Effects of exogenous melatonin on leaf antioxidants and antioxidant capacity of kiwifruit seedling under the stress of cadmium

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Abstract. Using one-year-old wild *Actinidia deliciosa* as the experimental material, the effects of melatonin (MT) on the antioxidant system of kiwifruit seedlings under heavy metal Cd stress were studied by root irrigating method. The results showed that after MT pretreatment, the contents of antioxidants (TPC, TFC, TFAC and ASA) increased in the leaves of *Actinidia chinensis* seedlings after Cd stress treatment, and the antioxidant capacity (DPPH, ABTS and FRAP) descended first and then rose.

Introduction

Cadmium (Cd) is a heavy metal that is toxic to humans and animals and plants^[1,2]. According to statistics, China's farmland cadmium pollution exceeds 13 thousand hm², involving 25 areas in 11 provinces^[3]. The study of cadmium pollution in orchard soil is also the focus of attention at home and abroad^[4]. At present, there are many reports about the harm of heavy metal pollution to plants. Kiwifruit belongs to *Actinidiaceae Actinidia Lindl* and is perennial vines, has a unique flavor, nutrient-rich, high Vc content and high economic value^[5]. Kiwifruit, as an important fruit tree resource in China has been a large number of planting^[6]. Fruit growth requires a certain amount of heavy metals (such as Cu, Zn, Fe, Mn and Mo), these heavy metals play a very important role on the growth of fruit trees; but beyond a certain limit, it will produce toxic effects on the fruit, through the formation of reactive oxygen species ROS) produces oxidative stress in plants^[7], leading to oxidative damage to cell membrane lipid peroxidation, protein, enzymes, nucleic acids, and even plant death^[8]. Therefore, it is great helpful to study the methods to improve the stress resistance of kiwifruit trees in order to maintain the normal physiological level and obtain green fruits. Melatonin (MT) is a neuroendocrine hormone secreted by pineal gland, which is N-acetyl-5-methoxytryptamine^[9], exists in the vast majority of biological organisms^[10]. A large number of studies have shown that MT can not only regulate the plant growth, but also improve the plant resistant abilities to biotic and abiotic stresses, such as, high temperature, low temperature, heavy metals, chemical pollution and biological oxidation of to a certain extent^[11].

In this study, wild *Actinidia deliciosa* seedlings were used to study the effects of exogenous melatonin on leaf antioxidants and antioxidant capacity of kiwifruit seedling under the cadmium stress. The results will helpful to solve heavy metal stress issues by using rational MT in the kiwi cultivation and provide a theoretical basis on anti-heavy metals stress of kiwi breeding.

Materials and methods

Materials and Treatment. Seeds of wild *Actinidia deliciosa* was collected from Qinling, Shaanxi Province, China in September 2015, and were placed in sand for 50 days at 4°C and 60-70% relative humidity in winter. kept at 4°C untill March, 2016. After variable temperature treatment at 4°C 16h, 24°C 8h for two weeks, seeds were placed at 25°C for 10-15 days to germinate. We began watering the seedlings at 2-day intervals with 1/2 Hoagland's nutrient solution at the two-true-leaf stage (pH adjusted to 6.5±0.1 with diluted HCl or NaOH).

When grown up with 9-10 true leaves, seedling was irrigated with $0.1\text{mol}\cdot\text{L}^{-1}$ MT solution for five times, two days once. Afterwards, seedlings were treated with $5\text{mg}\cdot\text{L}^{-1}$ (CdCl_2) for 15 days, irrigated once every two days. Middle leaves (seven to nine per plant) were sampled after 0, 3, 6, 9, and 12d. All collected tissues were immediately frozen in liquid nitrogen and stored at -80°C .

Enzymes Assay and Indicator Determination. AsA content determination method adopts Ma et al^[12]. TPC content determination method adopts Folin-Ciocalteu method^[13]. TFC content determination method adopts Jia et al^[14]. TFAC content determination method adopts p-DMACA method^[15]. DPPH, ABTS and FRAP determination method adopt Du Guorong^[16].

Data Analysis. Analysis of variance was performed using the SPSS software (SPSS, Inc., Chicago, IL, USA). Each treatment was replicated three times. Significant differences were detected using Duncan's test at the $P < 0.05$ level.

Results and Discussion

Antioxidant substances. After treatment with Cd solution, TPC content in leaves of kiwifruit seedling was increased first and peaked at the 9d with $2.97\text{mg}\cdot\text{kg}^{-1}$ and then decreased towards, which were significant higher than those in CK all over the time. However, when pretreated with MT, the TPC content of seedling significantly increased from 6 to 9 days and peaked at 9d, when compared with CK increased by 97.52% and then decreased towards. But The TPC content in the leaves of MT+Cd treatment higher than that of Cd all the time (Table 1).

After treatment with Cd solution, the AsA content in leaves of kiwifruit seedling was increased firstly and then decreased, and decreased slightly at 3d, then increased and peaked at the 9d, when compared with CK significantly increased by 31.71% and then decreased towards. However, when pretreated with MT, the AsA content of seedling significantly increased when compared with CK, by 93.71% at 9d, and compared with Cd increased by 47.07%. The AsA content in the leaves of MT+Cd treatment higher than that of Cd all the time (Table 1).

Table 1 Effects of exogenous melatonin on TPC content, AsA content, TFC content and TFAC content in kiwifruit under Cd stress

		0d	3d	6d	9d	12d
TPC ($\text{mgGAE}\cdot\text{kg}^{-1}$ FW)	CK	$1.61\pm 0.03\text{h}$	$1.61\pm 0.11\text{h}$	$1.60\pm 0.06\text{h}$	$1.61\pm 0.06\text{h}$	$1.61\pm 0.07\text{h}$
	Cd	$1.62\pm 0.05\text{h}$	$1.75\pm 0.03\text{g}$	$1.85\pm 0.01\text{f}$	$2.97\pm 0.05\text{d}$	$3.41\pm 0.01\text{b}$
	MT+Cd	$1.62\pm 0.04\text{h}$	$1.78\pm 0.08\text{g}$	$1.89\pm 0.01\text{e}$	$3.18\pm 0.05\text{c}$	$3.55\pm 0.10\text{a}$
AsA ($\mu\text{mol}\cdot\text{g}^{-1}$ FW)	CK	$6.90\pm 0.19\text{fg}$	$6.91\pm 0.24\text{fg}$	$6.95\pm 0.20\text{fg}$	$7.00\pm 0.12\text{f}$	$6.90\pm 0.21\text{fg}$
	Cd	$7.03\pm 0.26\text{f}$	$6.40\pm 0.28\text{h}$	$8.26\pm 0.28\text{e}$	$9.22\pm 0.21\text{d}$	$11.7\pm 0.09\text{c}$
	MT+Cd	$6.98\pm 0.17\text{fg}$	$6.66\pm 0.2\text{gh}$	$9.07\pm 0.06\text{d}$	$13.6\pm 0.06\text{b}$	$14.6\pm 0.23\text{a}$
TFC ($\text{mgRE}\cdot\text{kg}^{-1}$ FW)	CK	$6.38\pm 0.33\text{e}$	$6.40\pm 0.17\text{e}$	$6.40\pm 0.01\text{e}$	$6.30\pm 0.34\text{ef}$	$6.40\pm 0.02\text{e}$
	Cd	$6.40\pm 0.29\text{e}$	$5.66\pm 0.03\text{h}$	$6.09\pm 0.03\text{fg}$	$9.99\pm 0.13\text{d}$	$10.39\pm 0.2\text{c}$
	MT+Cd	$6.39\pm 0.01\text{e}$	$5.89\pm 0.15\text{gh}$	$6.52\pm 0.02\text{e}$	$11.1\pm 0.16\text{b}$	$11.83\pm 0.23\text{a}$
TFAC ($\text{mgCE}\cdot\text{kg}^{-1}$ FW)	CK	$0.64\pm 0.01\text{h}$	$0.63\pm 0.02\text{h}$	$0.64\pm 0.01\text{h}$	$0.64\pm 0.02\text{h}$	$0.64\pm 0.02\text{h}$
	Cd	$0.63\pm 0.01\text{h}$	$0.70\pm 0.01\text{g}$	$0.85\pm 0.01\text{e}$	$1.13\pm 0.01\text{b}$	$0.93\pm 0.00\text{d}$
	MT+Cd	$0.64\pm 0.01\text{h}$	$0.73\pm 0.01\text{f}$	$0.92\pm 0.01\text{d}$	$1.22\pm 0.00\text{a}$	$1.01\pm 0.01\text{c}$

Note: Data with the different letters indicate the difference is significant ($P < 0.05$).

The TFC content in the leaves of Cd and MT+Cd treatments showed a downward first and then upword trend and decreased to minimum at 3d with $5.66\text{mg}\cdot\text{kg}^{-1}$ and peaked at 12d with $11.83\text{mg}\cdot\text{kg}^{-1}$, increased significantly from 6 to 9d, when compared with CK increased by 58.57% and 76.03%. However, the TFC content in MT+Cd treatment higher than those in Cd all over the time (Table 1).

Under Cd stress, the TFAC content in leaves of kiwifruit seedling showed an upward trend and peaked at 9d , when compared with CK increased by 76.56% and then decreased towards. However, when pretreated with MT, the TFAC content of seedling significantly increased when compared with Ck, by 90.63% at 9d and the TFAC content in MT+Cd treatment higher than those in Cd all over the time (Table 1).

Antioxidant ability. The ABTS in the leaves of Cd and MT+Cd treatments was decreased firstly and then increased, increased significantly from 6 to 9d and peaked at 12d, when compared with CK

increased by 21.65% and 32.13%. The ABTS in Cd treatment lower than those in MT+Cd all over the time (Figure 1A).

Figure.1 Effects of exogenous melatonin on antioxidant ability of kiwifruit seedlings under Cd stress

Note: Data with the different letters indicate the difference is significant ($P < 0.05$).

Under Cd stress, the DPPH in leaves of kiwifruit seedling showed a downward trend and decreased minimum at 3d, when compared with CK decreased by 20.83% and then increased towards and peaked at 12d. However, when pretreated with MT, the DPPH of seedling significantly decreased when compared with Ck, by 15.03% at 3d and then increased. The DPPH in Cd treatment lower than those in Ck all over the time (Figure 1B).

After treatment with Cd solution, the FRAP in leaves of kiwifruit seedling was decreased firstly and then increased and decreased minimum at 3d, when compared with CK decreased by 29.21% and then increased and peaked at the 12d. But the FRAP in Cd treatment lower than those in Ck all over the time. When pretreated with MT, the FRAP of seedling significantly higher than those in Cd treatment, and peaked at 12d, when compared with CK increased by 6.45% (Figure 1C).

Conclusions

Secondary metabolites such as phenols may be involved in the scavenging of reactive oxygen species as the second line of defense under adverse circumstances^[17]. The results showed that the content of antioxidant substances in the leaves of kiwifruit seedlings decreased first and then increased after Cd stress, and increased significantly at 9-12d. It can be speculated that phenolic compounds as the second line of defense, opened the self-protection model. Under the action of exogenous MT, the TPC、TFC and TFAC contents continued increased. At the same time, the AsA content was also increased. DPPH, ABTS and FRAP are 3 common methods for the evaluation of antioxidant activity by colorimetric assay^[16]. In this study, these three methods were used to determine the antioxidant capacity of the leaves of kiwifruit seedlings under Cd stress. The results showed that the antioxidant ability of the seedlings was enhanced, while the exogenous MT treatment significantly increased the antioxidant capacity. The results indicated that exogenous MT could improve the antioxidant and antioxidant ability of kiwifruit and alleviate the damage of kiwifruit under stress conditions.

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