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# Signaling pathway responses of grape plants under drought stress and regulatory effects of exogenous salicylic acid

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**Introduction:** Grapes (*Vitis* spp.) are of significant economic importance globally, but their growth and yield are increasingly threatened by drought stress amid climate change. The drought sensitive cultivar *Vitis vinifera* ‘Muscat Hamburg’ is particularly vulnerable to water deficit, while the rootstock ‘1103P’ exhibits strong drought tolerance. Exogenous salicylic acid (SA) has been reported to enhance plant drought resistance, but its regulatory mechanisms involving key signaling pathways (ABA, Ca<sup>2+</sup>, MAPK) in grapes remain unclear.

**Methods:** Using ‘Muscat Hamburg’ as the test material and ‘1103P’ as the drought-resistant control, we applied moderate drought stress (soil relative water content: 35 ± 5%) and foliar sprayed four concentrations of exogenous SA (0.5, 1.0, 1.5, 2.0 mM) 24 h before stress induction. Physiological indicators (leaf relative water content, membrane permeability, oxidative damage markers, antioxidant enzyme activities, proline content) and key signaling molecules (endogenous SA, ABA, cytoplasmic Ca<sup>2+</sup>, MAPK activity) were measured, with correlation analysis conducted to explore associations between signaling pathways and physiological responses.

**Results:** Exogenous SA significantly alleviated drought-induced damage in ‘Muscat Hamburg’: it improved leaf relative water content, reduced membrane permeability and oxidative damage, enhanced SOD, POD, and CAT activities, and promoted proline accumulation. The 1.0 mM SA treatment showed the most pronounced effects. Additionally, exogenous SA upregulated endogenous SA content and increased key signaling molecules in the ABA, Ca<sup>2+</sup>, and MAPK pathways, indicating an associative regulatory relationship among these pathways under drought stress. Correlation analysis confirmed strong links between signaling molecules and physiological indicators.

**Discussion:** This study is the first to reveal the integrated association of SA with ABA, Ca<sup>2+</sup>, and MAPK pathways in grapes under drought stress at the physiological level. The findings demonstrate that exogenous SA enhances grape drought resistance by modulating multiple signaling pathways and coordinating osmotic adjustment and antioxidant defense systems. The optimal SA concentration (1.0 mM) provides a practical technical reference, while the regulatory mechanisms identified offer a theoretical basis for SA application in grape drought-resistant cultivation.

**KEYWORDS**

antioxidant system, drought stress, exogenous salicylic acid, grapevine, physiological regulation, signaling pathways

# 1 Introduction

Grape (*Vitis* spp.) is an economically important fruit crop widely cultivated throughout the world. Besides fresh consumption, its berries are extensively used for winemaking and processing, playing a key role in the global agricultural economy (Gambetta et al., 2020; Guo et al., 2025; Petoumenou and Liava, 2025). China has a large vineyard area and high grape production, making the promotion of superior materials crucial for the development of the grape industry. Among them, the ‘Muscat Hamburg’ grape (*Vitis vinifera* ‘Muscat Hamburg’) is highly favored by the market due to its attractive berry appearance, unique flavor, and good storage and transport properties. Its cultivation scale continues to expand, with Shaanxi Province emerging as a major production region for this cultivar.

In the context of global climate change, the frequency and intensity of drought stress are increasing, which has become a critical abiotic stress factor limiting grape growth, yield formation, quality improvement, and sustainable industry development (Li et al., 2025a; Carvalho et al., 2025; Jin et al., 2025). The ‘Muscat Hamburg’ grape is particularly sensitive to extreme climatic events such as drought, often exhibiting poor budbreak and significant yield reduction under stress conditions (Wang et al., 2025). Therefore, exploring effective technical approaches to alleviate drought stress is of great practical significance for ensuring stable fruit quality and yield and promoting the sustainable development of the grape industry.

Drought stress induces water deficit in grapevines, leading to stomatal closure, inhibition of photosynthesis, and growth arrest, with severe cases even resulting in plant death (Charaves et al., 2003; Gambetta et al., 2020; Lehr et al., 2025). Additionally, drought disrupts cell membrane structure and function, interferes with the balance of endogenous hormones and metabolic processes, and consequently impairs fruit quality (Pan et al., 2015; Hakeem et al., 2025). It also weakens physiological activity, causes cellular structural damage, and ultimately adversely affects subsequent growth and yield formation (Wang et al., 2003; Zhao et al., 2023). Hence, in-depth analysis of the physiological and molecular mechanisms underlying grapevine responses to drought stress, and exploration of effective strategies to enhance drought resistance, are essential for ensuring the sustainable development of the grape industry.

Salicylic acid (SA) is a widely present endogenous signaling molecule in plants, playing a central role in regulating plant growth, development, and responses to various abiotic stresses (Raskin, 1992; Khan et al., 2015). Studies have shown that exogenous SA application can enhance antioxidant capacity under stresses such as drought, high temperature, and low temperature, improve photosynthetic efficiency, and regulate the accumulation of osmotic adjustment substances, thereby alleviating stress-induced

damage (Sun et al., 2014; Wang et al., 2023; Diao, 2023). In grapevines, SA exhibits similar functions: for instance, foliar spraying of SA can increase antioxidant enzyme activity under heat stress, enhancing thermotolerance (Wang et al., 2003; Chen, 2006; Sun et al., 2014); under drought conditions, exogenous SA promotes the synthesis of osmotic regulators such as proline, thus improving plant drought resistance (Diao, 2023; Li et al., 2025b). However, most current research has focused on the overall stress resistance effects of SA in grapevines, while the specific signaling pathways and molecular mechanisms involved in its regulation of drought responses remain to be systematically elucidated.

Abscisic acid (ABA), calcium ions ( $\text{Ca}^{2+}$ ), and the mitogen-activated protein kinase (MAPK) cascade pathway are all key signaling components in grapevine responses to abiotic stress. Nevertheless, the synergistic interactions among these three remain unclear. ABA can enhance stress resistance by inducing stomatal closure, promoting root growth, increasing antioxidant enzyme activity, and stimulating proline accumulation (Beldapalazón et al., 2020; Kondo et al., 2024).  $\text{Ca}^{2+}$  acts as a second messenger, with changes in its intracellular concentration regulating downstream physiological responses during stress signal transduction, including stomatal movement, antioxidant defense, and osmotic adjustment (Song and Hong, 2001). However, whether SA participates in the drought response of grapevines by modulating  $\text{Ca}^{2+}$  signaling remains unknown. The MAPK cascade pathway, once activated by stress, regulates the expression of related genes and physiological responses through phosphorylation cascades, and its role in abiotic stress responses in grapevines has been confirmed (Pan et al., 2015). Yet, it is still unclear whether SA regulates drought resistance via the MAPK pathway, and the cross-talk mechanisms among ABA,  $\text{Ca}^{2+}$ , and MAPK signaling require systematic investigation.

This research takes the drought-sensitive cultivar ‘Muscat Hamburg’ (*Vitis vinifera* ‘Muscat Hamburg’) and drought-resistant ‘1103P’ as experimental materials, and for the first time systematically analyzes the association characteristics between SA and three core stress signaling pathways (ABA,  $\text{Ca}^{2+}$ , MAPK) in grape under drought stress, combined with physiological and biochemical indicators. The study clarifies the optimal concentration of exogenous SA for improving drought tolerance of ‘Muscat Hamburg’, and reveals the regulatory pattern of SA in coordinating grape osmotic adjustment and antioxidant defense systems. Different from previous studies that only focused on the physiological effects of SA on grape drought resistance, this study constructs a link between SA and key stress signaling molecules, which further enriches the physiological mechanism of SA regulating grape drought resistance and provides a more comprehensive theoretical basis for drought-resistant breeding and cultivation management in grapevines.

## 2 Materials and methods

### 2.1 Materials

Two grapevine materials exhibiting significant differences in drought tolerance were selected: the highly drought-resistant

**Abbreviations:** SA, salicylic acid; ABA, abscisic acid;  $\text{Ca}^{2+}$ , calcium ion; MAPK, mitogen-activated protein kinase; RWC, relative water content; REC, relative electrical conductivity; MDA, malondialdehyde; ROS, reactive oxygen species;  $\text{H}_2\text{O}_2$ , hydrogen peroxide;  $\text{O}_2^-$ , superoxide anion; Pro, proline; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; SRWC, soil relative water content; HPLC, high-performance liquid chromatography; ELISA, enzyme-linked immunosorbent assay; CDPKs, calcium-dependent protein kinases; FW, fresh weight.

rootstock '1103P' (*Vitis berlandieri* × *Vitis rupestris* '1103P') was used as a resilient reference control, and the currently predominant drought-sensitive cultivated cultivar 'Muscat Hamburg' (*Vitis vinifera* 'Muscat Hamburg') was the primary test material. Self-rooted (non-grafted) seedlings were employed throughout the experiment (Song and Hong, 2001). This experimental system eliminates grafting-induced interactive effects, enabling direct comparison of the intrinsic drought tolerance and independent responses to exogenous salicylic acid (SA) between the two genotypes. Given that the present study focuses on foliar SA application and its effects on shoot drought resistance, the use of self-rooted materials also avoids potential interference from rootstock-scion signal transduction. To minimize the influence of inherent genetic differences between the two genotypes on the experimental results, plants with uniform age (12-month growth cycle), vigorous and consistent growth (plant height 30–35 cm, stem diameter 0.5–0.6 cm), and free from pests and diseases were strictly selected for transplantation. This selection was consistent with practical grape production applications and facilitated reliable benchmarking using a drought-tolerant control (Song and Hong, 2001). Seedlings were transplanted individually into plastic pots (15 cm diameter, 20 cm height) filled with washed and disinfected quartz sand, with one plant per pot.

All plant cultivation and subsequent experimental operations were conducted in the laboratory of the College of Enology, Northwest A&F University. Plants were cultivated in an artificial climate chamber (Model: RXZ-500D, Ningbo Jiangnan Instrument Factory) under the following conditions: temperature  $25 \pm 2$  °C, relative humidity  $60 \pm 5\%$ , light intensity  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ , photoperiod 16 h/8 h (day/night) (Song and Hong, 2001). The quartz sand was watered daily with 1/2 Hoagland nutrient solution to maintain moisture (water content maintained at approximately 75% of field capacity). Subsequent stress treatments were applied after a 2-week acclimation period.

## 2.2 Experimental design

Referencing commonly used stress intensities in grapevine drought stress studies, this experiment selected a soil relative water content (SRWC) of  $35 \pm 5\%$ . This condition induces significant drought stress responses in grapevine plants while avoiding rapid plant mortality due to excessive stress. It also aligns with the internationally recognized moderate drought threshold (SRWC 30%–40%) proposed by Charaves et al. (2003). Preliminary trials further validated that under this stress intensity, the differences in drought resistance phenotypes between the '1103P' control and 'Muscat Hamburg' grapevines could be stably observed within 7 days.

To establish SA concentration settings, we referenced both the reported effective range of 0.1–2.0 mM for SA in alleviating drought stress in fruit trees (Khan et al., 2015; Diao, 2023) and our preliminary trial results; specific treatments are detailed in Table 1.

All treatments were applied via foliar spraying 24 hours prior to drought stress. For each plant, the corresponding concentration solution (distilled water or varying concentrations of SA solution)

TABLE 1 SA concentration treatment settings for grape plant drought stress.

No.	Treatment name	Soil relative moisture content	Foliar spray treatment 24 h before drought application
1	Normal Water Control (CK)	$75 \pm 5\%$	Pure water
2	Drought-stressed treatment (MD)	$35 \pm 5\%$	Pure water
3	T1 treatment	$35 \pm 5\%$	0.5 mM SA solution
4	T2 treatment	$35 \pm 5\%$	1.0 mM SA solution
5	T3 treatment	$35 \pm 5\%$	1.5 mM SA solution
6	T4 treatment	$35 \pm 5\%$	2.0 mM SA solution

The preparation method for SA solution is as follows: Accurately weigh salicylic acid (analytical grade, purity  $\geq 99.5\%$ , China National Pharmaceutical Group Chemical Reagent Co., Ltd.), dissolve it in a small amount of  $0.1 \text{ mol L}^{-1}$  NaOH solution, then add deionized water to adjust the volume to the desired concentration. Adjust the pH to 6.0 (matching the pH of 1/2 Hoagland nutrient solution).

was uniformly applied using a small sprayer (Deli DL581105A, Deli Group Co., Ltd., Zhejiang, China), ensuring thorough coverage of both leaf surfaces. Spraying was completed when fine droplets formed on the leaf surface without dripping.

Sampling occurred 7 days after drought stress initiation. The 3rd to 4th healthy functional leaves at the top of each plant were uniformly selected (these leaves have stable physiological activity and can serve as representative samples). First, the index content of fresh samples was determined; then, the leaves were quickly frozen and fixed in liquid nitrogen, and subsequently transferred to a  $-80$  °C refrigerator for storage. They were used for subsequent determination of physiological parameters such as osmoregulatory substances, antioxidant enzyme activities and ABA, MAPK and SA. Each treatment had 3 plants of consistent growth status planted (Sun, 2015).

## 2.3 Measurement parameters and methods

### 2.3.1 Physiological parameter measurements

Leaf Relative Water (RWC) (Bates et al., 1973; Song and Hong, 2001): Determined by the weighing method.

$$RWC = \frac{FW - DW}{TW - DW} \times 100\%$$

FW: Fresh Weight; DW: Dry Weight; TW: Total Weight.

Relative Electrical Conductivity (REC) (Charaves et al., 2003; Sun et al., 2014): Measured using a DDS-307 conductivity meter.

$$REC = \frac{R_1}{R_2} \times 100\%$$

$R_1$ : Initial Conductivity;  $R_2$ : Total Conductivity.

Osmoregulatory substances: Malondialdehyde (MDA) content was determined via the thiobarbituric acid (TBA) colorimetric method (Sun et al., 2014); superoxide anion ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ) contents were measured using conventional methods (Sun et al., 2014); proline content was assayed by the acidic indophenol colorimetric method (Bates et al., 1973).

Antioxidant enzyme activities: SOD activity was measured using the nitroblue tetrazolium (NBT) photoreduction method (Iqbal et al., 2025), POD activity was determined by the guaiacol method (Iqbal et al., 2025), and CAT activity was determined using ultraviolet spectrophotometry (Iqbal et al., 2025).

### 2.3.2 Signal pathways and SA-related indicator assays

ABA content determination: a 0.5 g frozen leaf sample was mixed with 5 mL of 80% methanol (containing 0.1% phosphoric acid), and ground into a homogenate on ice. The homogenate was extracted at 4 °C in the dark for 12 h. After extraction, the mixture was centrifuged at 4000 rpm for 15 min. The resulting supernatant was filtered through a 0.22 µm organic-phase membrane, and the filtrate was used for high-performance liquid chromatography (HPLC) analysis. For HPLC analysis, a C18 column (250 mm × 4.6 mm, 5 µm) was employed. The mobile phase consisted of methanol and 0.1% phosphoric acid at a volume ratio of 45:55, with a flow rate of 1.0 mL min<sup>-1</sup>. The detection wavelength was set at 254 nm, column temperature at 30 °C, and injection volume at 10 µL (Wang et al., 2003).

Cytoplasmic Ca<sup>2+</sup> concentration determination: the cytoplasmic Ca<sup>2+</sup> concentration was measured using the Fura-2/AM loading method. A fluorescence spectrophotometer was used for detection, with excitation wavelengths of 340 nm and 380 nm, and an emission wavelength of 510 nm. The Fura-2/AM probe has high specificity for Ca<sup>2+</sup> and no cross-reaction with other metal ions; the method was validated by measuring the Ca<sup>2+</sup> standard solution with known concentrations, and the linear correlation coefficient R<sup>2</sup> was 0.998. The limitation of this method is that it can only detect the total cytoplasmic Ca<sup>2+</sup> concentration and cannot distinguish the Ca<sup>2+</sup> concentration in different subcellular compartments (Zhao et al., 2023).

#### 2.3.2.1 MAPK activity determination

Mitogen-activated protein kinase (MAPK) activity was determined via enzyme-linked immunosorbent assay (ELISA). A plant MAPK activity detection kit (Beijing Solabio Co., Ltd.) was used, and all operations were performed strictly in accordance with the manufacturer's protocol. The kit targets the conserved region of plant MAPK protein and has cross-reactivity with most MAPK isoforms in grape, with a detection limit of 0.1 U mg<sup>-1</sup> protein and a precision CV < 5%; the method was validated by gradient dilution of the positive control, and the linear correlation coefficient R<sup>2</sup> was 0.995. The limitation of this method is that it cannot distinguish the activity of specific MAPK isoforms, nor can it directly verify the phosphorylation level of MAPK protein (Pan et al., 2015; Zhu et al., 2020).

#### 2.3.2.2 Endogenous SA content determination

A 0.5 g frozen sample was homogenized in 4 mL of a mixed solution of methanol and 0.1% glacial acetic acid (30:70, v/v) on ice. The homogenate was then extracted at 4 °C for 8 h. After extraction,

the mixture was centrifuged at 12,000 rpm for 20 min. The supernatant was purified using a C18 solid-phase extraction column, eluted with 5 mL of the same mobile phase (methanol-0.1% glacial acetic acid, 30:70, v/v), and filtered through a 0.22 µm membrane before HPLC analysis. HPLC separation was carried out on a C18 column (250 mm × 4.6 mm, 5 µm) with the aforementioned mobile phase, at a flow rate of 0.8 mL min<sup>-1</sup>. Detection was performed at 304 nm (Chen, 2015; Li et al. 2025a), and quantification was achieved using the external standard method with salicylic acid (SA) standard concentrations ranging from 0.1 to 10 µg g<sup>-1</sup>.

## 2.4 Data statistics and analysis

Data were statistically analyzed using SPSS 22.0 software (IBM Corporation, USA). All parameters had 3 biological replicates (each biological replicate contained 3 uniform plants, n=3 for biological repetition), and 3 technical replicates per biological replicate; leaves from the same biological replicate were pooled for determination to reduce individual plant variation. Results were expressed as mean ± standard deviation (SD). Significant differences among groups were assessed via two-way ANOVA followed by Duncan's multiple range test ( $P < 0.05$ ). Pearson correlation analysis explored links between signaling molecules and physiological indicators, with the sample size for correlation analysis being n=18 (6 treatments × 3 biological replicates); correlation coefficients (r) and exact P values were calculated, and significance was set at  $P < 0.05$  and  $P < 0.01$ . Figures were generated using GraphPad Prism 10.1.2 software (GraphPad Software, USA). Error bars represent SD of biological replicates; lowercase letters (a, b, c, etc.) denote intravarietal differences, asterisks (\*, \*\*, \*\*\*) and "ns" denote intervariatal differences. All units in the figures and tables were unified, and the unit description was added to the figure legends and table headers for clarity.

## 3 Results

### 3.1 Effects of drought stress and exogenous SA treatment on physiological parameters of grape one-year-old plants

#### 3.1.1 Responses of leaf relative water content and cell membrane permeability to drought stress and exogenous SA

Under normal water conditions (CK), both the drought-resistant rootstock '1103P' and the drought-sensitive cultivar 'Muscat Hamburg' exhibited turgid, deep-green leaves with no signs of wilting (Figure 1A). Physiologically, the leaf relative water content (RWC) in both cultivars remained above 80%, while the relative electrical conductivity (REC) was maintained at low levels, with no significant differences observed between the two genotypes (Figures 1B, C). These results indicate that under non-stressed conditions, both cultivars retained favorable water status and membrane integrity.

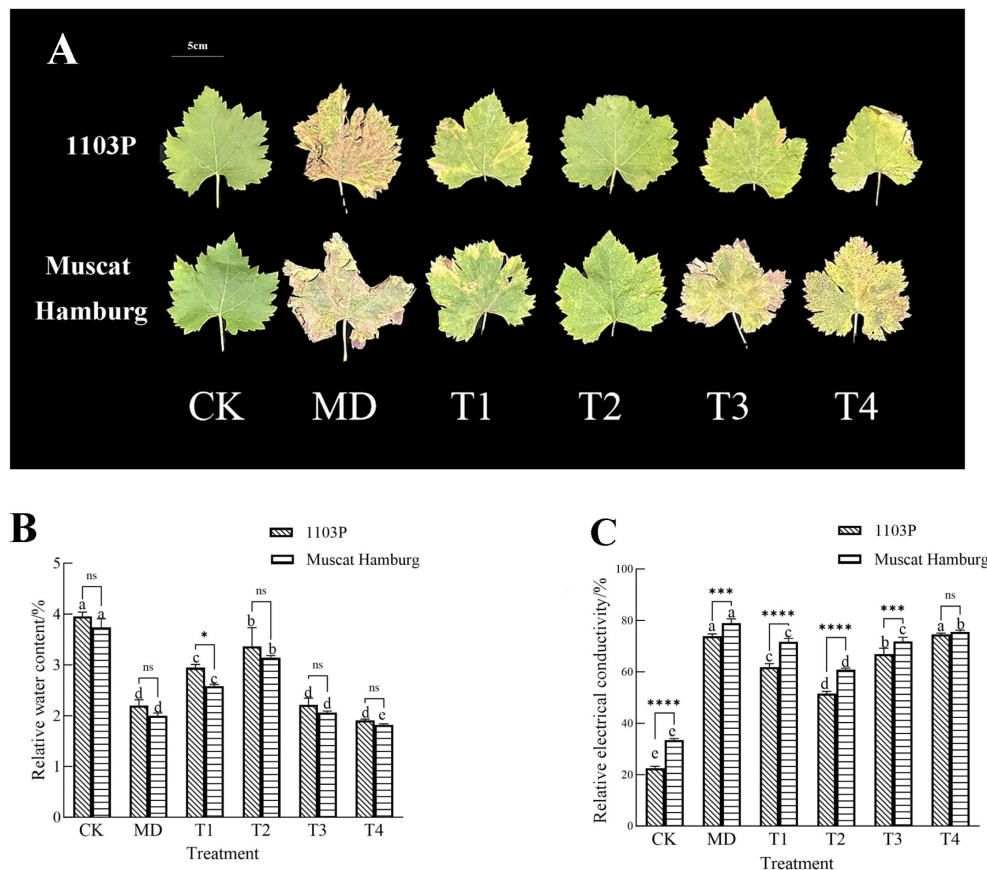


FIGURE 1

Effects of drought stress and exogenous SA on leaf relative water content and cell membrane permeability. CK: control group; MD: drought stress group; T1–T4: Treatment groups with different concentrations of exogenous salicylic acid (SA). (A) Phenotype of drought-stressed leaves of '1103P' and 'Muscat Hamburg' grapevines under different treatments. (B) Relative water content (RWC). (C) Relative electrical conductivity (REC). For the '1103P' control and 'Muscat Hamburg', differences between genotypes under the same treatment are indicated by asterisks (\*) and "ns" (no significant difference); different lowercase letters (a–e) above bars within the same genotype indicate significant differences among treatments ( $P < 0.05$ ).

Following drought stress (MD) treatment, RWC was significantly reduced and REC markedly increased in both cultivars ( $P < 0.01$ ). However, the extent of these changes was significantly greater in 'Muscat Hamburg' than in '1103P' ( $P < 0.05$ ; Figures 1B, C). Phenotypically, 'Muscat Hamburg' displayed severe wilting, loss of turgor, and leaf margin curling, whereas '1103P' maintained relatively better leaf condition despite visible stress symptoms (Figure 1A), demonstrating its superior drought tolerance.

Exogenous SA application alleviated drought-induced damage in a concentration-dependent manner. The 1.0 mM SA treatment (T2) was most effective, significantly increasing RWC and reducing REC compared to the MD group ( $P < 0.05$ ). Notably, T2 treatment restored RWC and REC in 'Muscat Hamburg' to levels statistically comparable to those of the drought-tolerant '1103P' (Figures 1B, C). Phenotypically, T2-treated 'Muscat Hamburg' plants showed recovered turgor, enhanced green coloration, and markedly reduced wilting, closely resembling the resistant control (Figure 1A). The alleviative effect of T2 was significantly stronger than that of T1 and T3 ( $P < 0.05$ ), while T4 treatment provided no significant improvement.

Drought stress markedly widened the phenotypic gap between the two cultivars in terms of leaf water status and membrane stability, while the optimal concentration (1.0 mM) of exogenous

SA narrowed this gap by enabling 'Muscat Hamburg' cultivar to converge physiologically and morphologically toward '1103P' grape, thereby enhancing its adaptability and drought resistance in water-deficient environments.

### 3.1.2 Responses of osmoregulatory substances and oxidative damage markers to drought stress and exogenous SA

Under well-watered conditions (CK), the leaf malondialdehyde (MDA) content of both the drought-resistant rootstock '1103P' and the drought-sensitive cultivar 'Muscat Hamburg' remained at low levels, with no significant difference between the two genotypes (Figure 2A), indicating intact cell membrane structures and minimal oxidative damage in the absence of stress. After drought stress (MD) treatment, the MDA content increased significantly in both cultivars ( $P < 0.01$ ), suggesting aggravated membrane lipid peroxidation. Notably, the accumulation of MDA was significantly higher in 'Muscat Hamburg' than in '1103P' ( $P < 0.01$ ), indicating that its cell membrane system is more sensitive to oxidative stress. Exogenous salicylic acid (SA) treatment suppressed MDA accumulation in a concentration-dependent manner, with the most pronounced effect observed under the T2 treatment (1.0 mM SA). In

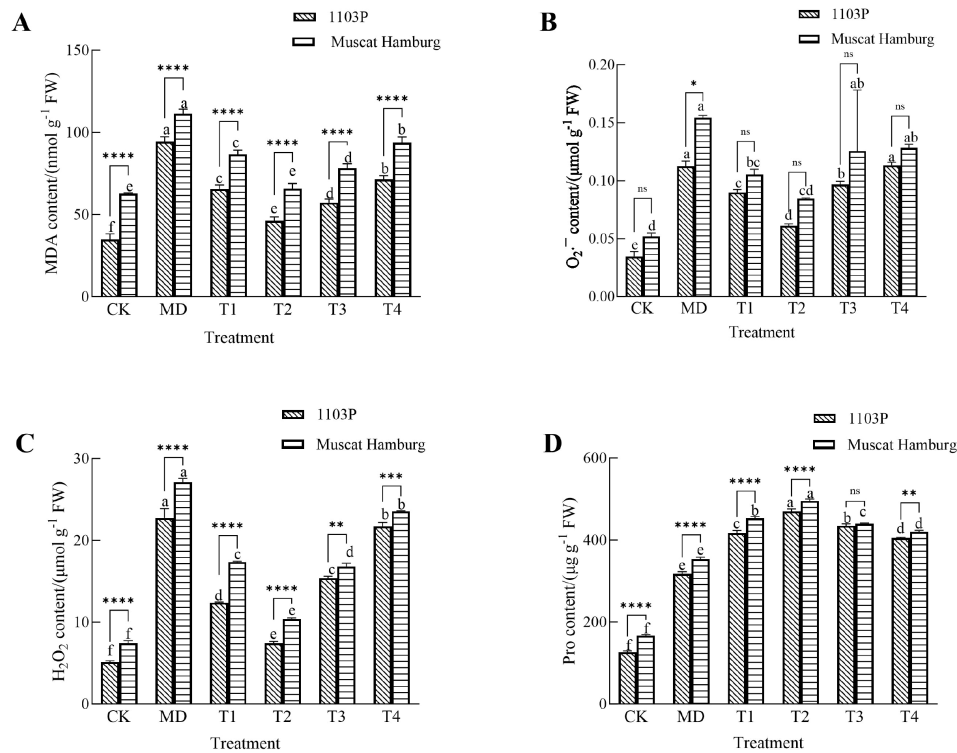


FIGURE 2

Effects of drought stress and exogenous SA treatment on oxidative damage indicators and osmotic regulatory substance contents in the '1103P' control and 'Muscat Hamburg' grapevine plants. (A) Malondialdehyde (MDA) content; (B) Superoxide anion ( $O_2^-$ ) content; (C) Hydrogen peroxide ( $H_2O_2$ ) content; (D) Proline content. CK: control group; MD: drought stress group; T1–T4: Treatment groups with different concentrations of exogenous salicylic acid (SA). Differences between different materials under the same treatment are indicated by asterisks (\*) and "ns" (no significant difference) to show the significance level; different treatment groups within the same material are marked with lowercase letters (a–e), where identical letters indicate no significant difference between groups, and different letters indicate significant differences ( $P < 0.05$ ).

'1103P', T2 treatment reduced MDA content by approximately 40% compared to the MD group, and this reduction was significantly greater than that in the T1, T3, and T4 treatments ( $P < 0.05$ ). In contrast, the T4 treatment did not exhibit a significant alleviating effect, suggesting that the mitigating effect of SA operates within an optimal concentration range. 'Muscat Hamburg' showed a response pattern to SA similar to that of '1103P', with significant mitigation observed within the 0.5–1.0 mM concentration range, while the 2.0 mM treatment had no notable effect. It is worth emphasizing that under the T2 treatment, the MDA content in 'Muscat Hamburg' decreased to a level statistically comparable to that of '1103P', indicating effective control of oxidative damage and a physiological state approaching that of the drought-resistant '1103P'.

The trends in superoxide anion ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ) contents were largely consistent with those of MDA (Figures 2B, C). Drought stress significantly promoted the accumulation of these reactive oxygen species (ROS) in both cultivars ( $P < 0.01$ ), with 'Muscat Hamburg' exhibiting higher accumulation than '1103P'. SA treatment, particularly at 1.0 mM, inhibited ROS accumulation. Under the T2 treatment, the difference in ROS levels between the two cultivars was markedly reduced, further supporting that an appropriate concentration of SA can alleviate drought-induced oxidative stress, leading the oxidative metabolism state of 'Muscat Hamburg' to approach that of the drought-resistant '1103P'.

In terms of osmotic adjustment, drought stress significantly promoted the accumulation of proline in the leaves of both

cultivars ( $P < 0.01$ , Figure 2D). Under both well-watered and drought conditions, the proline content of '1103P' was significantly higher than that of 'Muscat Hamburg', reflecting its inherently stronger osmotic adjustment capacity. Exogenous SA further enhanced proline synthesis, with the most notable increase under the T2 treatment, while the T4 treatment showed no significant difference from the MD group, indicating that excessively high SA concentrations may impede proline accumulation. Under T2 treatment, the proline content in 'Muscat Hamburg' increased to a level comparable to that of '1103P', demonstrating that SA application significantly improved its osmotic adjustment capacity, making its physiological characteristics closer to those of the drought-resistant '1103P'.

In summary, using the drought-resistant cultivar '1103P' as a reference, the effectiveness of the drought treatment applied in this study was confirmed. Moreover, it was demonstrated that 1.0 mM SA can significantly enhance the drought tolerance of 'Muscat Hamburg' by synergistically mitigating oxidative damage and improving osmotic adjustment.

### 3.1.3 Response of antioxidant enzyme activities to drought stress and exogenous SA

Under well-watered conditions (CK), the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in the leaves of both grape cultivars remained at stable

baseline levels (Figure 3), with no significant differences observed between the two genotypes. This indicates that their antioxidant systems were maintained in a basal metabolic state in the absence of stress.

Drought stress (MD) significantly enhanced the activities of all three antioxidant enzymes in both cultivars ( $P < 0.05$ ). In the drought-tolerant rootstock '1103P', SOD, POD, and CAT activities increased by 23.0%, 31.8%, and 48.3%, respectively, compared to the CK group. The drought-sensitive cultivar 'Muscat Hamburg' exhibited even more pronounced increases of 33.8%, 32.2%, and 46.3%, respectively. Despite this stress-induced upregulation, the SOD and CAT activities in 'Muscat Hamburg' remained significantly lower than those in '1103P' under MD treatment ( $P < 0.05$ ), reflecting its relative physiological vulnerability under oxidative stress.

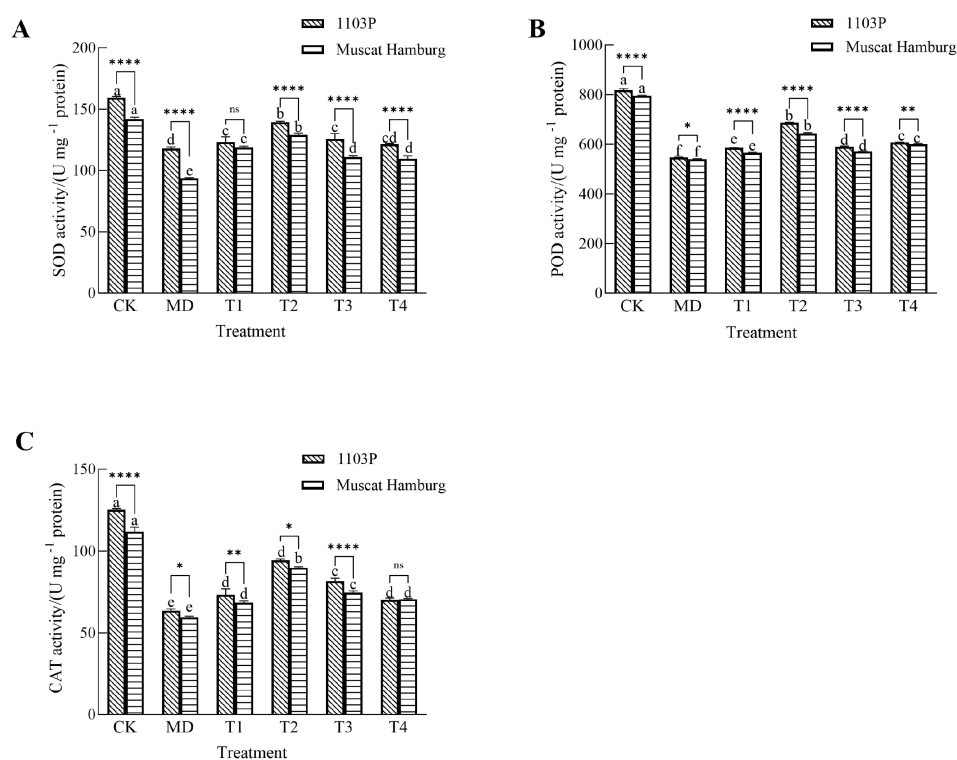
Exogenous SA application further enhanced antioxidant enzyme activities under drought conditions. In '1103P', the T2 treatment (1.0 mM SA) increased SOD, POD, and CAT activities by 20.0%, 18.0%, and 15.0%, respectively, compared to the MD group, with effects significantly stronger than those of T1 and T3 treatments ( $P < 0.05$ ). In contrast, the T4 treatment showed no significant difference from the MD group, indicating that the promotive effect of SA is confined to an optimal concentration range. Similarly, in 'Muscat Hamburg', the T2 treatment induced the most substantial upregulation, increasing SOD, POD, and CAT activities by 37.5%, 20.1%, and 51.0%, respectively, relative to the MD group ( $P < 0.05$ ).

Notably, under the T2 treatment, the activities of all three antioxidant enzymes in 'Muscat Hamburg' reached levels that were statistically indistinguishable from those in the drought-tolerant '1103P' (Figures 3A–C). This result demonstrates that an appropriate concentration of SA narrowed the physiological gap in antioxidant capacity between the two cultivars, enhancing the oxidative stress defense in 'Muscat Hamburg' and rendering its drought-responsive physiological traits closer to those of the tolerant rootstock '1103P'.

Drought stress enhanced the antioxidant defense capacity of grapevines by increasing the activities of SOD, POD, and CAT, and exogenous SA further strengthened this effect, with 1.0 mM identified as the optimal concentration. This treatment not only improved the antioxidant level of the drought-sensitive cultivar 'Muscat Hamburg' but also significantly reduced the physiological disparity between it and the drought-tolerant cultivar '1103P', providing a reliable theoretical basis for the application of SA in drought-resistant cultivation of 'Muscat Hamburg' grapevines.

### 3.2 Effects of drought stress and exogenous salicylic acid treatment on key molecules in the signaling pathways of annual grape plants

Exogenous salicylic acid (SA) exhibited a concentration-dependent associative regulation effect on ABA content. Under normal water conditions (CK), the abscisic acid (ABA) content in the leaves of the



**FIGURE 3** Effects of drought stress and exogenous SA treatment on antioxidant enzyme activities in the '1103P' control and 'Muscat Hamburg' grapevine plants. (A) Superoxide dismutase (SOD) activity; (B) Peroxidase (POD) activity; (C) Catalase (CAT) activity. CK: control group; MD: drought stress group; T1–T4: Treatment groups with different concentrations of exogenous salicylic acid (SA). Differences between different materials under the same treatment are indicated by asterisks (\*) and "ns" (no significant difference) to show the significance level; different treatment groups within the same material are marked with lowercase letters (a–e), where identical letters indicate no significant difference between groups, and different letters indicate significant differences ( $P < 0.05$ ).

drought-resistant rootstock '1103P' (control cultivar) and the drought-sensitive cultivar 'Muscat Hamburg' showed no significant difference (Figure 4A), both remaining at low levels. This indicates that under non-stressed conditions, endogenous ABA synthesis in both materials operates at a basal metabolic state. After drought stress (MD) treatment, the ABA content in both cultivars increased significantly ( $P < 0.001$ ), reaching  $170.7 \text{ ng g}^{-1}$  in '1103P' and  $184.94 \text{ ng g}^{-1}$  in 'Muscat Hamburg', with no significant difference observed between the two cultivars. This further confirms that ABA, as a core signaling molecule in drought response, can activate downstream osmotic adjustment mechanisms through its accumulation. Exogenous salicylic acid (SA) exhibited a concentration-dependent regulatory effect on ABA content (Figure 4A). T1 treatment showed no significant difference compared to the MD group; T2 treatment significantly increased the ABA content in both materials by 25.7%–28.3% compared to the MD group ( $P < 0.001$ ), reaching  $198.67 \text{ ng g}^{-1}$  and  $198.87 \text{ ng g}^{-1}$ , respectively, at which point the ABA level in 'Muscat Hamburg' converged with that of the drought-resistant control '1103P'; the promoting effect weakened with T3 treatment; T4 treatment showed no significant difference from the MD group. These results indicate that 1.0 mM SA promotes ABA synthesis in 'Muscat Hamburg', enabling the level of ABA signaling molecules in its ABA signaling pathway to approach that of the drought-resistant control.

Exogenous SA was associated with the regulation of cytosolic  $\text{Ca}^{2+}$  concentration in a concentration-dependent manner. As a key

second messenger in plant stress responses, the cytosolic  $\text{Ca}^{2+}$  concentration directly reflects the activation status of its signaling pathway (Figure 4B). Under normal water conditions, the  $\text{Ca}^{2+}$  concentration in '1103P' and 'Muscat Hamburg' showed no significant difference, maintaining a steady-state level. After drought stress, the  $\text{Ca}^{2+}$  concentration in both cultivars increased significantly ( $P < 0.001$ ), rising to  $482.0 \text{ nmol L}^{-1}$  in '1103P' and  $372.4 \text{ nmol L}^{-1}$  in 'Muscat Hamburg', representing increases of 164.3% and 108.0% compared to the CK group, respectively. A significant difference existed between the two cultivars ( $P < 0.05$ ), reflecting the disparity in their inherent drought resistance capacity. Exogenous SA regulation of cytosolic  $\text{Ca}^{2+}$  concentration also showed concentration dependence (Figure 4B). T1 treatment showed no significant difference from the MD group; T2 treatment significantly promoted  $\text{Ca}^{2+}$  accumulation, reaching  $577.27 \text{ nmol L}^{-1}$  and  $577.63 \text{ nmol L}^{-1}$ , respectively, elevating the  $\text{Ca}^{2+}$  response intensity in 'Muscat Hamburg' to a level comparable to '1103P'; the effect weakened with T3 treatment; T4 treatment showed no significant difference from the MD group. This demonstrates that 1.0 mM SA can significantly increase the cytosolic  $\text{Ca}^{2+}$  concentration in 'Muscat Hamburg', bringing the level of this key signaling molecule in the  $\text{Ca}^{2+}$  pathway closer to that of the drought-resistant control.

Mitogen-activated protein kinase (MAPK) transmits stress signals through phosphorylation cascades, and its activity reflects

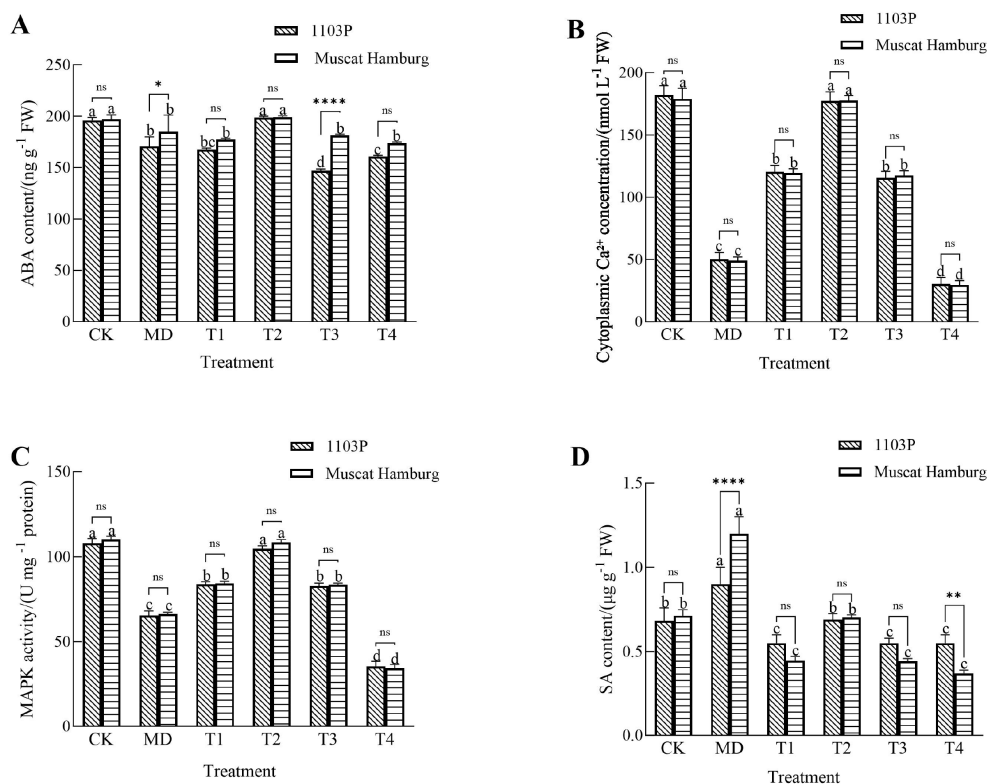


FIGURE 4

Effects of drought stress and exogenous SA treatment on key molecules in the Signaling pathway of grape plants. (A) Abscisic acid (ABA) content; (B) Cytosolic calcium ion ( $\text{Ca}^{2+}$ ) concentration; (C) Mitogen-activated protein kinase (MAPK) activity; (D) Endogenous salicylic acid (SA) content. CK: control group; MD: drought stress group; T1–T4: Treatment groups with different concentrations of exogenous salicylic acid (SA). Differences between different materials under the same treatment are indicated by asterisks (\*) and "ns" (no significant difference) to show the significance level; different treatment groups within the same material are marked with lowercase letters (a–e), where identical letters indicate no significant difference between groups, and different letters indicate significant differences ( $P < 0.05$ ).

the regulatory efficiency of this pathway, Exogenous SA was associated with the regulation of MAPK activity in a concentration-dependent manner (Figure 4C). Under normal water conditions, the MAPK activity in ‘1103P’ and ‘Muscat Hamburg’ showed no significant difference, remaining at a basal level. Drought stress induced a significant increase in MAPK activity in both cultivars ( $P < 0.001$ ), reaching 65.4 U mg<sup>-1</sup> protein and 66.2 U mg<sup>-1</sup> protein, respectively. Exogenous SA regulation of MAPK activity was also concentration-dependent (Figure 4C). T1 treatment showed no significant difference from the MD group; T2 treatment significantly increased the activity in both cultivars by 26.3%–28.5% compared to the MD group ( $P < 0.001$ ), reaching 104.63 U mg<sup>-1</sup> protein and 108.47 U mg<sup>-1</sup> protein, respectively, at which point the MAPK activity in ‘Muscat Hamburg’ showed no difference from ‘1103P’; the promoting effect weakened with T3 treatment; T4 treatment showed no significant difference from the MD group. This indicates that 1.0 mM SA can significantly increase the MAPK activity in ‘Muscat Hamburg’, raising the level of this key signaling molecule in the MAPK pathway to that observed in the drought-resistant cultivar.

Under normal water conditions, the endogenous SA content in the leaves of ‘1103P’ and ‘Muscat Hamburg’ showed no significant difference, remaining at low levels (Figure 4D). After drought stress, the endogenous SA content in both cultivars increased significantly ( $P < 0.05$ ), rising to 0.90 μg g<sup>-1</sup> in ‘1103P’ and 1.20 μg g<sup>-1</sup> in ‘Muscat Hamburg’, with a significant difference between the cultivars ( $P < 0.05$ ). Exogenous SA regulation of endogenous SA content also exhibited concentration dependence (Figure 4D): T1 treatment showed no significant difference from the MD group; T2 treatment significantly increased the endogenous SA content in both cultivars compared to the MD group ( $P < 0.001$ ), reaching 3.15 μg g<sup>-1</sup> and 3.36 μg g<sup>-1</sup>, respectively, promoting endogenous SA accumulation in ‘Muscat Hamburg’ to a level equivalent to ‘1103P’ and creating a synergistic signaling effect; the promoting effect weakened with T3 treatment; T4 treatment showed no significant difference from the MD group. These results demonstrate that 1.0 mM SA enhances the endogenous stress resistance signal level in ‘Muscat Hamburg’, bringing it closer to the drought-resistant control.

Integrating the response characteristics of key molecules across the three signaling pathways reveals that under drought stress, exogenous SA significantly enhances the drought response capacity of ‘Muscat Hamburg’ by being associated with the coordinated changes of the three core signaling pathways: ABA, Ca<sup>2+</sup>, and MAPK. Notably, under the 1.0 mM SA treatment, this sensitive cultivar exhibited a convergent trend with the drought-resistant rootstock ‘1103P’ across multiple signaling molecule levels, demonstrating a physiological enhancement of its drought resistance. The regulation by exogenous SA displayed distinct “pathway association characteristics”, not only elevating the levels of key molecules within individual pathways but also showing synergistic association changes between them. This provides an important theoretical basis and application potential for the drought-resistant cultivation of drought-sensitive grape cultivars such as ‘Muscat Hamburg’.

### 3.3 Correlation analysis of signaling molecules, endogenous SA, and physiological indicators

Pearson correlation analysis results showed that under 1.0 mM SA treatment, ‘Muscat Hamburg’ exhibited a convergent characteristic toward the drought-tolerant rootstock ‘1103P’ in the responses of multiple key physiological pathways and related indicators, systematically revealing the core regulatory role of SA in integrating plant signal transduction and physiological defense networks (Tables 2, 3).

Under drought stress, the relative water content (RWC) of leaves in ‘Muscat Hamburg’ was significantly negatively correlated with the contents of reactive oxygen species (O<sub>2</sub><sup>-</sup> and H<sub>2</sub>O<sub>2</sub>) ( $r = -0.94892$ ), and the relative electrical conductivity (REC) was also significantly negatively correlated with the activities of antioxidant enzymes (SOD, POD, and CAT) (e.g.,  $r = -0.90346$  between REC and SOD). This indicated that the cultivar suffered severe oxidative damage and significant decline in cell membrane system stability under drought conditions. However, after SA treatment, the strength and direction of the aforementioned correlations in ‘Muscat Hamburg’ were highly close to those of ‘1103P’, demonstrating that SA enhanced its reactive oxygen species scavenging capacity and cell membrane stability, making its performance in water retention and membrane protection approach that of the drought-tolerant control.

SA treatment not only improved the water and oxidative homeostasis of ‘Muscat Hamburg’ but also significantly enhanced the associative regulation efficiency between key signal molecules and physiological responses. After treatment, endogenous SA in this cultivar was positively correlated with abscisic acid (ABA) ( $r = 0.43625$ ,  $P < 0.05$ ) and showed a positive association trend with Ca<sup>2+</sup> and mitogen-activated protein kinase (MAPK), which was highly consistent with the correlation pattern of ‘1103P’ ( $r = 0.39886$ ,  $P < 0.05$ ). This suggested that SA may act as an upstream signal hub to synergistically strengthen downstream drought-resistant physiological responses through its association with plasma membrane Ca<sup>2+</sup> channel activity and MAPK phosphorylation cascade, in coordination with the ABA signaling pathway. Additionally, the correlation between SA and malondialdehyde (MDA), a product of membrane lipid peroxidation, in ‘Muscat Hamburg’ ( $r = 0.38208$ ,  $P < 0.05$ ) also approached the level of ‘1103P’ ( $r = 0.3998$ ,  $P < 0.05$ ) after SA treatment, further confirming that the function of SA in alleviating membrane lipid peroxidation was induced in this cultivar (Tables 2, 3).

This study further clarified the hub role of endogenous SA in the associative regulation of multiple drought-resistant signal pathways. As shown in Tables 2, 3, endogenous SA was not only positively correlated with ABA content (‘1103P’:  $r = 0.39886$ ,  $P < 0.05$ ; ‘Muscat Hamburg’:  $r = 0.43625$ ,  $P < 0.05$ ) but also showed positive associations with Ca<sup>2+</sup> and MAPK. This indicated that SA can be associated with the synchronous increase of Ca<sup>2+</sup> signaling and MAPK phosphorylation cascade-related molecule levels, thereby showing a synergistic association with the ABA pathway

TABLE 2 Correlation analysis of signaling molecules, endogenous SA, and physiological indicators in '1103P' grape plants.

Indicators	RWC	REC	MDA	O <sub>2</sub> <sup>-</sup>	H <sub>2</sub> O <sub>2</sub>	Pro	SOD	POD	CAT	ABA	Ca <sup>2+</sup>	MAPK
REC	-0.89176											
MDA	-0.78628	0.84138										
O <sub>2</sub> <sup>-</sup>	-0.96766	0.95277	0.88819									
H <sub>2</sub> O <sub>2</sub>	-0.94031	0.83748	0.91563	0.95221								
Pro	-0.5138	0.72609	0.26864	0.5309	0.29208							
SOD	0.89711	-0.9902	-0.8725	-0.97	-0.86362	-0.65008						
POD	0.85681	-0.98042	-0.8614	-0.93592	-0.81637	-0.65245	0.9871					
CAT	0.87064	-0.98687	-0.89881	-0.95937	-0.86485	-0.6383	0.99345	0.97606				
ABA	0.84328	-0.70995	-0.53013	-0.79753	-0.70197	-0.41036	0.75282	0.74544	0.67663			
Ca <sup>2+</sup>	0.90741	-0.78376	-0.87282	-0.92451	-0.98026	-0.24044	0.82226	0.74851	0.82761	0.6744		
MAPK	0.87412	-0.71689	-0.73367	-0.86537	-0.90867	-0.28189	0.7489	0.64446	0.75176	0.6461	0.96583	
SA	0.08068	-0.01192	0.3998	-0.01086	0.17562	-0.36416	0.02609	-0.02901	-0.04377	0.39886	-0.08185	0.10942

The significance levels for correlation analysis were set at  $P < 0.05$  and  $P < 0.01$ ,  $n=18$  (6 treatments  $\times$  3 biological replicates). The values in the table are Pearson correlation coefficients ( $r$ ); the closer the absolute value of the correlation coefficient is to 1, the stronger the correlation between the indicators.

to optimize downstream physiological defense responses. In summary, the significant negative correlation between cytoplasmic Ca<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> ( $r = -0.98026$  for '1103P',  $P < 0.01$ ;  $r = -0.9583$  for 'Muscat Hamburg',  $P < 0.01$ ) can serve as a potential molecular indicator for auxiliary screening of drought-tolerant grape germplasm. Combined with the optimal effect of 1.0 mM SA treatment confirmed in previous studies, the positive associations between SA and multiple signal pathways collectively support the feasibility of adopting SA pretreatment to systematically improve plant drought resistance in production. Furthermore, the significant positive correlation between MAPK and Ca<sup>2+</sup> in '1103P' ( $r = 0.96583$ ,  $P < 0.01$ ) points out the direction for subsequent research, where future work can focus on exploring Ca<sup>2+</sup> signal-regulated MAPK genes and their application potential in the genetic improvement of grape drought tolerance.

## 4 Discussion

This study systematically investigated the regulatory role of exogenous salicylic acid (SA) in enhancing the drought resistance of the drought-sensitive grape cultivar 'Muscat Hamburg'. Our results indicated that under moderate drought stress, exogenous SA significantly improved leaf relative water content, reduced relative electrical conductivity and malondialdehyde (MDA) content, while simultaneously increasing the activities of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), and promoting the accumulation of osmoregulatory substances like proline. These findings are highly consistent with previously reported beneficial effects of SA in alleviating abiotic stress in grapes and other crops (Sun et al., 2014; Khan et al., 2015; Diao, 2023). Khan et al. (2015) noted in

TABLE 3 Correlation analysis of signaling molecules, endogenous salicylic acid (SA), and physiological indicators in 'Muscat Hamburg' grape plants.

Indicators	RWC	REC	MDA	O <sub>2</sub> <sup>-</sup>	H <sub>2</sub> O <sub>2</sub>	Pro	SOD	POD	CAT	ABA	Ca <sup>2+</sup>	MAPK
REC	-0.92525											
MDA	-0.80331	0.79099										
O <sub>2</sub> <sup>-</sup>	-0.94892	0.9293	0.90114									
H <sub>2</sub> O <sub>2</sub>	-0.90411	0.85925	0.97617	0.94919								
Pro	-0.50829	0.72219	0.18081	0.4477	0.28578							
SOD	0.91893	-0.90346	-0.92775	-0.99544	-0.95707	-0.38727						
POD	0.86472	-0.978	-0.75641	-0.90155	-0.79866	-0.7263	0.88192					
CAT	0.90298	-0.97482	-0.88083	-0.94226	-0.90828	-0.59133	0.93359	0.9712				
ABA	0.83048	-0.73372	-0.65369	-0.68361	-0.73335	-0.36451	0.64741	0.67915	0.75782			
Ca <sup>2+</sup>	0.89264	-0.76941	-0.90047	-0.86401	-0.9583	-0.1983	0.86014	0.66216	0.80046	0.79477		
MAPK	0.86126	-0.713	-0.76616	-0.75604	-0.86268	-0.24463	0.73289	0.57475	0.70799	0.83512	0.96553	
SA	0.08872	-0.01008	0.38208	0.21456	0.2394	-0.33332	-0.29669	-0.03844	-0.09425	0.43625	-0.06688	0.1602

The significance levels for correlation analysis were set at  $P < 0.05$  and  $P < 0.01$ ,  $n=18$  (6 treatments  $\times$  3 biological replicates). The values in the table are Pearson correlation coefficients ( $r$ ); the closer the absolute value of the correlation coefficient is to 1, the stronger the correlation between the indicators.

their review that SA can enhance abiotic stress tolerance in various crops by activating the plant's antioxidant defense system, which aligns with the observed phenomenon of SA enhancing the antioxidant capacity in grapes in this study.

Under drought stress, plants often experience water deficit and oxidative damage, leading to increased membrane permeability and reactive oxygen species (ROS) accumulation (Charaves et al., 2003; Wang et al., 2023; Lehr et al., 2025). In this study, exogenous SA reduced relative electrical conductivity and MDA content, indicating its ability to maintain membrane integrity and reduce membrane lipid peroxidation. Notably, similar protective effects of SA have been observed in grapes under both high-temperature and low-temperature stress (Wang et al., 2003, Wang et al., 2023). Wang et al. (2023) found that SA treatment enhanced the heat tolerance of grape seedlings by increasing antioxidant enzyme activities, while Wang et al. (2023) reported that SA increased endogenous SA content and related gene expression in grape seedlings under low-temperature stress. These collective findings suggest that SA may enhance membrane stability under various abiotic stresses through a conserved mechanism.

The enhancement of antioxidant enzyme activities by exogenous SA further supports its role in strengthening the plant's antioxidant defense system. Our results are consistent with reports by Sun et al. (2014) and Wang et al. (2023), who found that SA treatment increased SOD and POD activities in grape seedlings under high-temperature and low-temperature stress, respectively. Sun et al. (2014) elaborated on the physiological mechanisms by which SA enhances heat tolerance in grape seedlings by modulating protein kinase properties, while this study further reveals the synergistic activation of multiple antioxidant enzymes by SA under drought stress, suggesting that SA may activate conserved antioxidant response pathways under different stress conditions.

Particularly noteworthy is that exogenous SA also promoted the accumulation of proline, a key osmoregulatory substance for maintaining cellular water balance and scavenging ROS (Bates et al., 1973). This is consistent with similar observations of proline accumulation following SA treatment in grape plants under drought stress reported by Diao (2023) and Li et al. (2025a). Diao (2023) systematically compared the effects of three exogenous hormones on the drought resistance of grape seedlings in their master's thesis, while Li et al. (2025a) revealed the molecular mechanisms of SA regulating endogenous SA accumulation in 'Beihong' grapes at the gene expression level. The synergistic increase in both proline and antioxidant enzymes highlights the dual role of SA in enhancing both osmoregulation and oxidative stress tolerance.

A key finding of this study is that under drought stress, exogenous SA was associated with the changes of multiple signaling pathways, including ABA,  $\text{Ca}^{2+}$ , and MAPK. The increase of endogenous ABA and  $\text{Ca}^{2+}$  levels, along with the rise of MAPK activity, suggests that SA may act as a core regulator integrating the associative changes of these pathways to coordinate drought defense responses. Similar cross-talk between SA, ABA, and  $\text{Ca}^{2+}$  signaling has been reported under stress conditions in other species (Song and Hong, 2001; Zhang et al., 2007; Beldapalazón et al., 2020). For instance, Cutler et al. (2010) systematically

elaborated the formation mechanism of the core ABA signaling network, while Zhang et al. (2007) detailed the mechanisms of  $\text{Ca}^{2+}$  signal transduction in plants. However, evidence for the associative regulation of these signaling pathways by SA in grapes remains limited. Our study is the first to confirm in grapes that SA can show coordinated association changes with three key signaling pathways—ABA,  $\text{Ca}^{2+}$ , and MAPK—at the physiological level, which is the main innovation of this study compared with previous research. This finding enriches the understanding of SA-mediated drought resistance in grapes and provides new insights into the associative activation characteristics of these pathways by SA in grapes, which may explain its broad-spectrum stress mitigation effects.

Correlation analysis further revealed close associations between signaling molecules and physiological indicators, particularly between  $\text{Ca}^{2+}$  and  $\text{H}_2\text{O}_2$ , and between MAPK and SOD. These relationships suggest that  $\text{Ca}^{2+}$  and MAPK signaling may play key roles in regulating ROS homeostasis and antioxidant defense, as previously proposed (Zhang et al., 2007; Pan et al., 2015). The stronger correlations observed in the drought-resistant rootstock '1103P' further support the view that efficient signal molecule response is a key determinant of drought resistance differences among genotypes (Song and Hong, 2001; Çakır and Kılıçkaya, 2015).

Despite these advances, this study has several limitations that need to be acknowledged: first, the experiment was conducted under controlled artificial climate chamber conditions, and the environmental factors (e.g., light intensity, temperature, humidity) are relatively stable, which is quite different from the complex and variable field conditions (e.g., wind, rainfall, soil heterogeneity). The effect of exogenous SA on grape drought resistance in the field may be affected by these environmental factors, and the optimal concentration may need to be adjusted according to the actual field conditions. Second, this study only used one drought-sensitive cultivar ('Muscat Hamburg') and one drought-resistant rootstock ('1103P') as experimental materials, and the results may not be fully applicable to other grape genotypes; future research needs to validate the findings across diverse grape cultivars and rootstocks. Third, this study only focused on the physiological level and the association of signal molecule levels, and did not analyze the molecular mechanisms such as ABA biosynthesis genes,  $\text{Ca}^{2+}$  channel regulation, and MAPK isoform specificity and phosphorylation level; these are the key directions for subsequent in-depth research.

Future research should first validate these findings under field conditions, and explore the combined application of SA with other plant growth regulators to improve the drought resistance of grapes. Second, expand the experimental materials to include more grape genotypes with different drought tolerance levels, to clarify the universal regulatory pattern of SA in grape drought resistance. Third, carry out molecular biology experiments to analyze the expression of key genes in ABA,  $\text{Ca}^{2+}$  and MAPK pathways, verify the phosphorylation level of MAPK protein and the activity of  $\text{Ca}^{2+}$  channels, and further reveal the molecular mechanism of SA regulating the associative changes of these signaling pathways under drought stress. Recent reports by Cao et al. (2025) on *CPK21* and *CPK23* conferring aluminum resistance through  $\text{Ca}^{2+}$ -dependent phosphorylation of *STOP1*, and Chen (2015)'s exploration of how  $\beta$ -aminobutyric acid regulates stomatal movement via ABA- and

SA-dependent signaling pathways, provide important references for in-depth analysis of the interaction between SA and other signaling molecules.

## 5 Conclusions

This study systematically investigated how exogenous SA enhances drought tolerance in the drought-sensitive grape cultivar 'Muscat Hamburg', using the resistant rootstock '1103P' as control. Under moderate drought stress, exogenous SA significantly improved leaf water status, reduced oxidative damage, enhanced antioxidant enzyme activities, and promoted proline accumulation, with 1.0 mM SA showing optimal efficacy. Furthermore, SA upregulated endogenous SA levels and was associated with the increased levels of key signaling molecules in ABA, Ca<sup>2+</sup>, and MAPK pathways, revealing their associative regulatory relationship under drought stress. Correlation analysis (n=18) confirmed close and significant associations between signaling molecules and physiological indicators, highlighting SA's integral role in coordinating drought defense mechanisms. This study is the first to reveal the integrated association of SA with three core stress signaling pathways in grape at the physiological level, which further enriches the physiological mechanism of SA regulating grape drought resistance. While this study provides valuable insights for drought-resistant viticulture, its limitations need to be fully recognized: the results were obtained under controlled laboratory conditions with only two grape genotypes, and lack of molecular mechanism verification. Future research should validate these findings across diverse grape germplasm and under field conditions, and carry out in-depth molecular biology experiments to explore the molecular mechanism of SA-mediated associative regulation of ABA, Ca<sup>2+</sup> and MAPK pathways. Further exploration of SA's synergy with other agronomic measures and molecular factors will facilitate developing more efficient and practical drought mitigation strategies for grape production.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

## Author contributions

KG: Conceptualization, Data curation, Software, Writing – original draft, Writing – review & editing. YZ: Software,

Writing – original draft. JC: Data curation, Writing – original draft. ZX: Resources, Supervision, Validation, Writing – review & editing. HZ: Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – review & editing. XW: Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – review & editing.

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## Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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