Effects of exogenous melatonin and abscisic acid on osmotic adjustment substances of 'Summer Black' grape under drought stress

Xinyu Niu¹, Cuomu Deqing¹, Dong Liang²

¹Plant Sciences College, Tibet Agriculture and Animal Husbandry University, Linzhi, 860000; ²Institute of Pomology and Olericulture, Sichuan Agricultural University, Chengdu, 611130

*Corresponding author, liangeast@sicau.edu.cn

Abstract. In this study, seedlings of grape ‘Summer Black’ were used as the experimental materials to investigate the effects of melatonin (MT) and abscisic acid (ABA) on osmotic adjustment substances under drought stress, by root irrigating 100μmol/L MT and sprayin 50μmol/L ABA solutions before or during the drought treatment. The results showed that the content of malondialdehyde and relative conductivity were significantly lower in the MT group than that in the drought control group, and the contents of soluble protein and hydrogen peroxide were significantly higher in the ABA treatment than that in the drought control group. However, regardless of the application of ABA or MT, the relative water content in leaves did not change much. The results suggested that the concentration of exogenous substances and the sampling time were unsuitable to improve drought tolerant at 18d, which provided a theoretical basis for MT and ABA appliance in grape planting.

1. Introduction
Grape is perennial deciduous vine and highly drought tolerant [1], which is one of the main cultivated fruit trees in China.

Plant growth regulators have the effect of improving plant stress resistance. Abscisic acid (ABA) plays an important role in plants. Spraying exogenous ABA can increase the antioxidant ability of the fruits distinctly [2]. Melatonin, a newly discovered strong antioxidant, has a positive effect on resisting abiotic stress. It can reduce the plasma membrane peroxidation level and maintain the integrity and function of cell membrane by increasing the activity and content of antioxidant enzymes in plants [3].

Although studies on the drought resistance of melatonin and abscisic acid to plants have been reported, the results are still very limited where there are few studies on grapes. Besides, related tests for applying various growth regulators under drought stress are rare. Therefore, it is of great theoretical and practical significance to clarify the ways of MT and ABA to improve the drought resistance of grapes.

2. Materials and methods

2.1. Experiment materials
The following experiments were conducted at Sichuan Agricultural University, Chengdu, China (30.67°N, 104.06°E). 1-yr-old Summer Black canes were collected from the vineyard of experimental
base of College of Horticulture, Sichuan Agricultural University and stored in sand that was maintained at an appropriate humidity and temperature during whole winter. In March 2017, these canes were cut into approximately 5 cm length with one bud each, and then rooted in plastic pots (13×15 cm) that were filled with a mixture of garden soil, vermiculite, and sand (1:1:1 (v/v)). They were placed in the greenhouse for 8 weeks at 70–80% relative humidity on a 27/18°C day/night cycle. Young grapevines that had developed 8–10 functional leaves were chosen for use in the experiment.

2.2. Experimental treatment
There are 5 groups in this experiment: normal water supply treatment (CK); drought control treatment (Dck); pretreatment with 100μmol/L MT root irrigation before drought treatment; 50μmol/L ABA leaf spray (ABA) plus drought treatment; 50μmol/L ABA leaf spray after 100μmol/L MT root irrigation (MT+ABA). First, seedlings were irrigated with 300ml melatonin solution or water for 4 times in the evening of every two days. Then begin to control water (no irrigation anymore) for 18 days, the next day of the last irrigation was set as 0d. ABA or water was sprayed on the leaves on the 0, 2, 4, 6, 8, 10d evening. The soil water content was measured every 2 days until reached moderate drought (field capacity < 60%). Leaves in the middle (3-6 node) were taken at 0d and 18d as samples for the following determination.

2.2.1. Determination of relative conductivity
The relative conductivity was measured by the method of Campos et al [4].

2.2.2. Determination of malondialdehyde content
The content of malondialdehyde was measured by the Supernatant which added 5ml of 5% TCA (trichloroacetic acid) and 2ml of 0.67% TBA (thiobarbituric acid) separately and centrifuged twice at 10000 r/min for 10 min.

2.2.3. Determination of hydrogen peroxide content
The content of hydrogen peroxide was determined by the method of Lin et al [5].

2.2.4. Determination of relative water content
The relative water content is determined by the method of Junfeng Gao et al [6].

3. Results and discussions

3.1. Change of malondialdehyde substances content
MDA is a product of membrane lipid peroxidation, and can be used to determine the degree of the resistance of plants indirectly. At day 18, the MDA content of the drought control was significantly higher than that of the CK group, by 35.9% higher. The MDA content which were spraying ABA and MT was significantly higher than that of the drought treatment, reaching 0.022mmol·g⁻¹FW. It indicated that under drought stress, the application of exogenous MT and ABA could promote the increase of MDA content after 18-day's water controlling in plants.
3.2. **Change of hydrogen peroxide substances content**

The accumulation of hydrogen peroxide (H$_2$O$_2$) will cause oxidative damage to cells, and it can reflect the ability of plants to resist stress to a certain extent. The content of hydrogen peroxide in the drought treatment was significantly improved compared with the normal treatment at day 18 (Figure 2). The content of H$_2$O$_2$ treated with the mixture of ABA and MT was significantly lower than that of drought treatment, which was 25.6% lower, reaching 122.2521mmol·g$^{-1}$FW. It suggested that the increase of hydrogen peroxide content in plants could be inhibited with adding exogenous MT+ABA under drought stress.

3.3. **Change of relative water content**

Relative water content is an important indicator of plant drought resistance. It can be seen from Figure 3 that there was no significant difference in the relative water content of each exogenous substances’ treatment. It suggested that under drought stress, exogenous substances had no significant effect on relative water content of plants.
3.4. Change of relative conductivity content
Relative conductivity is an important physiological and biochemical indicator reflecting the condition of the plant membrane system. At day 18, the relative conductivity of the exogenous ABA group and the exogenous MT group were both significantly higher than that of the drought control, separately increasing from 22.5% to 64.9% and 26.1% to 54.3% (Figure 4). There was no significant difference in the relative conductivity between the exogenous MT and ABA group and the drought group. It indicated that under drought stress, the relative conductivity of plants could be promoted by adding exogenous ABA and MT.

3.5. Change of Soluble protein content
Soluble protein content can reflect the ability of plants to resist stress to a certain extent. Compared with the drought treatment, the contents of soluble protein in the treatment of exogenous ABA and exogenous MT were significantly lower than that of drought control, which were respectively reduced by 9.2% and 9.6%. The exogenous ABA+MT treatment was slightly higher than the other exogenous substances, but it was still significantly lower than the drought control (Figure 5). It suggested that under drought stress, the application of exogenous ABA or MT alone could reduce the soluble protein content of plants, and the effect was better than spraying ABA+MT at the same time.
Figure 5. Effect of MT and ABA on soluble protein content in leaves under drought stress

4. Conclusions
In this test, there was no significant difference in the treatment of each group at the relative water content of 18d, which directly reflected the defect of the sampling point.

MDA is the final product of membrane lipid peroxidation damage. The level of MDA reflects the degree of damage of cell membrane [7]. In this experiment, the MDA content of grapes increased significantly, which was consistent with the studies of Chang Yongyi [8] and Wu Yingxia [9]. But the different treatments of spraying MT and ABA in the further increase of MDA content in grapes, which was inconsistent with the trend of rising-decreasing-rising of MDA content [10]. It suggested that the sampling time of this experiment was too late and the effect of 4 times of root irrigation in the pretreatment could not last for 18 days.

The content of H$_2$O$_2$ was significantly increased under the influence of drought stress, and it could significantly alleviate the drought stress of plants under the simultaneous application of exogenous ABA and MT. The content of H$_2$O$_2$ was significantly increased under the influence of drought stress, and it could significantly alleviate the drought stress of plants under the simultaneous application of exogenous ABA+MT. Similar to Wang Juan [11].

When the plants are subjected to drought stress, the membrane permeability is increased, and the relative conductivity is increased, which is consistent with the results of this experiment. But the application of exogenous MT or ABA alone resulted in the increase of relative conductivity of grape seedling leaves, and the increase of relative conductivity of tobacco seedling leaves under suitable water stress with suitable concentration of exogenous substances [12] was quite different, which indicated that the ABA concentration used in this experiment was unsuitable.

The content of soluble protein was closely related to the stress environment. The results showed that the soluble protein content decreased significantly under drought stress, which were opposite to the results of Perilla seedlings [13], further indicating the late sampling point and the collapse of the grape defense system.

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