

Effects of Exogenous Melatonin on Antioxidant Activity of Kiwifruit Leaves in Response to Drought Stress

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Abstract. In this study, the seedlings of kiwifruit were used as the research materials to investigate the effects of exogenous melatonin (MEL) on antioxidant activity in kiwifruit leaves in response to drought stress. The results showed that exogenous melatonin effectively decreased the MDA content of leaves of kiwifruit seedlings under drought stress, increased the content of DPPH, ABTS and FRAP, improved the antioxidant activity and enhanced the ability of scavenging ROS. The results also indicated that exogenous melatonin alleviated the damage of drought stress on kiwifruit seedlings and improved the plants stress resistance.

Introduction

The fruit of kiwifruit contains lots of vitamin C and has high nutritional value [1]. Melatonin firstly was found in mammals in 1958 [2]. In recent years, melatonin has been found in higher plants [3-5]. Abiotic stress is an important factor limiting crop yield, and drought stress is one of the major factors [6]. Plants subjected to drought stress on growth and other physiological processes are affected in different aspects, and even lead to death. Studies have shown that melatonin can relieve the damage of plants under drought stress [7]. At present, the effects of exogenous melatonin on the leaves of kiwifruit under drought stress are still very few. The aim of this study was to discuss the effects of exogenous melatonin on the antioxidant activity of kiwifruit seedlings under drought stress.

Materials and Methods

Materials and Treatment. The seeds of annual wild kiwifruit were placed at 4°C refrigerator for 60 days. After pregermination with heterotherm treatment at 4°C 10h/ 25°C 14h for a week, the germinated seeds were sown in 25°C incubator for two weeks until they grew to two-true-leaf stage. The seedlings were moved into plastic pots (diameter: 18 cm; height: 23 cm) filled with mixed soil consisting of peat substrate, pulverized coconut shell and perlite, with three seedlings per pot. We watered the seedlings at 2-d intervals with 1/2 Hoagland's nutrient solution from the two-true-leaf stage (pH adjusted to 6.5±0.1 with diluted HCl or NaOH).

Treatments began at 10-true-leaf stage, as follows: (1) Control (CK), plants were well-watered during the whole experimental time; (2) drought treatment (DR): seedlings were well-watered for 8 days, subsequently irrigation was withheld for up to 9 days; (3) melatonin and drought treatment (MTDR): seedlings were pretreated with 100µM melatonin solution for 4 times, two days once, then irrigation was withheld for up to 9 days. Each treatment was repeated three times. Sample their middle leaves (from five to eight per plant) after 0, 3, 6, and 9d of drought treatment. All seedlings leaves were immediately frozen in liquid nitrogen and stored at -80°C.

Physiological Indexes. Malonaldehyde (MDA) determination method adopted Li [8]. DPPH[•] free radical scavenging capacity (DPPH), ABTS^{•+} free radical scavenging capacity (ABTS) and iron ion reduction capacity (FRAP) were measured by Du [9]; The above indicators were set three times, and calculated the average value.

Data Handling. Software Excel 2010 was used to calculate the test data and plot. Statistical analysis was performed using software Excel SPSS.

Result and Analysis

MDA Content. The MDA content in the leaves of DR and MTDR treatments increased significantly, especially from 6 to 9 days and peaked at 9d were 21.78 and 15.05 $\text{nmol}\cdot\text{g}^{-1}$ FW respectively, when compared with CK increased by 181.95% and 61.65% respectively. However, the MDA content in the leaves of MTDR group was lower than that of DR group, especially at 9d (Fig.1).

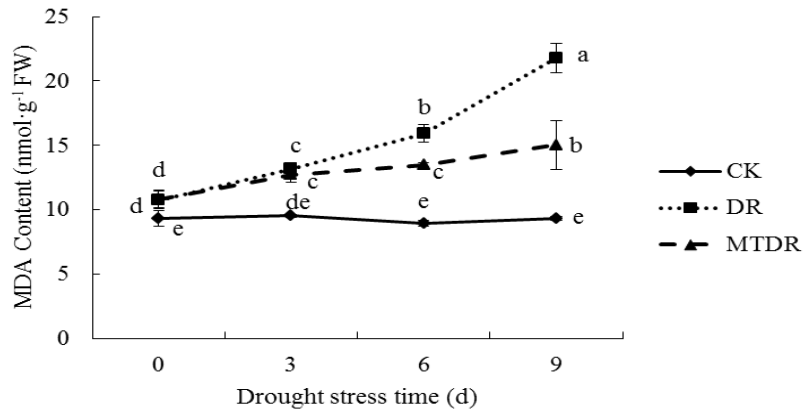


Fig. 1. Effect of exogenous melatonin on MDA content in leaves of kiwifruit seedlings under drought stress ($\text{nmol}\cdot\text{g}^{-1}$ FW). Note: Data with the different letters indicate the difference is significant ($P<0.05$).

DPPH Content. As seen from Fig.2, with the prolongation of drought stress, the DPPH content of DR increased and then decreased. At 0-6d, the DPPH content of DR increased gradually, and reached a maximum value of $3.14\ \mu\text{mol}\cdot\text{g}^{-1}$ FW, then decreased gradually. The DPPH content of MTDR showed an upward trend, and was always higher than that of CK and DR. The results indicated that exogenous melatonin treatment improved the DPPH content of kiwifruit seedlings drought copper stress.

ABTS Content. As shown from Fig.3, after drought stress treatment, the ABTS content of DR and MTDR all were increased all over the time, which were higher than that of CK at 3-9d, and peaked at 9d, to $14.55\ \mu\text{mol}\cdot\text{g}^{-1}$ FW and $14.67\ \mu\text{mol}\cdot\text{g}^{-1}$ FW, respectively. At 6-9d, the ABTS of DR and MTDR increased significantly. Therefore, at 6-9d, the ABTS content of MTDR were higher than that of DR. It could be concluded that drought stress increased the ABTS content of the leaves of kiwifruit seedlings, as well as exogenous melatonin increased the ABTS content of kiwifruit under copper stress, so then enhanced the antioxidant activity of plants.

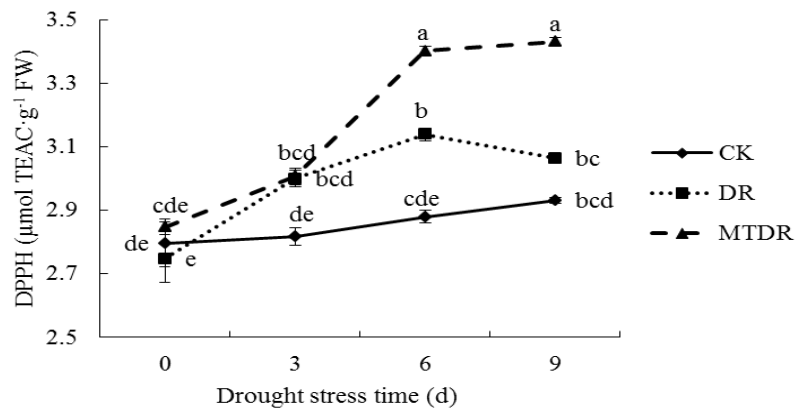


Fig. 2. Effects of exogenous melatonin on the DPPH content in leaves of kiwifruit seedlings under drought stress ($\mu\text{mol TEAC}\cdot\text{g}^{-1}$ FW). Note: Data with the different letters indicate the difference is significant ($P<0.05$).

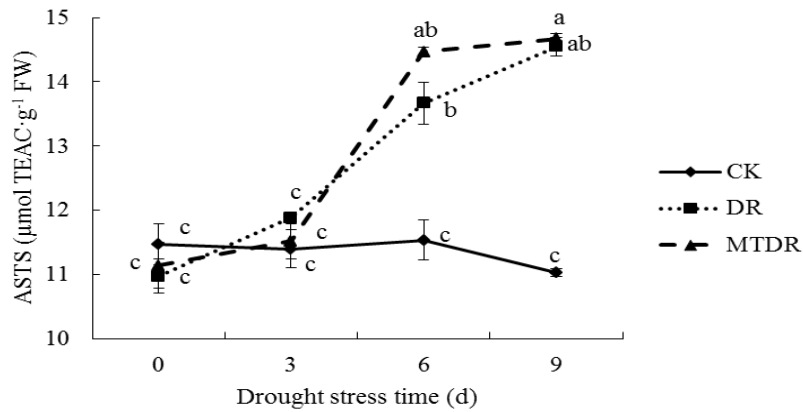


Fig. 3. Effects of exogenous melatonin on the ABTS content in leaves of kiwifruit seedlings under drought stress ($\mu\text{mol TEAC}\cdot\text{kg}^{-1}\text{ FW}$). Note: Data with the different letters indicate the difference is significant ($P<0.05$).

FRAP Content. Fig.4 is the change of the FRAP content of kiwifruit seedlings in the process of drought stress. When drought stress 0d, the change of the FRAP of each treatment was not obvious. With the prolongation of drought stress, the FRAP content of DR and MTDR showed an upward trend, which peaked at 9d, to $20.98 \mu\text{mol}\cdot\text{g}^{-1}\text{ FW}$, $22.18 \mu\text{mol}\cdot\text{g}^{-1}\text{ FW}$. At 3-9d, the FRAP content of DR and MTDR always were higher than that of CK. The FRAP content of MTDR was always higher than DR, at 6d and 9d. The results indicated that drought stress added the FRAP content of kiwifruit seedlings, and exogenous melatonin treatment improved the FRAP content of kiwifruit seedlings under drought stress, and enhanced plant antioxidant ability to heavy drought stress.

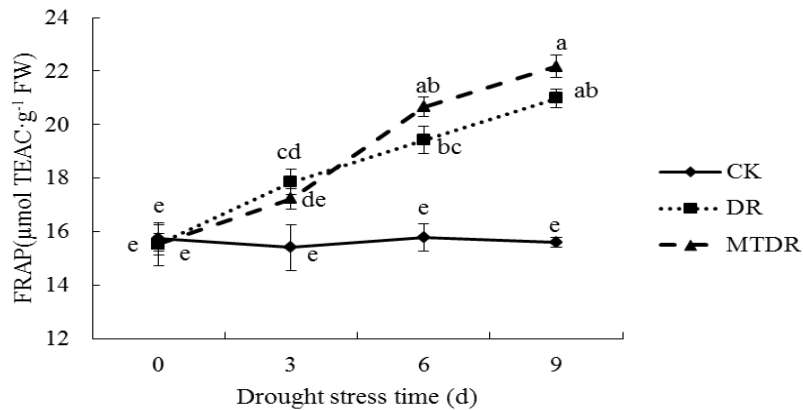


Fig. 4. Effects of exogenous melatonin on the FRAP content in leaves of kiwifruit seedlings under drought stress ($\mu\text{mol TEAC}\cdot\text{kg}^{-1}\text{ FW}$). Note: Data with the different letters indicate the difference is significant ($P<0.05$).

Conclusions

Drought is an important factor affecting the growth and development of crops, especially at seedling Stage [10-12]. Under drought stress, plant cells initiate a series of scavenging mechanisms of ROS that produce antioxidants, which can remove the excessive ROS, thereby alleviating oxidative stress. MDA reflects cell damage degree, therefore it can be related to the drought resistance of plants [13]. DPPH, ABTS, FRAP reflect the antioxidant activity of plants. The results show that exogenous melatonin treatment can increase the DPPH, ABTS and FRAP content of kiwifruit seedlings under drought stress, improve the ability to scavenge free radicals to enhance the antioxidant activity, and then advance the ability of plant adversity resistance. It is consistent with the results of Wang et al. [14] on apple leaves and Ye et al. [15] on wheat seedlings.

Acknowledgements

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References

- [1] J.V. Possingham: *Scientia Horticulture* Vol. 1-2 (1991), p. 171.
- [2] A.B. Lerner, J.D. Case and Y. Takahashi: *Am Chen Soc.* Vol. 80 (1958), p. 2592.
- [3] G.A. Bubenik: *Gastrointestinal melatonin: Digestive Diseases and Sciences* Vol. 47 (2002), p. 2347.
- [4] J. Zagajewski, D. Drozdowicz and I. Brzozowska: *Journal of Physiology and Pharmacology* Vol. 63 (2012).
- [5] R. Dubbels, R.J. Reiter and E. Klenke: *Journal of Pineal Research* Vol. 18 (1995), p. 30.
- [6] J.K. Zhu: *Annual Review of Plant Biology* Vol. 53 (2002), p. 271.
- [7] C.Q Jiang and Z.L. Zu: *Biotechnology Information* Vol. 31 (2015), p. 49, 50 (In Chinese).
- [8] H.S. Li: *Principles and Techniques of Plant Physiological and Biochemical Experiment* (Higher Education Press, China 2000).
- [9] G.R. Du: *Analysis of antioxidant activity and antioxidant activity of kiwifruit, persimmon and apple fruits* (Northwest A&F University, China 2009).
- [10] M.T. Harrison, F. Tardieu, Z. Dong, et al: *Global Change Biology* Vol. 20 (2014), p. 878.
- [11] D.B. Lobell, M.J. Roberts, W. Schlenker, et al: *Science* Vol. 344 (2014), p. 519.
- [12] C.L. Cao, S.X. Li: *Acta Agriculturae Nucleatae Sinica* Vol. 18 (2004), p. 404 (In Chinese).
- [13] Q. Sun, N.H. Cai and S. Chen: *Forum* Vol. 18-22 (2016), p. 21 (in Chinese).
- [14] P. Wang, X. Sun, C. Li, et al: *Journal of Pineal Research* Vol. 54 (2013), p. 299.
- [15] J. Ye, X.P. Deng, S.W. Wang, et al: *Journal of Triticeae crops* Vol. 35 (2015), p. 1282 (In Chinese).