

# Effects of Exogenous Melatonin on Physiological Indexes of Kiwifruit Seedlings under Low Temperature Stress

Xiu Wang<sup>1,a</sup>, Dong Liang<sup>1,2,b</sup>, Xiaojing Huang<sup>1,c</sup>, Xuwen Zhao<sup>1,d</sup>, Yunmei  
Wang<sup>1,e</sup>, Hui Xia<sup>1,2,f,\*</sup>,

1 College of Horticulture, Sichuan Agricultural University, Chengdu, Sichuan, China

2 Institute of Pomology and Olericulture, Sichuan Agricultural University, Chengdu, Sichuan, China

<sup>a</sup>347434587@qq.com, <sup>b</sup>liangeast@sina.com, <sup>c</sup>1301621709@qq.com,

<sup>d</sup>867396930@qq.com, <sup>e</sup>1336489390@qq.com, <sup>f</sup>susanxia\_2001@163.com

\*Corresponding author. Xiu Wang and Dong Liang contributed equally to this work.

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**Abstract:** To investigate the effects of melatonin (MT) on kiwifruit seedlings under low temperature (LT) stress, seedlings of *Actinidia eriantha* were primed with MT solution by root irrigation, relative electrolyte leakage (REL), malonaldehyde (MDA) content, chlorophyll content (ChI), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content and antioxidant enzyme activity were measured. The results showed that under LT stress, REL, MDA, H<sub>2</sub>O<sub>2</sub> content, ChI content and POD activity increased apparently in seedling leaves. However, MT application not only effectively reduced REL, H<sub>2</sub>O<sub>2</sub> content and MDA content, but also improved significantly the activity of SOD and CAT in leaves of kiwifruit seedlings under low temperature stress. The results suggested that exogenous MT alleviated the damage by low temperature stress in kiwifruit seedlings and thus improve the ability of plants to tolerance low temperature.

## Introduction

Low temperature, which is one of the main environmental factors, cause physiological disturbance of the thermophilic crop, resulting crop growth inhibition and yield reduction and even causing crop death [1]. Research on the cold resistance of crops has become an important aspect in crop stress resistance research. Kiwifruit is perennial vine, belonged Actinidiaceae family and *Actinidia* genus, and the fruit are rich in trace elements, amino acids and vitamin C [2]. However, information showed that kiwifruit has poor cold hardiness. Melatonin (MT), an indole derivative of tryptophan, was first extracted from the pine nut bodies by the American scientist Lerner in 1958 [3] and then largely used in plant recently. As a strong antioxidant, MT can react with free radicals to remove reactive oxygen species and protect cell structure. Meanwhile, MT has been used as a plant growth regulating substance to resist adverse effect of abiotic stresses, such as high temperature, low temperature, ultraviolet and ionizing radiation, heavy metals and chemical pollution, biological oxidation [4]. The aim of this study was to investigate physiological regulation mechanism of exogenous MT on kiwifruit seedlings under low temperature stress, so as to provide a reference for the use of MT in production.

## Materials and Methods

**Plant Preparation and Treatments.** Seeds of *Actinidia eriantha* were collected in September, 2016.

After stored at 4°C for two months, seeds were treated with variable temperature at 4°C 16h, 24°C 8h for two weeks, then incubated at 25°C to accelerate seed germination. The germinated seeds were planted in nursery disks and grown in a greenhouse. When grew to 2-3 leaves, seedlings were transplanted into the pots (23 cm × 18 cm), 3 plants per pot. All seedlings were uniformly watered daily and fertilized weekly with 1/2 strength Hoagland's solution.

When grew to 7-8 leaves, seedlings were primed with 100 μmol•L<sup>-1</sup> MT solution in 2-days-interval with 250 ml each pot. After 10 days, seedlings were transferred into climatic chamber for low temperature treatment at 6°C. Totally 3 treatments were set as follow: (1) CK: seedlings irrigated with water, grew at 25°C; (2) LT: seedlings irrigated with water, were treated with LT; (3) MT+LT: seedlings were irrigated with 100 μmol•L<sup>-1</sup> MT solution, were treated with LT. Each treatment group contained 45 seedlings. The stress period lasted 24 hours, and samples of leaves were collected at 0, 1, 4, 12 and 24 h during cold treatment and rapidly frozen in liquid nitrogen and stored at -80°C for biochemical assay.

**Physiological Indexes.** REL, MDA content and antioxidant enzyme activity (SOD, POD and CAT) were determined by the method of Li Hesheng [5], H<sub>2</sub>O<sub>2</sub> content was determined by the method of Hao et al [6], chlorophyll content was determined by the slightly improved method of Gao Junfeng [7].

**Data Analysis.** All Data was processed using Excel 2010 software and analysis of variance (ANOVA) was performed by the SPSS 20.0 and significant differences ( $P < 0.05$ ) between treatments were determined using Duncan's test. Data were expressed as mean ± SD.

## Results and Discussion

**REL and H<sub>2</sub>O<sub>2</sub> Content.** Under the condition of adversity, the plant will produce and accumulate H<sub>2</sub>O<sub>2</sub>, increase the membrane lipid peroxidation, produce MDA, seriously damage the biomembrane, increase the membrane permeability and relative electrolyte leakage [8]. In this study, the REL and H<sub>2</sub>O<sub>2</sub> content of MT + LT were significantly higher than those of CK and LT treatment at 0h (Figure.1). After 24 hours, there was no significant difference in the REL among three treatments. The REL of LT and MT+LT treatments both increased at first stage, reaching the maximum at 4h, then decreased. Interestingly, MT + LT treatment had significant lower REL than LT treatment at 12h (Figure.1A). In addition, the H<sub>2</sub>O<sub>2</sub> of LT treatment was significantly higher than that of CK at 1h. While the H<sub>2</sub>O<sub>2</sub> of MT+LT decreased obviously, whose was 55.02% lower than that of LT. However, from 4 to 24h, the difference between the three treatments was not obvious (Figure.1B). This suggested that the addition of melatonin restricted the accumulation H<sub>2</sub>O<sub>2</sub> and reduced the relative electrolyte leakage under low temperature stress.

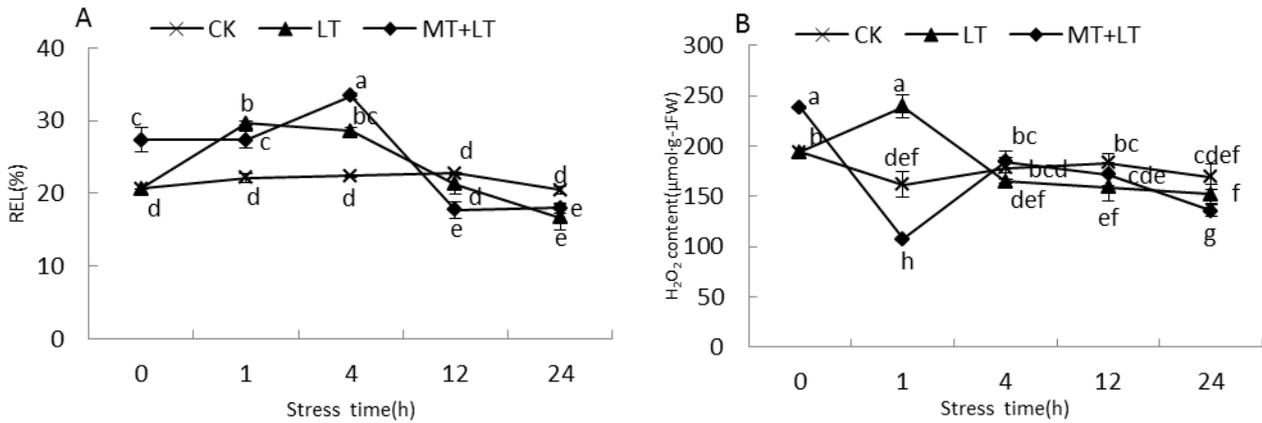


Fig.1 Effects of exogenous melatonin on REL (A) and H<sub>2</sub>O<sub>2</sub> content (B) in kiwifruit seedlings under low temperature stress

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

**MDA Content and Chlorophyll Content.** Compared with CK, the contents of MDA in LT treatment increased significantly at 1-12h. With the increase of stress time, the content of MDA in LT treatment increased firstly, reaching the maximum at 1h, then decreased. While MDA of MT + LT treatment increased overall, significantly increased at 24h, reaching the maximum. Compared with the LT treatment increased 55.43% (Figure. 2A). The results showed that MT reduced MDA content of kiwifruit seedlings in a certain time and alleviated the lipid peroxidation under low temperature stress. In addition, low temperatures hindered the synthesis of chlorophyll in plant cells. In this study, the chlorophyll content of LT and MT+LT was significantly increased compared with CK. Application of MT also increased the chlorophyll content of kiwifruit seedlings under low temperature stress (Figure. 2B).

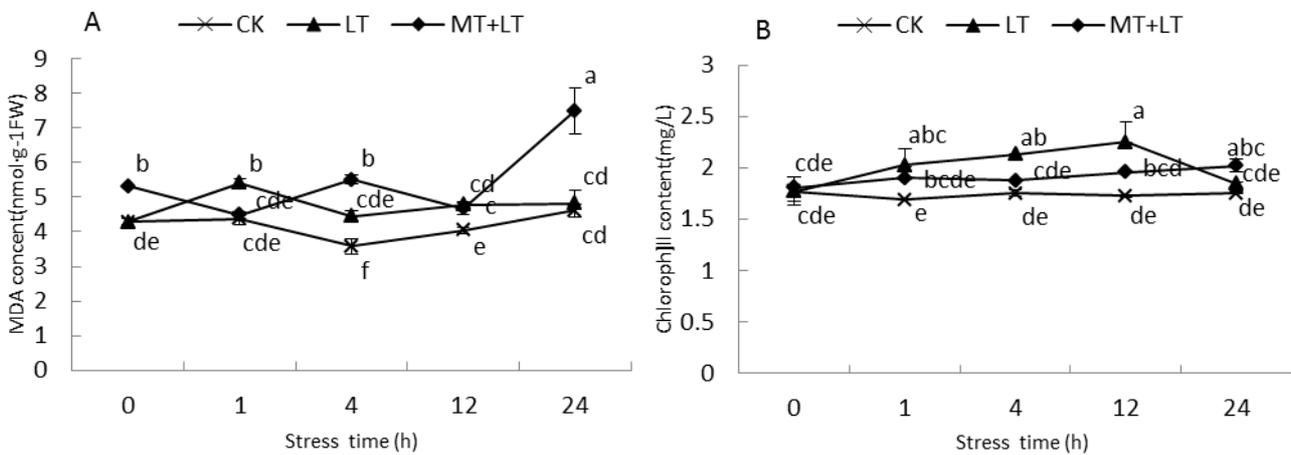


Fig.2 Effects of exogenous melatonin on MDA content (A) and chlorophyll content (B) in kiwifruit seedlings under low temperature stress.

**The Activity of SOD, POD, CAT.** SOD, POD, CAT are important protective enzymes in plant antioxidant system. SOD is the only enzyme in plant cells that can convert excess superoxide anion (O<sub>2</sub><sup>-</sup>) into H<sub>2</sub>O<sub>2</sub>, while both CAT and POD function to convert H<sub>2</sub>O<sub>2</sub> into water and oxygen [9,10]. In this study, as Fig 3 shown, low temperature decreased significantly the activities of SOD and CAT in leaves of seedlings, but increased POD activity. While application MT (MT+LT), activities of SOD and CAT were markedly increased compared with LT and CK. For example, at 4h, SOD activity increased by 897.17% and the CAT activity increased by 70.67% than LT (Figure.3 A and C). Besides, the POD activity of LT increased firstly, reaching the maximum at 1h, and then

decreased. POD activity of MT + LT significantly decreased by 79.63% compared with LT at 1h (Figure.3B). These results showed that application of MT significantly enhanced SOD and CAT activities and reduced POD activity under low temperature stress.

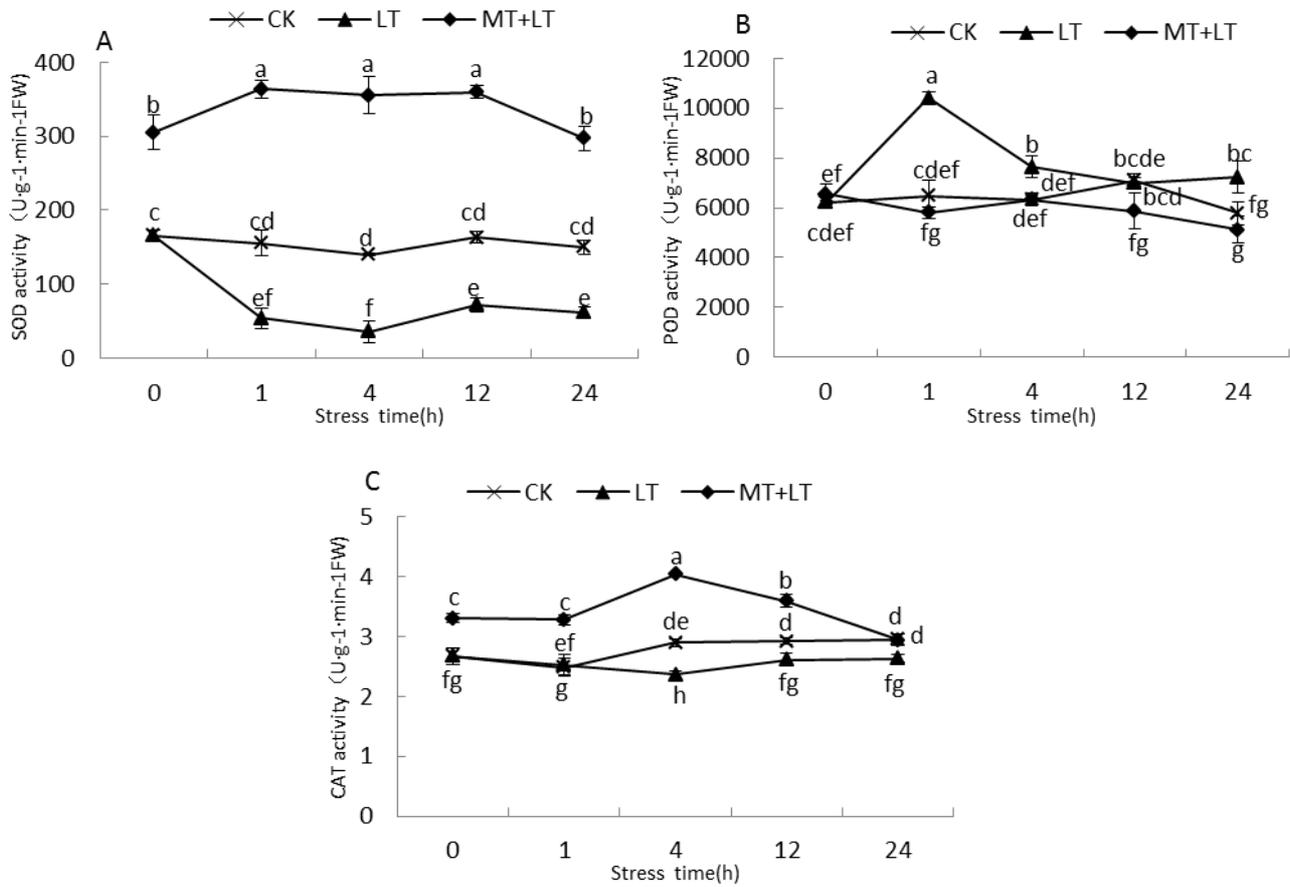


Fig.3 Effects of exogenous melatonin on SOD、POD and CAT activity in kiwifruit seedlings under low temperature stress

## Conclusions

The integrity of the cell membrane system plays an important role in plant growth. Many studies show that resistance of plant to adverse conditions will decrease when the cell membrane is damaged [11-13]. In this study, MT application decreased the REL, H<sub>2</sub>O<sub>2</sub> and MDA contents of kiwifruit seedlings in low temperature. Meanwhile MT increased SOD and CAT activities so as to relieve accumulation of REL, H<sub>2</sub>O<sub>2</sub> and MDA content. in seedlings. In summary, MT could protect the integrity of cell membrane, maintain the dynamic balance of physiological responses within the cell and alleviate the metabolic imbalance of reactive oxygen species. Thereby enhanced the antioxidant capacity and improved chilling tolerance of kiwifruit seedlings.

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