Effects of exogenous melatonin and abscisic acid on the antioxidant enzyme activities and photosynthetic pigment in 'Summer Black' grape under drought stress

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Abstract. In this study, 'Summer Black' grape seedlings were used as the experimental materials, to be applied with 100μmol/L melatonin (MT) and 50μmol/L abscisic acid (ABA) solutions to investigate the effects on antioxidant enzyme activities and photosynthetic pigment under drought stress. The results showed that the increase of chlorophyll content in grape leaves due to drought was reduced by the function of MT and ABA, while the content of carotenoid showed the opposite trend. The antioxidant capacity was measured with SOD, POD and CAT, and showed that MT, ABA treatment significantly increased the activity of SOD, but reduced activity of POD and CAT in the leaves. ABA had the greater effect, but MT seems impaired the influence of ABA. The results provided a theoretical basis for improving the yield and quality of grape.

1. Introduction
Grape is one of the main crops planted in the arid and semi-arid regions of northwest China. Drought stress is a pivotal stress [1], which affects not only the growth and development of grapes, but also its physiological and biochemical metabolism, which in turn affects its yield and quality.

Plant growth regulators are widely used to regulate plant growth and improve plant stress resistance. Melatonin (MT) is a small molecule of steroids and plays a major role in resisting adversity. Under drought stress, the accumulation of MT in plants increases, and the ability of plants to resist drought stress is improved[3]. Abscisic acid is an important endogenous hormone in plants and an important substance to initiate self-protection. Under drought conditions, plants accumulate ABA, which improves the adaptability and tolerance of plants to drought stress[3]. Therefore, it is of great theoretical and practical significance to study the influence of applying exogenous MT and ABA on 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). At winter pruning, 1-yr-old Summer Black canes were collected from the vineyard of experimental base of College of Horticulture, 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.

2.2. Experimental treatment
Uniform grape seedlings with 8–10 functional leaves were chosen and divided into 5 groups for treatment: normal water supply treatment (CK); drought control treatment (Dck); 100μmol/L MT root irrigation pretreatment (MT); 50μmol/L ABA leaf spray (ABA); 50μmol/L ABA leaf spray after 100μmol/L MT root irrigation (MT+ABA). First, seedlings were irrigated with 300ml 100μmol/L melatonin solution or water for 4 times, once in every 2 evenings. Then begin to control water (no irrigation anymore) for 18 days, the next day of the last irrigation was set as 0d. 50umol/L 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 the soil water content 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.

2.3. Determination of chlorophyll and carotenoid content
The chlorophyll content was determined according to the method by Lichtenthaler et al [4] with little modification.

2.4. Determination of superoxide dismutase activity
The activity of superoxide dismutase was determined by the method of Giannopolittis and Ries [5].

2.5. Determination of catalase activity
The activity of catalase was determined by the modified method of Kato and Shimizu [6], and the improvement was adding 0.2ml of the extract and 0.3ml of 0.1mol/L hydrogen peroxide into it.

2.6. Determination of peroxidase activity
The activity of peroxidase was determined by the method of Scebba et al [7], and a slight improvement was made on this basis.

3. Results and discussions

3.1. Change of chlorophyll content
Chlorophyll plays an important role in plant photosynthesis. The lack of water induced increase in chlorophyll content after 18 days' water controlling. The chlorophyll content of all exogenous substance groups was higher than that of CK group after 18d (Figure 1). With the addition of MT and ABA, the chlorophyll content was significantly lower than that of drought treatment, reaching 1.994mmol·g⁻¹FW. It indicated that under drought stress, the effect of spraying exogenous substances reduce the increase of chlorophyll content.
3.2. Change of carotenoid content
Carotenoids are a kind of important natural pigments and non-enzymatic antioxidant. At 18d, the carotenoid content in the drought group (Dck) was reduced by 30.7% compared with the CK (Figure 2), while ABA and MT treatments alleviated the decrease degree of it. With the addition of plant growth regulators, the carotenoid content was significantly higher than that in the drought group, which was 17.3% higher in ABA group. The results showed that MT and ABA can significantly alleviate the reduction of carotenoids.

![Figure 2. Changes of carotenoid content in leaves under drought stress](image)

3.3. Change of SOD activity
Superoxide dismutase shows the ability of plants to resist stress to a certain extent. Drought treatment significantly increased SOD activity up to 516.3625U·g⁻¹FW (Figure 3). Exogenous ABA has a significant effect in this respect, and the activity of SOD treated by exogenous ABA alone was 9.8% higher than that of the drought group at day 18. It suggested that under drought stress, exogenous ABA could promote the activity of SOD in plants, while spraying exogenous ABA+MT or MT alone had no significant effect on it.

![Figure 3. Changes of SOD activity in 'Summer Black' leaves under drought stress](image)

3.4. Change of CAT activity
The function of CAT is to remove hydrogen peroxide from physiological systems. It can be seen from Figure 4 that at day 18, under the drought treatment, CAT activity was as high as 15.8782 U·g⁻¹FW, which was significantly higher than that in the control. At the same time, the CAT activity of the external ABA+MT treatment and the external application of the exogenous MT was significantly lower than that in the drought control, which was 27.1% and 20.4% lower, respectively. It indicated that under drought stress, spraying of exogenous substances could significantly inhibit the increase of CAT activity in plants.
3.5. Change of POD activity
Peroxidase can protect enzyme activity or maintain high levels in stress, and scavenge free radicals, reactive oxygen species, and maintain cell membrane stability and integrity. The POD activity in the drought group was significantly reduced by 30.1% compared with the normal group at day 18. The POD activity in the ABA group was significantly different from that in the drought treatment (Figure 5), which was only 240.28 U·g\(^{-1}\)FW. It indicated that under the drought stress, the effect of spraying exogenous ABA alone was more significant.

4. Conclusions
In summary, drought stress has a great influence on the photosynthetic pigment content and antioxidase activity in plants.

Chlorophyll and carotenoids are closely related to photosynthesis \([8]\). In this experiment, the chlorophyll and carotenoid content of grape leaves were gradually reduced under drought stress, which was confirmed in Li’s research \([9]\), and exogenous ABA and MT can significantly inhibit the decrease of photosynthetic pigment content and delay the drought negative effects.

Drought stress can interfere with the equilibrium system of active oxygen production and elimination in plant cells. In this experiment, the activity of SOD in the exogenous ABA treatment group increased, but decreased significantly in other treatments, which was not completely consistent with the trend of the increase and decreased in SOD activity of Wang’s six varieties \([10]\). In addition, the CAT and POD activities in wheat \([11]\), maize \([12]\) and broad bean leaves under drought stress decreased to some extent under moderate and severe drought stress, which were different from the trends of this experiment. It suggested that the effect of exogenous ABA was significant, and reflected the problem of too late sampling time.

Besides, it is well known that the role of exogenous melatonin is high resistance to low promotion, and the concentration of the MT solution used for the pretreatment should be further studied.
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