Effects of exogenous melatonin on antioxidant capacity in *Actinidia* seedlings under salt stress

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Abstract. To investigate the alleviation of exogenous melatonin (MT) in *Actinidia* seedlings under 100 mM NaCl stress, one-year-old *Actinidia deliciosa* seedlings were treated with 0.1, 0.5 and 1μM of exogenous melatonin solution, respectively. The results showed that the antioxidant substance (ASA, TPC, TFC and TFAC) contents and antioxidative capacity (DPPH, ABTS and FRAP) of *Actinidia* seedlings under salt stress were significantly increased compared with the CK. At the same time, the antioxidant substance contents of *Actinidia* seedlings with MT pretreatment were significantly higher than those of CK and S, then the antioxidative capacity was improved, and the damage of *Actinidia* seedlings under salt stress was alleviated. And the treatment with 0.1μM MT solution was the most significant.

1. Introduction

Soil salinization is one of the major crises faced by agricultural development and ecological environment. The research on cultivation of fruit and saline is also the focus of attention at home and abroad. Salt stress causes ion toxicity, osmotic stress and oxidative stress, leads to metabolic disorders, seriously affects the growth and development of fruit trees [1]. Melatonin (MT) is a neuroendocrine hormone secreted by pineal gland, which is N-acetyl-5-methoxytryptamine [2], exists in the vast majority of biological organisms [3]. A large number of studies have shown that MT can not only regulate the plant growth [4], but also improve the resistance of plants to drought, salt damage, heavy metals, UV radiation, high temperature, chilling injury and other stress [5,6]. Plants produce phenolic substances that can remove free radicals, harden singlet oxygen, remove superoxide, inhibit lipid peroxidation and destroy free radical chain reaction, which has a strong antioxidant capacity and protect plants from damage [7]. *Actinidia* in plant taxonomy belongs to the Actinidiaceae, *Actinidia* Lindl plants, which is native to China's ancient wild vine fruit trees [8]. In the cultivation, *Actinidia* tree preference warm but intolerance to high temperature, drought and sald damage [9].

At present, the research on the salt tolerance of *Actinidia* has been reported [10, 11], but the study about effect of exogenous MT on antioxidant substance and antioxidant capacity of *Actinidia* under salt stress has not been reported. Therefore, in this study, we determined the antioxidant substance (ASA, TPC, TFC and TFAC) contents and antioxidant capacity (DPPH, ABTS and FRAP) of *Actinidia* leaves pretreated with different concentrations of exogenous MT and under NaCl stress, and screened the appropriate MT concentration, which provides a theoretical basis for rational use of MT to solve the problem of salt damage in *Actinidia* cultivation.
2. Materials and methods

2.1. Materials
The experiment was conducted in January to August 2016 in the artificial climate chamber of the horticultural college of Sichuan Agricultural University. The seeds of *A. deliciosa* were placed at 4°C for 2 months and poikilothermic treatment for 2 weeks. The germination seeds were planted in plastic pots filled with sand and then moved to a greenhouse with temperature of 25°C and humidity above 40%. We began watering the seedlings at 2-d intervals with 1/2 Hoagland’s nutrient solution at the two-true-leaf stage.

2.2. Experimental Design
Treatments began at 6-true-leaf stage, seedlings were divided into 5 groups for treatment, as follows: CK (running water), S (running water, 100 mM NaCl), T1 (0.1μM MT, 100 mM NaCl), T2 (0.5μM MT, 100 mM NaCl), T3 (1μM MT, 100 mM NaCl). Seedlings were irrigated with melatonin solution for corresponding concentration for five times, once a day. Then, seedlings were irrigated with 100 mM NaCl for 12 days, except for CK. Mature function leaves from the base up to 3 to 5 were collected and immediately frozen in liquid nitrogen and stored at −80°C.

2.3. Indexes measurement
The content of ascorbic acid (AsA) was determined by the method of Ma et al. [12]. The total phenolics (TPC) was determined using the Folin-Ciocalteu method [13]. The total flavonoids (TFC) was determined following [14].The total flavanols (TFAC) was determined using the slightly modified DMACA method [13]. The ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals was determined based on the method of [15], results were expressed as trolox equivalent antioxidant capacity. ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical cation (ABTS⁺) assay was based on the method of [16], results were expressed as trolox equivalent antioxidant capacity. The FRAP (ferric reducing antioxidant power) assay was done according to [17] with some modifications, results were expressed as trolox equivalent antioxidant capacity. The above indexes were repeated three times or more, and the average value was taken as the measured value of each treatment.

2.4. 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

3. Results and Discussion

3.1. Effects of exogenous melatonin on antioxidant substance contents in Actinidia seedlings under salt stress
AsA and phenolic substances are important antioxidants in plants, and have the effect of scavenging reactive oxygen species under stress. The contents of antioxidant substances of *Actinidia* seedlings under salt stress are all increased (Table 1). The content of AsA in T1 was the highest, reached 12.50 μmol/g, which was 2.18 times higher than that of CK. The AsA content of T2 and T3 were 1.87 times and 1.80 times higher than that of CK, respectively, but the difference was not significant.

The TPC of T1 and T2 increased by 35.20% and 30.58%, respectively, while the difference was not significant. S and T3 increased by 14.52% and 13.68%, respectively, and the difference was not significant. The TFC of T1 was highest, followed by T2, while the total flavonoids of T3 and S were significantly higher than CK, but the difference was not significant. The TFAC was T1> T2> T3> S> CK, and the difference was significant.
This indicated that MT pretreatment increased the amount of each antioxidant to varying degrees. It was possible that MT enhanced the activity of AsA synthase to accumulate AsA or promote AsA-GSH cycle to regenerate AsA. At the same time, MT activated the related enzymes activity of phenolic compounds and improved their content. Treatment with 0.1μM MT was the best.

Table 1. Effects of exogenous melatonin on contents of AsA, total phenolics, flavonoids and flavanols of Actinidia seedlings under salt stress

| Treatment | ASA (μmol ascorbic acid/g FW) | TPC (mg gallic acid/g FW) | TFC (mg rutin/g FW) | TFAC (mg catechin/g FW) |
|-----------|-----------------------------|--------------------------|--------------------|------------------------|
| CK        | 5.74±0.5d                   | 3.42±0.18c               | 9.33±0.23d         | 1.04±0.09e             |
| S         | 7.67±0.61c                  | 3.91±0.16b               | 10.26±0.27c        | 1.26±0.02d             |
| T1        | 12.50±0.54a                 | 4.62±0.18a               | 11.67±0.48a        | 1.83±0.06a             |
| T2        | 10.74±0.3b                  | 4.46±0.19a               | 11.06±0.01b        | 1.67±0.03b             |
| T3        | 10.33±1.01b                 | 3.88±0.18b               | 10.36±0.15c        | 1.47±0.06c             |

Note: AsA (μmol ascorbic acid/g FW); TPC (mg gallic acid/g FW); TFC (mg rutin/g FW); TFAC (mg catechin/g FW). Values are means of 3 replicates ± SD. Data with the different letters indicate the difference is significant (P<0.05)

3.2. Effects of exogenous melatonin on antioxidant capacity in Actinidia seedlings under salt stress

DPPH, ABTS and FRAP are the most common methods for determining the antioxidant capacity of plants. It can be seen from Table 2 that salt stress significantly increased DPPH free radical scavenging capacity, the largest increase in T1, reaching 38.25%, T2 and T3 were increased by 31.74% and 29.80%, respectively, and the difference was significant, while difference among S, T2 and T3 was not significant.

The ABTS·+ free radical scavenging ability values of each test group were not much different, but the difference is significant. Among all groups, T1 was the highest, reaching 14.63μmol•g⁻¹ TE, and there was no significant difference between T2, which reached 14.63μmol•g⁻¹ TE. T2 and T3 were not significantly different, but significantly higher than S and CK.

For FRAP, although S was higher than CK, the difference was not significant, indicating that salt stress had little effect on FRAP. MT pretreatment could significantly increase FRAP, T1 was the highest, 1.85 times higher than CK, T2 and T3 were 1.60 times and 1.68 times higher than CK, but the difference was not significant.

The comprehensive evaluation results of antioxidant capacity of Actinidia seedlings under salt stress determined by the three methods showed that MT pretreatment significantly enhanced antioxidant capacity, which might be a combination of improvement of antioxidant enzyme activity and antioxidant substances content.

Table 2. Effects of exogenous melatonin on antioxidant activity (µM TE/g FW) determined by DPPH, ABTS and FRAP of Actinidia seedlings under salt stress

| Treatment | DPPH (µM TE/g FW) | ABTS (µM TE/g FW) | FRAP (µM TE/g FW) |
|-----------|------------------|-------------------|------------------|
| CK        | 2.87±0.03d       | 14.41±0.04d       | 9.68±0.61c       |
| S         | 3.75±0.04bc      | 14.51±0.02c       | 10.54±0.48c      |
| T1        | 3.97±0.03a       | 14.63±0.01a       | 17.86±0.81a      |
| T2        | 3.79±0.01b       | 14.61±0.01ab      | 15.48±0.33b      |
| T3        | 3.73±0.02c       | 14.59±0.01b       | 16.23±0.76b      |

Note: Values are means of 3 replicates ± SD. Data with the different letters indicate the difference is significant (P<0.05)

References
[1] Chen S S and Lan H Y 2011 Signal transduction pathway of plant responses to salt stress J. Plant. Physiol. 47 119-28
[2] Posmyk M M and Janas K M 2009 Melatonin in plants Acta Physiol Plant 31 1-11
[3] Manchester L C, Montes A C, Boga J A, Andersen L P H, Zhou Z, Galano A, Vriend J, Tan D X and Reiter R J 2015 Melatonin: an ancient molecule that makes oxygen metabolically tolerable J. Pineal Res. 59 403-19
[4] Kolar J and Machackova I 2005 Melatonin in higher plants: occurrence and possible functions J. Pineal Res. 39 333-41
[5] Arnau M B and Hernández R J 2015 Functions of melatonin in plants: a review J. Pineal Res. 59 133-50.
[6] Byeon Y and Back K 2014 Melatonin synthesis in rice seedlings in vivo is enhanced at high temperatures and under dark conditions due to increased serotonin N-acetyltransferase and N-acetylserotonin methyltransferase activities J. Pineal Res. 56 189-95.
[7] Sakihama Y, Cohen M F and Grace S C 2002 Plant phenolic antioxidant and pro-oxidant activities: Phenolics-induced oxidative damage mediated by metals in plants Toxicology. 177 67-80
[8] Cui Z X 1993 Chinese Kiwifruit (Jinan: Shandong Science and Technology Press) P 64.
[9] Ma K, Wang L J, Wang Y L, Jiang W B and Gu P 1997 Study on salt resistance and salt tolerance of 18 kinds of fruit trees J. Fruit Sci. 14 1-5.
[10] Liu X F 1992 Comparative experiment on salt tolerance of Actinidia. Shaanxi J. Agric. Sci. 19
[11] Zhou L M, Wang F and Wang J 2009 Study on the screening of salt-tolerant mutants of kiwifruit by EMS mutagenesis Acta Agriculturae Boreali-occidentalis Sinica 18 330-5
[12] Ma F W and Cheng L L 2003 The sun-exposed peel of apple fruit has higher xanthophyll cycle-dependent thermal dissipation and antioxidants of the ascorbate–glutathione pathway than the shaded peel Plant Science 165 819-27.
[13] Jayaprakasha G K, Singh R P and Sakariah K K 2001 Antioxidant activity of grape seed (Vitis vinifera) extracts on peroxidation models in vitro Food Chemistry 73 285-90.
[14] Yong S P, Soon T J, Seong G K, Buk G H, Patricia A A and Fernando T 2008 Antioxidants and proteins in ethylene-treated kiwifruits Food Chem. 107 640-8.
[15] Brandwilliams W, Cuvelier M E and Berset C 1995 Use of a free-radical method to evaluate antioxidant activity. Food Sci. Technol.-LWT 28 25-30.
[16] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C 1999 Antioxidant activity applying an improved ABTS radical cation decolorization assay Free Radical Biology and Medicine 26 1231-7.
[17] Benzie I F F and Strain J J 1996 The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay Anal. Biochem. 239 70-6.