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Oxidation resistance of exogenous melatonin on leaves of kiwifruit seedlings under copper stress

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Abstract. In this study, the wild kiwifruit seedlings were used as the research object to investigate the oxidation resistance of exogenous melatonin on leaves of kiwifruit seedlings under copper stress, adopting the root irrigation method. The results showed that exogenous melatonin effectively increased the content of proline in leaves of kiwifruit seedlings under copper stress, enhanced the osmotic adjustment ability of plant cells, increased the antioxidant substances and improve the antioxidant capacity, reduced the toxicity of copper stress on kiwifruit and promote the tolerance of plants. The results indicated that exogenous melatonin could availably alleviate the toxicity of copper stress on kiwifruit seedlings and increase the tolerance of plants to heavy metal stress.

Introduction

Kiwifruit riches in Vc and has high nutritional value, known as "the king of fruits" [1]. Melatonin is a signal substance found in mammals, 1958 [2]. Studies have found that melatonin can enhance plant cells expansion in vitro, promote root growth and regeneration, maintain the integrity of the plant biofilm, and against adverse environmental damage to plants [3].

Copper is an important trace element in plant growth and development, which involved in many physiological processes of plants. But excessive copper will lead to plant poisoning, and affect the normal growth and development of plants [4]. At present, the effects of copper stress on plants are mainly studied by crops, and horticultural plants are less. Therefore, it is very important to study the effects of melatonin on plants under copper stress. This study was to investigate the physiological regulation mechanism of exogenous melatonin on kiwifruit under copper stress to provide guidance of application of exogenous melatonin in cultivation.

Materials and Methods

Materials and Treatment. The one-year-old wild *Actinidia deliciosa* seeds were placed at 4℃ for 55 days, then to 4℃ 16h and 24℃ 8h variable temperature treatment for two weeks. The seeds were placed at 25℃ for 10-15 days to germinate. Then the seeds are sown in the moist soil, and placed in the culture room with temperature of 20-25℃ and humidity above 40%. We watered one time every morning and evening, and 1/2 Hoagland nutrient solution every week for one time. When grown 3 true leaves, the plants were moved to pots containing perlite (10cm*10cm), 3 plants per pot.

When grown up with 9-10 true leaves, the seedlings with the same growth condition were divided into control group and treatment groups. The seedlings were treated with the 50 mL 0 and 0.1 μmol•L^{-1} MT solution in the root irrigating for 5 days, one day at a time. The seedlings were treated

with 15 μmol•L^{-1} $CuSO_4$. A total of 3 treatments: (1) CK; (2) T1: $CuSO_4$ (15 μmol•L^{-1}); (3) T2: MT (0.1 μmol•L^{-1})+$CuSO_4$ (15 μmol•L^{-1}). 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.

Physiological Indexes. The content of proline was determined by the method of acid ninhydrin [5]; The total phenolic content (TPC) was measured using a slightly modified Folin-Ciocalteu method [6]; The total flavonoid content (TFC) was measured using a slightly modified method of Jia et al [7]; The total flavanol content (TFAC) was determined using the p-DMACA method [8]; DPPH• free radical scavenging capacity (DPPH), $ABTS^{\bullet+}$ free radical scavenging capacity (ABTS) and ferric reducing antioxidant (FRAP) were measured by Du [9]; Determination of the ascorbic acid content (AsA) refered to Ma et al [10]. The above indicators w ere set three times repeats, and calculated the average.

Data Handling. Software Excel 2010 was used to calculate the test data and tabulation. Statistical analysis was performed using software SPSS. $P<0.05$ indicated a significant difference.

Results and Analysis

Effects of exogenous melatonin on proline content in leaves of kiwifruit seedlings under copper stress. As seen from Table 1, after Cu stress treatment, the content of proline was significantly increased all over the time when compared with control. The proline content of T1 and T2 increased first and then decreased, and peaked at 6d, to 104.92μg•g^{-1}FW and 128.94μg•g^{-1}FW, respectively, which were 55.67% and 91.31% higher than that of CK. With the MT pretreatment (T2), the proline contents were always higher than those of T1, increased by 20.69%、22.89%、12.63%、6.25% respectively at 3, 6, 9, and 12d. It can be concluded that exogenous melatonin can increase the proline content of kiwifruit under copper stress, enhanced the cell osmotic adjustment and improve the ability of plant adversity resistance.

Table 1 Effects of exogenous melatonin on proline content in leaves of kiwifruit seedlings under copper stress [μg·g^{-1}FW]

	0d	3d	6d	9d	12d
CK	66.99±5.48e	67.26±3.74e	67.40±3.99e	67.84±3.85e	67.26±5.20e
T1	67.23±4.35e	77.68±3.95d	104.92±5.70b	90.07±4.72c	77.16±3.78d
T2	67.90±2.40e	93.75±3.78c	128.94±5.16a	101.45±4.92b	81.98±4.79d

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

Effects of exogenous melatonin on antioxidant substances in leaves of kiwifruit seedlings under copper stress. As seen from Table 2, the TPC contents of T1 and T2 showed an upward trend, and were significantly higher than CK. At 12d, T1 and T2 reached the maximum, and were higher than CK by 194.41% and 253.42%. The change trends of TFC and TFAC in T1 and T2 were similar, all increased first and then decreased, and reached peaked at 6d. At 6d, compared with CK, the content of TFC increased by 20.16%, 33.28%; The content of TFAC respectively increased by 25%、50%. However, the TPC, TFC, TFAC of T2 were always higher than T1. Compared with CK, the content of AsA in T1 and T2 decreased firstly and then increased, and reached minimum at 3d, which were higher by 13.46%, 8.68%. At 12d, T1 and T2 respectively increased by 52.17%, 106.96%, compared with CK. It could be seen that exogenous melatonin increased the antioxidant content of kiwifruit leaves under copper stress.

Effects of exogenous melatonin on antioxidant ability of kiwifruit seedlings under copper stress. Table 3 are the change of antioxidant capacity (DPPH, ABTS, FRAP) of kiwifruit seedlings in the process of copper stress. With the prolongation of copper stress, the antioxidant capacity of the leaves of kiwifruit seedlings in the treatment group decreased and then increased. When compared with CK, at 0-6d, the DPPH content of T1 and T2 decreased gradually, which were 3.06% and 2.55%; Then the DPPH content of T1 and T2 increased gradually, which were 31.28% and 36.92%. The DPPH

content of T2 is always higher than T1. The trends of ABTS and FRAP were similar to DPPH. The results indicated that exogenous melatonin treatment improved the antioxidant ability of kiwifruit seedlings under copper stress, and enhanced plant resistance to heavy metal stress.

Table 2 Effects of exogenous melatonin on the TPC, TFC, TFAC and AsA content in leaves of kiwifruit seedlings under copper stress [mgGAE•kg^{-1} FW]

		0d	3d	6d	9d	12d
TPC	CK	1.61±0.03h	1.61±0.11h	1.60±0.06h	1.61±0.06h	1.61±0.07h
	T1	1.62±0.08h	2.39±0.11g	2.64±0.05f	4.19±0.18d	4.74±0.06b
	T2	1.65±0.06h	2.44±0.10g	3.20±0.09e	4.48±0.03c	5.69±0.10a
TFC	CK	6.38±0.33d	6.40±0.17d	6.40±0.01d	6.30±0.34d	6.40±0.02d
	T1	6.37±0.30d	6.90±0.09c	7.69±0.09b	6.44±0.07d	4.02±0.15f
	T2	6.39±0.06d	7.70±0.12b	8.53±0.14a	7.83±0.04b	5.70±0.19e
TFAC	CK	0.64±0.01ef	0.63±0.02ef	0.64±0.01ef	0.64±0.02ef	0.64±0.02ef
	T1	0.63±0.01ef	0.64±0e	0.80±0c	0.65±0e	0.62±0.01f
	T2	0.64±0ef	0.65±0.01e	0.96±0a	0.85±0.03b	0.76±0.01d
AsA	CK	6.90±0.19fg	6.91±0.04fg	6.95±0.20fg	6.91±0.09fg	6.90±0.05fg
	T1	6.67±0.08g	5.98±0.19i	7.00±0.18ef	7.49±0.30d	10.50±0.27b
	T2	6.76±0.03fg	6.31±0.08h	7.25±0.19de	9.13±0.30c	14.28±0.27a

Table 3 Effects of exogenous melatonin on the DPPH, ABTS and FRAP content in leaves of kiwifruit seedlings under copper stress [umol•kg^{-1} FW]

		0d	3d	6d	9d	12d
DPPH	CK	1.92±0.10e	1.95±0.02e	1.96±0.02e	1.94±0.02e	1.95±0.03e
	T1	1.95±0.01e	1.92±0.03d	1.90±0.05e	2.48±0.16c	2.56±0.13bc
	T2	1.96±0.03e	1.94±0.03d	1.91±0.08e	2.59±0.03ab	2.67±0.03a
ABTS	CK	8.52±0.08e	8.52±0.08e	8.54±0.09e	8.54±0.07e	8.59±0.05e
	T1	8.54±0.09e	5.83±0.08h	4.82±0.07i	10.49±0.10d	12.21±0.13b
	T2	8.57±0.05e	7.82±0.02f	6.93±0.08g	11.47±0.12c	14.39±0.12a
FRAP	CK	4.94±0.34ce	5.08±0.38c	5.15±0.18c	5.00±0.21c	5.12±0.45c
	T1	5.09±0.06c	4.59±0.04e	3.74±0.24h	8.48±0.26b	8.75±0.35b
	T2	5.16±0.06c	4.90±0.08ce	4.17±0.17f	8.54±0.33b	10.03±0.25a

Conclusions

Under stress, plant cells can accumulation of proline and other osmotic adjustment substances to protect the cell membrane, in order to maintain a high water potential and keep turgor pressure and water content [11]. The results showed that the content of proline increased significantly under the stress of copper. After spraying exogenous melatonin, the content of proline increased significantly. Maintaining the balance of inner and outer water potential prevents excessive cell loss and protects the plant cell membrane, to provide a guarantee for the mitigation of copper stress.

TPC, TFC, TFAC, AsA are important non enzymatic antioxidant substances in plants. The plant itself can protect the cell from oxidative damage by the non-enzymatic antioxidant system to eliminate excessive reactive oxygen species formed under adverse conditions [12]. When there are antioxidants for hydrogen production (such as phenols), the color of DPPH solution becomes shallow; $ABTS^{\bullet+}$ reacts with it and turn it into a colorless ABTS; And FRAP measures the antioxidant capacity of power by measuring the ability of Fe^{3+} to be reduced to Fe^{2+} [13]. The results showed that exogenous melatonin treatment increased the content of TPC, TFC, TFAC, AsA in leaves of kiwifruit seedlings, enhanced the antioxidant ability. It can be concluded that exogenous melatonin can increase the content of antioxidant substances and the antioxidant ability in plants, vigorously scavenging reactive oxygen species, to improve the ability of plants to resist heavy metal stress.

In conclusion, under the condition of copper stress, exogenous melatonin treatment can increase the content of proline in leaves of kiwifruit seedlings, increase cell permeability, improve antioxidant and antioxidant capacity, timely removal free radical, so as to enhance the ability of plants to resist heavy metals.

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