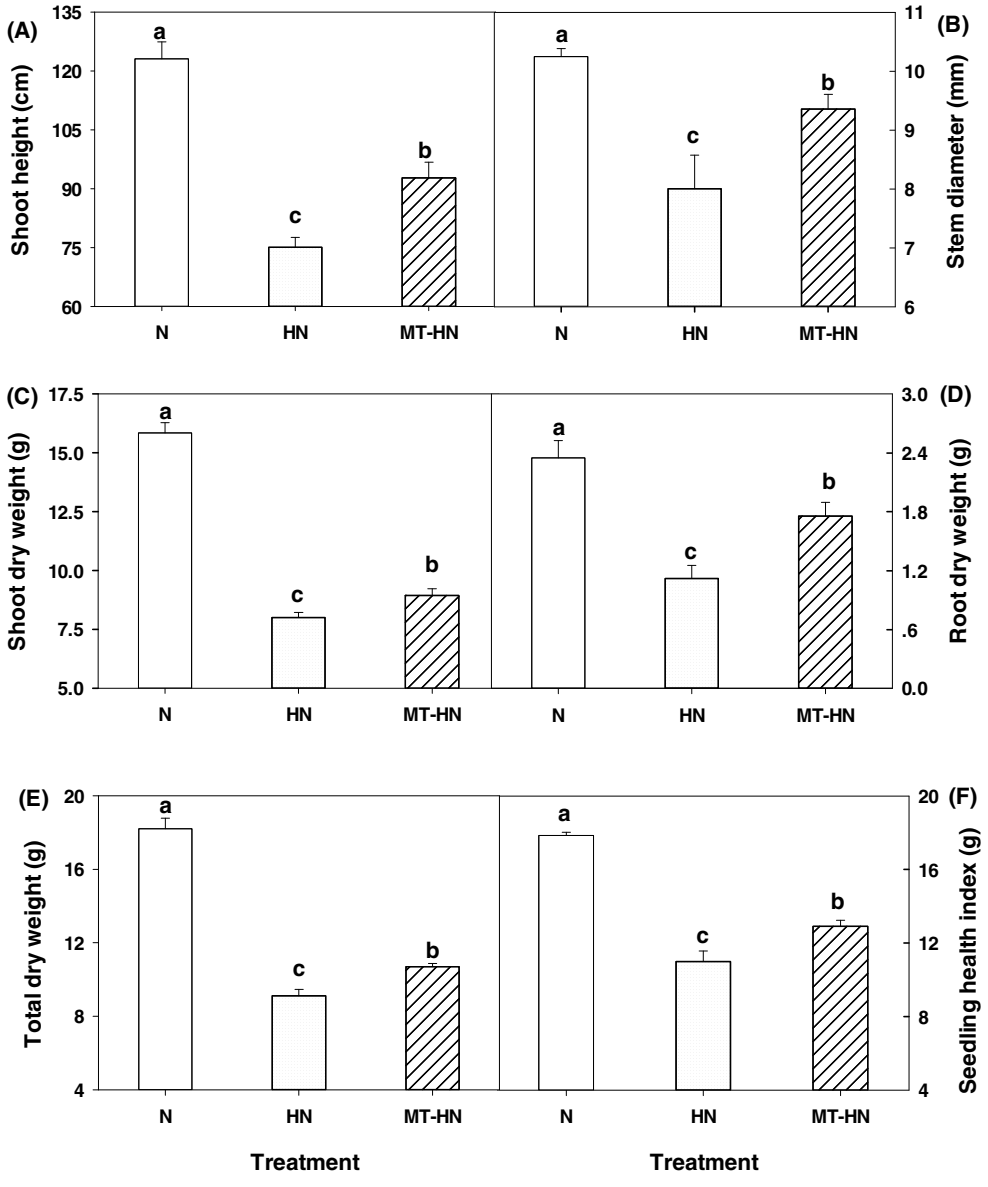

Fig. 2

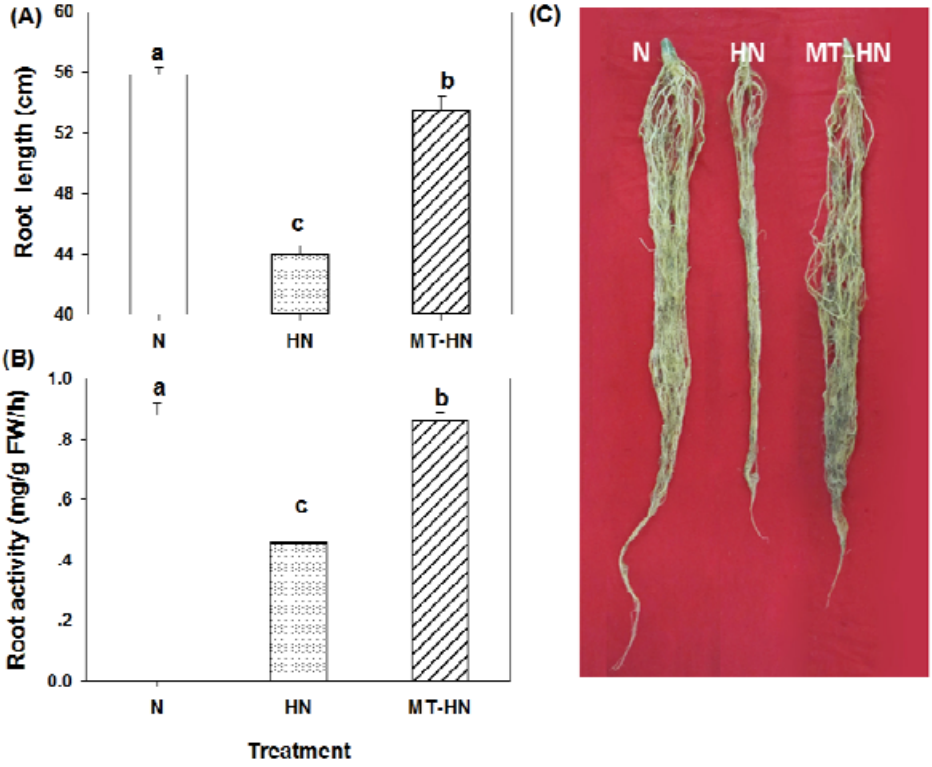

Fig. 3

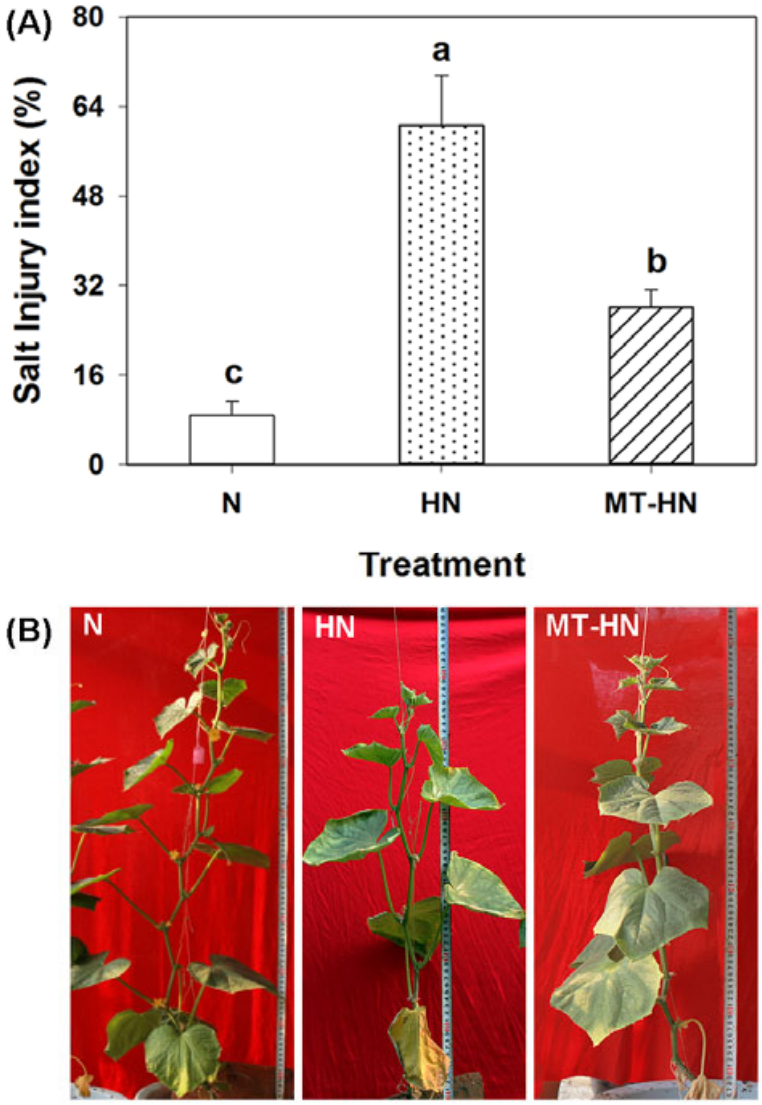

Fig. 4

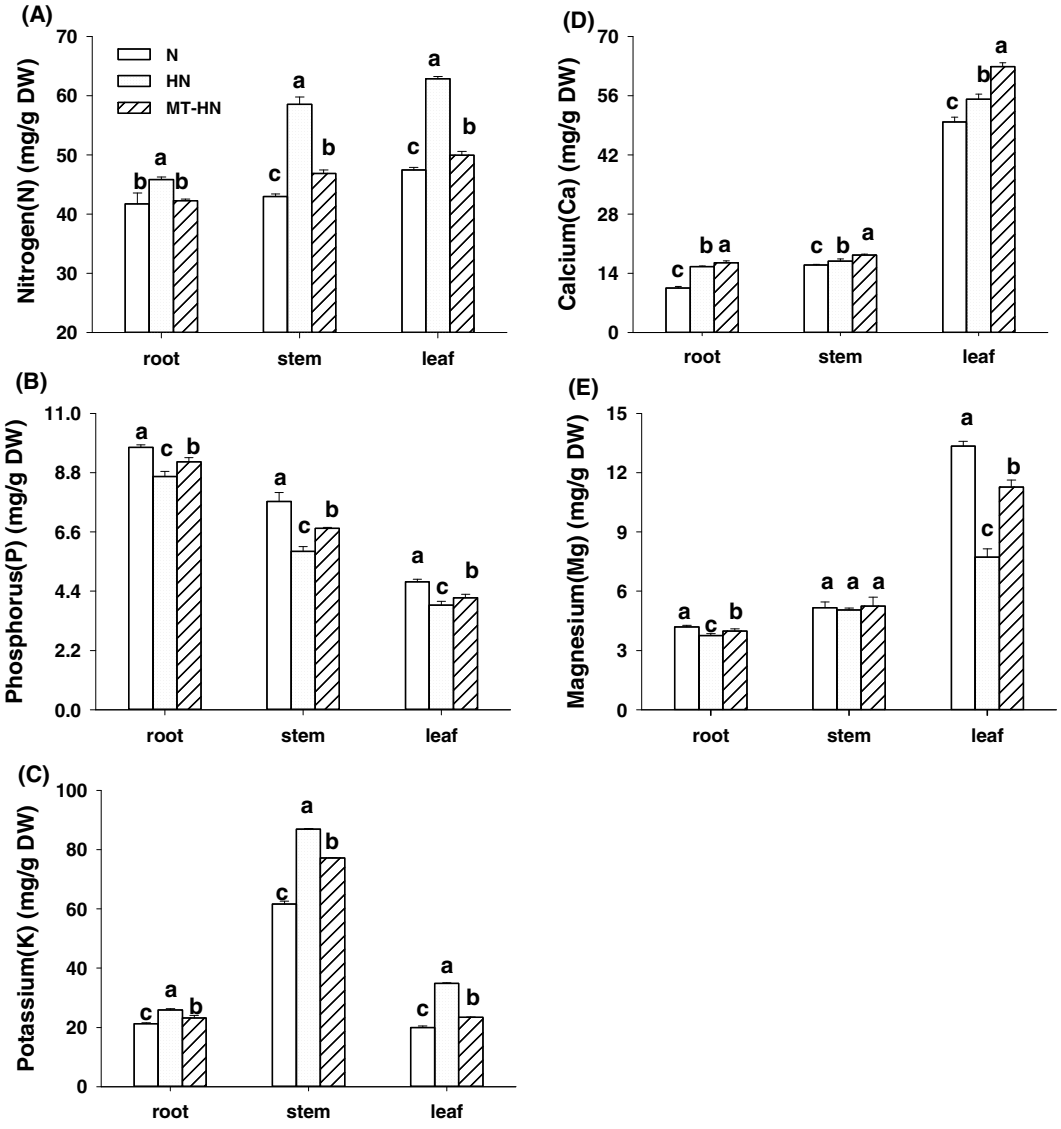

Fig. 5

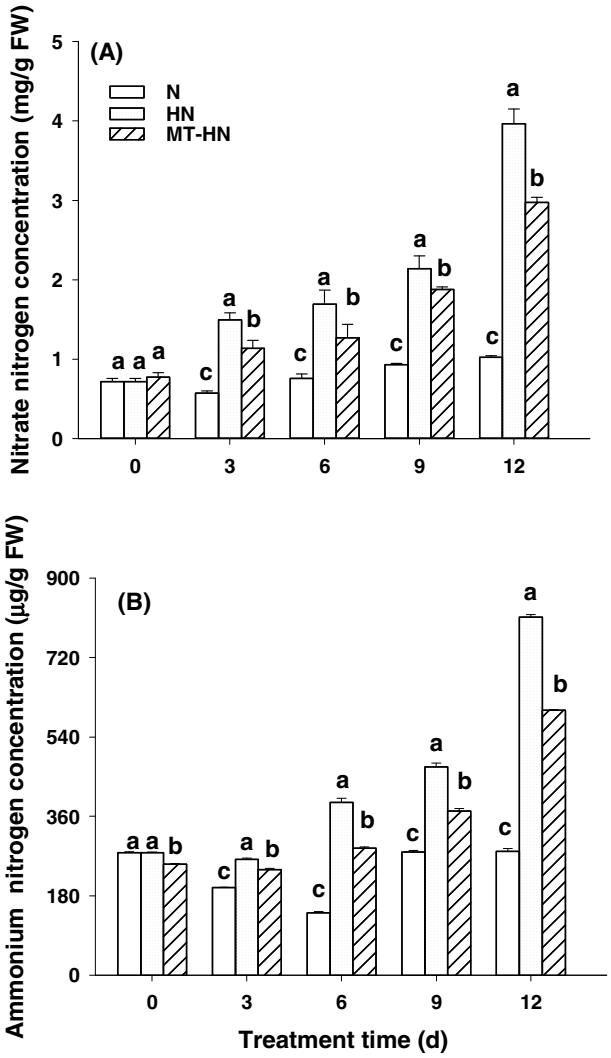

Fig. 6

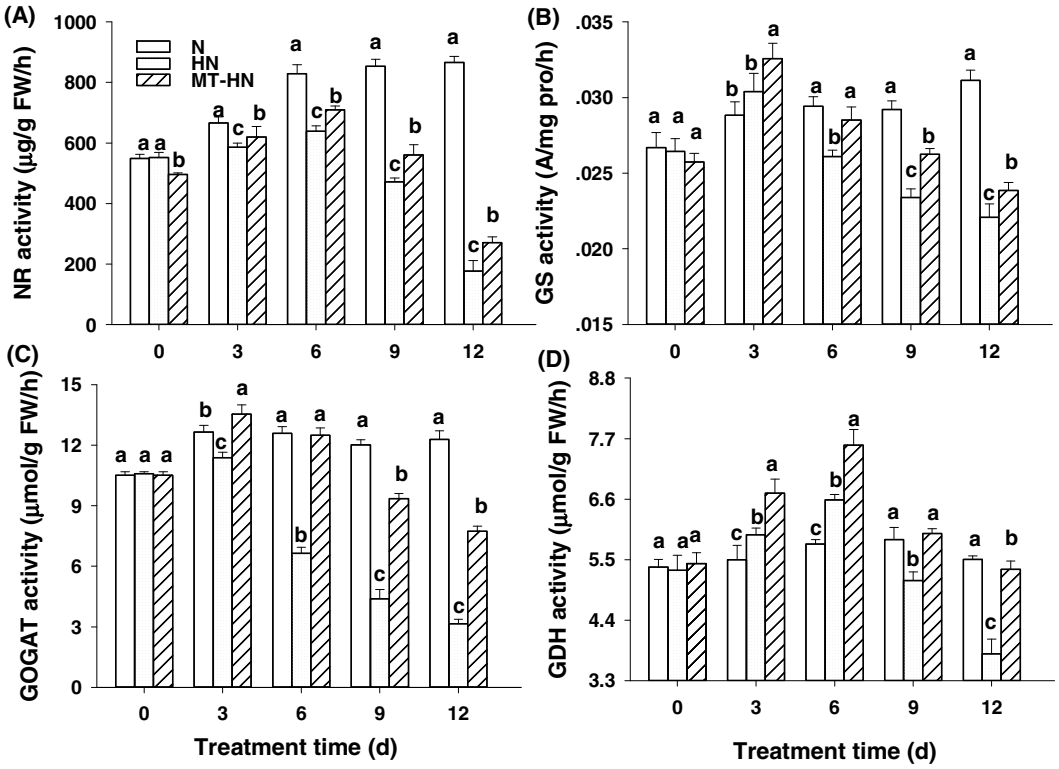

Fig. 7

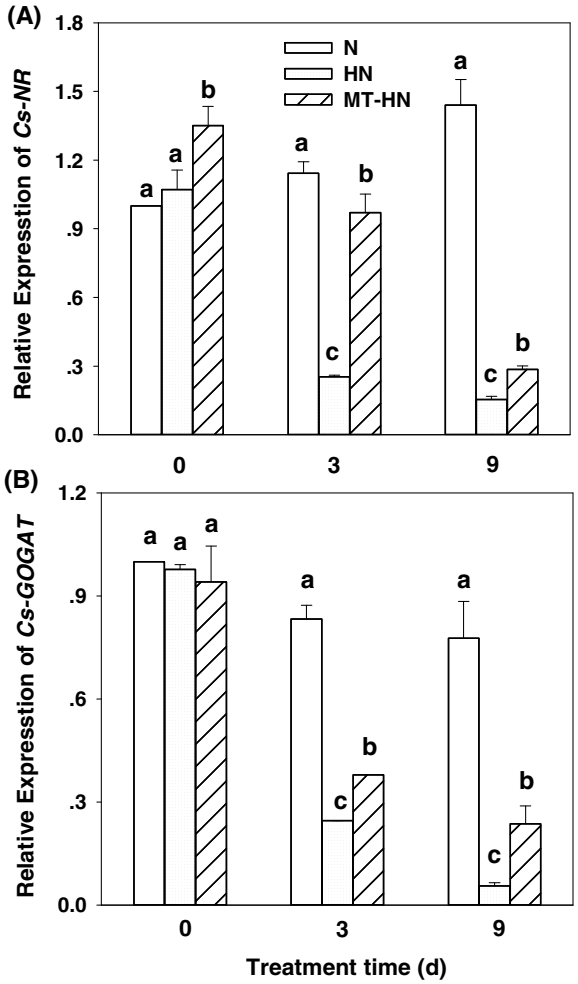

Fig. 8