

The Effect of Exogenous Melatonin on Kiwifruit Phenolic Compounds and Antioxidant Capacity under low light environment

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Abstract. Melatonin (N-acetyl-5-methoxytryptamine) is a low-molecular-weight molecule having an indole ring structure and considered as the best antioxidant substances. However, the role of melatonin in low light stress remains unknown. Our study investigated the possible role of melatonin on secondary metabolite and antioxidant capacity, involving total phenolics (TPC), flavonoids (TFC), flavanols (TFAC), FRAP, DPPH, and ABTS. The change trend of phenolic compounds and antioxidant capacity for different concentrations melatonin groups is basically the same. The results indicated melatonin significantly increased the phenolic compounds content and enhanced the antioxidant capacity compared to control group. In general, the 200 μM group had the markedly effect, 100 μM group followed, 50 μM group in the end.

Introduction

Light is an important signal for plant growth and development. Some researches had shown that weak light or shading could significantly affect growth and development of plants, resulted decrease in the yield and quality of plants [1, 2]. However, low light environment induce leaves senescence. The aging process topically accompanied with accumulation reactive oxygen species (ROS), which will damage the membrane system, such as chloroplast membrane. Moreover, in addition to activating the antioxidant system to respond to stress, plant cells also produce secondary metabolites to scavenge excessive ROS.

Since the discovery of melatonin, it has been confirmed the presence of which in almost all plant organs such as seeds, roots, stems, leaves, flowers and fruit [3]. Chloroplast and mitochondria had higher concentration melatonin than other parts of cell, due to the function of antioxidant attributed to melatonin can protect the chloroplast and mitochondria from damage of ROS. Previous research have been confirmed that exogenous melatonin application improved tolerance against cold stress [4], drought stress[5].

Furthermore, the role of exogenous melatonin in low light stress has not been testified, hence we sought to analyze TPC, TFC, TFAC, FRAP, DPPH, and ABTS. This study explored the change of secondary metabolite and antioxidant capacity of melatonin pretreated kiwifruit, which might have 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 (100 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 and 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 five 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 TPC, TFC, TFAC, FRAP, DPPH and ABTS. The methods of determining total phenolics (TPC), flavonoids (TFC) and flavanols (TFAC), were described by Wang [6]. The methods of Du [7] were applied to measure free radical scavenging ability, including DPPH, ABTS, and FRAP methods.

Results

Exogenous melatonin improved the content of phenolic compounds of kiwifruit under lowlight. Phenolic substances are secondary metabolites in plant cells, which have strong antioxidant capacity and scavenge the reactive oxygen species in the cells. In this study, it can be seen that melatonin can significantly increase the TPC, TFC, TFAC content in kiwifruit leaves than control group. The effect of melatonin treatment can be summed up as: 200 μM group > 100 μM group > 50 μM group. The TPC, TFC, and TFAC content in 200 μM group were 46.1%, 64.6% and 91.5% higher than control group, which in 100 μM group were 49%, 39.4% and 69% higher than control group, and which in 50 μM group were 29.8%, 19.8% and 9.8% higher than control group.

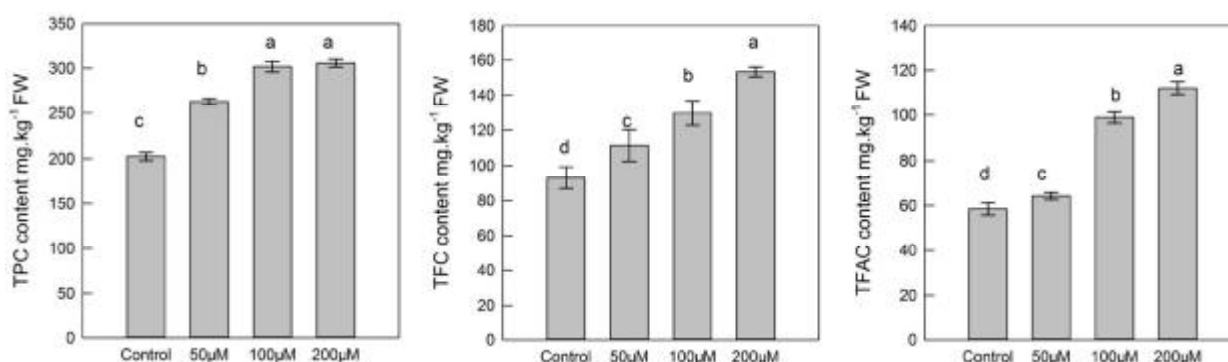


Figure 1. Effect of exogenous melatonin on phenolic compounds under weak light. FW means fresh weight (the same as below). Data are show as means \pm SE (n=9), different letters indicate significant differences at $p < 0.05$ level.

Exogenous melatonin enhanced the antioxidant capacity of kiwifruit under low light. Three different antioxidant methods were used to determine the antioxidant ability of kiwifruit leaves. The results showed that 50 μM group had no effect on the antioxidant ability of kiwifruit leaves, while the antioxidant capacity of 100 μM and 200 μM group was significantly improved. Regarding the FRAP, 200 μM group was 31.4% higher than control group, but 100 μM group just 21.7%. Besides, DPPH in 200 μM group also have marked difference with 100 μM group, but there was no significant difference between 100 and 200 μM group in ABTS and 200 μM group was just slightly higher than 100 μM group. The effect of melatonin treatment was as follows: 200 μM group > 100

μM group > 50 μM group.

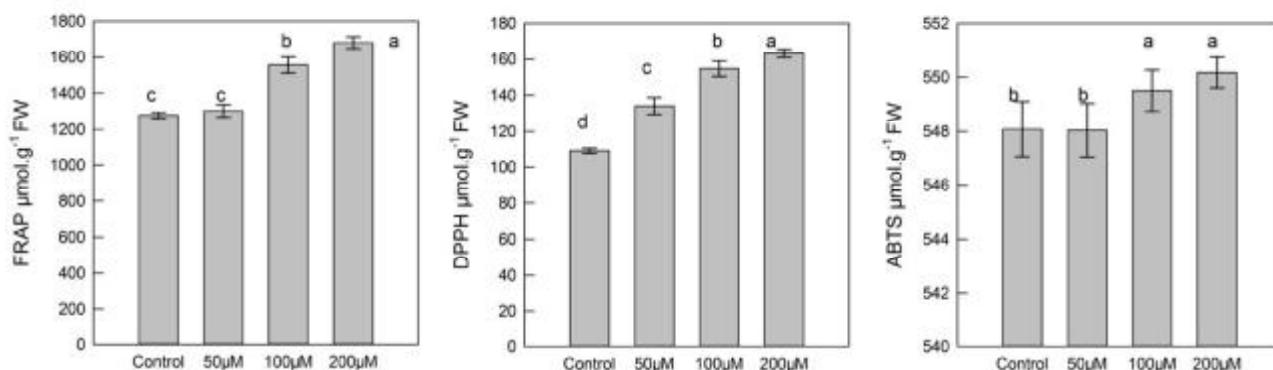


Figure 2. Effect of exogenous melatonin on antioxidant capacity under weak light. Data are shown as means \pm SE (n=9), different letters indicate significant differences at $p < 0.05$ level.

Discussion

As mentioned above, low light generates excessive ROS and accelerates the leaf senescence. In this process, the plants will activate the enzymatic or non-enzymatic mechanism to suppress the accumulation of ROS and delay senescence. In this study, melatonin induced the nonenzymatic antioxidant activities in kiwifruit seedlings, including TPC, TFC and TFAC. As shown in Figure 1, the content of TPC, TFC and TFAC in the melatonin application group significantly increased compared to the control. Additionally, Debnath et al. found that melatonin caused an increase in phenolic compound content to scavenge the ROS induced by acid rain [8].

DPPH, ABTS and FRAP were three typically used methods in antioxidant capacity measurement, which can comprehensively evaluate the antioxidant capacity of plants. Moreover, phenolic compounds are fundamental secondary metabolites in plant cells, which have strong antioxidant capacity and scavenge the reactive oxygen species in the cells. Due to the strong antioxidant capacity of phenolic compounds and melatonin, we detected that the higher antioxidant capacity in the melatonin application groups than the control group. This beneficial effect of melatonin was also found by Gao et al. [9]. The consequences supported the concept that melatonin may have a vital role in protecting the plants from any abiotic stress conditions and in overcoming damage from oxidative stress at the plant's cellular level.

Conclusions

Melatonin treatment significantly enhanced the content of TPC, TFC and TFAC, hence improved the kiwifruit antioxidant capacity, including FRAP, DPPH and ABTS. In conclusion, the 200 μM melatonin treatment showed the marked alleviation in kiwifruit seedlings under low light. Therefore, the results of the present study also provide new ideas for melatonin application in agricultural production under a low light environment.

Acknowledgements

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