

The Effect of Exogenous Melatonin on Kiwifruit Photosynthesis under low light environment

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Abstract. Melatonin is a ubiquitous indol molecule and has powerful functions in relieving abiotic stress responses. In our study, we used the kiwifruit seedlings as the materials and investigated the efficiency of different concentration melatonin (50 μ M, 100 μ M and 200 μ M) on protect photosynthesis compared with control under low light. The results indicated that each concentration of melatonin significantly alleviated the degradation of chlorophyll and carotenoid and had no significant difference. Attributing to the preservation of chlorophyll by melatonin, the melatonin application groups accumulated more soluble sugar, soluble protein and proline than control group. In general, combining the results of photosynthetic pigment, photosynthate and proline, 200 μ M of melatonin had the mostly significant effect on protecting photosynthesis.

Introduction

Light is one of the most important environmental factors in agricultural production. Plants can obtain energy from light and synthesize organic substances which ensure the normal reproduction and growth of plants [1-3]. Light is a main source of energy for plants. However, in agricultural production, it is usually due to shading or regional factors that lead to lack of light. Lack of light can directly induce leaf senescence, which resulted in the extreme production of ROS. Moreover, excessive ROS will destroy chloroplast structure and the chlorophyll content decrease, affect photosynthesis accordingly.

Melatonin (N-acetyl-5-methoxytryptamine) is a ubiquitous indol molecule, firstly discovered in 1958 [4, 5]. Since the discovery of melatonin, it has been extensively studied. It has been confirmed melatonin in almost all plant organs such as seeds, roots, stems, leaves, flowers and fruit[6]. Many researches indicate melatonin can act a growth regulator to govern plant growth (e.g. root growth [7]), or as a biostimulator: anti-senescence and anti-stress effects (e.g. drought [8], cold[9]).

Our study used the kiwifruit seedlings to investigate the effect of different concentration of melatonin on photosynthesis under low light. To better understand the effect of melatonin under lower light, we sought to analyze photosynthetic pigment, photosynthate and proline. The results may provided a potential suggestion in agriculture production.

Treatment and Methods

Treatment We used the healthy and uniform kiwifruit seedlings to assign two conditions for

pretreatment: (i) standard water supply and low light (control), (ii) solution of $50 \mu\text{mol}\cdot\text{L}^{-1}$ melatonin in water and low light (50 μM group), (iii) solution of $100 \mu\text{mol}\cdot\text{L}^{-1}$ melatonin in water and low light (10 μM group), (iv) solution of $200 \mu\text{mol}\cdot\text{L}^{-1}$ melatonin in water and low light (200 μM group). The pretreatment was conducted for 8 days in an open experimental field. During this period, the seedlings were treated with melatonin or water every 2 day by root irrigation (20 mL per pot). After the fifth irrigation, the seedlings were put under shade with 60% sunlight (the day was set as 0 days). The plants were sampled at day 0, 2, 4, 6, 8 between 10:00 and 11:00 h, by removing the fifth to ninth leaves upward along the stem from three trees per treatment. Every treatment used 15 pots, 3 seedlings per pot. The samples were quickly frozen after collection and stored in a cryogenic refrigerator at -80°C for subsequent index determination. All reactions were performed by using the leaf mixture of three kiwifruit seedlings with three technical and three biological replicates.

Determination of photosynthetic pigment, photosynthate and proline The measurement of chlorophyll and carotenoid referred to Lichtenthaler [10]. Content of soluble protein, soluble sugar and proline were determined using the method of Wang et.al [11]. The content of soluble sugar was determined using anthrone colorimetry method. Soluble protein content was measured by Coomassie brilliant blue G-250 method.

Results

Exogenous melatonin improved the photosynthetic pigment content under weak light. After 8 days low light treatment, we detected that the concentration of total chlorophyll of control group has been decreased, while the degradation of chlorophyll of melatonin application groups has been inhibited. From the figure 1, the results indicated the chlorophyll content of 50 μM , 100 μM and 200 μM group were significantly higher than control group. All the melatonin treatment groups chlorophyll a content were 25.7%, 29.4%, 24.7% higher than control group respectively, chlorophyll b were about 29%, 31.1%, 24.8% respectively, and total chlorophyll were 27%, 30.1%, 24.1% higher than control group respectively. Likewise, the change of carotenoid content was in accordance with chlorophyll. Each concentration of melatonin application also inhibited the carotenoid degradation. We observed that the carotenoid content of 50 μM , 100 μM and 200 μM group was 29.9%, 35.2%, 26.9% higher than control group respectively. However, there was no significant difference in melatonin application groups in chlorophyll and carotenoid content.

Exogenous melatonin enhanced the content of photosynthate and proline under weak light. Soluble sugar, soluble protein and proline are important osmotic regulators in plants. In our study, we detected melatonin enhanced the content of soluble sugar, soluble protein and proline in melatonin application groups compared to control group, and there was significant difference between melatonin application groups and control (Figure 2). Regarding soluble sugar, we found 200 μM melatonin had the most marked effect on the increase of soluble sugar content, and the effect both 50 μM and 100 μM groups were lower than 200 μM group. In addition, when monitored the soluble protein, the results showed 50 μM and 200 μM group accumulated more soluble protein than 100 μM group (about increased 10.4% and 10.7% respectively, but 100 μM increased just 5.2%). However, in terms of proline, the results presented as: 50 μM group > 100 μM group > 200 μM group > control, but there was no significant difference between 50 μM and 100 μM group.

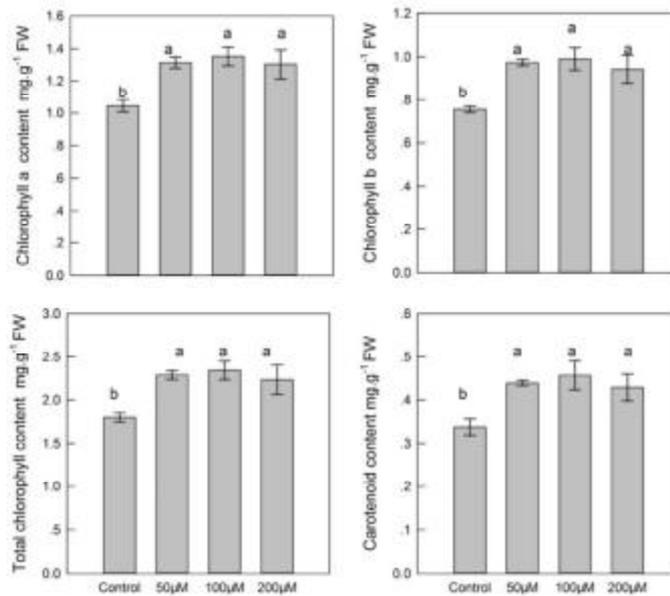


Figure 1. Effect of exogenous melatonin on photosynthetic pigment content under weak light. FW means fresh weight (the same as below). Data are show as means \pm SE (n=9), different letters indicate significant differences at $p < 0.05$ level.

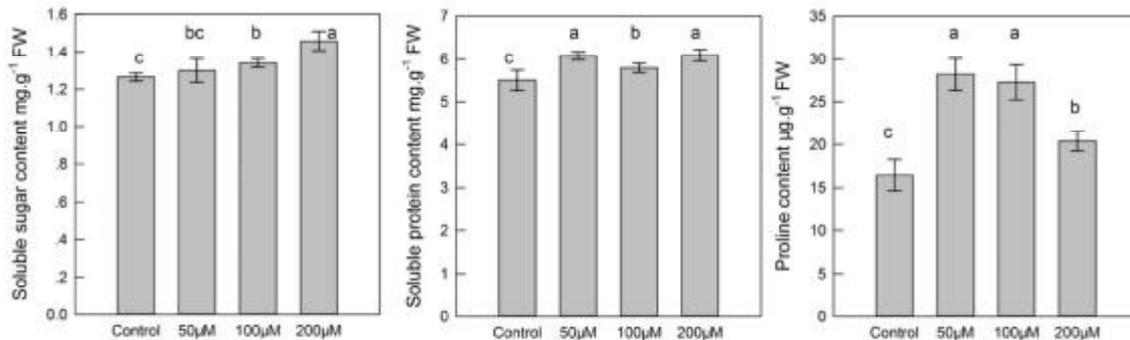


Figure 2. Effect of exogenous melatonin on photosynthate and proline under weak light. Data are show as means \pm SE (n=9), different letters indicate significant differences at $p < 0.05$ level.

Discussion

In field production, many factors will lead to leaf senescence, such as drought, high temperature, low temperature. However, low light is also an induction factor. As we all know, senescence generates excessive ROS. When these excessive ROS are not removed in time, the excessive ROS causes serious damage in the chloroplasts. In our study, the chlorophyll and carotenoid content in control group significantly reduced compared to melatonin application groups. It indicates that the exogenous melatonin application can greatly alleviate the reduction in the pigment content under low light. This beneficial effect of melatonin might be attributed to the decreased levels of ROS. Moreover, the result that melatonin protects the photosynthetic pigment from injury of ROS also demonstrated by Debnath et al. [12] But there was no difference in melatonin application groups, maybe it's because photosynthetic pigments are equally sensitive to melatonin at 50-200 μ M concentrations.

Furthermore, photosynthate and proline accumulation were markedly observed in melatonin

pretreatment seedlings. Wang et al. also found melatonin exhibited better preservation of soluble protein and soluble sugar in *Malus hupehensis* [13]. In control group, the soluble sugar, soluble protein and proline reduced attributed to the degradation of photosynthetic pigment. We found 200 μM group generated the most marked effect on the increase of soluble sugar and soluble protein, but this efficiency of melatonin reduced in proline. Proline is import osmotic regulators in plant. Maybe because the melatonin mitigated leave senescence, resulting less accumulation of proline. In general, 200 μM was the mostly optimum concentration.

Conclusions

In conclusion, our study demonstrated that melatonin protect photosynthesis of kiwifruit from injury of ROS under low light. Moreover, we confirm an optimum concentration of melatonin. The results of the present study also provide new ideas for agricultural production under low light.

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References

- [1] Wang, H.; Gu, M.; Cui, J.; Shi, K.; Zhou, Y.; Yu, J. Effects of light quality on CO_2 assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*: *J. Photoch Photobio B* Vol.96 (2009), p. 30-37
- [2] Tu, W.; Li, Y.; Zhang, Y.; Zhang, L.; Liu, H.; Liu, C.; Yang, C. Diminished photoinhibition is involved in high photosynthetic capacities in spring ephemeral *Berberoa incana* under strong light conditions: *J. Plant Physiol.* Vol.169 (2012), p. 1463-1470
- [3] Fu, W.; Li, P.; Wu, Y. Effects of different light intensities on chlorophyll fluorescence characteristics and yield in lettuce: *Sci Horti* Vol.135 (2012), p. 45-51
- [4] Lerner, A. B.; Case, J. D.; Takahashi, Y.; Lee, T. H.; Mori, W. Isolation of melatonin, a pineal factor that lightens melanocytes¹: *J. Am Chem Soc* Vol.80 (1958), p.
- [5] Lerner, A. B.; Case, J. D.; Heinzelman, R. V. Structure of Melatonin¹: *J. Am Chem Soc* Vol.81 (1959), p.
- [6] Arnao, M. B.; Hernández - Ruiz, J. Functions of melatonin in plants: a review: *J. Pineal Res* Vol.59 (2015), p. 133-150
- [7] Hernández-Ruiz, J.; Cano, A.; Arnao, M. B. Melatonin acts as a growth-stimulating compound in some monocot species: *J. Pineal Res* Vol.39 (2005), p. 137- 142
- [8] Cui, G. B.; Zhao, X. X.; Liu, S. D.; Sun, F. L.; Zhang, C.; Xi, Y. J. Beneficial effects of melatonin in overcoming drought stress in wheat seedlings: *Plant Physiol. Biochem.* Vol.118 (2017), p. 138-149
- [9] Ding, F.; Liu, B.; Zhang, S. Exogenous melatonin ameliorates cold-induced damage in tomato plants: *Sci Horti* Vol.219 (2017), p. 264-271
- [10] Lichtenthaler, H. K., Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In *Methods in Enzymology*, Academic Press: 1987; Vol. 148, pp 350-382.
- [11] Wang, X.; Huang, J. L., *The Principle and Technology of Plant Physiology and Biochemistry Experiment*. Higher Education Press: Beijing, China, 2015.
- [12] Li, C.; Wang, P.; Wei, Z. W.; Liang, D.; Liu, C. H.; Yin, L. H.; Jia, D. F.; Fu, M. Y.; Ma, F. W. The mitigation effects of exogenous melatonin on salinity-induced stress in *Malus hupehensis*:

J. Pineal Res Vol.53 (2012), p. 298-306

- [13] Wang, P.; Sun, X.; Chang, C.; Feng, F.; Liang, D.; Cheng, L.; Ma, F. 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 Vol.55 (2013), p. 424-434