Regulation of melatonin on chlorophyll fluorescence and nitrate accumulation in lettuce seedlings under excess nitrate stress

Xiaoting Zhou¹,², Tian Yang², Zhongli Jiang², Zhongqun He² and Zhirong Zou ¹*
¹ College of Horticulture, Northwest Agricultural & Forest University, Yangling, 712100, China
² College of Horticulture, Sichuan Agricultural University, Chengdu, 611130, China
*Corresponding author’s e-mail: zouzhirong2005@hotmail.com

Abstract. Excess nitrate stress has become a threat of environmental issues to crop cultivation in China. While measures of remediation and knowledge of the mechanisms underlying excess nitrate stress are limited. Previous studies reported that melatonin could improve the plant resistance to abiotic stress. Thus, we investigated the role of melatonin on the photosynthetic characteristic involved in nitrate assimilation under the excess nitrate stress in lettuce (Lactuca sativa). Lettuce plants were subjected to moderate excess nitrate stress (160 mM NO₃⁻) with/without supply of 1.0 μM melatonin. Results showed that adding of melatonin decreased the nitrate content both in leaves and roots. Melatonin also dramatically increased the fresh and dry weights in lettuce seedlings. According to the chlorophyll fluorescence parameters, exogenous melatonin inhibited the reduction in $\phi_{PSII}$, Fv/Fm, qP and rETR caused by excess nitrate stress, while reduced NPQ. In conclusion, exogenous melatonin could decrease the nitrate content in lettuce tissues and enhance the activity of PSII as well as the light energy conversion efficiency for carbon assimilation.

1. Introduction
Secondary salinization of soil has become one of the environmental problems to protected agriculture in China, the lack of rain and the high surface evaporation in protected cultivation has aggravated secondary salinization. Further, the salinized land is deteriorated by excessive fertilizer N and combined with a high Ca²⁺ concentration and major anions of NO₃⁻ (67-76% of total anions) in the soil surface layer (0–5 cm depth) [1-2]. The high concentration of nitrate causes a stress environment to the plants, resulting in a decrease in plant yield and quality. Excess nitrate stress induced severe oxidative damage and photosynthesis inhibition in cucumbers [1] and tomatoes [3]. Thus, works should be done to mitigate the excess nitrate stress.

A large number of studies have reported that melatonin can improve the adaptability of plants to abiotic and biological stresses [4-5]. Photosynthesis is highly susceptible to stress. Therefore, photosynthetic parameters can be used to preliminarily explore effect of melatonin on excess nitrate stress. Previous studies have shown that melatonin has a positive mitigation effect on photosynthesis under stress [6]. Exogenous melatonin can regulate stomatal opening and closing under stress and improve the adaptability of apple leaves to drought [7]. In addition, stress affects many processes of photosynthesis, such as electron transport, activity of light reflection centers and CO₂ fixation [8-9]. However, the specific regulatory mechanism of melatonin on different photosynthesis processes is still
unclear, and the response mechanism of melatonin to photosynthesis needs to be further clarified. At present, the effect of melatonin on excess nitrate stress is rarely reported. The purpose of this study was to elucidate whether melatonin could alleviate the growth inhibition of lettuce by alleviating photosynthesis under excess nitrate stress.

2. Material and methods
The experiments were conducted at climate-control chamber. Lettuce plants (Lactuca sativa cv. Da Su Sheng) seeds were germinated and grown for 25 days in cell flats filled with perlite. The 25-day-old seedlings were transferred to plastic tubs containing 40 L of hydroponic nutrient solution. Half-strength Japanese Garden formula for nutrient solution was used, with the final concentration of macro-elements shown as follows: NH₄⁺ (0.665 mM); NO₃⁻ (3.5 mM); P (0.33 mM); K(1.5); Ca(1.0 mM); Mg(0.5 mM); S (0.5 mM). The controlled environmental conditions include a relative humidity (RH) of 60–80%, temperature of 25/15°C (day/night), and 16/8 h photoperiod at a photosynthetic photon flux density of 300 μmol m⁻² s⁻¹. During the experiments, the nutrient solution was aerated using an air pump at an interval of 20 min to maintain the dissolved oxygen concentration at 8.0 ± 0.2 mg L⁻¹. The nutrient solution contained deionized water at a pH of 6.0 ± 0.1 and was changed every 3 days.

Treatments were administered in combination with the nutrient solution. The analytical pure of Ca(NO₃)₂ and melatonin were added into nutrient solution separately or combined, with final concentrations of NO₃⁻ (160 mM) according to Yuan [10] and melatonin (1 μM) according to the results of our previous experiments (data not shown). The 40-day-old seedlings, with 6 leaves in basic vegetative period, were treated as follows: (a) CK, control; (b) M, adding 1 μM melatonin; (c) S, adding 160 mM NO₃⁻; (d) M+S, adding 160 mM NO₃⁻ + 1 μM melatonin.

Leaves were sampled from 15 days stressed seedlings (40 days old after germination). The second fully expanded leaves from the top of the plants and roots were cleaned and sampled for nitrate content analysis. After 15 days of treatment, the fresh weight of each plant was recorded. Dry weights were determined by drying the tissues to achieve constant weight at 80 °C.

Nitrate content was expressed by nitrate nitrogen and determined by salicylic acid method [11]. Chlorophyll fluorescence in the second fully expanded leaf from the top of the plant was measured using a portable fluorometer (PAM 2500; Walz, Germany) after dark adaptation for 30 min according to the method previously reported by Kooten and Snel [12].

Data were analyzed by using one-way ANOVA. Letters indicate significant differences at \( p < 0.05 \) according to Duncan’s multiple range tests.

3. Results and Discussion

3.1. Effects of melatonin on fresh and dry weights in lettuce seedlings
After 15 days of treatment, excess nitrate stress significantly decreased the fresh and dry shoot and root weights compared with control (Table 1). However, the supply of melatonin mitigated the apparent decrease in weight. In addition, treatment with melatonin alone caused no significant weight differences except for a decrease in dry shoot weights when compared with control. The positive alleviation of melatonin to stress has also been reported in apples, cucumbers, soybeans and Cynodon dactylon (L). Pers induced by salt stress as the result of improvement of growth[13-15].

| Treatments | Fresh shoot (g/plant) | Fresh root (g/plant) | Dry shoot (g/plant) | Dry root (g/plant) |
|------------|-----------------------|----------------------|---------------------|-------------------|
| CK         | 34.705±1.258a         | 3.272±0.163a         | 1.375±0.102a        | 0.135±0.012a      |
| M          | 32.347±1.397a         | 2.988±0.110a         | 1.058±0.031b        | 0.129±0.007ab     |
3.2. Effect of melatonin on chlorophyll fluorescence

The effect of excess nitrate stress on photosystem II (PSII) activity of lettuce was determined by chlorophyll fluorescence parameters. Compared to control, excess nitrate stress significantly decreased \( \varphi_{\text{PSII}} \), \( F_v'/F_m' \), \( q_P \) and \( r\text{ETR} \), but significantly increased NPQ (Table 2). However, exogenous melatonin inhibited the reduction in \( \varphi_{\text{PSII}} \), \( F_v'/F_m' \), \( q_P \) and \( r\text{ETR} \), while reduced NPQ in nitrate-stressed plants. In addition, melatonin alone had no significant effects as compared to control. \( \varphi_{\text{PSII}} \) represents the actual photochemistry efficiency of the PSII reaction center, reduction of \( \varphi_{\text{PSII}} \) indicated less photosynthetic electrons transmitted for carbon fixation reaction as a result of the decrease of net photosynthetic rate. Thus, excess nitrate stress also affects photosynthesis through non-stomatal factors. Photochemical quenching coefficient (\( q_P \)) reflects the redox state of PSII primary electron receptor(QA). Reduction of \( q_P \) showed that QA is over-reduced and the excitation energy pressure is too high due to the damage of PSII, which can not transfer electrons smoothly, and the number of photochemical reaction centers used in photochemistry reaction was reduced. The decrease of \( F_v'/F_m' \) can also lead to the decrease of \( \varphi_{\text{PSII}} \), i.e. light energy was directly consumed through the thermal dissipation of antenna pigments Instead of being transmitted to the PSII light reaction center. NPQ represents non-photochemical quenching, which directly reflect the change of thermal dissipation of antenna pigments, and is an effective heat used to prevent light damage. However, the increase of heat dissipation mechanism will inevitably reduce the conversion efficiency of light energy and photosynthetic rate. Therefore, excess nitrate stress led to the increase of NPQ and the decrease of photochemical efficiency and photosynthetic rate of PSII. While under excess nitrate stress, the application of melatonin increased \( \varphi_{\text{PSII}} \), \( F_v'/F_m' \) and \( q_P \) and decreased NPQ. The results showed that melatonin could increase the activity of PSII, increase the photochemical efficiency of PSII, and help to improve the photosynthetic rate.

### Table 2. Effects of melatonin on fresh and dry weights in lettuce seedlings.

| Trements | \( F_v'/F_m' \) | \( \varphi_{\text{PSII}} \) | \( q_P \) | NPQ | \( r\text{ETR}(\mu\text{mol m}^{-2}\text{s}^{-1}) \) |
|----------|-----------------|-----------------|-------|-----|-----------------|
| CK       | 0.705±0.005a    | 0.578±0.022a    | 0.819±0.033a | 0.287±0.02c | 27.0±1.080c     |
| M        | 0.701±0.009a    | 0.571±0.013a    | 0.816±0.026a | 0.309±0.049c | 27.0±0.707c     |
| S        | 0.567±0.01c     | 0.457±0.008b    | 0.806±0.012b | 1.263±0.065a | 21.5±0.289a     |
| M+S      | 0.623±0.007b    | 0.544±0.009a    | 0.873±0.009a | 0.783±0.038b | 25.8±0.479b     |

\( F_v'/F_m' \), PSII maximum efficiency; \( \varphi_{\text{PSII}} \), PSII operating efficiency; \( q_P \), Photochemical quenching coefficient; NPQ, Nonphotochemical quenching; \( r\text{ETR} \), relative electron transfer rate. Values represent the mean ± SE (n = 4). Letters indicate significant differences at \( p < 0.05 \) according to Duncan’s multiple range tests. CK, control; M, 1 μM melatonin treatment; S, 160 mM NO\(_3\); M+S, co-treatment of 1 μM melatonin and 160 mM NO\(_3\).

3.3. Effects of melatonin on nitrate content in lettuce seedlings

As shown in Figure 1, excess nitrate stress significantly increased nitrate content in lettuce leaves and roots. Under excess nitrate stress, the nitrate content in leaves was 6.45 times higher than that of control. The exogenous melatonin decreased the nitrate content by 10.43% in leaves under excess nitrate stress, which was 5.77 only times higher than that in the control. The nitrate content in root tissue showed the same tendency of change. The nitrate content was also increased in stress-treated
roots while restrain by supply of melatonin. Thus, the results above showed that melatonin could reduce the nitrate level of lettuce. Nitrate absorption of plants is assimilated mainly by the reduced power of light energy conversion. Studies showed that there is energy competition in carbon and nitrogen assimilation. In this study, excess nitrate stress caused a sharp increase in nitrate content in plants. Under the high concentration of nitrogen, nitrogen assimilation is vigorous and consumes excessive energy. On contrary, reduced carbon assimilation capacity was reduces as result of a decrease in photosynthetic rate. However, exogenous melatonin decrease of nitrate content under excess nitrate stress and promoted the equilibrium mechanism of carbon and nitrogen assimilation.

4. Conclusion

The results of this study indicated that excess nitrate stress inhibited plant growth and photosynthetic processes in lettuce plants, with results of less efficient electron transport and a rise of nitrate accumulation. However, exogenous melatonin provided protection against excess nitrate stress by means of enhancement of photosynthetic characteristic and plant growth in lettuce leaves, while nitrates content was reduced as well. Collectively, the results of the current investigation indicated that exogenous melatonin plays an important role in improving the performance of lettuce plants under excess nitrate stress.

Figure 1. Effects of melatonin on nitrate content in lettuce seedlings under excess nitrate stress. A, nitrate content in lettuce leaf. B, nitrate content in lettuce root. Value is mean±SE (n=3), and values followed by the different letters show significant difference ($p<0.05$). CK, control; M, 1 μM melatonin treatment; S, 160 mM NO$_3^-$; M+S, co-treatment of 1 μM melatonin and 160 mM NO$_3^-$.

References

[1] Yuan, L.Y., Du, J., Yuan, Y.H., Shu, S., Sun, J., Guo, S.R. (2013) Effects of 24-epibrassinolide on ascorbate-glutathione cycle and polyamine levels in cucumber roots under Ca(NO$_3^-$)$_2$ stress. Acta Physiologiae Plantarum, 35: 253-262.

[2] He, F., Chen, Q., Jiang, R., Chen, X., Zhang, F. (2007) Yield and nitrogen balance of greenhouse tomato(Lycopersicum esculentum Mill.) with conventional and site-specific nitrogen management in northern China. Nutrient Cycling in Agroecosystems, 77: 1-14.

[3] Zhang, G.W., Liu, Z.L., Zhou, J.G., Zhu, Y.L. (2008) 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 Regulation, 56: 7-19.

[4] Hardeland, R. (2016) Melatonin in plants - diversity of levels and multiplicity of functions. Front Plant Sci., 7: 198.

[5] Shi, H., Chen, K., Wei, Y., He, C. (2016) Fundamental issues of melatonin-mediated atress signaling in Plants. Front Plant Sci., 7(198): 1124.
[6] Arnao, M.B., Hernandez-Ruiz, J. (2015) Functions of melatonin in plants: a review. J. Pineal. Res., 59(2): 133-150.

[7] Li, C., Tan, D.X., Liang, D., Chang, C., Jia, D., Ma, F. (2015) Melatonin mediates the regulation of ABA metabolism, free-radical scavenging, and stomatal behaviour in two Malus species under drought stress. J. Exp. Bot., 66(3): 669-680.

[8] Zhao, H., Ye, L., Wang, Y., Zhou, X., Yang, J., Wang, J., Cao, K., Zou, Z. (2016) Melatonin increases the chilling tolerance of chloroplast in cucumber seedlings by regulating photosynthetic electron flux and the ascorbate-glutathione cycle. Frontiers in Plant Sci., 7:1814.

[9] Zhou, X., Zhao, H., Cao, K., Hu, L., Du, T., Baluška, F., Zou, Z. (2016) Beneficial roles of melatonin on redox regulation of photosynthetic electron transport and synthesis of D1 protein in tomato seedlings under salt stress. Frontiers in Plant Sci., 7:1823.

[10] Yuan L.Y. (2013) Physiological mechanism of exogenous brassinolide in alleviating cucumber seedling injury under calcium nitrate stress. Agricultural University, Nanjing.

[11] Gao, J.F. (2006) Instruction for Plant Physiology Experiments. Higher Education Press, Beijing.

[12] Van Kooten, O., Snel, J.F., (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosyn. Res., 25(3):147-150.

[13] Li, C., Wang, P., Wei, Z., Liang, D., Liu, C., Yin, L., Jia, D., Fu, M., Ma, F. (2012) The mitigation effects of exogenous melatonin on salinity-induced stress in Malus hupehensis. J. Pineal Res., 53(3): 298-306

[14] Wang, L.Y., Liu, J.L., Wang, W.X., Sun, Y. (2016) Exogenous melatonin improves growth and photosynthetic capacity of cucumber under salinity-induced stress. Photosynthetica, 54(1): 19-27

[15] Wei, W., Li, Q.T., Chu, Y.N., Reiter, R.J., Yu, X.M., Zhu, D.H., Zhang, W.K., Ma, B., Lin, Q., Zhang, J. S., Chen, S.Y. (2015) Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. Journal of Experimental Botany, 66(3): 695-707.