THE PHYSIOLOGICAL MECHANISM OF EXOGENOUS 24-EPIBRASSINOLIDE IN REGULATING THE RESPONSE OF "SHINE MUSCAT" GRAPES TO LOW-TEMPERATURE STRESS

ZHANG, S. W.[1,2] – LIAO, W. Y.[3] – WU, Q. J.[3] – CHENG, J. H.[3] – SU, W. Z.[3] – LI, G. R.[3*]

[1]*Henan Open University, Zhengzhou 450046, China*

[2]*Henan Provincial Engineering Research Center for Rural Environmental Ecological Restoration and Planning Design, Zhengzhou 450046, China*

[3]*School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology, Xinxiang 453003, China*

**Corresponding author*
e-mail: ligrapes@hist.edu.cn

(Received 22nd Jun 2025; accepted 6th Aug 2025)

Abstract. To investigate how exogenous 24-epibrassinolide (EBR) regulates 0°C stress in annual potted self-rooted *Vitis labrusca* × *V. vinifera* "Shine Muscat" seedlings, 0.5 mg/L EBR was tested against a water control, with indices measured at 0, 60, 120, 180 h. EBR increased chlorophyll levels at 120 h and 180 h ($P < 0.001$). Net photosynthetic rate (Pn) and stomatal conductance (Gs) were 4.4% and 118.2% higher at 120 h ($P < 0.0001$), while transpiration rate (Tr) remained lower ($P < 0.0001$). EBR boosted proline (Pro) content by 37.6% and soluble protein (SP) content by 68.8% at 120 h ($P < 0.0001$), reduced electrolyte leakage (EL) at 180 h ($P < 0.0001$) and malondialdehyde (MDA) content at 120 h ($P < 0.01$) and 180 h ($P < 0.0001$), thereby alleviating membrane damage. It enhanced superoxide dismutase (SOD) activity by 20.0% at 180 h ($P < 0.01$) and peroxidase (POD) activity by 33.4% at 120 h ($P < 0.0001$), and activated the ascorbate-glutathione (AsA-GSH) cycle at 120 h ($P < 0.0001$), maintaining redox balance. Correlation analysis showed AsA-GSH-photosynthesis synergy ($P < 0.001$). In conclusion, spraying 0.5 mg/L EBR alleviated low-temperature stress in "Shine Muscat" grapes.
Keywords: *"Shine Muscat" grape, low-temperature stress, 24-Epibrassinolide, physiological indexes, ASA-GSH cycle*

Introduction

Grapes (*Vitis spp*.) are widely cultivated worldwide. Also, its cultivation area and production have occupied a significant position, especially in China. The cultivation of excellent varieties of grapes has increased their significance in the supply-side reform of the agricultural sector. "Shine Muscat" grapes (*Vitis labrusca* × *Vitis vinifera* Shine Muscat) are popular among consumers because of their beautiful appearance, strong aroma, sweet taste, storage resistance, and other characteristics. Recent years have witnessed a continuous expansion of the planting area nationwide. Henan Province has become one of the major planting areas. However, "Shine Muscat" grapes are susceptible to extreme climatic events such as winter freezes and spring "inversions." Low-temperature stress can lead to impaired branch sprouting, significantly reducing yield (Hou et al., 2023). Therefore, exploring effective strategies to mitigate the cold damage of "Shine Muscat" grapes is of great significance to safeguard the fruit quality and yield and promote the sustainable development of the industry.

Low-temperature stress leads to excessive production and accumulation of reactive oxygen species (ROS) in plant cells, thereby triggering membrane lipid peroxidation,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

which further destroys the components of the cell membrane and causes cell death, this aligns with the results reported by Amin et al. (2022). Plants have evolved a complete antioxidant defense system to counteract this oxidative damage. The most representative of this system is the ascorbate-glutathione (AsA-GSH) cycle (Wang et al., 2013; Li et al., 2024), comprising ascorbic acid (AsA), glutathione (GSH), and some key antioxidants such as SOD and ascorbate peroxidase (APX), which together scavenge cellular ROS and maintain biofilm stability.

Epibrassinolide (24-epibrassinolide, EBR) is a synthetic and highly active oleuropein lactone analogue that plays an essential regulatory role in plant response to adversity (Liu et al., 2020). EBR has been shown to enhance grapevine resistance through several pathways. Chen et al. (2019) discovered that EBR application reduced the mortality of grapevine leaves under low-temperature stress and was associated with the control of stomatal switching. Qiao et al. (2022) showed that EBR application to grapes increased leaf photosynthetic rate (Pn) and stomatal conductance (Gs), thus influencing the efficiency of photosynthetic utilization in grapes. Dong et al. (2025) found that EBR inhibited antioxidant coefficients in grape seedlings at low-temperature. Moreover, EBR reduces membrane damage caused by lipid peroxidation and maintains the stability of cellular water potential by activating antioxidant enzymes, attenuating electrolyte leakage (EL), and decreasing malondialdehyde (MDA) content in adverse environments. Lian et al. (2021) found that EBR application resulted in a significant decrease in MDA content and a significant increase in soluble protein (SP) content, suggesting the involvement of EBR in controlling the antioxidant enzyme system and the accumulation of nonenzymatic antioxidants. Fu et al. (2024) demonstrated that exogenous EBR treatment alleviated the damage caused by low-temperature adversity in grape seedlings. The aforementioned studies demonstrated that exogenous EBR increased the photosynthetic pigment content, photosynthetic ability of grape plants, and contents of enzymatic and nonenzymatic antioxidants, thus significantly enhancing the defense capacity of the leaves of grape seedlings against low-temperature stress. Previous studies have demonstrated the multifaceted regulatory role of EBR in response to low-temperature stress in plants. However, systematic studies on the mechanism underlying the effect of EBR on the AsA-GSH cycle in plants, especially grapes, are lacking (Jiang et al., 2021). In this study, we used 1-year pot-grown "Shine Muscat" grape self-rooted seedlings as experimental materials and explored the effects of exogenous EBR on the content of antioxidants and the activities of key enzymes in the AsA-GSH cycle of grapes. For this, the physiological and biochemical indexes of plants under low-temperature stress were determined. The aim was to reveal the physiological mechanism of EBR in enhancing the low-temperature resistance of grapes, thereby providing the theoretical basis and technical support for cultivating and managing cold-resistant "Shine Muscat" grapes.

Materials and methods

Plant material and treatments

Annual self-rooted seedlings of "Shine Muscat" grapes were obtained from the grape germplasm resource nursery of Henan Institute of Science and Technology, Xinxiang City, Henan Province. They were planted in pots with a diameter of 40 cm and a height of 35 cm. Each pot was filled with garden soil, perlite, and humus in a ratio of 1:1:1.1, and plants were planted in each pot. Also, 10 plants were replicated in each treatment.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

Based on the previous research results of Chen et al. (2019), Lian et al. (2021) and Qiao et al. (2022), and combined with the pre-test verification of this study, 0.5 mg/L EBR was determined as the treatment concentration suitable for the physiological needs of "Shine Muscat" grape under low-temperature stress. The test material was one-year potted "Shine Muscat" grape self-rooted seedlings. When the seedlings grew to the fifth to sixth true leaves, two groups of treatments were set up: the EBR treatment group was sprayed with 0.5 mg/L EBR, and the control group was sprayed with clean water. All the leaves were sprayed evenly on the front and back of the plant until there was slight drip on the leaf surface, and the treatment lasted for 3 days. After the treatment, the two groups were treated with 0°C low-temperature stress, and the samples were taken at 0, 60, 120 and 180 h respectively. Among them, the selection of 0°C low-temperature stress level was not only consistent with the temperature involved in extreme weather such as winter freezing and spring "inversions" injury, which were easy to encounter in the actual cultivation of "Shine Muscat" grapes, but also consistent with the temperature used in the study of low-temperature stress of grapes by Chen et al. (2019), which could ensure that the research results had direct reference significance for the solution of practical agricultural problems. Three to five fully stretched young leaves were used for determining photosynthetic fluorescence parameters. Finally, the samples were stored in a refrigerator at -80°C for evaluating indicators.

Measurement of photosynthetic characteristics

Gaseous photosynthetic parameters were measured daily from 09:00-11:00 a.m. using a Li-6800 photosynthesizer (LI-COR, USA), with fully expanded and functional leaves in the same position (Jiang et al., 2014). The chlorophyll content was determined referring to the method proposed by Yang et al. (2022).

Measurement of the contents of osmoregulatory substances

The electrolyte exudate (EL) (Jiang et al., 2014) was determined using a DDSJ-308F Conductivity Meter (Shanghai Yidian Scientific Instrument Co.). Malondialdehyde (MDA) content was determined by the thiobarbituric acid colorimetric method (Subramanian et al., 2023). Free proline (Pro) content was determined by the ninhydrin colorimetric method (Jiang et al., 2014). SP content was determined by the Coomassie Blue G-250 method (Yang et al., 2012).

Determination of oxidase activity

SOD enzyme activity was determined by SOD inhibition of nitrogen blue tetrazolium photochemical reduction (Toivonen et al., 1998). POD enzyme activity was determined by the guaiacol method (Zou et al., 2019). CAT enzyme activity was determined by potassium permanganate titration (Srivastava et al., 1973). APX enzyme activity was determined referring to Nakano's (1980) method.

Measurement of AsA-GSH cycle indices

Glutathione reductase (GR), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR) activities were determined referring to the methods proposed by Foyer et al. (1976) and Zhu et al. (2022). AsA content was determined referring to Zhang et al. (2017). Reduced glutathione (GSH) and oxidized

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

glutathione (GSSG) contents were determined as described by Rahman et al. (2006). The measurements were repeated three times for each indicator type.

Statistical analysis of data

Data were organized using Excel 2010, with results presented as mean ± standard deviation (mean ± SD). Statistical analyses and graph construction were performed using GraphPad Prism 10.1.2 software. For differences in indicators across various time points within the same treatment group, one-way analysis of variance (One-way ANOVA) followed by Duncan's multiple comparison test was applied; distinct lowercase letters (e.g., a, b, c) denote significant differences at the $P < 0.05$ level. For comparisons of indicators between the control group (CK) and EBR-treated group at the same time point, independent samples t-tests were conducted, where "ns" (not significant) indicates no significant difference, ** indicates $P < 0.01$, *** indicates $P < 0.001$, and **** indicates $P < 0.0001$. Pearson correlation coefficients were used to analyze correlations among the indicators.

Results and analysis

Effect of EBR treatment on the chlorophyll content of grapes under low-temperature stress

As the core pigment in plant photosynthesis, chlorophyll content is directly related to photosynthetic efficiency. Low-temperature stress often impairs photosynthetic capacity by disrupting chlorophyll synthesis mechanisms or accelerating its degradation. In this study, under 0°C low-temperature stress, the changing trends of chlorophyll a, chlorophyll b, and total chlorophyll content in "Shine Muscat" grapes showed significant differences between the control group (CK) and the EBR-treated group (*Fig. 1*).

In the control group (CK), the contents of chlorophyll a, chlorophyll b, and total chlorophyll all exhibited a "first increase then decrease" trend: there was a slight increase in the early stage of low-temperature stress (0-60 h), which may be an adaptive response of the plants by enhancing chlorophyll synthesis in the short term to cope with stress. However, with the extension of stress duration (60-180 h), all three significantly decreased ($P < 0.05$), indicating that prolonged low-temperature had damaged the chlorophyll synthesis system or accelerated its decomposition, resulting in impairment of the photosynthetic apparatus.

After treatment with 0.5 mg/L EBR, although the changing trends of chlorophyll a, chlorophyll b, and total chlorophyll were consistent with those in CK (first increase then decrease), their overall contents were significantly higher than those in CK at the same period, with particularly significant differences in the late stage of stress (120 h and 180 h) ($P < 0.01$ or higher). Specifically, chlorophyll a increased by 53.17% compared with CK at 120 h ($P < 0.0001$), and the increase reached 179.13% at 180 h ($P < 0.001$); chlorophyll b increased by 30.94% compared with CK at 120 h ($P < 0.01$), and by 167.39% at 180 h ($P < 0.001$); total chlorophyll increased by 46.18% compared with CK at 120 h ($P < 0.0001$), and by 175.78% at 180 h ($P < 0.001$).

Intra-group comparisons showed that the contents of chlorophyll a, chlorophyll b, and total chlorophyll in the EBR-treated group reached their peaks at 120 h, then slightly decreased but remained at a relatively high level ($P < 0.05$); while the CK group showed a significantly greater decrease after 120 h, dropping to a relatively low level at 180 h.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

In summary, EBR treatment can effectively maintain the chlorophyll content of "Shine Muscat" grapes under low-temperature stress by promoting chlorophyll synthesis or inhibiting its degradation, with an extremely prominent effect especially in the middle and late stages of stress (after 120 h). This provides a material basis for photosynthesis and helps alleviate the damage of low-temperature to the photosynthetic apparatus.

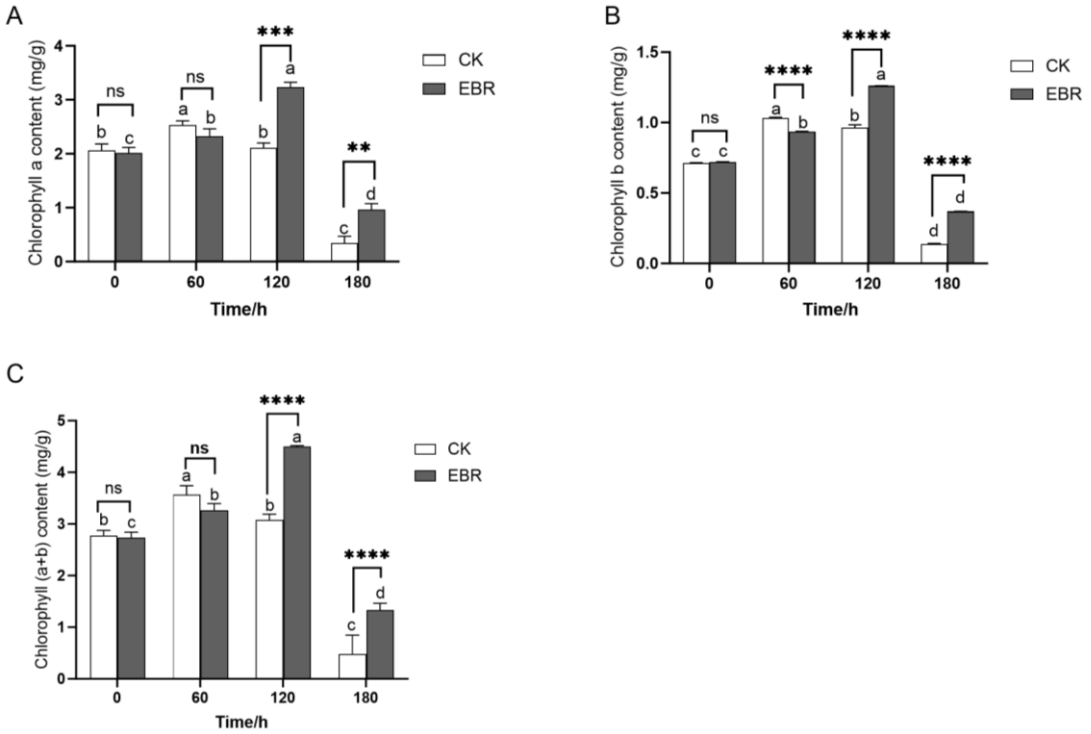

Figure 1. *Effect of exogenous EBR treatment on chlorophyll content of grapes under low-temperature stress. (A) Chlorophyll a content. (B) Chlorophyll b content. (C) Chlorophyll (a + b) content. CK, control group (sprayed with clear water); EBR, treatment group sprayed with 0.5 mg/L EBR. Different lowercase letters (e.g., a, b, c) indicate significant differences in indicators at different time points within the same treatment group at the $P < 0.05$ level. For indicators between the control group (CK) and the EBR treatment group at the same time point, "ns" (not significant) indicates no significant difference, ** indicates $P < 0.01$, *** indicates $P < 0.001$, and **** indicates $P < 0.0001$. The same applies below*

Effect of EBR treatment on the photosynthetic characteristics of grapes under low-temperature stress

Under low-temperature stress, significant differences in the photosynthetic characteristics of "Shine Muscat" grape leaves were observed between the control group (CK) and the 0.5 mg/L EBR treatment group (*Fig. 2*).

In the control group, the net photosynthetic rate (Pn), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci) all exhibited a "first increase then decrease" trend. This pattern may reflect the plant's self-regulatory mechanism, which temporarily enhances photosynthetic responses to cope with stress during the early stages of low-temperature. However, as the stress duration extended (60-180 h), damage to the photosynthetic system intensified, and all *indicators* showed a continuous decline. At 180 h, Pn and Gs were significantly lower than their peak values ($P < 0.0001$), indicating that the inhibitory effect

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

of low-temperature on photosynthesis gradually became significant. Meanwhile, the transpiration rate (Tr) in the control group increased continuously with prolonged stress, and was significantly higher at 180 h compared to 0 h ($P < 0.0001$), reflecting accelerated water loss from leaves under low-temperature conditions.

Treatment with 0.5 mg/L EBR significantly improved photosynthetic characteristics, with particularly marked differences in all indicators between the treatment and control groups at 120 h and 180 h ($P < 0.0001$). Specifically, at 120 h of treatment, the net photosynthetic efficiency was 4.38% higher than that in CK, stomatal conductance was substantially increased by 118.18%, and intercellular CO_2 concentration also showed a significant elevation ($P < 0.0001$). These results suggest that EBR regulates stomatal opening-closing mechanisms to significantly increase stomatal conductance, thereby promoting CO_2 uptake by leaves and providing sufficient substrates for the dark reaction of photosynthesis, which alleviates the low-temperature-induced inhibition of photosynthetic efficiency. Additionally, although Tr in the EBR treatment group peaked at 180 h, it remained significantly lower than that in the control group at the same time point ($P < 0.0001$), indicating that EBR can effectively reduce water loss under low-temperature stress, maintain cellular water balance, and ensure the stable operation of photosynthetic machinery.

Intra-group comparisons revealed that Pn, Gs, and Ci in the EBR treatment group reached their peaks at 120 h, followed by a slight decline; however, the magnitude of this decline was significantly smaller than that in the control group ($P < 0.05$). In contrast, the increasing trend of Tr was significantly gentler in the EBR treatment group compared to the control group.

In summary, EBR treatment enhances the photosynthetic capacity of "Shine Muscat" grapes under low-temperature stress by optimizing stomatal behavior (increasing Gs and promoting CO_2 utilization) and reducing water loss (decreasing Tr). The effect is particularly significant during the middle and late stages of stress (after 120 h), which significantly improves their adaptability to low-temperature environments.

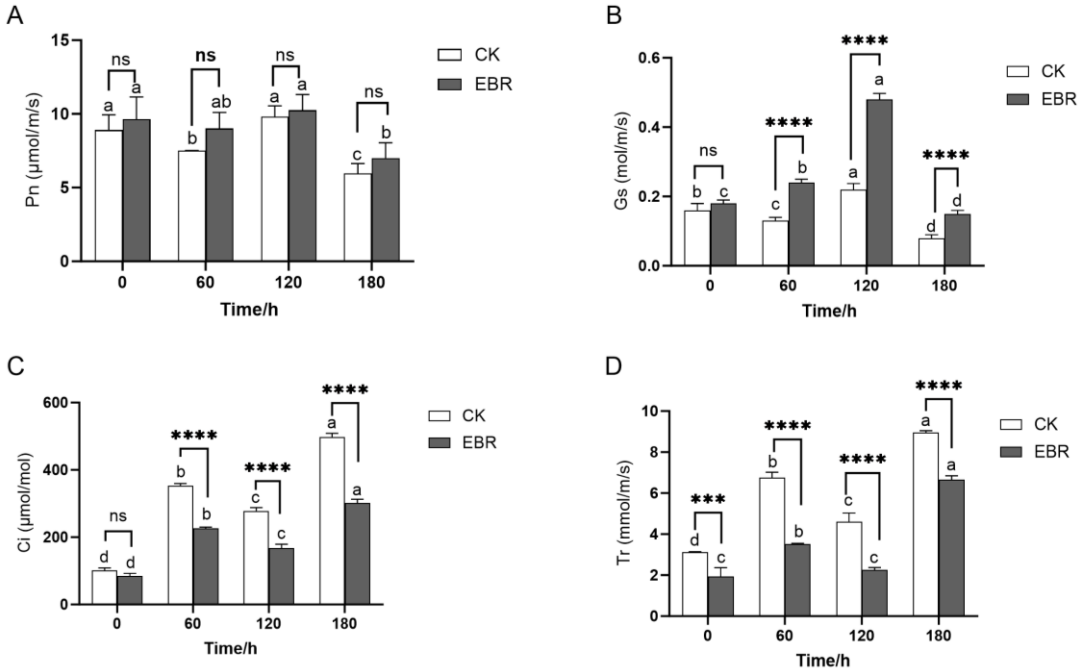

Figure 2. *Effect of EBR treatment on the photosynthetic characteristics of grapes under low-temperature stress. (A) Pn. (B) Gs. (C) Ci. (D) Tr*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

Effect of EBR treatment on the osmoregulatory substances in grapes under low-temperature stress

Under low-temperature stress, significant differences were observed in the osmotic adjustment substances and cell membrane damage-related indices of "Shine Muscat" grape leaves between the control group (CK) and the 0.5 mg/L EBR treatment group (*Fig. 3*).

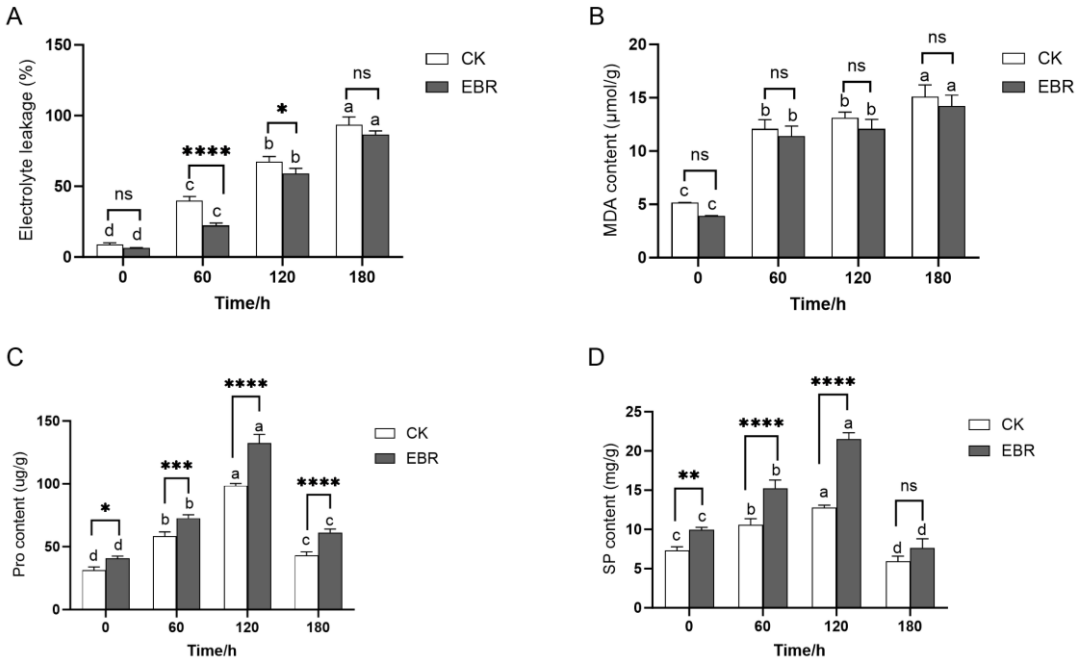

Figure 3. *Effect of EBR treatment on the osmoregulatory substances of grapes under low-temperature stress. (A) Electrolyte leakage. (B) MDA content. (C) Pro content. (D) SP content*

In the control group, both electrolyte leakage rate (EL) and malondialdehyde (MDA) content increased continuously with prolonged stress duration. Specifically, EL at 180 h was significantly higher than that at 0 h ($P < 0.0001$), while MDA content at 120 h and 180 h also showed significant increases compared with the 0 h level. These results indicated that low-temperature stress exacerbated cell membrane damage and progressively intensified membrane lipid peroxidation. In contrast, the contents of free proline (Pro) and soluble protein (SP) exhibited a trend of initial increase followed by a decrease: they increased slightly at 60 h, but then decreased significantly at 120 h and 180 h. This phenomenon may be attributed to the active accumulation of osmotic adjustment substances by plants in the early stage of stress to counteract adverse effects; however, as stress intensified, the capacity for synthesis declined, leading to reduced contents of these substances.

Treatment with 0.5 mg/L EBR significantly ameliorated the changes in osmotic adjustment substances, with particularly marked differences from the control group observed at 120 h and 180 h. Regarding cell membrane damage-related indices, the magnitude of increase in EL and MDA content was significantly lower in the EBR-treated group than in the control group. At 180 h, EL in the EBR treatment group was significantly lower than that in CK ($P < 0.0001$), while MDA content at 120 h and 180 h was also significantly lower than in CK ($P < 0.01$ and $P < 0.0001$, respectively). These

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

findings suggest that EBR can effectively alleviate low-temperature-induced membrane lipid peroxidation and maintain the structural stability of cell membranes. In terms of osmotic adjustment substance accumulation, the peaks of Pro and SP contents in the EBR group both occurred at 120 h. Specifically, Pro content was 37.61% higher than that in CK ($P < 0.0001$), and SP content was 68.78% higher than in CK ($P < 0.0001$). Furthermore, both Pro and SP contents in the EBR treatment group were significantly higher than those in the control group at all time points. These results indicate that EBR enhances cellular osmotic pressure and improves plant water retention capacity by promoting the synthesis and accumulation of osmotic adjustment substances, thereby alleviating cellular dehydration damage caused by low-temperature stress.

Intra-group comparisons revealed that the increasing trends of EL and MDA content in the EBR treatment group were significantly less pronounced than those in the control group. Additionally, after peaking at 120 h, the magnitude of decrease in Pro and SP contents in the EBR treatment group was significantly smaller than that in the control group ($P < 0.05$).

In summary, EBR treatment enhances the osmotic adjustment ability of "Shine Muscat" grapes under low-temperature stress by alleviating cell membrane damage (reducing EL and MDA) and promoting the accumulation of osmotic adjustment substances (Pro and SP). This effect is particularly significant in the middle and late stages of low-temperature stress (after 120 h), thereby significantly improving the adaptability of "Shine Muscat" grapes to low-temperature environments.

Effect of EBR treatment on grape oxidase activity under low-temperature stress

Under low-temperature stress, significant differences in antioxidant enzyme activities were observed in the leaves of "Shine Muscat" grapes between the control group (CK) and the 0.5 mg/L EBR treatment group (*Fig. 4*).

In the control group, the activities of superoxide dismutase (SOD) and catalase (CAT) increased continuously with prolonged stress duration, reflecting an active defense response in plants, whereby they enhance enzyme activities to scavenge excessive reactive oxygen species (ROS), though the magnitude of increase was limited. In contrast, the activities of peroxidase (POD) and ascorbate peroxidase (APX) exhibited a "first increase then decrease" trend: they increased slightly at 60 h, followed by a significant decline at 120 h and 180 h. This suggested that plants could counteract adversity by elevating enzyme activities in the early stages of stress; however, as stress intensified, the enzyme synthesis system became impaired, leading to a reduction in activity.

Following treatment with 0.5 mg/L EBR, antioxidant enzyme activities were significantly enhanced, with particularly marked differences from the control group at 120 h and 180 h. For SOD and CAT activities, both showed an upward trend with extended stress duration and remained significantly higher than those in the control group throughout. At 180 h, SOD activity was 20.0% higher than in CK ($P < 0.01$), and CAT activity was 39.1% higher than in CK ($P < 0.0001$). These results indicate that EBR can continuously activate the synthesis mechanisms of these two enzymes, thereby strengthening the scavenging capacity for superoxide anions and hydrogen peroxide. Regarding POD and APX activities, both peaked at 120 h: POD activity was 33.4% higher than in CK ($P < 0.0001$), and APX activity was 29.6% higher than in CK ($P < 0.0001$). Furthermore, at all time points, enzyme activities in the EBR treatment group were significantly higher than those in the control group, suggesting that EBR enhances ROS scavenging efficiency by promoting the activities of these two enzymes during critical stages of stress.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

Intra-group comparisons revealed that the increasing trends of SOD, CAT, POD, and APX activities in the EBR treatment group were significantly more pronounced at each time point compared to the control group. Additionally, after peaking at 120 h, the magnitude of the decrease in POD and APX activities in the EBR treatment group was significantly smaller than that in the control group ($P < 0.05$).

In summary, EBR treatment improves the ROS scavenging capacity of "Shine Muscat" grapes under low-temperature stress by systematically enhancing the activities of antioxidant enzymes (SOD, CAT, POD, and APX). This effect is particularly significant in the middle and late stages of stress (after 120 h), effectively mitigating oxidative damage and thereby significantly enhancing the adaptability of "Shine Muscat" grapes to low-temperature environments.

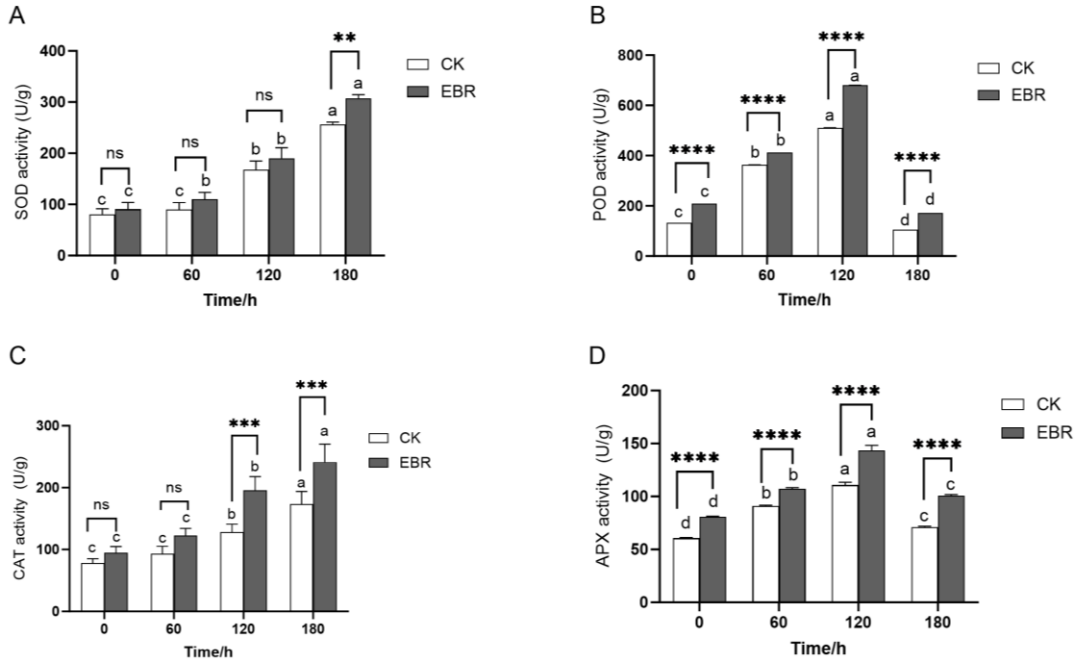

Figure 4. *Effect of EBR treatment on grape oxidase activity under low-temperature stress.* ***(A)*** *SOD activity. (B) POD activity. (C) CAT activity. (D) APX activity*

Effect of EBR treatment on AsA-GSH cycle in grapes under low-temperature stress

Under low-temperature stress, significant differences in the indices related to the ascorbate-glutathione (AsA-GSH) cycle were observed in the leaves of "Shine Muscat" grapes between the control group (CK) and the 0.5 mg/L EBR treatment group (*Fig. 5*).

In the control group, the content of ascorbic acid (AsA) increased continuously with prolonged stress duration, reflecting the adaptive mechanism by which plants counteract low-temperature stress through enhanced accumulation of non-enzymatic antioxidants. In contrast, the activities of dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR), along with glutathione (GSH) content and the GSH/GSSG ratio, exhibited a "first increase then decrease" trend: they increased slightly at 60 h, followed by a significant decline at 120 h and 180 h. This indicates that during the early stages of stress, plants can activate the activities of key enzymes in the AsA-GSH cycle to maintain redox homeostasis; however, as stress intensified, impairment of the enzymatic system led to a reduction in activity.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

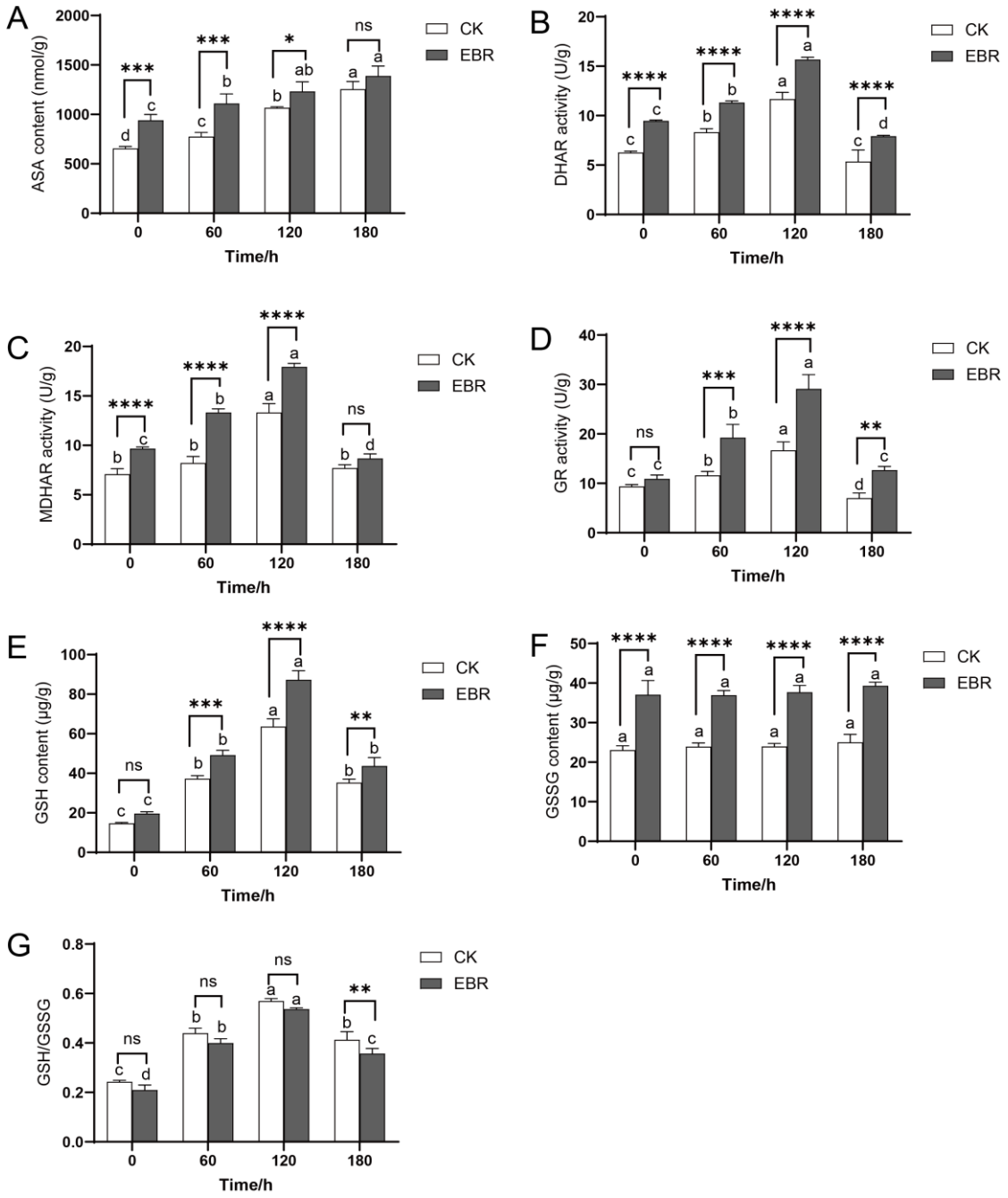

Figure 5. *Effect of EBR treatment on AsA-GSH cycle in grapes under low-temperature stress. (A) AsA content. (B) DHAR activity. (C) MDHAR activity. (D) GR activity. (E) GSH content. (F) GSSG content. (G) GSH/GSSG ratio*

Following treatment with 0.5 mg/L EBR, the operational efficiency of the AsA-GSH cycle was significantly enhanced, with particularly marked differences from the control group at 120 h and 180 h. For non-enzymatic antioxidants, AsA content increased continuously with extended stress duration, and at 180 h, it was 10.68% higher than in CK ($P < 0.0001$). This suggests that EBR promotes the sustained synthesis and accumulation of AsA, thereby strengthening non-enzymatic antioxidant capacity. Regarding key enzyme activities, DHAR, MDHAR, and GR all reached peak activity at

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

120 h: DHAR activity was 37.70% higher than in CK ($P < 0.0001$), MDHAR activity was 34.58% higher than in CK ($P < 0.0001$), and GR activity was 74.75% higher than in CK ($P < 0.0001$). Furthermore, at all time points, enzyme activities in the EBR treatment group were significantly higher than those in the control group ($P \leq 0.05$), indicating that EBR significantly enhances the catalytic efficiency of cycle enzymes during critical stress periods, accelerating AsA regeneration and reactive oxygen species (ROS) scavenging. In terms of glutathione metabolism, GSH content and the GSH/GSSG ratio also peaked at 120 h: GSH content was 37.22% higher than in CK ($P < 0.0001$), and the GSH/GSSG ratio was 35.00% higher than in CK ($P < 0.01$). These results demonstrate that EBR promotes GSH synthesis and maintains its reduced state, thereby reinforcing the protective effect on membrane structures.

Intra-group comparisons revealed that the increasing trend of AsA content in the EBR treatment group was significantly more pronounced than that in the control group. Additionally, after peaking at 120 h, the magnitude of the decrease in DHAR, MDHAR, and GR activities, as well as in GSH content and the GSH/GSSG ratio, was significantly smaller in the EBR treatment group compared to the control group ($P < 0.05$).

In summary, EBR treatment efficiently activates the AsA-GSH cycle by promoting AsA accumulation, enhancing key enzyme activities, and optimizing the redox state of glutathione. This effect is particularly significant during the middle and late stages of stress (after 120 h), effectively maintaining intracellular redox balance and thereby significantly strengthening the antioxidant defense capacity of "Shine Muscat" grapes under low-temperature stress.

Effect of exogenous EBR treatment on grapevines growth under low-temperature stress

Under normal growth conditions (25℃), The leaves of "Shine Muscat" grape were bright green in color, fully unfolded, and flat, without yellowing or curling. This indicated that the physiological activities in grapevines could proceed in an organized manner under 25℃ conditions. The growth of "Shine Muscat" grape was significantly inhibited after 120 h of low-temperature stress, and the leaves turned yellow and curled. This series of changes indicated that low-temperature damaged the cell membrane of the plants, impacting the normal physiological functions of the leaves. The slow growth of new shoots indicated that low-temperature restricted the growth and development of plants. Compared with LT 120-h treatment, "Shine Muscat" grape treated with EBR + LT 120 h (exogenous EBR treatment under low-temperature stress for 120 h) displayed significant improvement in leaf condition after exogenous EBR treatment, with less yellowing and curling and relatively stronger new shoot growth. This indicated that EBR treatment promoted the overall growth of grapevines better than LT 120-h treatment (*Fig. 6*).

Correlation analysis of various indicators in grape under exogenous EBR treatment

The Pearson correlation analysis results (*Table 1*) showed that the contents of chlorophyll a (Chl a) and chlorophyll b (Chl b) in grapes were significantly positively correlated with Pn and Gs (Chl a Pn: $r = 0.998$; Chl b Pn: $r = 0.732$; Chl a-Gs: $r = 0.965$; Chl b-Gs: $r = 0.843$, $P < 0.001$). Also, the contents showed a significant negative correlation with transpiration rate (Tr) (Chl a Tr: $r = –0.728$; Chl b Tr: $r = –0.643$, $P < 0.001$). These results indicated that an increase in chlorophyll content not only enhanced light energy capture and conversion efficiency but also optimized water use

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

efficiency by regulating stomatal behavior. The GSH content was significantly positively correlated with Pn ($r = 0.446$, $P < 0.001$) and stomatal conductance (Gs) ($r = 0.795$, $P < 0.001$), indicating that GSH may affect the CO_2 assimilation process and regulate plant photosynthetic performance by regulating stomatal opening. AsA content was significantly positively correlated with the activities of DHAR, MDHAR, and GR ($r = 0.553$, 0.575, and 0.491, respectively, $P < 0.001$) and significantly positively correlated with GSH content ($r = 0.298$, $P < 0.05$). At the same time, AsA content was also significantly positively correlated with net photosynthetic rate (Pn) ($r = 0.578$, $P < 0.001$), indicating a close relationship between the metabolic activity of AsA-GSH cycle and plant photosynthetic efficiency. A highly significant positive correlation ($r = 0.971$, 0.965, and 0.883, respectively, $P < 0.001$) was observed among the three key reductase activities of DHAR, MDHAR, and GR. This synergy indicated that the various enzymatic reaction links of the AsA-GSH cycle could maintain coordinated metabolic activity under EBR treatment, thus effectively maintaining the overall function of the circulatory system. This synergistic mechanism has important physiological significance for the timely clearance of ROS and maintaining cellular redox homeostasis. In summary, exogenous EBR treatment significantly increased the content of antioxidants such as AsA and GSH in grapevines by enhancing the activity of key enzymes in the AsA-GSH cycle (DHAR, MDHAR, and GR) (*Fig. 5*). A significant correlation was noted between the components of the AsA-GSH cycle and photosynthetic parameters, revealing the synergistic regulatory mechanism between the antioxidant system and photosynthesis. This synergistic effect helped ensure that grapevines maintained normal physiological functions under low-temperature stress (*Fig. 6*).

Figure 6. *Effect of exogenous EBR treatment on grapevines growth under low-temperature stress. 25°C was the optimal temperature for normal growth; LT 120 h referred to 120 h of low-temperature (0°C) stress; EBR + LT 120 h referred to 120 h of low-temperature (0°C) stress after spraying with 0.5 mg/L EBR*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

Table 1. *Correlation analysis of various indicators in grape under exogenous EBR treatment*

	Chlb	Chla+b	Pn	Gs	Ci	Tr	EL	MDA	Pro	SP	SOD	POD	CAT	APX	AsA	DHAR	MDHAR	GR	GSH	GSSG
Chla	0.982**	0.998**	0.805*	0.743*	–0.603	–0.728*	–0.537	–0.322	0.583	0.808*	-0.62	0.787*	–0.352	0.578	–0.389	0.765*	0.654	0.723*	0.446	0.137
Chlb	1	0.991**	0.785*	0.732*	–0.489	–0.643*	–0.428	–0.183	0.665*	0.833**	0.863**	0.649*	–0.314	0.649*	–0.323	0.806*	0.704	0.749*	0.542	0.086
Chla + b		1	0.802*	0.743*	–0.573	–0.706*	–0.507	–0.283	0.609*	0.819**	0.812**	0.601*	–0.342	0.601*	–0.371	0.780*	0.671	0.734*	0.476	0.123
Pn			1	0.728*	–0.806**	–0.925**	–0.546	–0.502	0.533	0.705*	–0.521	0.666*	–0.319	0.498	–0.253	0.769*	0.694	0.656*	0.374	0.241
Gs				1	–0.458	–0.642*	–0.067	0.002	0.865**	0.943**	–0.040	0.851**	0.25	0.850**	0.231	0.927**	0.927**	0.963**	0.795*	0.469
Ci					1	0.951**	0.776*	0.833*	–0.064	–0.339	0.567*	–0.188	0.378	–0.100	0.419	–0.387	–0.270	–0.317	0.105	–0.351
Tr						1	0.726*	0.718*	–0.289	–0.572	0.577*	–0.426	0.353	–0.309	0.317	–0.608*	–0.519*	–0.526*	–0.125	–0.390
EL							1	0.889**	0.307	–0.104	0.913**	0.028	0.807*	0.261	0.767*	–0.063	0.06	0.026	0.454	–0.029
MDA								1	0.422	0.096	0.728*	0.236	0.681*	0.408	0.692*	0.079	0.197	0.186	0.576	–0.036
Pro									1	0.888	0.203	0.940**	0.415	0.962**	0.431	0.912**	0.935**	0.928**	0.982**	0.303
SP										1	–0.156	0.943**	0.134	0.895**	0.19	0.967**	0.958**	0.975**	0.828	0.42
SOD											1	–0.128	0.938**	0.224	0.879*	–0.091	0.023	0.02	0.343	0.275
POD												1	0.104	0.905**	0.151	0.942**	0.924**	0.916**	0.883*	0.204
CAT													1	0.48	0.921**	0.182	0.269	0.312	0.531	0.518
APX														1	0.506	0.926**	0.921**	0.940**	0.948**	0.513
AsA															1	0.255	0.369	0.327	0.553	0.575
DHAR																1	0.971**	0.959**	0.839*	0.476
MDHAR																	1	0.965**	0.892*	0.441
GR																		1	0.883**	0.491
GSH																			1	0.298

*Indicated significant difference at $P < 0.05$ level, ** indicated extremely significant difference at $P < 0.01$ level

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

Discussion

The branches, vines, and roots of grapevines are prone to cold damage in the winter cold or early spring low-temperature environment. This triggers a delay in the spring budding, improper budding, inadequate growth of new shoots, abnormal flower bud differentiation, reduced fruiting rate, and other issues, significantly limiting the yield and quality of grapes in the industry. Various frost protection and remedial measures have been explored to effectively mitigate the negative effects of low-temperature on grape budding. EBR has been found to be highly effective in enhancing plant cold tolerance. Studies by Hui et al. (2013) and Zhao et al. (2022) confirmed that EBR treatment could enhance the cold resistance of grape seedlings and promote their growth and development.

One of the main manifestations of low-temperature stress significantly affecting the photosynthesis process in plants is that it leads to a decrease in chlorophyll content (Aazami et al., 2021). Zhao et al. (2018) found that the leaf chlorophyll content showed a significant decreasing trend with the intensification of the degree of low-temperature stress in mountain grapevine "Shuang Feng" and "Zuo Youhong" varieties. This decrease was mainly attributed to the low-temperature induced chlorophyll catabolism and peroxidative damage of chloroplast membrane lipids. The results of the present study revealed that the chlorophyll content of "Shine Muscat" grapevines was similarly reduced significantly ($P < 0.05$) after being subjected to low-temperature stress. This finding was consistent with the findings of Wang et al. (2010), Anwar et al. (2018), and Wan et al. (2021), indicating that the exogenous application of EBR could alleviate chlorophyll degradation caused by low-temperature stress.

Photosynthesis is the central process of energy and matter accumulation in plants. Sustained low-temperature stress impairs various aspects of photosynthesis, including gas exchange, carbon assimilation pathways, and cyclic electron transport (Tang et al., 2023; Faizan et al., 2021; Yang et al., 2019). This study found that low-temperature stress significantly reduced the net photosynthetic rate (Pn) and stomatal conductance (Gs) in "Shine Muscat" grapevine leaves, while causing a significant increase in the intercellular CO_2 concentration (Ci) and transpiration rate (Tr) ($P < 0.05$). The findings indicated that the cold damage severely impaired the photosynthetic apparatus of the leaves and reduced the photosynthetic efficiency of the leaf pulp cell, which was consistent with the findings of Tang et al. (2023), Faizan et al. (2021), and Yang et al. (2019). This indicated that the photosynthetic parameters of the plants were significantly improved after spraying EBR, suggesting that the exogenous application of EBR could effectively alleviate the damage caused by low-temperature stress to the photosynthetic system of grape seedlings.

Plants defend themselves against cold damage under low-temperature stress mainly through accumulating osmoregulatory substances and activating antioxidant enzyme systems (Zhang et al., 2023; Wang et al., 2024). The present study found that low-temperature stress resulted in a significant ($P < 0.05$) decrease in Pro and SP contents in "Shine Muscat" grapevines. Also, the changes in the activities of SOD, POD, and CAT reflected the course of the plant response to low-temperature stress. The results were consistent with those of Lian et al. (2021), Hui et al. (2013), and Faizan et al. (2021), indicating that exogenous application of EBR could significantly regulate the metabolic homeostasis in plants, promote the accumulation of osmoregulatory substances, enhance the activity of antioxidant enzymes, effectively scavenge excess intracellular ROS, and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

reduce cellular membrane permeability, thus significantly enhancing the low-temperature resistance in plants.

The AsA-GSH cycle is a key pathway for plant antioxidant defense, in which APX plays a central role in hydrogen peroxide scavenging. This study found that EBR treatment significantly increased the activities of APX, DHAR, MDHAR, and GR under low-temperature stress ($P < 0.05$), besides significantly upregulating AsA and GSH contents, which was in agreement with the findings of Huang et al. (2011), Luo et al. (2007), and Chen et al. (2019). This suggested that exogenous application of EBR could enhance the ability of plant cells to scavenge free radicals and alleviate the oxidative damage caused by low-temperature stress on grapevine plants by enhancing the metabolic efficiency of the AsA-GSH cycle, further revealing the vital role of EBR in enhancing the low-temperature resistance of plants.

Conclusion

EBR treatment effectively maintained photosynthesis, increased chlorophyll content, and promoted photosynthetic function in plants under low-temperature stress. Meanwhile, it prompted the accumulation of osmoregulatory substances, reduced the electrolyte exudate rate and malondialdehyde content, and attenuated cell membrane damage. Further, it significantly enhanced antioxidant enzyme activities, promoted the functioning of the AsA–GSH cycle, and reduced oxidative damage. In conclusion, spraying 0.5 mg/L EBR could effectively alleviate the adverse effects of low-temperature stress on "Shine Muscat" grapes and enhance their low-temperature tolerance.

Acknowledgments. This project was jointly supported by the Key R&D Projects of Henan Province, China (241111113200).

REFERENCES

[1] Aazami, M. A., Asghari-Aruq, M., Hassanpouraghdam, M. B., Ercisli, S., Baron, M., Sochor, J. (2021): Low temperature stress mediates the antioxidants pool and chlorophyll fluorescence in *Vitis vinifera* L. cultivars. – Plants 10(9): 1877.

[2] Amin, B., Atif, M. J., Meng, H., Ali, M., Li, S., Alharby, H. F., Majrashi, A., Hakeem, K. R., Cheng, Z. H. (2022): Melatonin rescues photosynthesis and triggers antioxidant defense response in Cucumis sativus plants challenged by low temperature and high humidity. – Frontiers in Plant Science 13: 855900.

[3] Anwar, A., Bai, L. Q., Miao, L., Liu, Y. M., Li, S. Z., Yu, X. C., Li, Y. S. (2018): 24-Epibrassinolide ameliorates endogenous hormone levels to enhance low-temperature stress tolerance in cucumber seedlings. – International Journal of Molecular Sciences 19(9): 2497.

[4] Chen, Z. Y., Wang, Y. T., Pan, X. B., Xin, Z. M. (2019): Amelioration of cold-induced oxidative stress by exogenous 24-epibrassinolide treatment in grapevine seedlings: toward regulating the ascorbate–glutathione cycle. – Scientia Horticulturae 244: 379-387.

[5] Dong, F. X., Li, X. Y., Liu, C., Zhao, B. X., Ma, Y., Ji, W. (2025): Exogenous 24-epibrassinolide mitigates damage in grape seedlings under low-temperature stress. – Frontiers in Plant Science 16: 1487680.

[6] Faizan, M., Faraz, A., Hayat, S., Bhat, J. A., Yu, F. (2021): Zinc oxide nanoparticles and epibrassinolide enhanced growth of tomato via modulating antioxidant activity and photosynthetic performance. – Biocell 45(4): 1081-1093.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

[7] Foyer, C. H., Halliwell, B. (1976): The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. – Planta 133(1): 21-25.
[8] Fu, X. K., Chen, X., Chen, Y. W., Hui, Y. R. (2024): Foliar Co-applications of nitrogen and iron on vines at different developmental stages impacts wine grape (*Vitis vinifera* L.) composition. – Plants 13(16): 2203.
[9] Hou, Y. J., Wong, D. C. J., Li, Q. Y., Zhou, H. M., Zhu, Z. F., Gong, L. Z., Liang, J., Ren, H. S., Liang, Z. C., Wang, Q. F., Xin, H. P. (2023): Dissecting the effect of ethylene in the transcriptional regulation of chilling treatment in grapevine leaves. – Plant Physiology and Biochemistry 196: 1084-1097.
[10] Huang, Z. M., Wu, J. C., Chen, W. J., Cai, L. Q., Xie, C. P., Lin, L. J., Huang, S. J., Ye, M. L. (2011): Effects of SA on the AsA-GSH cyclase system of young loquat fruits after low-temperature stress. – Scientia Silvae Sinicae 47(9): 36-42.
[11] Hui, Z. M., Wang, Z. Z., Hu, Y., Deng, M. M., Zhang, Z. W. (2013): 24 - Effects of brassinosteroids on antioxidant system and osmoregulatory substances in grape seedlings under low temperature stress. – Scientia Agricultura Sinica 46(5): 1005-1013.
[12] Jiang, H. Y., Li, W., He, B. J., Gao, Y. H., Lu, J. X. (2014): Sucrose metabolism in grape (*Vitis vinifera* L.) branches under low temperature during overwintering covered with soil. – Plant Growth Regulation 72(3): 229-238.
[13] Jiang, J. L., Hou, R. P., Yang, N., Li, L., Deng, J. R., Qin, G. W., Ding, D. K. (2021): Physiological and TMT-labeled proteomic analyses reveal important roles of sugar and secondary metabolism in Citrus junos under cold stress. – Journal of Proteomics 237: 104145.
[14] Li, Y., Li, G. Q., Gao, M. G., Xu, R. R. (2024): Bioinformatics and expression analysis of grapevine VvLBD35 and VvLBD36. – Shandong Agricultural Science 56(8): 23-29.
[15] Lian, H. S., Li, X. X., Lin, L. J., Liao, M. A. (2021): Effects of brassinolide on the growth of summer black grape seedlings. – Acta Agriculturae Zhejiangensis 33(10): 1889-1896.
[16] Liu, L. J., Ding, M. Y., Liu, X. N., Sun, Y. T., Wang, L. Y., Zhang, J. Z., Li, S. S. (2020): Induced effects of exogenous epigallocatechin lactones on cold resistance in wheat seedlings. – Molecular Plant Breeding 18(20): 6815-6821.
[17] Luo, Y., Tang, H. R., Zhang, Y. (2007): Effects of low temperature stress on SOD and AsA GSH circulating enzyme system in strawberry leaves. – Acta Horticulturae Sinica (6): 1405-1410.
[18] Nakano, Y., Asada, K. (1980): Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. – Plant and Cell Physiology 22(5): 867-880.
[19] Qiao, Z. C., He, Y., Dai, H. J., Liang, X. H. (2022): Effects of exogenous brassinolide on the photosynthetic characteristics and fruit quality of Merlot grapes. – Journal of Southern Agriculture 53(8): 2251-2260.
[20] Rahman, I., Kode, A., Biswas, S. K. (2006): Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. – Nature Protocols 1(6): 3159-3165.
[21] Srivastava, O. P., Huystee, R. B. (1973): Evidence for close association of peroxidase, polyphenol oxidase, and IAA oxidase isozymes of peanut suspension culture medium. – Canadian Journal of Botany 51(11): 2207-2215.
[22] Subramanian, S., Mitkus, E., Souleimanov, A., Smith, D. L. (2023): Lipo-chitooligosaccharide and thuricin 17 act as plant growth promoters and alleviate drought stress in Arabidopsis thaliana. – Frontiers in Microbiology 14: 1184158.
[23] Tang, Y. L., Zhang, J. J., Wang, L. J., Wang, H., Long, H. C., Yang, L. Y., Li, G. W., Guo, J. M., Wang, Y. C., Li, Y. L., Yang, Q. H., Shi, W. Y., Shao, R. X. (2023): Water deficit aggravated the inhibition of photosynthetic performance of maize under mercury stress but is alleviated by brassinosteroids. – Journal of Hazardous Materials 443: 130365.
[24] Toivonen, P. M. A., Sweeney, M. (1998): Differences in chlorophyll loss at 13°C for two broccoli (*Brassica oleracea* L.) cultivars associated with antioxidant enzyme activities. – Journal of Agricultural and Food Chemistry 46(1): 20-24.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463

[25] Wan, Q. W., Yang, N., Li, Y. M., Han, M. H., Lin, S. J., Teng, R. M., Zhuang, J. (2021): Effects of exogenous 24 epibrassinolide on photosynthetic characteristics of tea trees. – Journal of Tea Science 41(1): 58-70.
[26] Wang, J. J., Wang, S. W., Pan, Y., Li, Y. L., Li, S. D. (2024): Identification and comprehensive evaluation of cold resistance of wine grape germplasm in the northern foothills of Tianshan mountains in Xinjiang. – Journal of Fruit Science 41(10), 1933-1946.
[27] Wang, N., Xu, J., Wu, J., Yuan, M. L., Su, J. L. (2010): Effects of spraying different exogenous substances on some cold resistant physiological indicators of light wax seedlings under low temperature stress. – Journal of West China Forestry Science 39(1): 31-35.
[28] Wang, P., Li, Y. H., Zhang, X. M., Li, B. G., Yao, F. F. (2013): The effect of low temperature on the ascorbic acid glutathione cycle in the pistil of apricot kernels. – Acta Horticulturae Sinica 40(3): 417-425.
[29] Yang, H. Y., Wu, W. L., Lu, L. F., Huang, Z. J. (2022): Effects of drought stress on the physiological characteristics of leaves of 'Boni' thin shell walnuts. – South China Fruits (3): 135-140.
[30] Yang, P., Wang, Y., Li, J., Bian, Z. H. (2019): Effects of brassinosteroids on photosynthetic performance and nitrogen metabolism in pepper seedlings under chilling stress. – Agronomy 9(12): 839.
[31] Yang, Z. K., Wang, X. L., Long, S. H., Hao, Z. B., Shan, Z. Z., Zhou, F. F. (2012): Determination of protein content in soybean stems and leaves by Coomassie Brilliant Blue staining method. – Hubei Agricultural Sciences 51(20): 4610-4612.
[32] Zhang, L. P., Liu, H. F., Xin, H. P. (2023): Progress in the study of cold resistance mechanisms in grapes. – Journal of Fruit Science 40(2): 350-362.
[33] Zhang, X. Y., Zhang, G. C., Li, P. X., Yang, Q. Y., Chen, K. P., Zhao, L. N., Apaliya, M. T., Gu, X. Y., Zhang, H. Y. (2017): Mechanisms of glycine betaine enhancing oxidative stress tolerance and biocontrol efficacy of Pichia caribbica against blue mold on apples. – Biological Control 108: 55-63.
[34] Zhao, F. F., Guo, X. L., Liu, Y., Wang, Y. N., Yin, M. T., Dai, H. J. (2022): Effects of EBR spraying before winter on the physiological characteristics and germination of 'Meile' grape branches. – Acta Agriculturae Boreali - Occidentalis Sinica 31(3): 328-334.
[35] Zhao, Y., Wang, Z. X., Xu, P. L., Yang, Y. M., Liu, Y. X., Liu, H. S., Ai, J. (2018): Chlorophyll fluorescence characteristics and the relationship between reactive oxygen species metabolism and low temperature damage of mountain grape 'Shuangfeng' and 'Zuoyouhong'. – Acta Horticulturae Sinica 45(4): 650-658.
[36] Zhu, J., Li, C. Y., Sun, L., Cheng, Y., Hou, J. B., Fan, Y. T., Ge, Y. H. (2022): Application of γ-aminobutyric acid induces disease resistance in apples through regulation of polyamine metabolism, GABA shunt and reactive oxygen species metabolism. – Scientia Horticulturae 291: 110588.
[37] Zou, J. N., Jin, X. J., Zhang, Y. X., Ren, C. Y., Zhang, M. C., Wang, M. X. (2019): Effects of melatonin on photosynthesis and soybean seed growth during grain filling under drought stress. – Photosynthetica 57(2): 512-520.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94479463