Effects of melatonin on growth, non-photochemical quenching and related components in tomato seedlings under calcium nitrate stress

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Abstract: The effects of exogenous melatonin on the growth, chlorophyll fluorescence parameters and related components of distribution of heat dissipation in tomato seedlings under Ca (NO₃)₂ stress were studied in this experiment. The results showed that calcium nitrate stress inhibited the growth of tomato seedlings, reduced Y (II), Fv'/Fm', qP and ETR, and produced photoinhibition on tomato seedlings. Spraying exogenous melatonin could increase the growth of tomato seedlings under calcium nitrate stress. Melatonin regulated qP and ETR under calcium nitrate stress, reduced the ratio of Y(NPQ), Y(NO), and increased the ratio of Y(II). Further analysis of NPQ and its three components, which including qE, qT and qI, showed that NPQ and its three components were significantly increased in the stress treatment group, while melatonin could inhibit the increase. Thus, it indicated that exogenous melatonin could effectively alleviate the growth of tomato seedlings under calcium nitrate stress, maintain the reasonable distribution of light energy, and protect the photosystem II from light damage caused by excess light energy.

1. Introduction
Tomato is one of the important vegetables cultivated in greenhouse in China, which can play an important role in increasing farmers’ economic income and meeting people’s living standards[1]. However, due to the lack of rainwater leaching greenhouse, coupled with the problems of excessive application of fertilizer and unreasonable irrigation, the accumulation of soil salt in the facilities is a serious problem, which is prone to secondary salinization of soil[2]. Previous studies point out that the salt content of the soil in the facilities is generally 2-3 times more than that of the open soil, and the main anion and cation were NO₃⁻ and Ca²⁺, respectively, resulting the excess accumulation of calcium nitrate[3]. Studies have shown that the mechanism of damage caused by calcium nitrate stress on plants is different from NaCl. Calcium nitrate mainly causes the plant cells to lose water and produce physiological drought by osmotic stress, thus inhibiting plant growth and even causing plant death[4].

Melatonin (MT), a hormone substance, can not only play a variety of physiological regulation functions, but also alleviate the harm caused by various stress on plants. Most studies have shown that MT can remove free radicals in plants, alleviate the oxidative damage of plants caused by various abiotic stress[5]. In addition, MT can alleviate the chlorophyll degradation of tomato under salt stress, and prevent the damage caused by photoinhibition by regulating the electron transfer ability of photosystem II (PSII) reaction center[6].
The excessive light energy dissipated by plants in the form of heat energy can avoid or reduce the influence of light suppression on plants, and it is an important light protection mechanism of plants [7]. There are three components of NPQ: qE, qI and qT. Among them, qE is an NPQ component that depends on energy or proton gradient of the transmembrane, which can indirectly show the proton gradient of thylakoid membrane; qI is the NPQ component related to photoinhibition. qT is a NPQ component related to state conversion, reflecting the distribution of light energy between two optical systems [8]. However, the regulation of MT on NPQ and related components under salt stress or secondary salinization stress is still unclear.

The experiment analyzed the changes of light energy distribution and heat dissipation of tomato seedlings under calcium nitrate stress treated with exogenous MT by chlorophyll fluorescence analysis technology. The aim is to explore the regulation of MT on the light protection mechanism of plants under the calcium nitrate stress.

2. Materials and methods

The experimental material was ‘Lycopersicum esculentum cv. Jinpeng No.1’. After the seeds were soaked and germinated, the seedlings were raised with a 72-hole disk for 35 days under the growth chamber. Then the 35-day-old tomato seedlings with four leaves were selected and transplanted into plastic pots in the intelligent artificial climate chamber, with relative humidity (RH) of 60%-70%, temperature of 26/18°C (day/night), the light intensity was (200 ±25) μmol · m⁻² · s⁻¹, and the photoperiod was 12 h · d⁻¹. The concentration of calcium nitrate was 160 mmol·L⁻¹ for stress treatment. As for the treatments, the leaves were sprayed with 0 or 200 μmol·L⁻¹ of exogenous melatonin at weak light until the liquid was evenly covered with leaves, and treated once every 2 days. All indexes were measured on the 15th day after stress treatment. Each group was treated with 15 plants and repeated 3 times.

The plant height and stem diameter of tomato seedlings were measured with straight ruler, the plant fresh weight was measured after being washed with distilled water and sucking dry. The dry weight of plant was measured after deactivated at 105°C for 15min, drying at 75°C to constant weight.

Chlorophyll fluorescence parameters were measured by PAM-2500 portable chlorophyll fluorescence meter. The specific method for the determination of NPQ and its three components referred to Xu et al’s method [9], which was as follows: first, open the detection light to determine Fo, and then apply a saturated pulse light to measure Fm, which was recorded as A. Then turn on the applied light and added a continuous supersaturated light to the leaves every 2 min, where achieved a Fm’ value every time. The measured Fm’ value at the 6th application was recorded as B. After that, the action light and detection light were turned off, and the above process was repeated under dark recovery. The measured Fm’ value at 6 min after dark recovery was recorded as C, and the last measured Fm’ value under dark recovery was recorded as D [15]. NPQ and its three components can be calculated by the following formula: NPQ = (A - B) / B, qE = (C - B) / B, qT = (D - C) / B, qI = (A - D) / B.

Data statistics and analysis were carried out by Microsoft Excel 2016 software, and variance and significance were tested by SAS system program V8.

3. Results and Discussion

3.1 Effects of MT on growth index of tomato seedlings under calcium nitrate stress

It can be seen from table 1 that calcium nitrate stress significantly reduced the growth of tomato seedlings. In the non-stress treatment group, compared with CK, the growth of M increased, indicating that spraying exogenous MT can promote the growth of tomato seedlings. In the stress group, the plant height and stem diameter of M + N increased by 11.67% and 13.58% respectively compared with that of N. Similarly, the above-ground and under-ground fresh weight of M + N increased by 77.58% and 36.86%, respectively, when compared with N. And the dry weight of M + N also increased significantly compared to stress alone.
Table 1. Effects of melatonin on the growth of tomato seedlings under calcium nitrate stress

| Treatments | Height(cm) | Stem diameter (cm) | Fresh weight(g) | Dry weight(g) |
|------------|------------|--------------------|-----------------|---------------|
|            |            |                    | above-ground    | under-ground  | above-ground | under-ground |
| CK         | 33.62±5.24a| 0.48±0.03a         | 22.33±7.82b     | 3.31±1.05b     | 1.72±0.59b | 0.25±0.499b |
| M          | 38.30±3.08a| 0.55±0.31a         | 33.45±8.26a     | 4.43±1.01a     | 2.33±0.38a | 0.30±0.039a |
| N          | 26.56±4.55c| 0.40±0.06b         | 10.88±1.97d     | 2.50±0.67      | 1.44±0.13c| 0.24±0.013c |
| M+N        | 29.66±7.27b| 0.45±0.08c         | 19.31±14.88c    | 3.42±1.25      | 1.67±0.18b| 0.29±0.010ab|

Values are means ± standard errors. Means with the same letter within each column are not significantly different at p < 0.05. CK, control; M, melatonin treatment; N, calcium nitrate stress; M+N, melatonin with calcium nitrate stress.

3.2 Effects of MT on chlorophyll fluorescence parameters of tomato seedlings under calcium nitrate stress

From Table 2, there was a trend of reduction on Y(II), Fv/Fm', qP and ETR under calcium nitrate stress, while no significance in Fv/Fm, reflecting the inhibition of photosynthesis electron transport. While addition of melatonin increased Y(II). qP was also increased, indicating that the efficiency of excitation energy capture by open PSII reaction centers was enhanced by MT, and MT promoted the ETR as well. In addition, in the treatment of stress, Y( NPQ) and Y( NO) increased, while melatonin could prevent the increase in Y( NO). According to the light distribution of PSII, photochemical energy in PSII was composed of Y(NPQ), Y(NO) and Y(II). The prevention of increase in Y( NPQ), Y(NO) indicated the higher proportion of photochemical energy in Y(II). Thus, MT could enhance the photosynthesis electron transport under the calcium nitrate stress.

Table 2. Effects of melatonin on chlorophyll fluorescence parameters of tomato seedlings under calcium nitrate stress

| Treatments | Fv/Fm | Fv'/Fm' | Y(II) | Y(NPQ) | Y(NO) | qP | ETR |
|------------|-------|---------|-------|--------|-------|----|-----|
| CK         | 0.80±0.008a | 0.63±0.009a | 0.51±0.007ab | 0.21±0.018bc | 0.28±0.010b | 0.81±0.004a | 58.43±0.851ab |
| M          | 0.79±0.003a | 0.65±0.027a | 0.56±0.035a | 0.17±0.038c | 0.28±0.005b | 0.86±0.020a | 63.63±3.968a |
| N          | 0.79±0.010a | 0.56±0.014b | 0.39±0.014c | 0.29±0.011a | 0.32±0.020a | 0.69±0.038c | 44.20±1.637c |
| M+N        | 0.80±0.010a | 0.60±0.005ab | 0.46±0.007b | 0.26±0.006ab | 0.29±0.003b | 0.76±0.013b | 52.07±0.762b |

Values are means ± standard errors. Means with the same letter within each column are not significantly different at p < 0.05.

3.3 Effects of MT on NPQ and related components in tomato seedlings under calcium nitrate stress

Figure 1 showed the NPQ and its three components of four treatment groups. Among the treatment groups, qE accounted for a large proportion, while qT and qI accounted for a relatively small proportion. There was no significant difference in NPQ and three components between M and CK in non-stress treatment group. While NPQ and three components in the stress treatment group were significantly increased, and the increase range of M+N was less than that of stress alone(N).
Figure 1. Effects of melatonin on NPQ and related components in tomato seedlings under calcium nitrate stress

4. Conclusion
It can be concluded that light energy distribution and heat dissipation of tomato seedlings under calcium nitrate stress changed dramatically, which caused photoinhibition and less growth. Exogenous melatonin could alleviate the inhibition of photosynthetic electron transport chain, which is manifested as a high proportion of Y(II) and less Y(NPQ) and Y(NO).

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